P001 / #2058

Topic: *AS01.1 Living with parasites, living without parasites*

COMPARATIVE SINGLE CELL TRANSCRIPTOMIC ANALYSIS OF THE MURINE CNS IN RESPONSE TO T. BRUCEI BRUCEI AND T. BRUCEI GAMBIENSE INFECTIONS

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Introduction: Chronic infection with Trypanosoma brucei, the causative agent of Human African trypanosomiasis (HAT), induces gliosis and neuroinflammation in the central nervous system (CNS).

Methods: Disease outcome varies greatly depending on the parasite subspecies; rhodesiense HAT is considered more aggressive, whereas gambiense HAT often causes a milder, subclinical infection. In this study, we used single cell transcriptomics to investigate CNS responses in murine models of infection with trypanosome causing subclinical or clinical infections (T. b. gambiense and T. b. brucei, respectively).

Results: We analysed ~20, 000 cells from the hypothalamus of naïve and infected mice and identified a total of 10 cell clusters with an ~500 genes/ cell and ~1,000 transcripts/cell, including microglia, astrocytes, vascular-associated cells, T and B cells. Of these, B cell with a regulatory phenotype (Ighm, Cd79a, Cd79b, Il10) and microglia with a robust pro-inflammatory phenotype (Il1a, Tgfbr1, Ifngr1, Il6ra, Il10ra) were abundant in T. b. brucei infection compared to T. b. gambiense. These observations are consistent with a reduced gliosis and neuroinflammation in T. b. gambiense infections compared to T. b. brucei infections. Furthermore, T. b. gambiense infection induces a progressive change in circadian activity without changes in clinical scoring, whereas T. b. brucei infection induces clinical symptoms that precedes changes in circadian behaviour.

Conclusions: Taken together, our data suggest that changes in circadian behaviour in T. b. gambiense infection may arise due to lowgrade CNS inflammation. Given the lack of clinical symptoms and based on the transcriptional and behavioural findings presented here, we propose that infection with T. b. gambiense is an ideal model to study how trypanosomes interfere with circadian rhythms without the need for pharmacological interventions. Our work provides important insights into mechanisms underlying the immunological responses of the CNS to different parasite subspecies, opening new avenues to investigate the molecular basis of HAT-associated circadian disorders.

Keywords: infection, CNS, West Africa

P002 / #1739

Topic: *AS01.1 Living with parasites, living without parasites*

LIVING WITH PARASITES: CONSERVATION AND UTILIZATION

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Introduction: Parasitic biological resources (living parasites, cells, genes, and data information) are essential materials for the development of biotechnology, human and animal health, and R&D in life sciences. Advances in molecular biology provide tremendous opportunities to acquire and transform these biological resources and to use them for the benefit of human beings. This is the time for us to invest and recover these biological resources, to explore them, and to make them accessible to scientific, economic and medical benefits.

Methods: Parasite Resource Bank (PRB) was established as a part of the National Biological Resources Bank in April 2005 and is operated by Prof. Keeseon S. Eom's team at the School of Medicine, Chungbuk National University. Currently, PRB has a research network with 27 overseas institutions on five continents, with a stockpile of more than 200,000 resources. In 2020, PRB expanded to the International Parasite Resource Bank (iPRB) with offices around the world to reach the researchers on the globe. So far, the iPRB is the world's unique parasite biobank which distributes parasite resources of frozen, living, fixed and also data information as well.

Results: In 2021, iPRB took the role of Caenorabditis elegans and Nematodes Bank (CeNbank) as well under the support of Korean government.

Conclusions: In the near future, a policy should be developed in response to the Nagoya Protocol and to activate the operational parameters in which iPRB functions and a systematic biobank integrated management system that reflects the unique characteristics of parasitic research resources. For operation of parasitic big data, strengthening the global cooperation on parasitic resource network and fostering domestic and foreign experts are necessary.

Keywords: living, Parasites, conservation, utilization, resources

P003 / #1341

Topic: *AS01.1 Living with parasites, living without parasites*

THE KUBIC FLOTAC MICROSCOPE: A NEW TOOL FOR HELMINTH EGGS DIAGNOSIS IN ANIMALS AND HUMANS

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Introduction: In last decades, several (semi-)automated systems have been developed to overcome gaps and limitations (i.e., human errors and time for analysis) of Faecal Egg Count (FEC) in veterinary and human fields. In this study a new automated system for diagnosis of helminth eggs in animals and humans is presented, the Kubic FLOTAC Microscope (KFM).

Methods: The KFM is a compact, portable digital microscope designed to analyse faecal samples prepared with the Mini-FLOTAC/FLOTAC in both field and laboratory settings. This system can be remotely controlled via software by smartphone, tablet or PC, or via internet it is possible to transfer the captured pictures to other laboratories, that could be very useful to create a network or to support operators directly in the field. Moreover, an Artificial Intelligence (AI) based predictive model was developed to perform an automated recognizing and counting of eggs. For this purpose, a dataset with 20,152 objects was used including gastrointestinal nematode eggs from ruminants, Trichuris vulpis and Toxocara canis eggs from dogs and Ascaris lumbricoides, Ancylostoma duodenale and Trichuris trichiura eggs from humans.

Results: The KFM combines the high sensitivity, accuracy and precision of the Mini-FLOTAC/FLOTAC techniques with a reliable AI system that was able to recognize the 99.0% of the eggs analysed.

Conclusions: Therefore, the KFM is a promising automated system for a rapid and accurate FEC to improve the diagnosis of veterinary and human parasitic infections.

Disclosure: GC invented the KFM, all the authors participated in the KFM development and validation, but this tool is not commercialized.

Keywords: Humans, automated system, diagnosis, Helminths, animals

P004 / #1042

Topic: *AS01.1 Living with parasites, living without parasites*

HIGH TOXOCARA CATI PREVALENCE AND ABUNDANCE IN WILD EURASIAN LYNX (LYNX LYNX) IN FINLAND, 1999–2015

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Introduction: We investigated prevalence and abundance of Toxocara cati in free-ranging, wild Eurasian lynx (Lynx lynx) in Finland throughout a time period when the estimated population size increased substantially.

Methods: The material for the study were T. cati nematodes collected from the intestines of 2756 lynx from Finland in 1999–2015.

Results: Toxocara cati was found in 2324 (84.3%) of the examined lynx. The prevalence was not dependent on the population size, but was high throughout the years, varying from 77.2% (95% CI 70.4–84.0) to 92.3% (95% CI 84.8–99.8). The infection was highly prevalent in both sexes and all age groups. The abundance of T. cati was significantly lower in older lynx ($p < 0.001$) than in younger lynx, and old females harbored a significantly higher number of T. cati nematodes ($p < 0.001$) than males of the same age group. There was a positive relationship between T. cati abundance and presence of cestodes ($p < 0.001$). The parameter k for parasite aggregation indicated highly aggregated parasite distribution.

Conclusions: The results showed that the zoonotic parasite T. cati was highly prevalent and abundant parasite throughout the study period, regardless of the changing host population size. Our study yielded new information about host-parasite dynamics, and illustrate that Eurasian lynx contribute to the circulation of T. cati in Finland. Reference: Virta, M., Huitu, O., Heikkinen, J., Holmala, K., & Jokelainen, P. (2022). High Toxocara cati prevalence in wild, free-ranging Eurasian lynx (Lynx lynx) in Finland, 1999–2015. International Journal for Parasitology: Parasites and Wildlife. 17, 205-210. https://doi.org/10.1016/j.ijppaw.2022.02.004

Keywords: Toxocara cati, Eurasian lynx, host-parasite dynamics

P005 / #358

Topic: *AS01.1 Living with parasites, living without parasites*

HUMAN ANTI-TOXOPLASMA ANTIBODIES ATTACH STRONGLY TO BREAST CANCER CELLS

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Introduction: Toxoplasma gondii (T.gondii) is the most common intracellular parasite in human population. The anti-cancer effect of this parasites has been shown which may be due to the presence of commons antigens between the parasite and cancer cells. In the this work the reaction of Toxoplasma positive human sera and Toxoplasma negative human sera with cell surfaces of 4T1 and MCF7 cell lines have been investigated.

Methods: 4T1 and MCF7 cells were harvested from cell cultures and treated with either human Toxoplasma Positive or negative sera. The reaction of the sera was then detected using flow cytometry method.

Results: Toxoplasma positive sera but not Toxoplasma negative ones reacted very sharply with both breast cancer cell lines.

Conclusions: Anti-T. gondii antibodies react strongly with breast cancer cells. These antibodies may be used for selective cancer immunotherapy in future.

Keywords: Mcf7 cells, 4T1 cells, toxoplasma, antisera

P006 / #1370

Topic: *AS01.2 Climate change, changing world, biodiversity, environments, sustainability*

CASE STUDY OF MALARIA IN AN INFECTIOLOGY DEPARTMENT

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Introduction: Malaria is a common vector-borne parasitic disease in Africa. The objectives of the study are to draw up an epidemiological assessment and to describe the different clinical, therapeutic and evolutionary aspects of the cases.

Methods: The retrospective study concerns adult patients hospitalized in the infectious diseases department for malaria in 2019. Patient data collected from the DPI (computerized patient file) relating to epidemiological data (stay in an endemic area, chemoprophylaxis), clinical, diagnostic (thick gout, blood smear) and therapeutic (antimalarials). An input mask is created and data analysis on Epi Info software version 6.

Results: We collected 59 cases of malaria, 56 men and 03 women, Average age: 25 years (Extremes: 15-41 years). Nationality of patients: Mali (25), Niger (17), Cameroon (8), Burkina-Faso (5) and Chad (4). Average duration of appearance of signs: 19 days (Extremes 4 days-2 months). No antimalarial prophylaxis taken (Significant factor: $p<0.01$). Uncomplicated malaria was frequent (44) versus severe malaria (11), pernicious access (04). Plasmodium Falciparum was the most frequent species (56) versus P.vivax (03). Parasitaemia was low (44), high (15). The average length of stay was 07 days (Extremes: 5-18 days): risk factor ($p<0.01$). Antimalarials were used according to the clinical forms: Mefloquine (44), Quinine IV (8) and Artesunate IV (7). Early treatment allowed a favorable outcome (56 cases) ($p<0.01$) and three deaths from infections associated with intensive care were recorded.

Conclusions: Malaria is a public health problem. Plasmodium falciparum is the most common species. Chemoprophylaxis is a determining factor. Early diagnosis and treatment provide a better prognosis.

Keywords: P.falciparum, simple malaria attack, pernicious attack, antimalarials

P007 / #745

Topic: *AS01.2 Climate change, changing world, biodiversity, environments, sustainability*

APPLICATIONS OF EXPLAINABLE AI APPROACH TO STUDY ENVIRONMENTAL FACTORS AND OVITRAP INDEX IN DENGUE TRANSMISSION HOTSPOTS

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Introduction: Dengue fever is one of the most important mosquito-borne diseases which brings huge burden in sub-tropical and tropical regions. The ecological complexity of dengue transmission is comprised of the interaction among virus, mosquito vector, and host. Multiple environmental conditions play critical roles to affect the spatial and temporal distributions of dengue transmission and vector abundance. Ovitrap is an economical method for surveillances of the seasonal dynamics of Aedes population. The Ovitrap index reflect both vector abundance and oviposition behavior which could be influenced by multiple environmental conditions.

Methods: Understand the environmental impacts on oviposition can help public health worker to modify vector control strategy to reduce transmission. However, the ecological phenomena between environment and vector abundance usually have non-linear patterns. In this study, we attempt to apply explainable AI approach to integrate XGBoost and Shapley Additive exPlanations (SHAP) method to evaluate the Ovitrap index by climate parameters in Tainan City of Taiwan throughout 2018-2021.

Results: The results indicated that the temporal peak of Ovitrap index appear one month earlier than the emergence of indigenous dengue cases historically. The SHAP values indicated that 27-degree C is a critical threshold for high Ovitrtap index. In addition, the interactions between precipitation and temperature at specific lag weeks demonstrate different scenarios on Ovitrap index.

Conclusions: The explainable AI approach can delineate clear associations between Ovitrap index and specific climate conditions. The information will be beneficial to resources allocation and control strategy modification.

Keywords: Ovitrap index, XGBoost, Dengue

P008 / #861

Topic: *AS01.2 Climate change, changing world, biodiversity, environments, sustainability*

CONTAMINATION OF THE SOIL IN SERBIA WITH PATHOGENIC PROTOZOA AND HELMINTHS AS A HUMAN AND ANIMAL PUBLIC HEALTH RISK

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Introduction: Contact with soil is an important risk factor for the spread of various parasitic diseases. Also, the presence of parasitic life forms which can remain viable in the soil for long periods is an important indicator of environmental contamination. We performed a first large-scale study of parasites in surface soil in Serbia.

Methods: A total of 1130 samples (250 g each) of surface soil were collected along a longitudinal SE-NW transect of Serbia of approximately 500 km, each taken 0.5 km apart, on three occasions between 2012 and 2018. Detection of parasitic (oo)cysts, ova and larvae was carried out using flotation with NaCl and $ZnSo_4$ (with and without Lugol staining), sedimentation (native, and with modified Ziehl-Neelsen staining), and the Baermann and Fülleborn methods. Mapping was performed using GIS.

Results: Even 91% of the samples (1029/1130) were positive for at least one parasite species. A total of 35 different taxa were identified, with Toxocara spp. detected in almost half (49%) of the samples, followed by Trichostrongylidae, Strongyloides spp., Ascaris spp., Dictyocaulinae, Protostrongylinae, and Fasciola spp. in 27%, 22%, 17%, 16%, 15% and 14%, respectively. Importantly, taenid-type ova were detected in over 11% of the samples. Of the protozoa, Eimeria spp. was detected in 16%, Balantidium spp. in 4.8%, Cryptosporidium spp. in 1.1%, and Giardia spp. in 0.7% of the samples. Epidemiological risk appraisal of these findings showed that the hazard was significant (medium and high) in less than 11% of the locations.

Conclusions: The contamination of soil with pathogenic protozoa and helminths is widespread in Serbia, but for most locations the epidemiological risk was estimated to be low.

Keywords: soil, pathogenic protozoa and helminths, parasite detection in soil, Environment

P009 / #600

Topic: *AS01.2 Climate change, changing world, biodiversity, environments, sustainability*

TRANSMISSION OF SCHISTOSOMES : SCALING UP THE COMPETENCE CONCEPT TO THE ECOSYSTEM LEVEL

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Introduction: Understanding the transmission dynamics of pathogens is urging. They primarily depend on the spatial and temporal distribution of their hosts and on their hosts' competence (i.e. the propensity to amplify pathogens at levels that are transmitted to other hosts). This prompted us to develop the concept of 'ecosystem competence' defined as the propensity of an ecosystem to amplify or mitigate an incoming quantity of pathogens that can be ultimately transmitted to their definitive hosts. We develop this concept using African schistosomes as model.

Methods: We conducted a deep literature review to document all biotic and abiotic factors of freshwater ecosystems that influence the transmission of schistosomes and identify technical tools to study 'ecosystem competence'. We also developed an eDNA-based metabarcoding tool to characterize trematode communities. Finally, we combined several eDNA tools with epidemiological and malacological monitoring at 9 transmission sites in Senegal to explore the effect of mollusk and trematode communities on the transmission of Schistosoma haematobium.

Results: Our literature review led to a complex picture of ecosystem competence with a crucial role of trematode and mollusk communities on the transmission dynamics of Schistosomes. Preliminary results from the field indicate low prevalences of S. haematobium in Bulinus truncatus and the presence of some trematode species that could interact with S. haematobium.

Conclusions: The 'ecosystem competence' concept and the developed eDNA-based tools will help a better assessment of emerging or transmission risk of schistosomiasis. We discuss the next technical developments that will improve the quantification of ecosystem competence.

Keywords: Schistosome, transmission, Ecosystem, biodiversity, One Health

P010 / #44

Topic: *AS01.2 Climate change, changing world, biodiversity, environments, sustainability*

PARASITISM BY METACERCARIAE MODULATES THE MORPHOLOGICAL, ORGANIC AND MECHANICAL RESPONSES OF THE SHELL OF AN INTERTIDAL BIVALVE TO ENVIRONMENTAL DRIVERS

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Introduction: Environmental variation alters biological interactions and their ecological and evolutionary consequences. In coastal systems, trematode parasites affect their hosts by disrupting their life-history traits. However, the effects of parasitism could be variable and dependent on the prevailing environmental conditions where the host-parasite interaction occurs.

Methods: This study compared the effect of a trematode parasite in the family Renicolidae (metacercariae) on the body size and the shell organic and mechanical characteristics of the intertidal mussels Perumytilus purpuratus, inhabiting two environmentally contrasting localities in northern and central Chile (ca. 1600 km apart).

Results: Congruent with the environmental gradient along the Chilean coast, higher levels of temperature, salinity and pCO_2, and a lower pH characterise the northern locality compared to that of central Chile. In the north, parasitised individuals showed lower body size and shell resistance than non-parasitised individuals, while in central Chile, the opposite pattern was observed. Protein level in the organic matter of the shell was lower in the parasitised hosts than in the non-parasitised ones regardless of the locality. However, an increase in polysaccharide levels was observed in the parasitised individuals from central Chile.

Conclusions: These results evidence that body size and shell properties of P. purpuratus vary between local populations and that they respond differently when confronting the parasitism impacts. Considering parasitism and identifying its effects on host species faced with environmental drivers is essential to understand and accurately predict the ecological consequences of climate change.

Keywords: trematode metacercariae, productivity/upwelling, Environmental variability

P011 / #933

Topic: *AS01.2 Climate change, changing world, biodiversity, environments, sustainability*

COMPARISON OF THE RT-QPCR TARGETING HSP70 MRNA AND FLOW CYTOMETRY FOR ASSESSMENT OF CRYPTOSPORIDIUM INACTIVATION BY SOLAR WATER DISINFECTION (SODIS)

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Introduction: Solar water disinfection (SODIS) is a simple, environmentally sustainable and inexpensive point-of-use treatment for improving the microbiological quality of drinking water. This work compares the techniques of reverse transcription-quantitative PCR (RT-qPCR) targeting heat induced hsp70 mRNA and inclusion/exclusion of the fluorogenic vital dye propidium iodide (PI) determined by flow cytometry to evaluate the Cryptosporidium survival in water samples exposed to solar radiation.

Methods: Quartz tubes containing 3 mL of distilled water were spiked with 1.5×10^6 oocysts of Cryptosporidium parvum, put into a reaction-jacketed borosilicate beaker filled 400 mL of distilled water and exposed to simulated solar light at intensities of UVA+B radiation of 30 and 40 W/m^2 and 30 and 40 °C, respectively. Every 2 h, aliquots of the samples were taken and incubated at 22 °C during 48 h. Then, the oocyst viability was determined by RT-qPCR, previous induction of the expression of hsp70 mRNA and isolation of mRNA by the oligo(dT)$_{25}$ method, and by inclusion/exclusion of the fluorogenic vital dye propidium iodide and flow cytometry.

Results: By RT-qPCR, log reductions in the oocyst viability of 2.46±0.00 and 4.80±0.36 were determined after 6 h of exposure to simulated solar light at UVA+B radiations of 30 W/m^2-30 °C and 40 W/m^2-40 °C. However, by flow cytometry, log reductions of 0.28±0.11 and 0.71±0.01 were observed after 6 h of exposure at 30 W/m^2-30 °C and 40 W/m^2-40 °C, respectively.

Conclusions: The RT-qPCR method targeting heat induced hsp70 mRNA is a more useful tool towards assessing the inactivation of Cryptosporidium oocysts by SODIS in comparison with flow cytometry since RT-qPCR method can discriminate viable from inactivated C. parvum oocysts over four orders of magnitude.

Disclosure: This study was funded by the European Union's Horizon 2020 Research and Innovation Programme (grant agreement number 820718).

Keywords: SODIS, survival, *Cryptosporidium*, hsp70 RT-qPCR, Flow cytometry

P012 / #934

Topic: *AS01.2 Climate change, changing world, biodiversity, environments, sustainability*

INFLUENCE OF THE PARTICLE SIZE IN THE REMOVAL OF CRYPTOSPORIDIUM PARVUM OOCYSTS FROM WATER BY GRANULAR ACTIVATED CARBON

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Introduction: Granular activated carbon (GAC) filters are commonly used as adsorption systems to remove micro-pollutants and natural organic matter. Depending of the process conditions, these filters could also eliminate pathogenic microorganisms at some extend. The aim of this study was to evaluate the capability of several fractions of granular activated carbon (GAC) in the removal of Cryptosporidium oocysts from water.

Methods: General test water was spiked with 10^5 oocysts of C. parvum per litre and filtered through a column loaded with a bed height of 35 cm of fresh GAC fractions corresponding to U.S. standard mesh sizes 14 (size range 1.70-1.40 mm), 18 (size range 1.18-1.00 mm) or 25 (size range 0.85-0.71 mm) at a flow rate of approximately 0.1 L/min. The filtrates were passed through nitrocellulose membranes (pore size, 3 µm) and the membranes were placed in re-sealable polyethylene bags and washed with phosphate buffered saline. The samples were centrifuged at 2000×g for 15 min and the number of oocysts was quantified in the sediments by a direct immunofluorescence test.

Results: Oocyst removal efficiencies of 0.70±0.18, 1.06±0.27 and 1.93±0.61 Log reductions were determined for GAC fractions of U.S. standard mesh sizes 14, 18 and 25, respectively, observing statistically significant differences ($P<0.001$) between the fraction 25 and the other two fractions evaluated.

Conclusions: Considering the results of the experiments and the filtration conditions, the oocysts of C. parvum were removed significantly by using GAC fraction 25. Therefore, GAC adsorption filters can be additional serious barriers against this waterborne protozoan parasite in water treatment facilities.

Disclosure: This study was funded by the European Union's Horizon 2020 Research and Innovation Programme (grant agreement number 820718).

Keywords: Adsorption, Water treatment, Cryptosporidium, Granular activated carbon

P013 / #734

Topic: *AS01.2 Climate change, changing world, biodiversity, environments, sustainability*

HIGHER TEMPERATURES REDUCE THE NUMBER OF TRYPANOSOMA CRUZI PARASITES IN THE VECTOR TRIATOMA PALLIDIPENNIS

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Introduction: Relatively little is known about how pathogens transmitted by vector insects are affected by chang- ing temperatures analogous to those occurring in the present global warming scenario. One expectation is that, like their ectothermic vectors, an increase in temperature could reduce their fitness. Here, we have investigated the effect of high temperatures on the abundance of Trypanosoma cruzi parasites during infection in the vector Triatoma pallidipennis.

Methods: We subjected the Chagasic bug Triatoma pallidipennis to two strains of the parasite Trypanosoma cruzi, Morelos and Chilpancingo. Previous studies have indicated that these strains differentially affect the fitness of Triatoma pallidipennis. Once infected, the fifth instar bed bugs were distributed into three groups with different temperatures, 20, 30 and 34° C, and the resulting parasites were counted when the bed bugs reached the adult stage.

Results: The number of parasites increased linearly with time at 20 °C and, to a lesser extent, at 30 °C, especially in the Chilpancingo compared to the Morelos strain. Conversely, at 34 °C, the number of parasites of both strains decreased significantly compared to the other two temperatures.

Conclusions: These results suggest negative effects on the abundance of Trypanosoma cruzi in Triatoma pallidipennis at high temperatures. This is the first evidence of the effect of high temperatures on a pathogenic agent transmitted by an insect vector in the context of global warming. Further tests should be done to determine whether this pattern occurs with other triatomine species and Trypanosoma cruzi strains.

Keywords: Triatoma pallidipennis, Trypanosoma cruzi, Global warming, Temperature, Parasites

P014 / #860

Topic: *AS01.2 Climate change, changing world, biodiversity, environments, sustainability*

SITE DISTRIBUTION ANALYSIS OF MITES FROM RHYNCHOPHORUS FERRUGINEUS OLIVIER, 1790 (COLEOPTERA: CURCULIONOIDEA): FIRST REPORT IN PORTUGAL

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Introduction: The red palm weevil (RPW), Rhynchophorus ferrugineus Olivier, 1790, is native to Southeast Asia and has been spread beyond its native habitat, becoming a significant pest of the Canary Island date palm (Phoenix canariensis). This weevil is associated with mites whose interaction type is yet to be unravelled. We aimed to document the presence and distribution of mites on RPW in Northern Portugal.

Methods: Pheromone traps were placed across 4 districts of Northern Portugal (Porto, Aveiro, Braga, and Viana) from July 2021 to January 2022, amounting to 174 adult weevils. Weevils were frozen, dissected and inspected for mites. Mites were counted and identified under a microscope.

Results: The prevalence in RPW was 100%. All body parts of the host were associated with mites, but the highest average intensity was found under the elytra with 308 mites per weevil (mpw). While the abdomen had average 72 mpw and the remaining body parts only 24 mpw. We found 5 species and 2 undetermined species of mites: Centrouropoda sp., Curculanoetus rhynchophorus, Uroobovella sp., Acarus sp. and Mesostigmata Type 1 were more prevalent on the wings and elytra, while Nenteria sp. and Mesostigmata type 2 were, respectively, more prevalent on the head and antenna and neck.

Conclusions: Our study indicated a 100% prevalence and a high parasitic diversity of RPW-associated mites. It also showed the wings and elytra as the main infection sites. Our findings suggest a possible compromise of RPW fitness due to high-intensity levels of mites per weevil.
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Keywords: invasive, Phoenix canariensis, mite, rhynchophorus ferrugineus, phoresis

P015 / #891

Topic: *AS01.2 Climate change, changing world, biodiversity, environments, sustainability*

IDENTIFICATION OF PATHOGENIC FUNGI ASSOCIATED WITH THE RED PALM WEEVIL-MITE COMPLEX IN PORTUGAL

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Introduction: The red palm weevil (RPW), Rhynchophorus ferrugineus Olivier, 1790, native of Southeast Asia, has spread to become a pest of the Phoenix canariensis palm tree. This weevil, and its associated mites, are known to carry fungi. We aim to detail the fungi species present on the RPW and its associated mites in Portugal.

Methods: RPW specimens were collected via pheromone traps in northern Portugal from July 2021 to January 2022 in 4 districts. Fungal spores and mycelium from weevils and mites with visible fungal growth were placed in potato dextrose agar plates and strains were isolated through serial plating. Fungal species were identified based on morphology and ITS2 rDNA sequences.

Results: A total of 4 fungal genera were found, i.e. Scopulariopsis sp., Alternaria sp., Fusarium sp., and Penicillium sp.. Scopulariopsis sp. is known to protect the tick Dermacentor variabilis against Metarhizium anisopliae infection while, on the other hand, it can also be pathogenic to other mites. Alternaria sp. and Fusarium sp. are plant and human pathogens, and Penicillium sp. has been found to grow better on chitin rich-medium, indicating its potential as an entomopathogen.

Conclusions: Our findings suggest that the RPW and its parasitic mites can be vectors of fungi that can be pathogenic to plants, humans, and their own parasites. On the other hand, they can carry fungi that may compromise their own populations, suggesting prospective novel biocontrol agents. In general, the study of fungi associated with the RPW-mite parasitic complex could be a research field with broad potential in both the field of biocontrol and pathogen spread. Acknowledgements: FCT - PTDC/ASP-PLA/6228/2020, PTDC/ASP-PLA/6228/BI_Lic_2021-019, CEECIND/03501/2017, UIDB/04423/2020, UIDP/04423/2020.

Keywords: mycobiome, rhynchophorus ferrugineus, fungi

P016 / #906

Topic: *AS01.2 Climate change, changing world, biodiversity, environments, sustainability*

WORLD DISTRIBUTION OF MITES ASSOCIATED WITH RHYNCHOPHORUS FERRUGINEUS OLIVIER, 1790 (RED PALM WEEVIL)

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Introduction: Rhynchophorus ferrugineus Olivier, 1790, commonly known as Red Palm weevil (RPW), is a major pest of palm trees and is native to South-East Asia. Upon its arrival to Europe, it has shown preference for the Canary Island date palm (Phoenix canariensis). This weevil has interactions with several organisms. Although mites associated with the RPW are often considered phoretic organisms, several studies have shown that some mites might cause harm to this weevil's life cycle. We aim to review and document the world distribution of mite species in RPW.

Methods: 36 scientific papers from 1981 to 2020 about mites associated with RPW were analyzed. Information about the mites, such as family, genera, species, locality and year of the collection, was compiled in a summary table.

Results: According to our analysis, associated mites were found in 18 countries, Egypt being the one with the highest species diversity (31 species). A total of 49 mite species, from 20 different families were identified. The most diverse families were Uropodidae with 6 species, and Urodinychidae and Laelapidae with 5 species each. Centrouropoda almerodai and Uroobovella marginata were reported in 8 and 7 different localities respectively.

Conclusions: We found that the same mite species are associated with RPW in several different localities. Our results suggest that mites have been reallocated from their original location while attached to the RPW. Acknowledgements: FCT - PTDC/ASP-PLA/6228/2020, PTDC/ASP-PLA/6228/BI_Lic_2021-019, UIDB/04423/2020, UIDP/04423/2020.

Keywords: rhynchophorus ferrugineus, mite, world distribution

P017 / #1300

Topic: *AS01.2 Climate change, changing world, biodiversity, environments, sustainability*

INSIGHTS ON THE ACAROFAUNA OF BUMBLEBEES (BOMBUS SPP) - AN ICELANDIC PERSPECTIVE

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Introduction: Bees are important pollinators for natural and agricultural ecosystems. In exchange for pollination, plants provide the bees with food in the form of pollen and nectar. Mites have been observed on bumblebees (Bombus spp) and in their nests for centuries. The ecological relationship between bumblebees and mites varies in form and complexity and may range from phoresy to parasitism. Iceland is an island that lies in the Atlantic Ocean, where the acarofauna of domestic bumblebees is poorly known. The aim was to identify mite species associated with bumblebees, examine their role in the nests and evaluate if potentially harmful species are present.

Methods: In spring 2017, fifty-three bumblebee queens were collected in the Greater Reykjavík area. Moreover, a few bees were collected in the East of Iceland. Each queen and associated mites were immediately fixed in 70% EtOH. The mites were then collected and fixed on a slide in Hoyer‘s medium to be microscopically-identified based on morphology.

Results: Five mite species were identified, two of which have previously been recorded from Iceland. Most are mutualists while others are considered kleptoparasites. When in low numbers, they present positive effects for bumblebee nests. High infestations, on the contrary, may be harmful to bumblebees causing food depletion within nests.

Conclusions: All five bumblebee species in Iceland are believed to have arrived by means of anthropogenic importation. With rising temperatures and continuing importation, an establishment of additional bumblebee species in Iceland, together with their tiny counterparts, is expected within the next decades. Future research on bumblebee mites throughout Iceland is necessary along with areas close to major importation sites such as ports and airports.

Keywords: Global warming, Acari, Bombus spp., Kleptoparasitism

P018 / #920

Topic: *AS01.2 Climate change, changing world, biodiversity, environments, sustainability*

TRANSCRIPTOMIC DIFFERENCES IN L3 AND L4 STAGE LARVAE OF ANISAKIS SIMPLEX S. S. FROM TWO DIFFERENT GEOGRAPHIC POPULATIONS – MRNA AND LONG NON-CODING RNA EXPRESSION PATTERNS

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Introduction: Anisakis simplex is a parasitic nematode of marine mammals. Humans can become infected by eating sea food contaminated with parasitic larvae. The ability of parasites to adapt to the environment or host is influenced by gene flow between populations and internal genetic drift. Therefore, estimating transcriptomic differences between populations is key to understanding the microevolutionary process in these taxa.

Methods: In this study, we employed high-throughput (Illumina) sequencing and bioinformatics methods to characterize the transcriptomes of two Anisakis simplex stages, L3 and L4, from two different geographical regions (Baltic and Atlantic) and described populational differences between the developmental stages.

Results: An average of 65,000,000 reads for each stage from different geographic regions were obtained from the generated cDNA libraries and assembled into ~61,000 transcripts. Comparative analyses identified 1,216 upregulated and 201 downregulated differentially expressed genes (DEGs) between Baltic and Atlantic L3 larvae. Furthermore, 2,532 up- and 2,519 downregulated DEGs were identified between Baltic and Atlantic L4 larvae. In addition, approximately 450 up- and 100 downregulated lncRNAs were identified. An analysis of the interactions between the identified mRNAs and lncRNAs was performed.

Conclusions: To our knowledge, the results presented here show for the first time the differences between developmental stages of A. simplex s. s. from two geographic populations. The results obtained could be of great value in systems biology to determine adaptations to environmental differences as well as to different hosts. Funding source: This work was supported by National Science Centre of Poland, grant no. 2018/31/B/NZ9/01683.

Keywords: Anisakis simplex, mRNA, long non-coding RNA, populational differences

P019 / #1375

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

ASCARIS INFECTION BUT NOT HOOKWORM IS ASSOCIATED WITH INCREASED WHEEZING ODDS IN CHILDREN AGED 6-14 YEARS OLD IN COMÉ, BÉNIN

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Introduction: Wheezing is a major symptom of asthma. Biomass combustion produces high levels of indoor pollution. We investigated association between helminth infection and wheezing (asthma) in Comé, Benin, taking into account the role of indoor air pollution.

Methods: We conducted a cross sectional survey among 964 children aged 6-14 years old in Comé, from August 26 to October 25, 2020, using an electronic questionnaire. It included the wheezing module of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire, a module on allergy predisposing factors and a module on exposure to indoor air pollution. Data on helminth infection were from the baseline Deworm3 study of the Deworm3 longitudinal cohort, conducted in April 2018. Association was assessed using logistic regression.

Results: The prevalence of current wheezing was 4.8%. The prevalence of helminths infection was 5.7%, with 3.2% of infection by roundworms and 1.8% of infection by hookworms. The main cooking fules used were wood (62.4%) and charcoal (53.7%). Univariate logistic regression showed an association between Ascaris and wheezing. Crude Odds ratio =6.9 ; 95%CI (2.8-17.2). Hookworm was not associated with wheezing. Crude OR=1.3 ; 95%CI (0.2-9.9). After ajusting on cooking fuels, tobacco smoking, allergen exposure, ascaris infection remained associated with wheezing ; adjusted odds ratio (aOR) =4.3 ; 95%CI (1.5-12.0). Other variables remaining in the final model were overweight aOR=9.7 ; opened versus Improved cookstove aOR=3.9 ; palm cakes for fire induction aOR= 3.4 and contact with house animals (dogs, cats, rodents) aOR= 2.5.

Conclusions: Deworming children or eliminating helminths could decrease the burden of wheezing in low income countries.

Keywords: indoor air pollution, Ascaris, asthma, wheezing

P020 / #700

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

MAPPING THE DISTRIBUTION OF THE ECTOPARASITE HESPEROMYCES VIRESCENS ASSOCIATED WITH HARMONIA AXYRIDIS LADYBIRDS IN EUROPE USING CITIZEN SCIENCE DATA

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Introduction: The harlequin ladybird *Harmonia axyridis* is considered one of the world's worst invasive alien species. Native to Asia, it was introduced as a biocontrol agent against aphids and scale pests in many countries and has since spread and established on all continents, including Europe. The fungal ectoparasite *Hesperomyces virescens* causes mortality of its ladybird hosts, which has sparked an interest for its potential to control invasive populations of *H. axyridis*. Our work aims to map the distribution of *He. virescens* associated with *H. axyridis* in Europe.

Methods: A database was created with records of *He. virescens* on *H. axyridis* in Europe. Records were obtained from online citizen science projects (iNaturalist, BugGuide) and photo-sharing platforms (Flickr) as well as from the published literature. To encourage citizen science contributions of *He. virescens* associated with *H. axyridis*, a social media campaign (Facebook, Twitter and Instagram) was initiated.

Results: The first record of *He. virescens* on *H. axyridis* in Europe was reported in Belgium in winter 2007. Since then the fungus has been observed on *H. axyridis* in 21 European countries. A dedicated project was launched in iNaturalist. A distribution map of curated records is under development and will be made available through https://beetlehangers.org/.

Conclusions: Citizen science data are increasingly important to monitor invasive alien species and their interactions with natural enemies, such as the ectoparasitic *He. virescens*.

Keywords: Harmonia axyridis, ectoparasitic fungi, citizen science, Laboulbeniales

P021 / #985

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

EXTERNAL CHALLENGES AND CO-INFECTION WITH TRY. CRUZI ENHANCES TOLERANCE WHILE DIMINISHED RESISTANCE AGAINST TRI. SPIRALIS ON LABORATORY RATS.

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Introduction: There are two intrinsically different defence strategies that the host implements in order to face parasite infection. A host may resist infection by fighting the intruder or it may tolerate its presence by minimizing the damage on health condition. The aim was to evaluate the effect of different scenarios of external challenges (food restriction, social conflict and co-infection with Trypansoma cruzi) on defence strategy against the nematode Trichinella spiralis on laboratory rats.

Methods: We used a total of 32 adult male rats (Rattus norvegicus, WISTAR/Cmedc) in each of two experiments that lasted for ten weeks. The experiments were defined by the combination of parasites: Tri. spiralis alone or co-infecting with Try. cruzi. After four weeks of exposure to food restriction (FR), social conflict (SC), both (FR+SC) or control (C), rats were inoculated with parasites. Tolerance was quantified as the slope of the regression between body weight change and Tri. spiralis intensity. Resistance was quantified as total Tri spiralis intensity.

Results: FR rats were less tolerant in mono- infection compared to co-infection. On the other hand, resistance was higher in SC rats, but only in absence of Try. cruzi.

Conclusions: FR had a negative effect on health condition, which influenced its capacity to tolerate nematodes as parasite intensity enhanced. However, the effect of Tri. spiralis intensity on host health was attenuated in co-infection. Moreover, Try. cruzi presence reduced resistance in SC rats, while enhanced tolerance in FR rats. Taken together, these results may indicate a shift on defence strategies in rats exposed to nematodes depending on the type of external challenge and on concomitant infections.

Keywords: Food restriction, Social conflict, resistance, Tolerance, Rodents

P022 / #564

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

ORTHOHPI 2.0: A RENEWED AND EXTENDED INTERACTIVE RESOURCE OF PREDICTED HUMAN-PARASITE PROTEIN-PROTEIN INTERACTION NETWORKS

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Introduction: The study of molecular human-parasite interactions is essential to understanding parasitic infection and adaptation and contributes to the development of new treatments. To this end, we developed OrthoHPI (PMID:30815000), a resource that provides homology-derived predictions of host-parasite protein-protein interactions (PPI). This work renews and extends OrthoHPI by integrating new versions of databases, predictors, and proteomes of 24 eukaryotic parasites such as apicomplexa and trypanosomatids allowing the comparison of the resulting networks.

Methods: The method uses orthology to predict host-parasite PPIs from intra-species interactions. Our approach filters human proteomes according to expression on tissues relevant to the parasites and it uses host and parasite proteins located in defined subcellular compartments. We collect orthologous proteins for both filtered proteomes and transfer the intra-species interactions to the human-parasite system.

Results: The resulting PPI networks were evaluated by linking enriched biological processes and pathways in these networks with the specific biology of each parasite. We identified relevant human and parasite proteins that may have a critical role in the molecular crosstalk. To make these predicted interactomes available, we built an interactive web interface.

Conclusions: We provided 24 predicted human-parasite interactomes and highlighted biological processes, pathways, and tissue-specific interactions that may be essential in the life cycle of the parasites.

Keywords: biological networks, host–parasite interactions, Data integration, systems biology

P023 / #459

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

INFLAMMASOME AND ROS SIGNALING CASCADES DURING MICROFILARIAE-INDUCED EOSINOPHIL ETOSIS.

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Introduction: Eosinophils and their cytotoxic granules are important for protective immunity against filariae. The release of those granules can be mediated through extracellular DNA trap cell death (ETosis), where intracellular DNA is explosively released, entrapping pathogens and supporting their killing. The aim of this study was to identify eosinophil ETosis (EETosis) in response to filariae and its underlying mechanism.

Methods: In vitro co-culture assays with Litomosoides sigmodontis microfilariae (MF) were done with bone marrow-derived eosinophils from immunocompetent mice and mice deficient for caspase-1, AIM2 or NOX2. Using fluorescence and electron microscopy, DNA quantification, chemiluminescence and fluorescence assays, DNA release and reactive oxygen species (ROS) production was analyzed. In vivo, MF coated with and without DNA traps were injected intravenously into naïve mice and blood MF clearance was observed.

Results: The in vitro results of the present study demonstrate that MF trigger DNA release by eosinophils in a dectin-1-dependent manner. Analyzing the underlying signaling cascade revealed that MF-induced EETosis is dependent on the AIM2 inflammasome including caspase-1 signaling. Furthermore, we demonstrate that MF trigger intracellular ROS production and release DNA traps in an NADPH oxidase-dependent manner. MF motility in vitro is inhibited by traps and in vivo MF that were covered by eosinophil DNA traps were removed faster from the peripheral circulation than MF that lack DNA trap coating, suggesting a potential role of ETosis in MF clearance.

Conclusions: In summary, these results identify EETosis as an important mechanism in protective immunity against MF and reveal the underlying signaling mechanism during eosinophil DNA release.

Keywords: Inflammasome, ROS, Filariae, Eosinophils, ETosis

P024 / #1249

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

VIRAL ENTRY INTO CATERPILLAR BRAINS TO UNDERSTAND VIRUS-INDUCED HYPERACTIVITY

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Introduction: Parasitic alteration of host behaviour can be caused by a broad range of organisms. Only a few of these parasites are known to manifest and alter these behavioural-changes from the central nervous system (CNS) itself, and little is known about the mechanisms behind these alterations. Neuroparasitology intertwines the existing fields of neurology, biology and parasitology – covering the cases of parasitic manipulation of the CNS. As a means of answering questions in a broader neuroparasitological sense, we studied insect-specific baculoviruses, for which genes of interest can be easily modified genetically.
After infection by baculoviruses, infected caterpillars climb the vegetation in “tree-top”-disease, and/or express hyperactivity, followed by complete liquefaction of the caterpillars and release of virus progeny. Both behavioural alterations are thought to increase the chance of transmission to susceptible hosts. Previous studies have shown that for a subset of baculoviruses the viral protein tyrosine phosphatase (PTP) is required to induce hyperactivity.

Methods: Here, we studied baculoviral entry into the CNS of 3rd and 4th instar Spodoptera exigua caterpillars using fluorescently tagged Autographa californica multiple nucleopolyhedrovirus (AcMNPV).

Results: Using different viral constructs (with PTP, without PTP or with a catalytically inactive PTP) we show that the enzymatic function of PTP is not mandatory for CNS-entry, neither is the presence of PTP as such. Viral infections are detectable earlier in the progression of infection in the trachea and the more caudally located ganglia.

Conclusions: Furthermore, we elaborate on the observed patterns of localization of the different virus-constructs within the CNS.

Keywords: parasitic manipulation of host behaviour, Insect central nervous system, virus, neuroparasitology, Autographa californica mulitiple nucleopolyhedrovirus

P025 / #364

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

A FINE-SCALE PHYLOGENETIC ASSESSMENT OF THE DIGENEAN TREMATODES PARASITISING THE POTAMIDID GASTROPOD PIRENELLA CINGULATA (GMELIN, 1791) FROM THE PERSIAN GULF

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Introduction: The Persian Gulf is considered as one of the most anthropogenically impacted regions and known for its strong seasonal fluctuations of environmental conditions with highest temperature extremes during the summer season. Despite these harsh conditions, the Persian Gulf supports a diverse and endemic fauna. In order to better understand the transmission of trematodes in natural habitats exhibiting extreme environmental conditions we carried out a comprehensive survey assessing the larval trematode diversity in the horn snail, P. cingulata, along the coast off Iran.

Methods: A total of 1,745 P. cingulata were sampled at eight distinct locations along the coast of Iran. Partial fragments of the mitochondrial cox1 and nuclear 28S rRNA genes were amplified and used for molecular identification and phylogenetic reconstruction. The population genetic structures of the snail host and the most abundant digenean trematodes were further characterised.

Results: A total of 361 isolates of larval digenean trematodes recovered from the horn snail P. cingulata were characterised both, morphologically and molecularly. Phylogenetic analyses based on Bayesian inference provide evidence for a rich trematode fauna comprising 26 species of 8 families. The host populations exhibited significant isolation by distance in contrast with the parasite populations.

Conclusions: We provide the first comprehensive characterisation of the digenean diversity in the horn snail P. cingulata from the Persian off Iran. Our study highlights the importance of molecular systematics in the assessment of larval trematode diversity and their life-cycle elucidations in this unique system. This knowledge comprises an important baseline to build a framework to model host-parasite dynamics over time.

Keywords: Cercariae, Diversity, phylogenetics, Trematoda, Persian Gulf

P026 / #582

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

ELUCIDATING THE MOLECULAR SIGNALS OF AVIRULENCE EFFECTORS INVOLVED IN THE TRAFFICKING MECHANISM OF PLANT PATHOGENIC OOMYCETE PHYTOPHTHORA INFESTANS.

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Introduction: PI3P plays a central role in pathogen virulence in parasite such as Plasmodium falciparum, by binding to the PEXEL motif in the effector protiens leading to their translocation into the host RBC. Similarly, in the case of oomycetes RxLR class of cytoplasmic effectors have been proposed to bind PI3P. Plant hosts contain very low levels of PI3P, thus parasite PI3Kinases play a crucial role in fulfilling the need for PI3P. Also, cytoplasmic and apoplastic effector proteins follow different routes of transport even though both contain signal sequences. Thus, the basis of the variation in their trafficking routes and the role played by PI3P in their differential trafficking is an important question. Therefore, this study aims to elucidate functional interaction of PI3P with effector proteins and identify the cellular-sorting pathway in an effort to understand Phytophthora pathogenesis.

Methods: Level of expression and localization of PiPI3K proteins and the amount of PI3P generated was analysed. Phytophthora transgenic lines expressing cytosolic and signal-peptide containing FYVE acting as PI3P biosensor to differentiate between cytosolic and luminal PI3P within P. infestans were generated and analysed by live cell imaging. Density gradient analyses from transgenic cell lines followed by LCMS/MS for determination of its vesicle cargo constituents was done.

Results: ER associated localization of PiPI3Kinase was observed through IFA. Developed double transgenic lines shows PI3P to selectively associate with cytoplasmic effector protein (Avr3a) but not with apoplastic effector (EPICI) in P.infestans.

Conclusions: PI3P plays a crucial role in the trafficking RxLR class of cytoplasmic effectors thus contributing to the pathogen's virulence.

Keywords: Effector proteins(Avr3a, EpicI), Trafficking signals (RxLR, DEER), PI3P, vesicles, PI3Kinases

P027 / #336

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

IN VITRO MODEL AND QUANTITATIVE PROTEOMICS FOR THE STUDY OF THE EARLY HOST-PARASITE INTERACTIONS IN FASCIOLOSIS

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Introduction: Fasciolosis represents a major human and animal health problem, as well as an economic concern for livestock industry. Although the life cycle of its main causative agent, Fasciola hepatica, has been extensively studied, the events governing the first contact between the juvenile stage of the parasite and its vertebrate host remain unclear due to the scarcity of models to study this process.

Methods: We developed an in vitro model in which F. hepatica Newly Excysted Juveniles (FhNEJ) were excysted and put into contact with a Mouse Primary Small Intestinal Epithelial Cell (mPSIEC) culture for 3h, a time similar to that required for FhNEJ to migrate through the intestinal wall. After this time, tegumental and somatic fractions were obtained from FhNEJ, whereas cytosolic and membrane proteins were extracted from mPSIEC, and each fraction was characterized using SWATH-MS-based proteomics against negative controls consisting in FhNEJ and mPSIEC cultured alone.

Results: 210 and 133 differentially expressed proteins were found in FhNEJ and mPSIEC, respectively. Functional annotation revealed that the main biological events triggered in FhNEJ after co-culturing with mPSIECs included proteolysis, along with metabolic and cytoskeletal adaptions, while mPSIECs showed an abundance of differentially expressed ribosomal proteins, together with changes in vesicle transport and adhesion processes.

Conclusions: Even a short stimulation is enough to trigger proteomic changes in FhNEJ and mPSIEC. Further analysis of these results is expected to provide a starting point for the development of new anthelmintic vaccines. Funding: PID2019-108782RB-C22 and PID2019-108782RB-C21 by MICINN, AEI y FEDER, and CLU-2019-05 and CL-EI-2021-01 by JCYL and European Union ERDF.

Keywords: Fasciola hepatica, In vitro model, Host-parasite interactions, Proteomics

P028 / #1131

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

DEVELOPMENT OF AN IN VIVO MODEL TO STUDY THE MIGRATION PROCESS CARRIED OUT BY THE FASCIOLA HEPATICA JUVENILES IN FASCIOLOSIS BY QUANTITATIVE PROTEOMICS

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Introduction: Fasciolosis is a zoonotic disease responsible for significant economic losses in livestock production, as well as a worldwide health concern. Although Fasciola hepatica life cycle is well known, molecular interactions governing the migratory process undertaken by the juvenile parasites from the small intestine to the liver through peritoneum in the vertebrate host remain to be addressed. Therefore, the aim of this work was to establish a mouse in vivo model of infection to identify key parasite molecules during migration of F. hepatica juveniles.

Methods: F. hepatica metacercariae were employed to orally infect C57BL/6 mice. These were sacrificed on alternate days post-infection and juvenile migrating flukes were dissected out of the peritoneal cavity and liver. Tegument and soma protein extracts from these parasites as well as from control F. hepatica juveniles were isolated and submitted for SWATH-MS-based proteomics.

Results: The in vivo model allowed us to identify the days post-infection when the number of F. hepatica juveniles recovered in the host peritoneum and liver were maximum. These corresponded to 24 hours for the peritoneal cavity (9.38% of recovery rate) and 8 days post-infection for the liver (21.19% of recovery rate).

Conclusions: We have developed an in vivo model that allows the obtainment of the juvenile migrating forms of F. hepatica on their way through the host peritoneum and liver. A quantitative proteomic analyses of these parasites is being performed, which could provide a better understanding of the parasite-host interactions in the early stages of fasciolosis. Funding: PID2019-108782RB-C22 and PID2019-108782RB-C21 by MICINN, AEI y FEDER, and CLU-2019-05 and CL-EI-2021-01 by JCYL and European Union ERDF.

Keywords: Fasciola hepatica, in vivo model, Host-parasite interactions, Proteomics

P029 / #1698

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

LONG-TERM BIOMONITORING OF PARASITES IN BANK VOLES IN POLAND - THE GOOD, THE BAD AND THE EFFORT

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Introduction: Rodents, can pose a significant threat to the health of humans, livestock, and wildlife because they are hosts for a wide range of pathogens and, constitute essential reservoir hosts for life-threatening zoonoses. Short-term cross-sectional studies are useful as a starting point to obtain a comprehensive ecologic picture, long-term monitoring and a multisite approach are crucial to identify rodent species that can serve as reservoirs for zoonotic pathogens.

Methods: We conducted a multisite, long-term study on bank voles in northeastern Poland. Our study sites are located in the Mazury Lake District region in the northeast corner of Poland. Our objectives were to monitor the prevalence/seroprevalence and abundance of a wide group of parasites in the four abundant vole species found in the region (M. glareolus, M. arvalis, M. agrestis, and A. oeconomus) and to assess variation in their ecology dynamics attributable to both intrinsic and extrinsic factors that were quantified.

Results: We report an analysis of intrinsic and extrinsic factors on the seroprevalence, prevalence and abundance of a broad group of pathogens (both zoonotic and nonzoonotic). While some pathogen species have fluctuated markedly (e.g., some helminths and hemoparasites) or have even become locally extinct in our study sites, others have shown relative stability from year to year.

Conclusions: Results of our long-term biomonitoring provide a significant and novel contribution to our understanding of the ecology of parasites within vole populations. We underline the role of long-term studies that are necessary to comprehensively reveal the status of parasites in wildlife and assess the risk of possible infection, outbreaks, or spillovers.

Keywords: Rodent-borne diseases, Host-parasite interactions, biodiversity, Biomonitoring

P030 / #787

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

PLASMODIUM PARASITES HITCHHIKING ON HOSTS' RHYTHMS: DO EMBODIED OSCILLATORS INFLUENCE DISEASE SEVERITY?

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Introduction: Malaria (Plasmodium) parasites replicate rhythmically within the hosts' red blood cells. This synchronised replication of parasites coupled with damage caused by the host's rhythmic immune responses is responsible for the severity of disease symptoms. Furthermore, a key aspect of parasite rhythms is that when parasites reach a certain stage, which occurs at a particular phase of host rhythms, they withdraw from the circulation to "sequester" in the host's organs. It is assumed, sequestration allows parasites to avoid clearance by the spleen which captures parasites within red blood cells in circulation. However, despite that sequestration occurs at a particular time of day, the role of host rhythms in influencing opportunities to sequester and its benefits have been overlooked.

Methods: Here, we use a rodent malaria model to determine how light-entrained and food-driven host rhythms influence the accumulation of parasites in host organs, as a marker for sequestration. We quantified parasites' relative abundance across organs by RT-qPCR

Results: We find that patterns of accumulation vary within host tissues, with the biggest ratio of parasite content found in the liver and least in the spleen. Further, accumulation rhythms correlate with light-entrained rhythms (parasite ratio in livers with matching daily rhythms with feeding rhythms have a shared pattern), whereas in the lungs parasites align with food-driven rhythms, and this pattern is consistent in a reversed light schedule. We were unable to detect any changes in parasite accumulation in the spleen, this is not unexpected because we did not address the inflammation window

Conclusions: Our findings reveal previously unknown spatial complexity in the roles of rhythms in the interactions between host and parasite

Keywords: hosts' rhythms, plasmodium, sequestration

P031 / #963

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

SPATIO-TEMPORAL DYNAMICS OF SNAIL INTERMEDIATE HOSTS OF SCHISTOSOMIASIS IN THE KIMPESE REGION

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Introduction: Schistosomiasis is a parasitosis caused by Schistosoma. A freshwater snail is the intermediate host of humans. The DR Congo is one of the endemic countries. Knowing the geographic distribution of the intermediate host snails of schistosomiasis is a very important step in control. The aim of this study is to describe the spatial and temporal distribution of these snails, to determine the infectivity of these snails as well as the environmental characteristics of the sites concerned.

Methods: This descriptive malacological study took place from August 2020 to December 2021, in the Kimpese region in DR Congo. The snails were collected from 72 selected water points in the 25 villages. Their geographical distribution was made through a map developed using QGIS 3.18 software. The time series graphs were developed using Excel software. A sample of snails benefited from PCR.

Results: 172,491 snails were collected, including 5,908 intermediate hosts, including 3,813 Biomphalaria spp and 2,095 Bulinus spp. An increase in snails is observed over time with a drop in September to November 2020. There is a spatial heterogeneity in the distribution of its snails. 15 of sites had genus bulinus ssp and 16 genus biomphalaria ssp. Of the 323 molluscs that benefited from PCR, 5% were infected with schistosomiasis and 32.5% with other trematodes.

Conclusions: The spatial heterogeneity of intermediate host snails observed is explained by several factors relating to their habitat, their nutrition, the characteristics of the aquatic environment, the presence of competing or predatory species. This study allowed us to draw a map of the distribution of intermediate host snails, it will be completed by the realization of the PCR in order to determine the infectivity as well as the species in question.

Keywords: hosts, schistosomiasis, Kimpese, dynamics, snail

P032 / #854

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

PARASITES, VECTOR AND THE HOST. SOME DATA FROM RAPTOR BIRD NESTS

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Introduction: Trypanosomatidae parasites can be found in various vertebrate and invertebrate hosts, including humans. Trypanosomatids are subdivided into two groups based on the type of their life cycle: monoxenous trypanosomatids develop in one host, while dixenous parasites develop in two different hosts, one of which serves as a vector. Almost one fifth of all known dixenous parasites Trypanosoma spp. are found in birds, however the biology and life cycles of most avian Trypanosoma species remain unknown. Only few vector species of avian trypanosomes have been determined so far.

Methods: This study aimed to get information on the prevalence of trypanosomatids in wild-caught biting midges (Ceratopogonidae, Culicoides) collected nearby the nests of raptor birds. Parous Culicoides females were collected using UV light traps located nearby nests of raptor birds in Lithuania in June and July 2021. Molecular methods were applied to estimate the infections of biting midges by trypanosomatids.

Results: In total 1289 parous wild caught biting midge females were investigated. Culicoides pictipennis and C. festivipennis accounted for the vast majority of investigated species. We have detected trypanosomatids in more than 6% Culicoides females. Trypanosoma everetti was the dominant species among the dixenous trypanosomatids. Monoxenous parasite Crithidia brevicula was detected in several Culicoides females. More than half (57 %) of infected biting midges belong to C. pictipennis species.

Conclusions: This study helped to reveal the main Culicoides species found nearby raptor bird nests, showed that they are infected with avian blood parasites and may play as their potential vectors. This research was funded by a grant (No. S-MIP-20-57) from the Research Council of Lithuania.

Keywords: Culicoides, Trypanosoma, Vectors, raptor birds

P033 / #1377

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

HYBRIDISATION OF TRYPANOSOMA CRUZI INSECT STAGES IN AXENIC CULTURE

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Introduction: Hybrid lineages of Trypanosoma cruzi are prevalent in several Chagas disease endemic areas, but the mechanism of sexual reproduction in this parasite is still obscure. The aims of this study were to develop an in vitro system for routine production of experimental hybrids and use it to assess whether hybridisation is driven by canonical meiosis or parasexual mechanisms.

Methods: Diverse T. cruzi strains were engineered to constitutively co-express different combinations of fluorescent protein and drug resistance reporter genes. In vitro crosses were conducted by mixed axenic epimastigote culture and by co-infection of mammalian cells. Hybrids were identified based on dual fluorescence or dual drug resistance phenotypes. Parent and hybrid genomes were sequenced to determine inheritance patterns.

Results: Dual fluorescent amastigotes were observed in mammalian cell intra-lineage co-infections, 1x TcI, 1x TcIII, but no hybrid parasites grew out under dual drug selection. Four of 10 intra-lineage crosses in axenic epimastigote cultures produced hybrid parasites, 1x TcI, 2x TcII and 1x TcIII. None were recovered from any of 16 inter-lineage crosses. In terms of kinetics, hybrid production required at least 4 days of co-culture and the frequency increased up to 10 days after mixing. We calculated that the minimum frequency of mating competent parasites was approximately $5x10^{-6}$ in the TcIII x TcIII cross. DNA content and genome sequence analysis of 3 hybrid clones from 3 independent crosses was consistent with a parasexual mechanism.

Conclusions: Production and analysis of T. cruzi hybrids in mixed epimastigote cultures indicates sexual reproduction involves a parasexual mechanism and likely occurs in the insect vector, in line with related trypanosomatids.

Keywords: Trypanosomes, Sexual reproduction, chagas disease, evolution

P034 / #254

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

THE ROLE OF TOXOPLASMA GONDII INFECTION IN INDUCTION OF HYPERALGESIA IN MICE

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Introduction: Toxoplasma gondii is a neurotropic parasite that is considered one of the world's most successful pathogens. Nociceptive pain results from direct activation of pain nerve fibers, either due to chemical or mechanical mediators. Neuropathic pain refers to pain that is generated or sustained by the conditions that damage the nervous system, including various direct nerve injuries and diseases such as diabetes, alcohol abuse, zoster, HIV, Lyme disease, or conditions involving the central nervous system. We hypothesized that in Toxoplasma gondii infection, communication among immune cells promotes neuroinflammation through cytokine networks and induces pain sensitivity under conditions of neuropathic pain.

Methods: The animal model of Toxoplasma infection was established by the intraperitoneal inoculation of 20-25 tissue cysts from Tehran strain of T. gondii to BALB/c mice. Amitriptyline (20 mg/kg, i.p., 1/day) administrated to animals for 7 days before behavioral tests. Pain behavioral tests including tail flick, hot plate, and formalin test were evaluated in all the groups. The mRNA levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 were examined by real-time PCR.

Results: revealed that T. gondii induce hyperalgesia in the infected mice, whereas amitriptyline showed a promising effect against the hyperalgesia induced by Toxoplasma infection. The mRNA levels of the aforementioned cytokines significantly ($P < 0.05$) increased in the infected mice compared to the uninfected ones.

Conclusions: Obtained findings suggested that T. gondii infection could promote neuroinflammation through cytokine networks and induced hyperalgesia in BALB/c mice, whereas amitriptyline as an analgesic drug reverses them.

Keywords: Toxoplasmosis, Neuroinflammation, Cytokine, Pain

P035 / #1460

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

DIFFERENTIAL SUSCEPTIBILITIES OF AFRICAN TRYPANOSOMES TO APOLIPOPROTEIN L1 (APOL1) VARIANTS

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Introduction: Trypanosoma brucei causes human and animal African trypanosomiasis in sub-Saharan Africa. Humans resist animal-infective T.b.brucei due to Apolipoprotein L1 (APOL1)-containing trypanolytic factors in their sera. The human-infective subspecies, T.b.gambiense and T.b.rhodesiense, are refractory to APOL1-mediated lysis by different mechanisms. However, two genetic variants of APOL1, G1 and G2, found at high frequency in African populations, are associated with resistance to human trypanosomiasis. G2 is associated with protection from T.b.rhodesiense but susceptibility to T.b.gambiense, while G1 mitigates severity of T.b.gambiense infection. How these different variants of APOL1 interact to influence the trypanolytic potential of human serum is not fully understood

Methods: We developed a high-throughput flow cytometry-based method to assess the lytic potencies of sera from 360 individuals with different APOL1 genotypes, against the three subspecies of T. brucei in vitro.

Results: No serum from any genotype lysed T.b.gambiense. All genotypes could lyse the animal-infective T.b.brucei, although the lytic potency decreased with increasing load of the G2 allele. Sera from G2-carrying individuals were able to lyse serum resistance associated protein (SRA)-expressing T.b.rhodesiense. Unexpectedly, G1/G1 sera was also able to lyse T.b.rhodesiense. ELISA-determined serum APOL1 levels were not significantly different across genotypes

Conclusions: We conclude that the G2 variant has a selective advantage of lysing both T.b.brucei and T.b.rhodesiense, with a trade-off of decreased potency to animal-infective T.b.brucei. In contrast, increased trypanolytic potential of APOL1 is not the mechanism of G1 protection from severe T.b. gambiense disease.

Keywords: Trypanosomiasis, APOL1, lytic potency, African, evolution

P036 / #1612

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

TRICHINELLA IN THE ARCTIC AND SUBARCTIC: CURRENT SITUATION AND CHALLENGES

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Introduction: *Trichinella* spp. are important zoonotic nematodes that have been reported across the globe. Importantly, several outbreaks of human trichinellosis associated with consumption of game meat have occurred in the communities from Arctic and subarctic regions, highlighting the public health significance of these parasites in the region.

Methods: We conducted a review focusing on the knowledge on epidemiology of *Trichinella* parasites in the Arctic and subarctic regions, with the aim to inform discussions on challenges and solutions for their control.

Results: In the Arctic and subarctic regions, *Trichinella* persist due to its sylvatic cycles; cannibalism, predation and scavenging are considered as importance routes of transmission in wild animals. The *Trichinella* species circulating in the region include *T. nativa*, *T. britovi*, *Trichinella* T6 and *T. chanchalensis*.

Conclusions: Monitoring the situation over time and space is needed to identify areas with high parasite prevalence and intensity. Understanding and controlling *Trichinella* requires a One Health approach and adaptation of approaches to changes in the environment, animal populations and human actions. *Trichinella* parasites thrive in the Arctic and subarctic regions and remain a public health issue also in the future.

Keywords: Arctic, Epidemiology, Zoonosis

P037 / #413

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

DAILY RHYTHMS OF HOST AND PARASITE INFLUENCE ARTEMISININ EFFICACY

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Introduction: Circadian rhythms are a well known contributor to treatment success in several non-communicable diseases. However, chronotherapy (administering drugs at the optimal time of day) against infectious diseases is just gaining ground. Malaria parasites have rhythms in their developmental cycle, which are generally aligned to their host's daily rhythm. Whereas drug sensitivity varies throughout parasites' developmental cycle, data are inconsistent, and contribution of host circadian rhythms has not been investigated. By experimentally disentangling host and parasite rhythms, we determine the impact of parasite stage and host rhythms on artemisinin sensitivity of malaria parasites.

Methods: Mice kept in either a standard (light at day) or reversed light regime (light at night) were infected with Plasmodium chabaudi, with parasites either aligned or misaligned with host rhythms. Artemisinin was administered when parasites were either at ring stage or at trophozoite stage to determine the impact of parasite stage and the alignment of parasite and host rhythms on drug sensitivity.

Results: Rings were less sensitive to artemisinin than trophozoites were, and this stage-specificity was especially pronounced when parasites were misaligned with hosts rhythms. Compared to parasites that were aligned to their host's rhythm, misaligned ring stage parasites were less and misaligned trophozoites were more sensitive to artemisinin. Moreover, higher haem levels improved artemisinin efficacy in aligned infections.

Conclusions: We show that parasites' developmental stage and their temporal alignment with the host affect P. chabaudi's sensitivity to artemisinin in vivo. Malaria chronotherapy should consider both host and pathogen rhythms, as well as their interaction.

Keywords: drug efficacy, Malaria, chronotherapy, biological rhythms

P038 / #503

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

THE INTERACTION BETWEEN FASCIOLA HEPATICA JUVENILES AND THE HOST FIBRINOLYTIC SYSTEM AS A POTENTIAL EARLY-STAGE INVASION MECHANISM

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Introduction: Owing to its broad range of substrates, plasmin, the central enzyme of the fibrinolytic system, is exploited by different parasite species for tissue migration. During Fasciola hepatica infection, newly excysted juveniles (FhNEJ) emerge in the duodenum and migrate towards their definitive location, the intra-hepatic biliary ducts. Crossing of the intestinal wall by FhNEJ can be regarded as a 'point of no return' in fasciolosis, thus a deeper understanding on how this tissue barrier is overcome is crucial for the development of more effective control strategies against this parasite. The present work is aimed at understanding whether FhNEJ coopt the functions of the fibrinolytic system of the host, which would serve as a mechanism to support FhNEJ migration during early fasciolosis.

Methods: The capability of FhNEJ tegument (FhNEJ-Teg) to bind the plasmin zymogen plasminogen (PLG) and enhance plasmin generation was analyzed by ELISA and chromogenic assays. PLG-binding proteins in FhNEJ-Teg were identified by 2D-MS analysis, and the interactions were validated using FhNEJ recombinant proteins.

Results: Our in vitro assays reveal that FhNEJ-Teg contains proteins that bind PLG and enhance plasmin generation, and we identify 33 protein isoforms that serve as potential PLG-receptors. Among others, juvenile-specific cathepsins L3, B2 and B3 stand out as novel PLG-binding proteins in FhNEJ.

Conclusions: FhNEJ express proteins on their tegument surface that bind PLG and stimulate its activation to plasmin, which could facilitate traversal of the intestinal wall and contribute to the successful establishment of the parasite within its mammalian host. Funding: RTI2018-093463-J-I00 by MCIU, AEI and FEDER, and CLU-2019-05 and CL-EI-2021-01 by JCYL and European Union ERDF.

Keywords: Fasciola hepatica, Host-parasite interaction, fibrinolytic system

P039 / #1308

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

EXPLORING THE INTERACTION BETWEEN FASCIOLA HEPATICA JUVENILES AND COMPONENTS OF THE MAMMALIAN INTESTINAL BASAL LAMINA

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Introduction: Fasciolosis caused by Fasciola hepatica is a major disease of livestock and an emerging zoonosis worldwide. Infection by F. hepatica occurs through ingestion of metacercariae, which release in the duodenum the newly excysted juvenile flukes (FhNEJ). In order to reach their definitive location inside the bile ducts, FhNEJ need to cross the intestinal epithelium, which is outlined by the basal lamina, a collagen- and laminin-rich structure. FhNEJ express proteases that can bind to and cleave collagen, but whether they can also interact with additional components of this tissue remains to be elucidated. The aim of this study was to determine whether the tegument of FhNEJ contains proteins capable of binding laminin and/or fibronectin.

Methods: FhNEJ tegument proteins (FhNEJ-Teg) were extracted in vitro, and their capability to bind to laminin and fibronectin was analyzed by ELISA. Two-dimensional electrophoresis coupled to mass spectrometry analysis were used to identify potential laminin-binding proteins in FhNEJ-Teg, and the observed interactions were validated using FhNEJ recombinant proteins.

Results: FhNEJ bind laminin but not fibronectin at their tegument surface, and we identify 14 protein spots containing isoforms that potentially serve as laminin-binding proteins. Among these, cathepsin L3 stands out as the most prominent protease with laminin-binding properties within the FhNEJ-Teg extract.

Conclusions: FhNEJ express proteins at their tegument surface that are capable of binding laminin, a major component of the intestinal basal lamina, which would be employed to support FhNEJ migration through host tissues. Funding: RTI2018-093463-J-I00 by MCIU, AEI and FEDER, and CLU-2019-05 and CL-EI-2021-01 by JCYL and European Union ERDF.

Keywords: Fasciola hepatica, laminin, basal lamina, Host-parasite interaction

P040 / #1315

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

FASCIOLA HEPATICA JUVENILES EXPRESS PROTEINS AT THEIR TEGUMENT SURFACE THAT MODULATE SARS-COV-2 CELL ENTRY

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Introduction: Helminth parasites including Fasciola hepatica have adapted to their mammalian hosts during long co-evolution processes by establishing host-parasite relationships that modulate different physiological routes within the host. Previous data from our lab showed that F. hepatica juveniles (FhNEJ) could modify molecular routes in epithelial cells related to vesicle-mediated transport and the innate anti-viral response, which could potentially be relevant during viral infections. Therefore, the aim of the present study was to determine whether FhNEJ molecules can regulate pathways that influence cellular entry of the SARS-CoV-2 coronavirus, responsible for the COVID-19 pandemic outbreak.

Methods: We screened for the potential of different FhNEJ-derived molecules to inhibit viral entry by using SARS-CoV-2 pseudotyped viral particles, and validated the results using genuine SARS-CoV-2 infections.

Results: FhNEJ tegument and somatic extracts, together with the protein KTSPIDP, inhibited viral cell entry in the pseudotyped infection screen; and this capability was confirmed for the tegument FhNEJ antigenic extract via genuine SARS-CoV-2 infection.

Conclusions: FhNEJ express proteins at their surface that are capable of inhibiting SARS-CoV-2 cell entry. Additional experiments are being performed to identify the molecules responsible for such effects, which could potentially encourage the use of F. hepatica-derived molecules in a safe, synthetic format as therapeutic agents against SARS-CoV-2 and other emerging respiratory viruses.
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Keywords: Fasciola hepatica, SARS-CoV-2, Host-parasite interaction

P041 / #765

Topic: *AS01.3 Ecology, evolution, host-parasite interactions*

DISCOVERY OF NOVEL MICRONEMAL MOLECULES AND CYTOSKELETAL STRUCTURES AND IMPLICATION OF THEIR ROLES IN THE INVASION OF THE ZOONOTIC CRYPTOSPORIDIUM PARVUM

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Introduction: Cryptosporidium parvum is a globally distributed zoonotic protozoan parasite of medical and veterinary importance. It infects the enterocytes, causing moderate to severe, sometime fatally, watery diarrhea in humans and animals. Despite of importance, the molecular mechanism of cryptosporidial invasion remains poorly understood.

Methods: Various approaches including bioinformatic analysis, immunofluorescence assay (IFA), immunogold electron microscopy (IEM), molecular cloning and expression, biochemical and cell biological assays, as well as in vitro invasion experiments for the identification and functional investigation of molecules involved in parasite invasion of host cells.

Results: We have identified several novel proteins stored in the micronemes of sporozoites, including adhesive molecules and peptidases, and investigated their roles in the parasite invasion. We also discovered that Cryptosporidium possesses unique tubulin-based structures that differ from the microtubular structures of other apicomplexans in the sporozoites and undergo transformation during the parasite invasion.

Conclusions: Cryptosporidium sporozoites possess a number of unique secretory molecules and cytoskeletal structures to facilitate the interactions with and the invasion of host cells.

Keywords: micronemes, parasite invasion, secretory proteins, Cryptosporidium parvum, structural proteins

P042 / #1169

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

ZOONOTIC TRANSMISSION OF INTESTINAL HELMINTHS IN THE PHILIPPINES

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Introduction: Intestinal helminths (IHs) are highly prevalent in humans and animals worldwide. Many IHs are transmitted through faecal contamination of the environment. In other instances, infection occurs through meat consumption. The ZooTRIP project aims to assess the contribution of zoonotic transmission and environmental reservoirs to the human IH infection burden in the Philippines, and determine effective strategies for IH control.

Methods: A cross-sectional household-based study was undertaken in 8 municipalities on Mindanao Island. Household members (10-60 years) provided faecal samples and completed a questionnaire gathering demographic and socio-economic data. Faecal samples were collected from companion animals and livestock, as well as environmental samples. Parasitological and molecular methods are used for parasite diagnosis. Data are being integrated into mathematical transmission models.

Results: Initial data from 663 human participants revealed a soil-transmitted helminth prevalence of 22.8% and Schistosoma japonicum prevalence of 8.3%. Samples from 91 dogs, 136 pigs, 146 water buffalo and 18 cattle revealed 16 species of zoonotic helminths, overall prevalence of 52.8%. Analysis is ongoing to confirm IH species present and determine extent of zoonotic transmission.

Conclusions: The project will provide an evidence-base for enhanced control and elimination strategies for intestinal helminths considering zoonotic and environmental reservoirs.

Keywords: One Health, Environment, zoonoses, Intestinal helminths, Schistosoma japonicum

P043 / #905

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

PROGRESS ON THE DEVELOPMENT OF A SOURCE-ATTRIBUTING DIAGNOSTIC TOOL OF TOXOPLASMA GONDII INFECTIONS

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Introduction: The environmental transmission route of Toxoplasma gondii infection, through the ingestion of oocysts, is considered more relevant than earlier thought. However, discrimination between oocysts- and tissue cysts-driven infections is still a challenge. We evaluated the source-attributing potential of a panel of T. gondii-specific proteins following a screening workflow.

Methods: A well characterized sera panel from 24 pigs experimentally infected with type II or III T. gondii oocysts or tissue cysts was used (sampled weekly till 6 weeks post-infection (wpi)). Six oocyst wall and twelve sporozoite-specific predicted proteins, few previously described, were first tested by Western blot (WB) with sera from three oocyst infected pigs, which were bled weekly up to three wpi. Proteins showing no reactivity or that were recognized prior to infection were discarded. Next, remaining candidates were analyzed by WB using the whole sera panel.

Results: The sporozoite-specific protein CCp5A resulted a promising antigen candidate in the screening. However, when it was tested by WB with a broader panel of sera, this protein could hardly discriminate between oocysts- and tissue cysts-driven infections, except for an earlier recognition by sera from pigs infected with type III oocysts.

Conclusions: Despite a restrictive workflow was employed, other additional factors are influencing protein recognition and complicate the interpretation of results. Further on-going work with other candidates will be also presented. Acknowledgements: TOXOSOURCES (One Health EJP, GA No. 773830), UCM-Santander/2018 predoctoral fellowship and Silvia Jara Herrera and Carmen San Juan Casero for their technical assistance.

Keywords: Serology, source-attributing, diagnosis, toxoplasma, OneHealth

P044 / #1069

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

WHAT DOES THE FOX HAVE?: DISSEMINATED CYSTICERCOSIS CAUSED BY TAENIA CRASSICEPS, DETECTED FROM AN IMPORTED RED FOX VULPES VULPES IN REPUBLIC OF KOREA

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Introduction: Reintroduction of species is artificial effort to restore damaged ecosystem, but is always challenged by the potential pathogens that possible to introduced together. Despite the red fox, Vulpes vulpes is deeply involved in wide variety of Korean cultures, this canid animal was considered as extinction from South Korea several decades ago. Foxes are known as important host that carry a range of pathogens including parasites, and the quarantine is one of the most important procedure for reintroduction project. In the present study, we describe a case of disseminated cysticercosis detected from an imported red fox in the Korea during its quarantine progresses.

Methods: A red fox was introduced from China to Korea in November, 2012 for species reintroduction project. During the quarantine procedure, abnormal masses were detected from ventral and dorsal side of the body by palpation. The following ultrasonography reveals the cyst formation in subcutaneous. The fox was euthanized, and the cysticerci were found from wide range of subcutaneous regions with internal organs. And adults were recovered from the small intestine. They were identified by using morphological and molecular comparison.

Results: Cysticercus was ovoid and harbored single scolex which armed with two rows of spines. And the molecular analysis based on mitochondrial cox1 partial gene sequences obtained from both of adult worm and metacestode also represented the pathogen is T. crassiceps.

Conclusions: Taenia crassiceps is an alien species that has not been reported in Korea before. If the quarantine procedures don't work properly and the animal is released into the wild Korea, it could return to great hazard. In the present study, we describe the case of cysticercosis of imported red fox caused by T. crassiceps.

Keywords: Species restoration, Quarantine, Taenia crassiceps, Disseminated cysticercosis, Vulpes vulpes

P045 / #1627

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

INFECTIVITY OF CRYPTOSPORIDIUM PARVUM CAN BE ALTERED BY FREE LIVING AMOEBAE

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Introduction: From 2004 until 2016, *Cryptosporidium* was responsible for 60% (524/905) of all reported waterborne outbreaks caused by protozoan parasites. In water, several microorganisms such as free living Amoebae could interact with *Cryptosporidim* species. Phagocytosis ability of free living amoebae is not really known regarding *Cryptosporidium* oocysts. The aim of this study was to describe interactions between free living Amoebae and *Cryptosporidium* oocysts in an aqueous environment *in vitro*.

Methods: *C. parvum* oocysts and free living amoebae (*Acanthamoeba castellanii* or *Vermamoeba vermiformis*) were co-incubated in both planktonic and biofilm water conditions. Potential interactions were evaluated over time (0, 1, 3, 24, 72, 168 hours). Encystment and survival of free living amoebae were evaluated by microscopy using trypan blue vital coloration. Oocysts counting was done microscopically. Remanence of oocysts infectivity was studied by CC-qPCR: a method based on cell culture associated with quantitative PCR.

Results: Numbers of *C. parvum* oocysts and/or free living Amoebae decreased over time whatever the studied conditions (planktonic or biofilm). Encystement ability of free living Amoebae was not altered by the presence of oocysts over time. In contrast, a marked decrease in oocyst infectivity was observed in both planktonic and biofilm conditions when *C. parvum* and *A. castellanii* were co-incubated. An effect which was reversible but not observed by co-culturing *C. parvum* oocysts and *V. vermiformis*.

Conclusions: While phagocytosis of *C. parvum* oocysts by *A. castellanii* was not clearly observed *in vitro,* such interactions resulting in a decrease of oocysts infectivity. Further elucidation of mechanisms which modulate *C. parvum* infectivity will be of interest.

Keywords: Cryptosporidium, free living amoebae, Interaction

P046 / #1436

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

OH-HARMONY-CAP WP4: HARMONISING PROTOCOLS FOR THE DETECTION AND CHARACTERIZATION OF MODEL MICROORGANISMS, INCLUDING CRYPTOSPORIDIUM SPP.

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Introduction: OH-Harmony-CAP is a project that aims to 1) collect information on current capabilities, capacities, adaptabilities and interoperabilities, at both the National Reference Laboratory and the primary diagnostic levels, across Europe and 2) quantitatively describe current and best practices and develop harmonized protocols for the detection and characterization of model organisms (Shiga toxin- and enterotoxin producing Escherichia coli, Cryptosporidium spp. as well as for the detection of antimicrobial resistance in Salmonella and Campylobacter.

Methods: Laboratory protocols were collected from laboratories operating in Public Health and Veterinary/Food Safety areas. Evaluation tables, one for each model organism, were created to facilitate comparisons and discussions among expert groups on possibilities to rank them. Differences between matrices and test purposes were taken into account.

Results: The outcome of the exercise varied depending on the model organism. For Cryptosporidium spp., ranking was not possible due to some encountered difficulties: i) few collected protocols, ii) different matrices iii) different purposes of the protocols (detection only/typing/ etc.). Thus, a decision tree was designed for Cryptosporidium spp., to inform the selection of the best protocol for a given situation.

Conclusions: In addition to the protocol selection, this exercise allowed to develop a global process and to list improvements and lessons learned to be included in a technical report. Acknowledgements: This work is part of the OH-Harmony-CAP project, funded by the European Union's Horizon 2020 Research and Innovation programme under grant agreement No. 773830: One Health European Joint Programme.

Keywords: Cryptosporidium, detection, typing, harmonized protocols

P047 / #1018

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

DETECTION OF B-TUBULIN GENE (ISOTYPE 3) IN FASCIOLA GIGANTICA FOR THE FIRST TIME AND COEXISTENCE OF F. GIGANTICA AND ASPERMATIC FASCIOLA IN BANGLADESH

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Introduction: Fasciola gigantica is a food-borne zoonotic trematode that affect around 80% livestock of Bangladesh. Many flukicides are being used in Bangladesh, however 120 flukes had been recovered from a single goat liver in spite of treatment. This indicates possibility of drug resistance in flukes. This study aimed to explore the flukicidal resistance in F. gigantica.

Methods: PCR, multiplex PCR and RFLP were performed in this study to explore the β-tubulin genes.

Results: Molecular characterization of β-tubulin isotype 3 gene in F. gigantica isolates from goat in Bangladesh was carried out. A total of 55 F. gigantica isolates were collected from 55 goats from eight regions of Bangladesh. PCR was performed on β-tubulin isotype 3 gene and 19 out of 55 isolates showed the same band profiles. Ten samples were randomly selected and DNA sequence of a 935 bp coding fragment of β-tubulin isotype 3 was performed that indicate polymorphism in Fasciola. This β-tubulin isotype 3 gene polymorphism of F. gigantica isolates from goat in Bangladesh have been identified for the first time. The molecular characterization of F. gigantica was also carried out on the basis of multiplex PCR and PCR restriction fragment length polymorphism (PCR RFLP) analysis of β-tubulin isotope 3 gene and sequence analysis as well for confirmation. Multiplex PCR showed band at 510 bp for F. gigantica and at 510 and 240 bp for aspermatic Fasciola where as PCR RFLP profile obtained from AluI restriction enzyme revealed two fragments of 708 bp and 544 bp. Multiple sequence alignment results β-tubulin isotope 3 gene also showed much more polymorphic sites in F. gigantica from Bangladesh.

Conclusions: All these results revealed that aspermatic Fasciola was responsible for resistance against flukicidal drugs in Bangladesh.

Keywords: Aspermatic Fasciola, Bangladesh, β-tubulin gene, Fasciola gigantica, Multiplex PCR

P048 / #1007

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

CONTAMINATION BY PARASITES IN PUBLIC SQUARES IN NOVA FRIBURGO AND NATIVIDADE, RJ, BRAZIL

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Introduction: It is estimated there are more than one billion individuals are infected with soil-transmitted helminthiasis (HTS) worldwide. HTS are mainly acquired through exposure to soil, water or food contaminated with feces. Children represent the main group at risk due to poor hygiene, geophagy and onicophagy habits. They are often infected after exposure to contaminated soil during recreation in parks and squares. HTS affects the nutritional balance of the child population, generating complications such as intestinal obstruction, rectal prolapse, neurological disorders and physical and mental impoverishment. The proximity between humans and pets, such as dogs and cats, is aggravating from an epidemiological point of view. The objective was to analyze the presence of HTS in the soil of different public leisure areas in the cities of Natividade and Nova Friburgo, RJ - Brazil and to analyze the convenient environmental condictions for development of parasites forms in these regions.

Methods: The samples were processed and analyzed by the method of Hoffman, Pons and Janer.

Results: 37.1% (26/70), with 22.8% (16/70) contaminated with A. lumbricoides eggs, 7.2% (5/70) with Hookworm eggs, 7.2 % (5/70) with Toxocara sp. eggs and 12.8% (9/70) samples with the presence of rhabdidoid larvae. Nova Friburgo presented greater contamination in the deep samples this represent 32% (12/50), Natividade presented 35% of the total (7/20).

Conclusions: The results highlight the need for review strategies to combat HTS in view of possible damages to children and the quality of life of the population. The implementation of actions aimed at protecting and maintaining the cleanliness of public recreation areas, educational actions to the population and campaigns of deworming of wandering animals.

Keywords: environmental contamination, Epidemiology, One Health, Helminths transmitted by soiL

P049 / #996

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

LIPID UPTAKE IN PARASITES – A TROJAN HORSE FOR DRUG DELIVERY

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Introduction: Many parasites rely on the uptake of host nutrients for their survival. Interference with the nutrient uptake mechanism can lead to the death of the parasite and hence it is a popular approach to drug design. We were wondering whether it would also be possible to exploit the essential uptake of lipids for a more efficient delivery of existing and experimental anti-parasitic drugs.

Methods: Using the malaria parasite Plasmodium falciparum as a model system, we tested various fluorescently labelled lipids for the efficient uptake into parasites. We then replaced the fluorophores with the anti-malarial compound primaquine and optimised the linkage between the lipid carrier and the drug cargo. The efficacy of these lipid-drug conjugates was then tested in various assays and dose-response experiments against different life-cycle stages.

Results: Coupling of existing or novel anti-malarial drugs increased the efficacy 4- to 20-fold compared to uncoupled drugs in all life-cycle stages tested. At the same time a significant decrease in toxicity against host cells was observed. The conjugates also showed beneficial effects on drug-resistant parasite lines compared to the drug alone. We further explored the efficacy of conjugated anti-malarial and other anti-parasitic drugs and observed similar improvements against other apicomplexa, as well as protozoa and helminths.

Conclusions: This novel drug delivery system improves efficacy and reduces toxicity of a vast array of existing and novel drugs. It is applicable to a wide range of parasitic diseases including protozoan and helminth parasites.

Disclosure: The presented technology is part of a filed provisional patent application.

Keywords: Lipids, Leishmania, plasmodium, drug delivery, toxoplasma

P050 / #538

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

DIROFILARIA REPENS IN BALTIC AND NORDIC COUNTRIES: DO MEDICAL DOCTORS AND VETERINARIANS KNOW THAT THE PARASITE IS ZOONOTIC?

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Introduction: Dirofilaria repens has been spreading northwards in Europe. We summarize results from two questionnaire surveys on knowledge about the parasite being zoonotic, conducted among medical doctors and veterinarians in the region.

Methods: Survey I targeted veterinarians in the whole region in 2017, and surveyed knowledge on D. repens being zoonotic. Survey II targeted medical doctors and veterinarians in Finland in 2019, and surveyed knowledge on the ability of D. repens to infect humans and dogs.

Results: A third (34%, 41/121) of the veterinarians participating in Survey I selected statement “D. repens is zoonotic” to be true. In Survey II, 3% (6/198) of medical doctors and 61% (37/61) of veterinarians correctly indicated that D. repens can infect humans, while 8% (16/198) of medical doctors and 89% (54/61) of veterinarians correctly indicated that the parasite can infect dogs.

Conclusions: Although the two surveys are not directly comparable, the proportion of veterinarians knowing that D. repens is zoonotic seemed to be higher in Survey II. While cross-sectoral One Health knowledge was evident, need to increase knowledge about the zoonotic nature of the emerging vector-borne parasite was particularly clear among medical doctors. Tiškina V., Jokelainen P. 2017. Vector-borne parasitic infections in dogs in the Baltic and Nordic countries: A questionnaire study to veterinarians on canine babesiosis and infections with Dirofilaria immitis and Dirofilaria repens. Vet Parasitol. 244:7-11. Mikola N., Oborina V., Jokelainen P. 2020. Knowledge About Emerging Zoonotic Vector-Borne Parasites Dirofilaria immitis and Dirofilaria repens in Finland: Questionnaire Survey to Medical Doctors and Veterinarians. Vector Borne Zoonotic Dis. 20(1):27-32.

Keywords: Dirofilaria repens, cross-sectoral, One Health, questionnaires

P051 / #246

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

MOLECULAR PATHOGEN-SCREENING AND BARCODING OF KEDS (DIPTERA: HIPPOBOSCIDAE): POTENTIAL VECTORS OF HUMAN AND VETERINARY PATHOGENS IN CENTRAL EUROPE?

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Introduction: Hippoboscid flies (Diptera: Hippoboscidae), also known as keds or louse flies, are blood-feeding ectoparasites which currently receive growing attention as potential vectors of pathogens. Here, we present results of the molecular pathogen-screening and barcoding of keds collected in Austria and Germany.

Methods: Keds (n=294) were collected from cattle, sheep, red deer and dogs in various locations in Austria and Germany between 2015 and 2019. DNA from 284 keds was screened to detect Trypanosomatida, Piroplasmida, Anaplasmataceae, Filarioidea, Borrelia spp. and Bartonella spp. Barcoding of keds was done by sequencing a region within the mitochondrial COX I gene.

Results: Four species of keds were identified based on their morphology: Hippobosca equina (n=66), Lipoptena cervi (n=120), L. fortisetosa (n=3) and Melophagus ovinus (n=105). Bartonella spp. DNA was detected in all M. ovinus specimens and in 74% and 20% of L. cervi and H. equina, respectively. Trypanosoma spp. DNA was present in 87% of M. ovinus and in 5% of each H. equina and L. cervi. Borrelia spp., Onchocercidae and Wolbachia DNA was identified in <6% of the keds. Barcoding revealed three distinct genotypes of H. equina and one genotype of M. ovinus.

Conclusions: Keds parasitizing domestic and wild animals in Austria and Germany were demonstrated to harbour various pathogens which warrants their study as potential vectors of infectious agents of human and veterinary importance. Acknowledgements: Financial support was from the Austrian Federal Ministry of Education, Science and Research (Austrian Barcode of Life - Hochschulraum-Strukturmittel).

Keywords: Barcoding, Molecular pathogen-screening, Keds, Vector-borne pathogens

P052 / #1314

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

BLASTOCYSTIS IN HUMANS AND WARM-BLOODED ANIMALS IN POLAND: GENETIC DIVERSITY AND ZOONOTIC POTENTIAL

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Introduction: Blastocystis spp. is one of the most frequently detected protozoa in the large intestine of humans and animals. Blastocystis spp. is a complex of species, in which 28 subtypes (ST) have been described so far, including at least 9 isolated from humans.

Methods: Material from 74 species of animals (including humans) has been examined – a total of 1413 samples. Microscopic examinations of stool samples and culture were conducted to detect Blastocystis. A fragment of the gene encoding the small subunit of the ribosome was amplified by PCR. The PCR products were sequenced, the obtained sequences were compared to the sequences deposited in the GenBank database. Phylogenetic analysis was performed with the use of Bayesian inference. The allelic diversity of the gene encoding the rRNA of the small ribosome subunit was investigated for the first time in Poland.

Results: The presence of Blastocystis detected in 156 samples. The following facts have been proven: – the occurrence of Blastocystis spp. in humans, 20 species of other mammals and 13 species of birds; – for the first time in Poland, the occurrence of Blastocystis spp. in 18 species of mammals and 12 species of birds; – a large variety of subtypes occurring in humans and warm-blooded animals in Poland (11 subtypes, including 8 subtypes detected in humans). Two new alleles of the gene encoding the small ribosome rRNA have been described. Most of detected subtypes (7 out of 11) are zoonotic.

Conclusions: The detection of other subtypes in Poland than those identified so far should be expected in the future.

Keywords: Subtypes, Blastocystis, Poland, Human, animals

P053 / #769

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

EFFICACY OF AN AEROSOL-RESISTANT PEPSIN POWDER IN LABORATORIES TESTING FOR THE PRESENCE OF TRICHINELLA LARVAE IN MEAT

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Introduction: Trichinellosis is an important worldwide foodborne zoonosis. The gold standard Trichinella test for meat intended for human consumption is the artificial digestion method. Handling and dispensing of conventional pepsin powder present significant safety risks for analysts. The use of pepsin powder that is resistant to aerosolization should alleviate these safety concerns.

Methods: The aim of this study was to evaluate the efficacy of an aerosol-resistant pepsin powder in the artificial digestion method according to EU directive 1735/2015 and ISO/IEC 17043:2010. Proficiency samples of pork diaphragm containing low numbers of viable Trichinella spiralis larvae were tested independently in two laboratories.

Results: The results revealed that aerosol-resistant pepsin was simple and convenient to use, and showed good solubility and high larval recovery that exceeded the requirements of the EU Directive.

Conclusions: The efficacy of the aerosol-resistant pepsin was very good; it is safe for analysts, and could be of use with confidence in laboratories performing official Trichinella control testing.
Acknowledgment: We are very grateful to: Blagoje Milosavljevic, INEP technician for help in laboratory work; Ministry of Education Science and Technological Development of Serbia, contract numbers: 451-03-68/2022-14/ 200019 and 451-03-9/2021-14/200143.

Keywords: Trichinella, Pepsin, Digestion method

P054 / #812

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

TRICHINELLA PTS IN SERBIA AND SOUTHEASTERN EUROPE

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Introduction: In some southeastern Europe (SEE) Trichinellosis is one of the most important foodborne zoonotic diseases. The first report of swine infection was in 1918 in Serbia. Detection of Trichinella presence was initially made at slaughter by trichinoscopy. From 1984 artificial digestion was adopted for use in Serbia in preventing human trichinellosis. Modern pork production systems, implemented control measures, artificial digestion method have eliminated farm pork as a source for Trichinellosis. All participants successfully passed the testing. Next Serbian PTs were in 2021 and 2022. Control of Trichinella QA system in veterinary subjects testing for Trichinella presence in meat samples and regularly participation in PTs are needed to achieve safe food for consumers.

Methods: National reference laboratories for Trichinellosis (NRLT) from Serbia and Southeastern European countries participate regularly in PT organized by EURLP, Rome. For the first time in Serbia proficiency test (PT) for the detection of Trichinella larvae in meat by Magnetic Stirrer Method (MSM) was organized in 2017

Results: All participants successfully passed the testing. Next Serbian PTs were in 2021 and 2022.

Conclusions: Control of Trichinella QA system in veterinary subjects testing for Trichinella presence in meat samples and regularly participation in PTs are needed to achieve safe food for consumers.
Acknowledgment: Ministry of Education Science and Technological Development of Serbia, contract numbers: 451-03-68/2022-14/ 200019 and 451-03-9/2021-14/200143

Keywords: Trichinella, Proficiency test, Serbia, SEE

P055 / #823

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

TRICHINELLA INFECTION IN SERBIA AND SOUTHEASTERN EUROPE

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Introduction: In Serbia and some Southeastern Europe (SEE) countries Trichinellosis is one of the most important foodborne zoonotic diseases. In Serbia first human case was in 1923 in Zemun. Modern pork production systems, implemented control measures, artificial digestion method have eliminated farm pork as a source for trichinellosis.

Methods: Epidemiological data from the last decades indicate that the number of human cases as well as the number of infected animals has decreased significantly in Serbia as well as in other SEE countries.

Results: Over the years, pork was the most frequent source of human Trichinellosis in Serbia. Cases generally occurred in family outbreaks and risk is linked to untested backyard pork consumption. Meat and meat products offered to relatives and friends may be source of infection with Trichinella when backyard pigs are raised without any compliance with hygienic rules and animals are not veterinary tested. In most numbers of outbreaks in Serbia T. spiralis were the etiological agent of infection but in 2016 we had a large outbreak provoked by consumption of wild boar meat containing T britovi larvae. In addition to T. spiralis, three more species present in Europe are reported in SEE countries also (T. britovi, T. nativa and T. pseudospiralis).

Conclusions: According to epidemiological data it is important that hunters and consumers of backyard pigs and wild game meat should be educated about the risk associated with consumption of untested meat. Also, full integration of veterinary and public health efforts, i.e. the one health concept is very important in all countries. Acknowledgment: Ministry of Education Science and Technological Development of Serbia, contract numbers: 451-03-68/2022-14/ 200019 and 451-03-9/2021-14/200143.

Keywords: Trichinella, Prevalence, Distribution, Serbia, SEE

P056 / #811

Topic: *AS01.4 One-health, multidisciplinary, cross-border, cross-species, cross-cutting*

GARLIC DERIVED METABOLITES ATTENUATE INFLAMMATION INDUCED BY ENTERIC PARASITE INFECTION

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Introduction: Bioactive phytonutrients are promising options towards parasite infection because of low resistance. Garlic-derived propyl-propane thiosulfonate and propyl-propane thiosulfinate (PTSO-PTS) are beneficial to human and animal production. However, the abilities of PTSO-PTS to affect mucosal immune responses during enteric parasite infection remains largely unknown. We explored the immunomodulatory effects of PTSO-PTS in macrophages and epithelial cells as well as helminth infected mice, and the underlying interaction of dietary compounds, host and helminth is discussed.

Methods: ELISA was conducted to study inflammatory cytokines production in cells. Relative reactive oxygen species (ROS) production was tested. The RNA-sequencing was also performed to investigate the cellular activity of PTSO-PTS and more details in murine model with Trichuris muris (T.muris) infection. qPCR was used for gene expression analysis.

Results: PTSO-PTS decrease proinflammatory cytokine production in LPS-stimulated macrophages. PTSO-PTS modulated the transcriptomic response of LPS-activated macrophages, especially immune defense and interferon signaling. In murine epithelial cells exposure to T.muris antigens, PTSO-PTS exerts immunomodulatory effects. In T.muris infected mice, pathways related to metabolism, inflammation, and immune function are regulated by PTSO-PTS, but no effects on worm burden and type 1 or type 2 immune responses.

Conclusions: PTSO-PTS has anti-inflammatory function and they can induce a varied transcriptomic response including anti-inflammatory and antioxidant responses in vivo and in vitro. Our data suggest that PTSO-PTS may exert immunomodulatory and antioxidant activity , and more details should be studied.

Disclosure: A.B. is an employee of Pancosma/ADM. The other authors declare no conflict of interests.

Keywords: dietary compound, enteric parasite infection, inflammation, Trichuris muris

P057 / #382

Topic: *AS01.5 Other studies related to living with parasites*

UNRAVELLING THE BIOSYNTHETIC PATHWAY OF PHOSPHORYLCHOLINE IN C. ELEGANS

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Introduction: The attachment of phosphorylcholine on carbohydrates (PC-glycans) is a common modification for nematodes including C. elegans. The presence of PC appears to be important for the development of nematodes and for immunomodulation by parasitic nematodes. However, the biosynthetic pathway of PC glycans is not completely understood. A major component of the pathway, the identity of the PC transferring enzyme(s) (PC-transferases), has yet to be elucidated. There are strong indications suggesting that fukutin-related genes potentially encode for this PC-transferase.

Methods: In this study, we examined whether three selected fukutin-related genes of C. elegans (W02B3.4, T07A5.1, T07D3.4) are involved in the biosynthetic pathway of PC glycans. With CRISPR/Cas9 technology we created C. elegans knock-out lines for each fukutin-related gene. Furthermore, we co-expressed the C. elegans fukutin-related genes in Nicotiana benthamiana for subcellular localisation and functional characterisation.

Results: C. elegans fukutin-related gene knock-out resulted in no significant reduction of PC. Interestingly, one mutant line of W02B3.4 showed an increase of PC due to a potentially introduced signal peptide, indicating that the W02B3.4 gene could encode for a PC-transferase. Co-expression of C. elegans fukutin-related genes in N. benthamiana did not result in PC transfer, but they appear to localise in the medial Golgi.

Conclusions: These findings indicate that fukutin-related genes could be involved in the PC biosynthesis. Yet, the PC glycosylation in other systems remains challenging. These findings will contribute to our understanding of the pathway for PC-glycan biosynthesis, offering potential opportunities for design and synthesis of PC-glycan therapeutics.

Keywords: immunomodulation, Phosphorylcholine, Glycosylation, Helminths

P058 / #1161

Topic: *AS01.5 Other studies related to living with parasites*

IMPACT OF COVID-19 RESTRICTIONS ON INCIDENCE OF INTESTINAL PARASITIC INFECTIONS IN DENMARK: A REGISTER BASED STUDY

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Introduction: Intestinal parasitic diseases is a major public health problem worldwide. We have noticed that the incidence of intestinal parasitic parasite infections is reduced in Denmark during COVID-19 pandemic. The implications of the strategies implemented to prevent COVID-19 transmission on other infectious diseases are unclear. This study aimed to determine the incidence of intestinal parasitic infections in Denmark in the period before and during COVID-19 pandemic.

Methods: Clinical microbiological diagnosis for Giardia lamblia, Cryptosporidium spp., Entamoeba histolytica, Cyclospora cayetanensi, and other intestinal parasites in the period of March 2019 to February 2021 are extracted from all Departments of Clinical Microbiology in Denmark.

Results: Incidence of Giardia lamblia, Cryptosporidium spp., Entamoeba histolytica, Cyclospora cayetanensi, and other intestinal parasites are reduced in the period of March 2020 to February 2021 in comparison of March 2019 to February 2020. Figure 1 shows the incidence of Giardia Lamblia from March 2019 to February 2021).

Conclusions: COVID-19 public health interventions may be considered to decrease the incidence of Giardia lamblia, Cryptosporidium spp., Entamoeba histolytica, Cyclospora cayetanensi, Nematodes and Helminths in Denmark. The most likely explanation for these reductions is the concurrence of social restrictions, physical distancing, personal hygiene awareness and international and domestic border closures in response to the COVID-19 pandemic.

Keywords: restrictions, Covid-19, Intestinal Parasitic Infections

P059 / #1466

Topic: *AS01.5 Other studies related to living with parasites*

FEMALE GENITAL SCHISTOSOMIASIS IN AUYO, JIGAWA STATE, NIGERIA

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Introduction: Female genital Schistosomiasis (FGS), a pathology caused by chronic urogenital schistosomiasis (US), is a neglected disease with low awareness. Up to 56 million women/girls in sub-Sahara Africa are infected. The disease has been overlooked within national neglected tropical disease control programs. Nigeria has the highest burden of US, yet studies on FGS are paltry. This study aimed at determining prevalence of FGS in Auyo Jigawa, Nigeria.

Methods: A cross-sectional survey, carried out in Auyo, Jigawa State Nigeria. About 157 people consented to participate. Urine microscopy was used to determine prevalence of urogenital schistosomiasis while Colposcopic examination of the genitals was performed on sexually active participants only to determine FGS prevalence (following the guidelines outlined in WHO FGS pocket atlas). A structure pretested questionnaire was administered face to face to evaluate the healthcare givers knowledge on FGS.

Results: In all, among the 157 consenting participants, 132 were eligible and included in the study. The participants were between 5 and 50 years of age. Amongst which, 79% were adults of fifteen and above years old. Prevalence of urogenital schistosomiasis was 19% and that of FGS was 17%. Most common FGS symptom observed were abnormal blood vessels, contact bleeding and grainy sandy patches. Among the community health workers, none knew about FGS, its clinical manifestations or the potential associated complications.

Conclusions: In conclusion, the prevalence of both urogenital schistosomiasis and female genital schistosomiasis was high in Auyo Jigawa State, Nigeria. Many of the participants and the community health workers were unaware of FGS and its complication. The infection was frequently misdiagnosed and not treated.

Keywords: Neglected disease, Female Genital Schistosomiasis, Urogenital schistosomiasis

P060 / #768

Topic: *AS01.5 Other studies related to living with parasites*

LOSS OF SAM50 IN GIARDIA INTESTINALIS AND OTHER EUKARYOTES

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Introduction: Sam50 is a transmembrane β-barrel of the mitochondrial outer membrane. It is the key molecule of the sorting and assembly machinery complex involved in the biogenesis of β-barrel proteins. Since most of the mitochondrial proteins are encoded in the nucleus and translated on cytoplasmic ribosomes, the mitochondria rely on the protein import mechanisms for which Sam50 is essential. While most of the studies consider Sam50 to be universally present in all eukaryotic lineages, some studies have suggested that the human parasitic protist Giardia intestinalis might not possess the Sam50 orthologue.

Methods: In order to gather evidence for the presence/absence of Sam50, we modelled 3D structures of all G. intestinalis proteins using the homology modelling approach. The models were subsequently evaluated whether any of them corresponds with known Sam50 structures. Additionally, we conducted a large-scale analysis using the Hidden Markov models based searches to identify more possible organisms without Sam50.

Results: Despite the presence of β-barrel proteins such as Tom40, none of our bioinformatic and structure-based searches revealed the presence of Sam50 homologue in G. intestinalis and some other related Metamonads. Moreover, our searches were able to identify other unrelated eukaryotes lacking Sam50. Our data suggest that structure of β-barrels from these organisms might have adapted to the lack of Sam50.

Conclusions: We gathered large evidence that there are eukaryotes lacking Sam50. We propose an alternative mechanism of β-barrel biogenesis for these organisms.

Keywords: Sam50, β-barrel biogenesis, Giardia intestinalis

P061 / #983

Topic: *AS01.5 Other studies related to living with parasites*

UNUSUAL PRESENTATION OF PARASITIC DISEASES

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Introduction: Rare cases are detected unexpectedly. Ancylostomiasis in a baby, a case of fake leishmaniasis, a nodule in the upper eyelid and a case with chronic diarrhoea and a case with intestinal myiasis

Methods: Ω In case of the baby: upper endoscopy was done and showed 2 whitich worms (1.4 cm in length). In the fake cutaneous leishmaniasis, smear was done, stained and examined microscopically, nested PCR and histopathology were done for diagnosis. In the case with an eyelid nodule, the nodule was extracted, opened and a thread-like live worm came out of the nodule. Transverse section of the worm was examined. In the case with diarrhea, mal absorption and electrolyte imbalance, stool samples were examined. In intestinal myiasis live dark maggots came out in fecal samples and were diagnosed microscopically.

Results: Ω The first case was diagnosed as ancylostomiasis. In the case with suspected leishmaniasis, microscopic examination of the smears showed no amastigotes, PCR was negative for ssRNA of Leishmania and histopathology showed that it was a lymphoma of the skin. The worm extracted from the eyelid nodule was diagnosed as Dirofilaria conjunctivae. Stool analysis of the case with diarrhea, malabsorption and electrolyte imbalance showed all stages of Capillaria philippinensis. The dark tiny worms in the stool were diagnosed as larvae of Clogmia albipunctata

Conclusions: Ω Unusual parasite (an animal parasite, not known to infect man), unusual site of infection (ectopic parasite), unusual geographic area, unusual age for the infection, unusual stage of the parasite, unusual clinical presentation, or unusual response to medical treatment. Data should be documented properly and honestly and should be published as they will be the guidelines for others in their diagnosis

Keywords: Ancylostomiasis, Parasitic infections, Dirofilariasis, Leishmaniasis, Ancylostomiasis, Parasitic infections,, Dirofilariasis

P062 / #1393

Topic: *AS01.5 Other studies related to living with parasites*

CRYPTOSPORIDIUM PARVUM IN CALVES IN DENMARK: HIGH PREVALENCE AND FEW SUBTYPES

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Introduction: The zoonotic Cryptosporidium parvum has been reported shed by calves in many countries, and zoonotic transmission can occur by direct contact as well as via contamination of water or food. We investigated Cryptosporidium spp. in calves in Denmark, with focus on C. parvum.

Methods: In March 2020, faecal samples were collected from 140 calves from 13 separate farms in Denmark. The samples were tested for the presence of Cryptosporidium DNA using real-time PCR and metabarcoding of small subunit ribosomal DNA (SSU rDNA). Samples positive for Cryptosporidium were subject to species identification (SSU rDNA) and typing (glycoprotein 60, gp60).

Results: Preliminary results indicate that Cryptosporidium spp. were commonly shed by calves in Denmark. Cryptosporidium spp. DNA was detected in 101 (72%) of the samples by real-time PCR, and in 46 (33%) of the samples by the metabarcoding approach. Cryptosporidium parvum was identified in 71 (51%) of the samples, and preliminary gp60 typing data indicated circulation of few subtypes, predominantly IIaA15G2R1. Other Cryptosporidium species identified were C. bovis and C. ryanae.

Conclusions: Despite the sample may not represent the population well, the study yielded relevant epidemiological baseline data. A high proportion of the investigated calves shed C. parvum, and the predominant subtype was the one that has been commonly identified in human samples. References: Stensvold et al. 2015. Cryptosporidium infections in Denmark, 2010–2014. Dan Med J 62:A5086. Thomas-Lopez et al. 2020.Veterinary Students Have a Higher Risk of Contracting Cryptosporidiosis when Calves with High Fecal Cryptosporidium Loads Are Used for Fetotomy Exercises. Appl Environ Microbiol. 86(19):e01250-20.

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Keywords: Cryptosporidium parvum, Zoonosis, Denmark

P063 / #1704

Topic: *AS01.5 Other studies related to living with parasites*

CHARACTERIZATION OF NAEGLERIA GRUBERI MITOCHONDRIAL T2SS BY SPATIAL PROTEOMICS

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Introduction: Type II protein secretion systems (T2SS) are molecular machines that promote specific transport of folded proteins in Gram-negative bacteria through a dedicated channel across the outer membrane. Recently, homologues of core T2SS components were discovered in mitochondria of several unicellular eukaryotic lineages, including both parasitic and free-living *Naegleria* species. This project attempts to characterize the proteome of *N. gruberi* with the focus on the mitochondrial sub-compartments, the role of T2SS pathway and the involvement of other cellular compartments.

Methods: Differential centrifugation, western blot analysis, transmission electron microscopy, DC-LOPIT.

Results: Differential centrifugation method was developed for separation of cellular organelles. Ten different cellular fractions have been analyzed by DC-LOPIT and the results are used for spatial proteomics.

Conclusions: This study provides the first insight into the overall proteome of cellular organelles in *Naegleria* species and the role T2SS in the biology of these eukaryotes.

Keywords: T2SS, Mitochondria, DC-LOPIT

P064 / #948

Topic: *AS01.5 Other studies related to living with parasites*

INVESTIGATION OF IN-VITRO MECHANICAL TRANSMISSION OF ACANTHAMOEBA BY ANOPHELES STEPHENSI

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Introduction: Acanthamoeba is a microscopic free-living ameba that can cause eye, skin, and central nervous system infections. The amoeba can be found in water and soil all across the world. The amoeba can be transmitted through contact lenses, cuts, and skin sores. Most people will contact Acanthamoeba at some point in their lives.

Methods: In this study, Acanthamoeba was isolated from an Acanthamoeba keratitis patient. After confirmation by morphological and molecular methods, the amoeba was amplified using a PYG culture medium. 150 Anopheles stephensi reproduced in the insectarium were placed next to the cysts and trophozoites of Acanthamoeba for 30 minutes. They were then transferred back to the cage, and 5 of them were cultured in the non-nutrient agar seeded with (E. coli). The rest were taken out of the cage (5 per day) and cultured for eight days. Five Anopheles were cultured in the non-nutrient agar before exposure to the parasite to ensure that they were not contaminated with amoebae. In addition, the midgut of 5 of them was isolated and cultured in the same way as before and every day. After 30 min exposure to amoeba, five of them were washed with phosphate-buffered saline and were cultured in the non-nutrient agar mediums.

Results: In the control group, Anopheles were not infected with amoebae before contact. Anopheles were not cleared of amoebae after washing and became positive. Anopheles tested positive for parasites every eight days. But their midgates were not infected in any of the studied groups.

Conclusions: Given the prevalence of Free-living amoeba in nature, Anopheles and perhaps other insects that need further investigation can transfer amoebae mechanically, and high-risk individuals, such as contact lens wearers, need to be informed.

Keywords: Acanthamoeba, Anopheles stephensi, Mechanical transmission

P065 / #449

Topic: *AS01.5 Other studies related to living with parasites*

ORIGIN AND EVOLUTION OF CYST WALL PROTEIN 1 IN GIARDIA INTESTINALIS AND OTHER METAMONADA

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Introduction: The formation of an infectious cyst is a key part of life cycles of many parasitic organisms, including Giardia intestinalis. Encystation of Giardia occurs in the lower parts of gastrointestinal tract and is induced by increased pH and higher bile concentration. During encystation, unique organelles known as encystation-specific vesicles (ESVs) originate from the ER. ESVs serve for the accumulation and transport of the cyst wall material to the surface of the cell. The wall is composed of a fibrous matrix, containing three paralogous cyst wall proteins (CWPs) and a Giardia-specific β-1,3GalNAc homopolymer. The aim of our project is to utilize CWP1 knock-out line to study (i) the function of this essential cyst component via the re-introduction of its truncated forms and (ii) the ability of CWP homologues from free living and parasitic metamonads to restore Giardia cyst wall formation leading to a viable cyst.

Methods: CRISPR/Cas9 system gene deletion, encystation, excystation, microscopy

Results: Modified versions of Giardia CWP1 and full length CWP homologues from free-living and parasitic representatives of Metamonada group were successfully expressed in CWP1 knock-out line. Their localization was observed by fluorescence and electron microscopy. Interestingly, some of the constructs formed a sparse fibrous envelope around cyst but the cysts remained viable and able to re-establish a culture upon excystation.

Conclusions: This work aims to provide a new insight into the biology of Giardia cysts as well as the origin of encystation in Metamonada.

Keywords: Giardia, Cyst Wall Protein 1, CRISPR/Cas9, Encystation, Metamonada

P066 / #947

Topic: *AS01.5 Other studies related to living with parasites*

GET GIARDIA TAILS: THE GUIDED ENTRY OF TAIL-ANCHORED PROTEINS PATHWAY IN GIARDIA INTESTINALIS.

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Introduction: Tail anchored (TA) proteins carry a single C-terminal transmembrane domain that anchor them to organelle membranes. This topology enables TA proteins to mediate interaction among the compartments in processes such as vesicular transport, apoptosis and protein translocation. TA proteins are targeted post-translationally to the ER membrane by the Guided Entry of TA proteins (GET) pathway, which is well studied in mammals and yeast. Here, we used the parasitic protist Giardia intestinalis as a model organism for the characterization of the GET pathway. In addition to giardia-specific information, our aim is to define the evolution of the GET pathway in eukaryotes.

Methods: Affinity purification; microscopy; phylogenetics; structural biology

Results: We identified all components of the GET pathway in Giardia intestinalis (giGet1-5, giSgt2) and proposed their conserved role in post-translational transport. Based on GRAVY and AGADIR scores potential GET pathway substrates were determined. The functional binding of TA proteins by giGet3 and giSgt2 was confirmed by an in vitro binding assay. Moreover, the interactome of s Get components revealed the involvement of Get components in the other cellular pathways such as protein degradation. Purified giGet3 was used to define the structural rearrangements during the catalytic cycle of TA protein insertion.

Conclusions: We identified all members of the GET pathway in G. intestinalis, confirmed their functionality and defined the general structural properties of Get3. This study shows the conservation of the GET pathway in all eukaryotes and confirmed that giardia is a suitable model organism for revealing general mechanisms of protein transport in the eukaryotic cell.

Keywords: Giardia intestinalis, endoplasmic reticulum, tail-anchored protein transport, Get pathway

P067 / #944

Topic: *AS01.5 Other studies related to living with parasites*

IMPORTANCE OF UDP-N-ACETYLGLUCOSAMINE 4'-EPIMERASE IN G. INTESTINALIS CYST-WALL FORMATION

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Introduction: On the surface of the Giardia intestinalis cysts is a thick fibrillar wall providing the necessary protection against external environment. The cyst wall is composed of proteins and carbohydrates. The major carbohydrate is a unique β (1-3) N-acetylgalactosamine homopolymer (known as Giardan). It is synthesized from glucose by cytosolic enzymes induced during encystation. This study focuses on functional characterization of UDP-N-acetylglucosamine 4'-epimerase (UAE) , that is involved in Giardan synthesis. For that purpose, CRISPR/Cas9 system was used for targeted deletion of the corresponding gene from the G. intestinalis genome.

Methods: CRISPR/Cas9 system gene deletion, encystation, proteomics, microscopy

Results: A stable cell line was obtained in which all four copies of UAE gene were successfully deleted. Encystation-specific vesicles were detected in the encysting mutant cells and typical morphological changes such as oval shape formation, flagella resorption or presence of four nuclei were observed. However, these cyst-like stages had thin surface layer and were unable to form typical thick cyst wall with fibrillar appearance at their surface.

Conclusions: The results show that mutant cells are able to initiate encystation and cell differentiation but no viable cysts are produced. In conclusion, UAE has an indispensable role in Giardan synthesis and its absence affects the assembly of the complete cyst wall and the achievement of environmental resistance.

Keywords: Giardia intestinalis, Encystation, cyst-wall formation, N-acetylgalactosamine

P068 / #713

Topic: *AS01.5 Other studies related to living with parasites*

NEOCARIDINA DAVIDI AS AN ALTERNATIVE OOCYST FILTER CRYPTOSPORIDIUM SPP. FROM WATER

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Introduction: Neocaridina davidi is a species of shrimp, with high resistance to environmental influences, living in streams and ponds in the wild. Their food source is leftovers from other animals, dead plant parts and other waste. In this experiment, we monitored the ability of N. davidi to filter Cryptosporidium spp. from water.

Methods: 120 shrimps were divided into four aquariums (A, B, C, n = 30), including the control (K), while drinking water with the same parameters was infected with different concentrations of faeces (A: 8g; B: 4g; C: 1g) with a known number of oocysts (24 oocysts per gram of faeces) of the parasite Cryptosporidium parvum genotype IIaA11G2R1. We took 30 pieces of shrimps from each aquarium and processed them in 3-time intervals (6 hours, 12 hours and 24 hours). We processed the shrimps whole and isolated the DNA using a tissue isolation kit. Nested PCR detected C. parvum, targeting the region of the GP60 gene.

Results: Gel electrophoresis showed the presence of C. parvum in 81 shrimp samples. The parasite was not found in the aquarium with the control group; there were 30/30 positive shrimps in the group A. In group B 29/30 and group C, there were 22/30 positive shrimps, while shrimps showed the highest values of positivity at 12 hours.

Conclusions: The presence of C. parvum was confirmed by sequencing, which demonstrated the filtering capabilities of N. davidi. Therefore, shrimps can be used to quickly diagnose the presence of protozoa in a small amount of water.
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Keywords: Neocaridina davidi, water, Nested PCR, Cryptosporidium spp.

P069 / #319

Topic: *AS01.5 Other studies related to living with parasites*

EVALUATION OF A NEW ELISA TEST FOR THE DIAGNOSIS OF ANISAKIDOSIS IN HUMAN

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Introduction: Anisakidosis is the infestation of humans by larvae of nematodes of the Anisakidae family, parasites in the adult state of the digestive tract of marine mammals. Human contamination occurs after ingestion of raw or undercooked sea fish. The larvae can no longer evolve in humans but their presence causes digestive and allergic manifestations. The diagnosis of anisakidosis is difficult: Clinic is non-specific. Serology is of little contribution in the digestive form. Serology is more contributive in the diagnosis of the allergic form by the detection of anti-Anisakis IgE. However, there are multiple cross-reactions depending on the allergen used. The study consists of evaluating the performance of a new commercial ELISA kit developed for the serological screening of digestive anisakidosis.

Methods: Samples 128 sera were tested: 38 samples from patients with a confirmed diagnosis of anisakidosis (digestive, allergic, combined 2 forms), 43 samples from patients submitted to an anisakidosis serology request, and 47 samples from patients suffering from other parasitic diseases or other pathologies. Method The ELISA Anisakidae kit (Bordier, Switzerland) was adapted to the Evolis immunoanalysis machine (Bio-Rad, France) according to the protocol recommended by the manufacturer.

Results: The sensitivity and the specificity of the kit were 97.4% (IC95: 86.2-99.9) and 84.4% (IC95: 75.3-91.2), respectively. Cross-reactions were observed with sera from patients with loasis, anguillulosis, alveolar echinococcosis, distomatosis, trichinellosis and toxocariasis. The technique was repeatable and reproducible.

Conclusions: Our study showed the kit is suitable in the screening of digestive or allergic anisakidosis.

Disclosure: Bordier ELISA Anisakidae kits were provided by the manufacturer; however, the authors were independent in the exploitation of the results.

Keywords: anisakis, Anisakidae, Serology, Diagnostic, ELISA

P070 / #1609

Topic: *AS01.5 Other studies related to living with parasites*

MAINTAINING LOW BACTERIAL CONCENTRATIONS OF DERMATOPHAGOIDES FARINAE

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Introduction: Symbiotic bacteria in house dust mites pose a risk of immunological side effects in the clinical use of immunotherapeutic agents. In this study, we investigated the duration for which the bacterial concentration in *Dermatophagoides farinae* could be kept low with antibiotic treatment, and whether the allergenic properties of the mite changed under antibiotics treatment.

Methods: *D. farinae* was cultivated in the presence of antibiotics in an autoclaved medium. After subsequent subcultures without antibiotics, the mites were harvested, and the extract was prepared. The amounts of bacteria, lipopolysaccharides (LPS), and a major allergen (Der f 1) were measured. Human bronchial epithelial cells and mice were treated with the *D. farinae* extract to assess the allergic airway inflammation.

Results: After several weeks of antibiotic treatment, the number of bacteria and LPS decreased by 150-fold and 150,000-fold, respectively. The concentration of Der f 1 remained unchanged by antibiotics treatment. The secretion of interleukin (IL)-6 and IL-8 from the human airway epithelial cells decreased when treated with the extract of antibiotics-treated *D. farinae* compared to that of antibiotics-untreated *D. farinae.* A mouse asthma model was developed using antibiotics-treated *D. farinae.* We observed that the level of lung function, airway inflammation, and serum specific immunoglobulin were not different for the mouse asthma model developed using antibiotics-treated *D. farinae* than the model developed using antibiotics-untreated *D. farinae*.

Conclusions: We developed *D. farinae* with a low bacterial content, which was sufficient to induce allergic sensitization and an immune response. This method will be used to develop more controlled allergy immunotherapeutic agents.

Keywords: lipopolysaccharides, Dermatophagoides farinae, house dust mite, asthma, Microbiome

P071 / #1421

Topic: *AS02.1 Malaria*

POPULATION GENETIC ANALYSES OF PFS230 IN MALARIA PARASITE ISOLATES AMONG GHANAIANS SUGGESTS HIGH FIELD EFFICACY OF CURRENT VACCINE CANDIDATES.

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Introduction: The prodomain plus the first cysteine motif domain (CMD1) of Pfs230 (herein referred to as the 'vaccine region') are the components transmission blocking vaccine candidates under development, whose designs are mostly based the 3D7 laboratory strain. This design stratergy neglects the possible impact of parasite population genetics on efficacy of future field vaccine trials. This study assessed the genetic diversity of the vaccine region of Pfs230 in Ghanaian parasite isolates and identifying any associations with MSP2 diversity.

Methods: The gene encoding the vaccine region of Pfs230 of malaria parasites from patients in Ghana was amplified by PCR, deep sequenced and assessed for population genetics parameters. MSP2 genotyping of the parasites were done by PCR and subsequent agarose gel electrophoresis.

Results: A total of 9 variants, including one deletion and 8 SNPs were identified. The CMD1 domain had the highest diversity and sequence differentiation was observed across the the country. No destabilizing mutations were identified within the recently reported binding site for a human monoclonal antibody with potent transmission blocking activity. Out of 13 circulating haplotypes observed, the 3D7 haplotype was the third most prevalent. Neutrality tests suggested the overall vaccine region was not under selection. None of the identified haplotypes were exclusive to any MSP2 allele.

Conclusions: The 3D7 sequence of P. falciparum was not the most prevalent haplotype in Ghana although identified variants have been reported not to render a potent monoclonal antibody inefective. Circulating Pfs230 haplotypes are not associated with any particular MSP2 clone.

Keywords: Vaccine, Pfs230, Malaria, transmission

P072 / #1697

Topic: *AS02.1 Malaria*

CHARACTERISTICS OF MALARIA OR BACTERAEMIA IN CHILDREN WITH SICKLE CELL DISEASE

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Introduction: The characteristics of malaria may overlap with other causes of fever in SCD patients. The aim of this study was to characterise the features of malaria and bacteraemia in febrile SCD children.

Methods: Children with SCD presenting with an acute febrile illness were recruited in Accra, Ghana. A detailed clinical examination, blood smears, total white blood cell (WBC) and differential counts, blood and urine cultures were done.

Results: A total of 637 SCD children aged 1 to 12 years with acute febrile illness, 146 febrile non-SCD and 82 SCD children in steady state, were enrolled. Malaria parasitaemia was present in 54 (8.5%) and 60 (41.1%) febrile SCD and non-SCD children, respectively. A positive blood culture was present in 35 (5.5%) febrile SCD patients. Admission parasite density and haemoglobin were lower, while total WBC, neutrophil, lymphocyte, and platelet counts were higher in SCD malaria patients, compared with non-SCD malaria patients. Among SCD patients, haemoglobin and platelet counts were lower, while total WBC, neutrophil and lymphocyte counts were higher in those with malaria compared with those in steady state. Among SCD patients with fever, admission temperature was higher, while haemoglobin and platelet count were lower in those with malaria compared with those with negative parasitaemia; and admission temperature, total WBC and neutrophil counts were comparable, while haemoglobin and platelet counts were lower, in those with malaria compared with those with a positive blood culture. Fever and vaso-occlusive pain syndrome were predominant symptoms but differentially clustered.

Conclusions: Acutely ill SCD patients exhibit diverse clinical and laboratory characteristics, with SCD patients with malaria exhibiting distinct features.

Keywords: Sickle cell disease, bacteraemia, Children, Malaria

P073 / #1655

Topic: *AS02.1 Malaria*

MOLECULAR EPIDEMIOLOGY AND K13-PROPELLER GENE POLYMORPHISM OF PLASMODIUM FALCIPARUM FROM MALARIA MESOENDEMIC REGION OF NIGERIA

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Introduction: The emergence of resistance to artemisinin derivatives in western Cambodia is threatening to revert the recent advances made toward global malaria control and elimination. The aim of this research was to examine the present status of the *Plasmodium falciparum* K13 propeller gene polymorphism(k13) as molecular marker for artemisinin resistance *P. falciparum* in Yobe State, Nigeria.

Methods: Total of 161 thick and thin blood smears from patients with monomalaria infection were screened microscopically, the samples were analyzed using Nested PCR protocol. Positive samples were sequenced and single nucleotide polymorphism analysis was carried out to detect possible mutations.

Results: The result of the present study showed that 29.2% (47/161) are positive for *P. falciparum* K13 gene. This is highest among subject aged 0-10 years with 46.7% (n=45). Analysis of single nucleotide polymorphisms revealed 88 novel polymorphisms on K13. Twenty six of which are Nonsynonymous. The phylogenetic analysis indicated that all the samples cluster together with the reference sequences except 3571195 sample 12 that cluster with 3571224 sample 3 and 3569033 sample 36 that cluster with 3569041 sample 43 indicating that they are more closely related with each other than with the reference sequences.

Conclusions: The current findings of this study show that mutations strongly linked to artemisinin resistance are not present in Yobe State, at this time, though other mutations closely related to the validated mutations, such as the Y458H, F493I, and S560N, may have the same consequences. Their significance in terms of artemisinin resistance, however, needs to be proven further.

Keywords: molecular-epidemiology, K13-Propeller-gene Polymophisms, Malaria molecular epidemiology

P074 / #705

Topic: *AS02.1 Malaria*

DIAGNOSTIC ACCURACY OF A SINGLE HRP2/PLDH (PAN) RAPID DETECTION TEST AND PAIRED BLOOD FILM TO EXCLUDE IMPORTED MALARIA AT SHEFFIELD CHILDREN'S HOSPITAL NHS FOUNDATION TRUST

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Introduction: Misdiagnosis of Malaria could result in significant morbidity and mortality. Rapid, accurate and accessible detection of falciparum and non-falciparum malaria parasites through the use of local combined HRP2/pLDH (Pan) Rapid Detection Test (RDT) with a paired blood film has an important role in addressing this. However, there are known limitations to the efficacy of RDTs such us its lack of validation for the recognition of Knowlesi malaria, currently prevalent in some countries of Southeast Asia.

Methods: Retrospective study of 196 presumed cases of imported Malaria seen at SCH between 2014 and 2019. All of them had at least one RDT, blood film and Full blood count to exclude disease. Hospital numbers were facilitated by our local Haematology lab. The data was collected from local electronic records. Data analysed using Microsoft Excel and MedCalc software.

Results: Overall, a single RDT was diagnostically accurate in all 17 cases of confirmed Malaria (100.00%; 95% CI 98.15%-100.00%) of which 15 had a positive paired blood film (98.99%; 95% CI 96.40%-99.88%). All confirmed cases seen at SCH travelled from African countries and presented with symptoms and abnormal FBC.

Conclusions: Serial combined HRP2/pLDH (Pan) RDT and blood films do not seem to increase the overall diagnostic accuracy. We propose the use of a single RDT to rule out malaria in children travelling from non-plasmodium knowlesi endemic countries. A multi-centre approach may be required to provide a larger sample size to power the study.

Keywords: blood film, imported malaria, malaria RDT, Children

P075 / #1368

Topic: *AS02.1 Malaria*

ELEVATED CLEARANCE AND DISTRIBUTION VOLUME OF DESETHYLAMODIAQUINE IN PAEDIATRIC SICKLE CELL DISEASE PATIENTS TREATED WITH ARTESUNATE-AMODIAQUINE

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Introduction: There is limited information on the safety or efficacy of recommended antimalarial drugs in patients with sickle cell disease (SCD), a population predisposed to worse outcomes if affected by acute malaria. Artesunate-amodiaquine (ASAQ) is used in the treatment of uncomplicated malaria in a number of countries, and is also used for treatment of uncomplicated malaria in SCD patients. There is paucity of data on the pharmacokinetics (PK) of amodiaquine or artesunate or the metabolites of these drugs in SCD patients.

Methods: A population PK modelling approach was used to analyze plasma desethylamodiaquine (DEAQ) concentrations obtained between 64 and 169 hours after oral administration of ASAQ in paediatric SCD patients with acute uncomplicated malaria (n=16). The PK parameters were compared with those of concurrently recruited non-SCD paediatric patients with acute uncomplicated malaria (n=13), and with DEAQ concentrations of a historical paediatric population treated with ASAQ (n=103) from the same study setting.

Results: The median DEAQ concentrations were lower in the SCD group compared to the non-SCD group; although the difference was not statistically significant. A two-compartment model best described the plasma DEAQ concentration-time data. The estimated population clearance of DEAQ was higher in the SCD patients (67 L/h, 21% relative standard error (RSE) compared with the non-SCD population (15.5 L/h, 32% RSE). The central volume of distribution was larger in the SCD patients compared with the non-SCD patients (4400 L, 43% RSE vs. 368 L, 34% RSE).

Conclusions: The data suggest altered DEAQ disposition in SCD patients with acute uncomplicated malaria, probably reflecting anticipated pathophysiological changes.

Keywords: Desethylamodiaquine, Sickle cell disease, Malaria, Clearance

P076 / #913

Topic: *AS02.1 Malaria*

BOTH PARASITE AND HOST FACTORS DRIVE THE SPONTANEOUS POST-PARTUM CLEARANCE OF P. FALCIPARUM PARASITES

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Introduction: African pregnant women with peripheral P. falciparum parasitemia spontaneously loss the parasites few hours after delivery. We characterized var gene transcript profiles and VAR2CSA-specific and non-VAR2CSA-specific antibody responses among Ghanaian women from a period near delivery through to the post-partum period to gain insight on the role of parasite and host factors that drive spontaneous parasite clearance.

Methods: Seventeen (17) out of 377 pregnant women in-labour diagnosed with peripheral malaria parasitemia were followed longitudinally after delivery to a point when they had cleared the parasites (8-16 hours, 24-48 hours, and 48-72 hours post-partum). Ten (10) controls/aparasitemic women were also followed longitudinally.

Results: Peripheral parasitemia declined by 99.3%/99.7% within 24-48 hours post-partum as quantified by microscopy/PCR. Var gene transcription analysis revealed a heterogeneous population of pre-delivery parasites, with a majority predominantly expressing var2csa and a few predominantly expressing ABC var groups. Within the post-partum period both var2csa and ABC var group transcripts declined/diminished significantly. Antibody trend analysis revealed VAR2CSA-specific IgG responses were stable while non-VAR2CSA-specific IgG responses subtly increased from pre-delivery to 72 hours post-partum. In control women, both VAR2CSA-specific and non-VAR2CSA-specific IgG responses remained stable within the post-partum period.

Conclusions: Put together, our data suggest the absence of a placental sequestration focus and Placental malaria-induced boosting of non-VAR2CSA PfEMP1-specific immunity drives rapid clearance of parasites in the post-partum period.

Keywords: Var gene transcription, Post-partum, placental sequestration focus, Parasite Clearance, PfEMP1-specific immunity

P077 / #486

Topic: *AS02.1 Malaria*

MALARIA-VISCERAL LEISHMANIASIS CO-INFECTION AND ASSOCIATED FACTORS AMONG MIGRANT LABORERS IN WEST ARMACHIHO DISTRICT, NORTH WEST ETHIOPIA

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Introduction: Malaria and leishmaniasis are the two largest parasitic killers in the world. Due to geographical overlap of these diseases, malaria-visceral leishmaniasis co-infections occur in large populations. The aim of this study was to determine malaria-visceral leishmaniasis co-infection and their associated factors among migrant laborers.

Methods: Community based cross-sectional study was conducted from October–December 2016. Standardized questionnaire was used to collect socio-demographic data. Capillary blood was collected for Giemsa stained blood film examination to detect and identify Plasmodium parasites. Recombinant kinensin (rk39) antigen test was performed to detect anti-leishmania donovani antibody. Data was entered, checked for completeness and analyzed using SPSS version-20 statistical software. Chi-square test was applied and a P-value < 0.05 was considered as statistically significant.

Results: A total of 178 migrant laborers were included in this study. 74.2% belong to the age group 15–29. Seroprevalence of visceral leishmaniasis was 9.6% and 22.4% of migrant laborer were malaria infected. The prevalence of malaria-VL co-infection was 2.8%. Of the total migrant laborer, 47.8% used bed nets, of them 1.2% were malaria-VL co-infected; 72.5% used outdoor sites as usual sleeping site, among them 3.1% were malaria-VL co-infected; 60.1% were migrants, of which 2.8% were malaria-VL coinfected. All variables were not significantly associated with malaria-visceral leishmaniasis co-infection.

Conclusions: Prevalence of malaria-visceral leishmaniasis co-infection was low and it is not significantly associated with residence, number of visits, bed net utilization and outdoor sleeping habit even if both diseases are prevalent in the study area.

Keywords: Visceral Leishmaniasis, Malaria, co-infection

P078 / #257

Topic: *AS02.1 Malaria*

MALARIA AND DENGUE AMONG SOMALI REFUGEES IN MUKALLA.YEMEN

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Introduction: Background: Hadhramout governorate of Yemen is in a pre-elimination phase of malaria. The presence of migrants, refugees and displaced persons is considered one of the challenges for malaria elimination. the aim of the study is to investigate the status of malaria, dengue fever among the Somali refugee population in Mukalla city of Hadhramout at eastern Yemen.

Methods: it was a cross-sectional community-based study, sample size was calculated to be 512 Somali refugees living in Mukalla city. Sociodemographic and morbidity related data were collected through well-structured questionnaire. Blood samples were collected and investigated for malaria (rapid diagnostic test and confirmed by blood films) and also use ELISA for dengue virus antibodies determination.

Results: Findings: The total sample of the survey is 512 people . About 6.3% of the sample members said that they contracted malaria during 2021. And 3.7% of their family members had contracted malaria. dengue fever is common (13.9%), followed by malaria (6.3%), severe watery diarrhea or suspected cholera (5.5%), then hepatitis (jaundice) (2.3%). All malaria tests were negative, while the dengue yest was positive in 460 of the total sample (89.8%), all of which are IgG type. Only 20 out of the 512of particpants own a bed net, (4%) and only six people who own nets slept under the net at night (6/20, 30%).

Conclusions: Although the rapid examination of the malaria parasite among the sample of Somali refugees in Mukalla did not show any infection during the survey, the statement of some respondents that they had contracted malaria must be taken into account. A clear rise in IgG dengue virus antibodies, indicates that dengue fever spreads in a seasonal or epidemic manner.

Keywords: Malaria, Dengue, Refugee

P079 / #736

Topic: *AS02.1 Malaria*

THE DEVELOPMENT AND CHARACTERISATION OF NOVEL ANTIMALARIAL CLASS TARGETING A PUTATIVE MITOCHONDRIAL CARRIER PROTEIN

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Introduction: Malaria has long been heralded a “preventable and curable disease.” However, parasite resistance against all currently available antimalarials, including the first-line treatment Artemisinin combination therapy (ACT), threatens our efforts to control the disease. An urgent need has arisen towards the development of antimalarials with novel mechanisms of action.

Methods: In collaboration with Janssen Pharmaceuticals and Medicines for Malaria Venture, a high-throughput screen was undertaken against the asexual blood stage of Plasmodium falciparum, identifying several novel antimalarial classes. One of these series is the focus of the present studies and is mediated by an unknown mechanism of action. Medicinal chemistry optimisation has generated potent nanomolar inhibitors which have been used to characterise the series’ activity in parasites phenotypically. A complement of proteomic, genomic and biomolecular techniques has been employed to characterise the target of this antimalarial series.

Results: The series was identified to act with a slow to moderate rate of kill, arresting parasites at the trophozoite stage. Potency is maintained in P. falciparum multidrug resistant strains, but reduced activity is observed in P. knowlesi, indicating a species difference in the target. Target identification studies have uncovered a putative mitochondrial protein as the molecular target of the series.

Conclusions: Using a multidisciplinary approach, we have identified a novel antimalarial class with potent activity against asexual P. falciparum parasites. This series has enabled identify a previously untargeted protein, a putative mitochondrial carrier of unknown function. Work is ongoing to biochemically and structurally define this putative protein.

Keywords: plasmodium, Therapuetics, Antimalarial

P080 / #945

Topic: *AS02.1 Malaria*

DEEPER INTO HEMOZOIN FORMATION PATHWAY OF PLASMODIUM FALCIPARUM

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Introduction: Plasmodium falciparum malaria remains a public health problem, accounting for half millions of lives yearly. Artemisinin-combination therapies are the front-line treatment against malaria, which efficacy is now threatened by parasite's-acquired resistance. Most antimalarial drugs target the symptomatic intra-erythrocytic stage of infection. During parasite development inside the human erythrocytes, parasites digest hemoglobin as a source of nutrients. This causes the release of toxic free heme, which the parasite detoxifies into inert crystals, called hemozoin. It is known that parasites with abnormalities in this pathway are committed to death. However, despite its importance, the orchestration of hemoglobin digestion into hemozoin formation has not been entirely resolved, and its players have not been identified.

Methods: Through reverse genetics, we explored the role of P. falciparum aspartic proteases and other genes thought to be involved in the formation of hemozoin and their capacity to modulate drug response.

Results: We believe that a deeper understanding of this pathway is of great interest and relevant for the identification of new targets, both for diagnosis and for the development of antimalarial drugs. Furthermore, it will also provide insights into drug resistance, overall contributing for better malaria control and management.

Conclusions: V. Baptista thanks FCT for the SFRH/BD/145427/2019 grant. Maria Isabel Veiga thanks FCT for her contract funding provided through 2020.03113.CEECIND. Susana Catarino thanks FCT for her contract funding provided through 2020.00215.CEECIND.

Keywords: Antimalarial drug resistance, Hemozoin formation, Malaria, Plasmodium falciparum

P081 / #952

Topic: *AS02.1 Malaria*

OPTICAL SPECTROPHOTOMETRY INTO HEMOZOIN: TOWARDS SENSITIVE MALARIA DIAGNOSIS

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Introduction: The lack of prompt and sensitive diagnosis hampers malaria control and elimination, highlighting the need for next generation technology alternative for the established optical microscopy and Rapid Diagnostic Tests (RDTs) as means of in situ point-of-care malaria parasite detection. Hemozoin (Hz), a byproduct of hemoglobin (Hb) degradation inside human infected red blood cells (RBCs), has been extensively explored as a malaria biomarker. Along with malaria parasite maturation inside the RBCs, Hb and Hz proportion is inversely related, which originates specific optical spectra of healthy and infected samples.

Methods: Herein, we characterized the optical spectra of Plasmodium falciparum-infected RBCs, aiming the development of an innovative diagnostic device, detecting malaria without finger prick blood sampling, measuring directly in patients' skin.

Results: Absorbance and reflectance spectrophotometry demonstrate their potential by increasing the limit of detection (LoD: 12 parasites/µL of RBCs) when compared with microscopy or RDT (LoD: 50-200 parasites/µL of RBCs).

Conclusions: This sensitivity, coupled with the possible integration into a low-cost, fast, and non-invasive diagnostic device meets the growing clinical demands for malaria control and elimination.
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Keywords: Optical Spectrophotometry, Hemozoin, diagnosis, Malaria

P082 / #448

Topic: *AS02.1 Malaria*

IDENTIFICATION OF PEPTIDES MIMICKING PROTECTIVE ANTIBODY EPITOPES IN THE MALARIA ANTIGEN PFCYRPA AS POTENTIAL VACCINE CANDIDATES.

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Introduction: The Plasmodium falciparum (Pf) antigen CyRPA has recently emerged as a promising target for blood stage malaria vaccines. Our group has generated a panel of monoclonal antibodies (mAbs) directed against PfCyRPA and identified those that can inhibit the growth of Pf in vitro. In this study, we aimed at inducing these selected inhibitory antibodies at high titers in vivo through vaccination. To this end, we utilized phage display to identify short peptides mimicking the epitope of the inhibitory mAbs and used these peptides as immunogens in mice.

Methods: A library of 12 amino acid-long random peptides displayed on M13 phages was used in the biopanning with growth inhibitory PfCyRPA-specific mAbs. Phages displaying peptides binding the inhibitory mAbs were isolated and sequenced. The identified peptides were synthesized and the binding to the target mAb was confirmed by ELISA. Balb/c mice were vaccinated with selected epitope mimics and blood samples were collected at several time points after immunization. The serum antibody response of the mice was measured by ELISA

Results: We identified 13 peptide mimics specific for two different inhibitory PfCyRPA mAbs. Using ELISA, 7 out of the 13 peptides were confirmed to bind the target mAb and were selected for vaccination of mice. However, we could not detect serum antibodies binding to PfCyRPA in the peptide-immunized mice

Conclusions: We have identified 7 linear peptides mimicking the epitopes of neutralizing antibodies on PfCyRPA. We have not yet succeeded in inducing the target neutralizing antbodies in vivo by peptide-vaccination. To improve the immunogenicity of the peptides, we are currently expressing them as virus-like particles (VLPs)-based vaccines.

Keywords: Malaria vaccine, peptide mimics, Antibody epitopes

P083 / #595

Topic: *AS02.1 Malaria*

THE ROLE OF THE STRESS GRANULE RESIDENT PROTEIN 7-HELIX-1 IN THE REGULATION OF TRANSLATIONAL CONTROL DURING HUMAN-TO-MOSQUITO TRANSMISSION OF PLASMODIUM FALCIPARUM

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Introduction: Due to the change of hosts the malaria parasite Plasmodium falciparum constantly needs to adapt to changing environments. As a means to pre-adapt to the human-to-mosquito transmission, gametocytes store inactive transcripts coding for proteins needed in the mosquito stages in stress granules (SGs) and initiate translation only after transmission. We recently described a novel SG component of female gametocytes, 7-Helix-1, which interacts with known ribonucleoproteins and forms a complex with mRNAs.

Methods: We characterized 7-Helix-1 by expression analyses and gene knock out studies. The interaction of 7-Helix-1 with proteins and mRNAs was analyzed by co-immunoprecipitations and BioID experiments. We further monitored the phosphorylation status of the translation initiation factor eIF2α upon stress induction.

Results: Expression analyses revealed that 7-Helix-1 localizes to cytosolic SGs. 7-Helix-1 interacts with known ribonucleoproteins such as CITH, DOZI and Puf2 and further forms a complex with repressed mRNAs. Assembly of SGs is usually initiated by phosphorylation of the translation initiation factor eIF2α. While eIF2α phosphorylation can be induced in Plasmodium gametocytes by treatment with sodium arsenite, 7-Helix-1-KO gametocytes show a decreased eIF2α phosphorylation, indicating impaired SG formation. Using BioID we aim at identifying the proteome of SGs in WT and 7-Helix-1-KO parasites. First results confirmed known SG components and interaction partners of 7-Helix-1 in WT gametocytes and further revealed novel putative SG constituents.

Conclusions: We hypothesize that 7-Helix-1 is involved in the regulation of translation at the onset of gametogenesis via its interaction with ribonucleoproteins and transcripts in SGs.

Keywords: transmission, gametocyte, translation

P084 / #1137

Topic: *AS02.1 Malaria*

PLASMODIUM FALCIPARUM EBA-181 MEROZOITE LIGAND – SEARCHING FOR ERYTHROCYTE RECEPTOR.

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Introduction: Erythrocyte binding-like (EBL) ligands play crucial role in the attachment of Plasmodium falciparum merozoites to human erythrocytes by binding to specific receptors. Four functional P. falciparum EBL proteins were identified: EBL-1 which binds glycophorin B (GPB), EBA-175 and EBA-140 that target the glycophorin A (GPA) and C (GPC), respectively, as well as EBA-181 which was the subject of our studies due to the fact that its receptor remained not identified. Previously, it was shown that EBA-181 recognizes unknown erythrocyte membrane protein of molecular mass 33 kDa, which is sensitive on chymotrypsin treatment but it is not glycophorin B. Moreover, it was suggested that the recombinant domain of EBA-181 (aa. res. 945-1097) interacts with 10 kDa fragment of 4.1 protein, known from its vertical interaction with glycophorin C/D.

Methods: In our studies, we used far-Western blotting method with erythrocyte membranes and the recombinant binding region of EBA-181 obtained in bacterial, insect and mammalian cells.

Results: We have not observed any binding of the EB1-181 to erythrocyte membrane proteins, including: GPA, GPB, GPC, band 4.1, band 3, CD 44 and CD 55 receptor or any other protein. Due to failure to pin down erythrocyte protein receptor we focused on membrane glycolipids. Indeed, we have noticed the recombinant EBA-181 binding, on TLC plate, to blood group P1 glycosphingolipid, with terminal Galα1→4Galβ structure.

Conclusions: The revealed interaction between EBA-181 ligand and P1 antigen needs to be evaluated further in details. The P1 antigen would be the first known glycolipid receptor for P. falciparum EBA merozoite ligands.

Keywords: Plasmodium falciparum, EBA-181 merozoite ligand, host erythrocyte receptor

P085 / #1469

Topic: *AS02.1 Malaria*

EFFICACY AND EFFECTIVENESS OF CHLOROQUINE PLUS PRIMAQUINE IN PLASMODIUM VIVAX INFECTIONS IN THE COLOMBIAN AMAZON

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Introduction: Plasmodium vivax causes half of the cases of malaria in Colombia, with an annual parasite index of 4.07/1,000 in 2021, among a total of 66,655 cases. In some regions of the world, therapeutic failure of P. vivax to chloroquine -CQ-, the most widely used antimalarial for these infections, has been documented. In the Colombian Amazon, no studies have been carried out in the last 20 years to evaluate the usefulness of the standard treatment of 3 doses of chloroquine plus 14 doses of primaquine -PQ-. A multicenter study was carried out in 2021-2022 to determine the usefulness of this combined treatment.

Methods: Participants with Plasmodium vivax mono-infection diagnosed by thick blood smear were included in Tarapacá, Pedrera and Puerto Nariño localities. The therapeutic efficacy evaluation was carried out with supervised treatment of chloroquine (1,500mg/total-dose) and primaquine (210mg/total-dose) with clinical and parasitological follow-up until day 28. The participants in the effectiveness evaluation received the same treatment without supervision. Diagnosis confirmation by PCR will be made.

Results: Among 60 patients with P. vivax mono-infection, 100% parasitological efficacy was found for the chloroquine plus primaquine regimen on day 28 of follow-up; Likewise, among 60 patients, 100% effectiveness was found for the same therapeutic scheme on the 28th day of follow-up. No adverse effects to the treatment provided were documented. The results must be confirmed with molecular diagnosis at the end of follow-up.

Conclusions: These results maintain the trend of the country where only a therapeutic failure of less than 2% has been documented to the CQ plus PQ scheme, in other regions of Colombia. Surveillance of relapses with complementary studies is necessary.

Keywords: Chloroquine, primaquine, Vivax, Efficacy, effectiveness

P086 / #1711

Topic: *AS02.1 Malaria*

CONSTRUCTION OF PALM GENE KNOCKOUT STRAIN OF PLASMODIUM YOELII BASED ON CRISPR/CAS9 TECHNIQUE

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Introduction: Plasmodium yoelii (P. yoelii) PALM gene is of importance in pre-erythrocytic stage. Knock out PALM gene could generate a parasite line as attenuated vaccine.

Methods: Specific sgRNA was designed based on P. yoelii 17XNL PALM gene sequence, and the upstream and downstream homologous arms were amplified by PCR. The fragments and sgRNA were cloned to pYCm-golden-Blue plasmid to construct the CRISPR plasmid pYCm-PLAM-KO. The recombinant plasmid was transfected into P. yoelii 17XNL, and the parasites were injected into the mice though tail vein. The mice were fed with 6mg/ml pyrimidine water every day for drug selection, and the parasitemia was examined by blood smear every 2 days. After parasitemia was detected, genomic DNA was extracted. PALM gene knockout was confirmed by PCR, and further by gene sequencing. PLAM knockout parasite line was constructed by limited dilution method. After 6 days, blood samples were collected every 2 days for microscopic examination of parasites, and monoclonal PLAM knockout P. yoelii strains were confirmed by PCR.

Results: The 600bp PALM homologous arms were successfully amplified by PCR. PCR fragments and sgRNA were successfully ligated with vector, and the pYCm-PLAM-KO recombinant plasmid was constructed and electrotransfected with schizont stage P. yoelii . The mice inoculated with transfected schizont parasite, and parasites were detected in mice after 8 days pyrimidine drug pressure screening. PCR showed that PALM gene was successfully knocked out, and sequencing results further confirmed that PLAM gene knockout strain of P. yoelii was successfully obtained.

Conclusions: P. yoelii PALM gene knockout strain was successfully constructed, it could bed used for the development of live attenuated preerythrocyte malaria vaccine.

Keywords: CRISPR/Cas9, Gene knockout, Plasmodium yoelii, PALM gene

P087 / #1714

Topic: *AS02.1 Malaria*

PLASMODIUM INFECTION SUPPRESSES COLON CANCER GROWTH BY INHIBITING PROLIFERATION AND PROMOTING APOPTOSIS IN MICE

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Introduction: In this study, we investigated the anti-colon cancer effect of *Plasmodium* infection and its related mechanisms using the mouse model of colon cancer.

Methods: An experimental model was established by intraperitoneal injection of *Plasmodium yoelii* 17XNL-infected erythrocytes into mice with colon cancer. The size of tumors was observed dynamically in mice, and the expression of Ki67 detected by immunohistochemistry was to analyze tumor cells proliferation. Apoptosis was assessed by terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) staining, and the expression of apoptosis concerned proteins, including Bax, Bcl-2, Caspase-9, Cleaved Caspase-3, were detected by western blot and immunohistochemistry respectively. Transmission electron microscopy (TEM) was used to observe the ultrastructural change of colon cancer cells.

Results: *Plasmodium* infection reduced the weights and sizes of tumors and decreased the expression of Ki67 in colon cancer-bearing mice. Furthermore, *Plasmodium* infection promoted mitochondria-mediated apoptosis in colon cancer cells, as evidenced by the increased proportion of TUNEL-positive cells, the up-regulated expression of Bax, Caspase-9, and Cleaved Caspase-3 proteins, and the down-regulated expression of Bcl-2 protein. In colon cancer cells, we found destroyed cell nuclei, swollen mitochondria, missing cristae, and the decreased number of autolysosomes.

Conclusions: *Plasmodium* infection can play an anti-colon cancer role in mice by inhibiting proliferation and promoting mitochondria-mediated apoptosis in colon cancer cells, which may relate to mitochondrial biogenesis and mitophagy.

Keywords: plasmodium, Colon cancer, Mitochondrial apoptosis, Mitophagy, Mitochondrial biogenesis

P088 / #297

Topic: *AS02.1 Malaria*

DRUG-INDUCED CALCIUM DISTRIBUTION IN MALARIA INFECTED ERYTHROCYTES TRIGGERS HALLMARKS OF ERYPTOSIS

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Introduction: In order to reach the objective set by the World Health Organization to decrease malaria cases by 2030, antimalarial drugs with novel modes of action are required. We have discovered a novel mechanism of action of chloroquine (CQ) which involves features of programmed cell death in the parasite, mainly characterised by disruption of calcium homeostasis, triggering eryptosis. This study aimed to identify the mediators and outcomes of eryptosis due to calcium redistribution in drug-treated infected red blood cells (iRBCs).

Methods: Plasmodium falciparum mid-late trophozoites were incubated with antimalarial drugs. Late-stage parasites were enriched via MACS and analysed using flow cytometry, electron microscopy and western blot. Moreover, we employed ultracentrifugation to isolate extracellular vesicles (EVs) and mass spectrometry for protein profiling.

Results: Our data showed that CQ-treated iRBCs have higher levels of phosphatidylserine externalization on the plasma membrane dependent on intracellular calcium distribution. iRBCs diameter measurements from scanning electron micrographs indicated cell shrinkage post-CQ treatment. Furthermore, we demonstrated that calcium distribution in iRBCs exposed to CQ triggers calpain-mediated cleavage of cytoskeleton leading to knob-associated histidine-rich protein loss and EV release. Interestingly, the proteomic analysis of EVs from CQ-treated iRBCs revealed 2 main protein clusters which might have unique roles in recipient cells.

Conclusions: Our results shed new insights into a novel drug-induced cell death mechanism which targets the parasite and specific components of the infected host RBC.

Keywords: Calcium, Eryptosis, Chloroquine, Malaria

P089 / #1311

Topic: *AS02.1 Malaria*

INFECTION OF NEOTROPICAL ANOPHELES SPP. MOSQUITOES BY PLASMODIUM CYNOMOLGI SPOROZOITES OBTAINED FROM INFECTED MACACA MULATTA

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Introduction: Relapses of Plasmodium vivax caused by dormant liver hypnozoites compromise malaria eradication efforts. The infection of Macaca mulatta (Rhesus monkeys) by Plasmodium cynomolgi is a well-established model for studying hypnozoites. Three South American Anopheles species and also Aedes aegypti were investigated for susceptibility to P. cynomolgi infection.

Methods: Plasmodium cynomolgi blood stages were inoculated intravenously in M. mulatta. Infected monkey blood containing gametocytes was fed to different mosquito species: Anopheles aquasalis, An. albitarsis, An. darlingi and Aedes aegypti females, using standard membrane feeding assays. After 7 days the intestines were dissected to check oocyst numbers and on day 14 the salivary glands were dissected to obtain sporozoites, which were used to infect a new Rhesus monkey.

Results: Anopheles albitarsis were not susceptible to P. cynomolgi. Aedes aegypti produced oocysts on day 7, but on day 14 sporozoites were not observed. Anopheles aquasalis and An. darlingi females were susceptible to P. cynomolgi, producing oocysts on day 7 and sporozoites on day 14 visualized by microscopy, but in general low sporozoite numbers were obtained (9,000 and 2,100 sporozoites per mosquito, respectively). Rhesus monkeys were inoculated with sporozoites obtained from An. aquasalis, however blood samples collected from these monkeys were negative for parasitemia, by both microscopy and PCR, and inoculated Rhesus blood fed to An. aquasalis females failed to induce infection.

Conclusions: Optimization of the specific procedures for An. aquasalis and eventually for An. darlingi infection by P. cynomolgi is necessary for establishing the parasite complete cycle using these neotropical Anopheles mosquitoes.

Keywords: Malaria, Plasmodium cynomolgi, Neotropical Anopheles, Macaca mulatta, Hypnozoites

P090 / #1406

Topic: *AS02.1 Malaria*

RED BLOOD CELL BCL-XL IS REQUIRED FOR PLASMODIUM FALCIPARUM SURVIVAL: INSIGHTS INTO HOST-DIRECTED MALARIA THERAPIES

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Introduction: Antimalarial drug resistance is an ongoing problem threatening progress towards malaria elimination. Antimalarial treatments are urgently needed for drug-resistant malaria infections and host-directed therapies (HDT) represent an attractive strategy with untapped targets and low propensity for resistance. In addition, drug repurposing can lead to a substantial decrease in the time and resources required to develop novel antimalarials. Host BCL-$_{XL}$ is a major target in anti-cancer therapy and is essential for the development of numerous intracellular pathogens. We hypothesised that red blood cell BCL-$_{XL}$ is essential for Plasmodium development and this study tested this hypothesis.

Methods: Six BCL-$_{XL}$ inhibitors were assessed for their anti-Plasmodium activity via parasite growth assays. Subcellular localisation of Bcl-$_{XL}$ was determined by fluorescent microscopy and western-blot. Bcl-$_{XL}$ molecular complexes were identified by immunoprecipitation of Bcl-$_{XL}$ followed by mass spectrometry.

Results: All BCL-x_L inhibitors tested impaired proliferation of P. falciparum 3D7 parasites in vitro at low concentrations. Western blot analysis and immunofluorescence microscopy assays revealed that BCL-x_L is transferred from the host RBC to the parasite upon infection. Immunoprecipitation of BCL-$_{XL}$ coupled with mass spectrometry identified that BCL-$_{XL}$ forms unique molecular complexes with human μ-calpain in uninfected RBCs, and with human SHOC2 in infected RBCs.

Conclusions: Inhibition of host erythrocyte Bcl-XL impairs the development of P. falciparum parasites. These results open exciting perspectives for the development of host-directed antimalarial therapies and drug repurposing efforts.

Keywords: Host-parasite interaction, Bcl-XL, Malaria, host-directed therapy, P falciparum

P091 / #825

Topic: *AS02.1 Malaria*

EVALUATING THE EFFECTS OF SYNCHRONIZATION AND PARASITES CULTURE-ADAPTATION ON THE SUSCEPTIBILITY OF P. FALCIPARUM PARASITES TO ANTIMALARIALS

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Introduction: Plasmodium falciparum parasites can dynamically shift from synchronous to asynchronous in in vitro cultures, leading to a corresponding shift in the patterns of response to antimalarials. Furthermore, asynchronous clinical isolates are known to harbour more diverse parasite genotypes than synchronous ones. Therefore, this study evaluated the effects of sorbitol and Percoll synchronization of P. falciparum parasites on their susceptibility to antimalarials.

Methods: Using in vitro growth inhibitory assays, this study evaluated the response of asynchronous clinical P. falciparum parasites to antimalarials and compared it to those synchronized with sorbitol and Percoll.

Results: From the data, the sorbitol synchronized parasites were more susceptible to the antimalarials compared to the Percoll synchronized parasites. We also observed that the response of the asynchronous and synchronous parasites to the antimalarials varied with respect to the antimalarial compound, the life cycle stage of the parasites, the synchronization method and between the clinical isolates and laboratory strains. Furthermore, the clinical isolates had increased sensitivity to the antimalarials during long-term culturing activities.

Conclusions: Therefore, during compound screening activities, it is important to consider the effect of synchronization and long-term culturing of clinical isolates on their response to antimalarials. Achieving this will provide a broader analysis of the potency of antimalarial compounds before they are further developed for clinical use.

Keywords: Sorbitol, Clinical Isolates, P. falciparum, Synchronization, Percoll

P092 / #1363

Topic: *AS02.1 Malaria*

THE INTRACELLULLAR CALCIUM DYNAMICS AND CELLULAR CONSEQUENCES OF LOW-MICROMOLAR LEVEL OF CHLOROQUINE ON THE MALARIA PARASITE PLASMODIUM FALCIPARUM

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Introduction: Previous studies in our group have unveiled a novel mode of action in which low-micromolar level of chloroquine (CQ) permeabilized the parasite's digestive vacuole (DV) membrane, leading to calcium redistribution, mitochondrial depolarization, and DNA degradation. These phenotypes implicate an alternative cell death mechanism triggered by low-micromolar CQ. We aim to (1) investigate the source(s) of Ca^{2+} release and CQ-induced phenotypic outcomes and (2) to identify novel Ca^{2+} redistributing screening compounds from the MMV Pathogen Box library.

Methods: High-throughput and high-content screening using an imaging flow cytometer was performed to select compounds which redistribute intracellular calcium, as measured by the Ca^{2+} probe Fluo-4 AM.

Results: By applying a series of organellar calcium efflux inhibitors, mitochondrial calcium (Ca^{2+mt}) was shown to be implicated in initiating this cascade of cell death phenotypes. 3 MMV hit compounds were subjected to further assays. 10 analogues were synthesized from the top hit and one analogue emerged as the top candidate drug based on its efficacy.

Conclusions: The work presented here uncovered an unexpected importance of Ca^{2+mt} in triggering cell death phenotype in P. falciparum, mechanistic insight into calcium dysregulation as a novel pathway target provides invaluable opportunities for therapeutic interventions against malaria.

Keywords: mitochondrial depolarization, cell death, Plasmodium falciparum, Chloroquine, calcium redistribution

P093 / #1371

Topic: *AS02.1 Malaria*

A RAPID SENSITIVE, IMAGING FLOW CYTOMETRY-BASED METHOD FOR THE DETECTION OF ZOONOTIC MALARIA PARASITES

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Introduction: Naturally acquired human infection of zoonotic malaria caused by Plasmodium knowlesi is now a major emerging disease, especially in Southeast Asia. Other two non-human primates infectious agents, P. cynomolgi and P. inui are also capable of transmission to human. These zoonotic transitions bare major impact on the current state of mankind health. New tools and methods will be needed to understand the biology of these zoonotic malaria.

Methods: We are optimizing the in vitro growth conditions for routinely cultured in laboratory settings of the zoonotic malaria P. cynomolgi. To monitor the progression of adaptive culture in real life we will collect cell samples periodically and observe the event by high-content imaging flow cytometer (ImageStream X MKII (ISX)). The digital data can be analyzed using the accompanying software (IDEAS) that supports statistical analyses giving the overall study an unbiased character.

Results: This approach will study the intracellular structures of the parasite cells representing alterations in organelle biogenesis. An example represents the Plasmodium digestive vacuole (DV) that is known to undergo morphological changes through the erythrocyte development and it is unique to each Plasmodium spp. We will characterize the DV morphology of all studied Plasmodium. Similarly, we will also evaluate other Plasmodium intracellular structures including endoplasmic reticulum (ER), Golgi stack, nucleus, mitochondrion and plastid.

Conclusions: This experimental strategy is design to generate a baseline set up that can be applicable for detection of zoonotic malaria across multiple hosts.

Keywords: zoonotic malaria, Plasmodium knowlesi, P. cynomolgi

P094 / #725

Topic: *AS02.1 Malaria*

IMMUNE-MODULATORY EFFECT OF FASCIOLA HEPATICA IN A MODEL OF CEREBRAL MALARIA

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Introduction: Malaria, mostly caused by Plasmodium falciparum is associated with the development of cerebral malaria; a complication characterized by increased levels of pro-inflammatory cytokines that induce an augmented expression of adhesion molecules in the vascular endothelium, these favors the adhesion of erythrocytes, leukocytes, and platelets which reduces the blood flow; causing a state of hypoxia and damage in endothelial cells enabling irreversible lesions mainly in the brain. Encouraging strategies are based on the concept of counteracting the above mention, thus modulating the inflammatory response would avoid this complication. In our group of work, we are interested in helminth´s immune-modulatory associated effects. The purpose of this research was to study the effects related to the administration of the excretory/secretory products of Fasciola hepatica (FhE/S) on cerebral malaria-associated-pathology in a murine model.

Methods: We infected C57BL/6 mice with Plasmodium berghei ANKA (PbA), administering or neither the FhE/S products and establishing different parameters associated with the pathophysiology in the experimental groups.

Results: The course of the infection was altered by the administration of the FhE/S products avoiding the development of cerebral malaria in the infected mice and extending their survival rate, which was also accompanied by a decrease in serum levels of IL- 6, TNF-α, IFN-γ, and MCP-1, as well as an increased number of $F4/80^{+}$ $CD206^{-}$ and $Arg\text{-}1^{-}$ leukocytes in the spleen of infected mice.

Conclusions: In conclusion, the administration of these FhE/S products prevented the triggering of the immunological events associated with cerebral malaria.

Keywords: Malaria, Immunology, immunomodulation, Fasciola hepatica, Cerebral malaria

P095 / #878

Topic: *AS02.1 Malaria*

RECRUITMENT OF COMPLEMENT REGULATORY PROTEINS BY PLASMODIUM FALCIPARUM MEROZOITES CAN BE BLOCKED BY MEROZOITE SPECIFIC ANTIBODIES

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Introduction: Antibodies play a crucial role in malaria immunity. Antibodies activating the complement system have been associated with protection from clinical malaria. Prior studies suggested that P. Falciparum merozoites can recruit human complement regulatory proteins to their surface to evade complement activation. The aim of this study was to investigate complement evasion strategies and the ability of antibodies to impact the recruitment of complement regulatory proteins.

Methods: Growth Inhibition Activity assays were performed on plasma samples from malaria exposed individuals in the presence and absence of normal human serum. The recruitment of complement regulatory proteins to the merozoite surface was quantified by flow cytometry, as was the ability of antibodies to impact this recruitment. Antibodies towards a panel of merozoite antigens were detected by ELISA and confocal microscopy was used to visualize FH and C4BP on the merozoite surface.

Results: Parasite growth was not impacted by active complement. Deposition of complement regulatory proteins Factor H (FH) and C4BP to the surface of merozoites was confirmed by flow cytometry. Naturally acquired antibodies as well as a monoclonal antibody targeting Pf92 were able to decrease FH deposition on the surface of merozoites.

Conclusions: We hereby show that merozoites recruit FH and C4BP, but that this apparent immune evasion strategy can be circumvented by antibodies specific for certain merozoite antigens.

Keywords: Antibodies, Malaria, Complement system

P096 / #603

Topic: *AS02.1 Malaria*

IN VITRO PHARMACODYNAMIC DRUG-DRUG INTERACTION OF M5715 – PYRONARIDINE A NEW ANTIMALARIAL COMBINATION USING P.FALCIPARUM FIELD ISOLATES

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Introduction: Development and spreading of drug resistant parasite substantially threatens malaria control and elimination. Antimalarial combination therapies have the potential to minimize the risk of drug resistance but require intensive preclinical studies to determine the optimal combination (e.g partners). In addition, too often, these studies fail to bridge the insidious gap between research and clinical trials in field setting. Aiming to bridge this gap by improving the current in vitro models to assess pharmacometrics drug-drug interaction (DDI) parameters and optimally select dose ratio and dosing schedule.

Methods: To assess DDI interactions for the M5717 and Pyronaridine, a new antimalarial combination about to entering Phase 2 clinical trials in Africa, we implemented new in vitro assays allowing the use of P. falciparum field isolates parasites in endemic countries. The parasite viability data generated with this assay are mathematically modelled allowing the extrapolation of different interaction values.

Results: In order to inform the dose rate and dosing regiments for the Phase 2 clinical trial the interactions values derived from the in vitro assay are used to simulate possible treatment effects scenarios in individual patients.

Conclusions: This is not only streamlining the development of novel antimalarial combination therapies but can also transform the development of new drug combinations in other therapeutic areas and meaningfully improve how new medicines are enhanced and used, in accordance with the 3R's principle.

Keywords: Drug-drug interaction, Pharmacodynamic, Plasmodium falciparum, New antimalarial combinations

P097 / #1259

Topic: *AS02.1 Malaria*

PULMONARY PROTEOMIC IN EXPERIMENTAL MALARIA-ASSOCIATED ACUTE RESPIRATORY DISTRESS SYNDROME

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Introduction: Malaria is a significant parasitic disease, and Plasmodium spp. infections can lead to a severe respiratory condition, like acute respiratory distress syndrome (ARDS), characterized by acute inflammation, alveolar endothelium and lung parenchyma injuries, dysfunction of the pulmonary alveolar-capillary barrier. Given several existing pathogenesis-related gaps and the absence of biomarkers for malaria-associated ARDS, this work aimed to identify the panel of proteins in mice that develop this syndrome and recognize possible biomarkers.

Methods: The lungs of a well-defined and controlled murine model of malaria-associated ARDS (DBA/2 infected by P. berghei ANKA) were analyzed by quantitative proteomic analysis.

Results: More than 150 proteins were regulated in ARDS, being the majority upregulated between 7- and 9-days post-infection. Proteins involved in complement activation, extracellular matrix organization, and migration of neutrophils, were identified to be regulated, confirming preliminary results. This large-scale approach allowed us to identify several novel candidate proteins to model the pathogenesis of malaria-associated ARDS.

Conclusions: Identifying novel regulated enzymes involved in biochemical and cellular signaling processes will help elucidate other molecular pathways besides potential biomarkers of malaria-associated ARDS. The work presented shed new light previously unanticipated on this disease, but the validation of these proteins is needed to confirm the involvement of these processes. Identifying this protein signature is crucial for early diagnosis tools and prognosis and deepening understanding of malaria-associated ARDS.

Keywords: pulmonary, murine model, Malaria, Proteomic, ARDS

P098 / #788

Topic: *AS02.1 Malaria*

AN EVOLUTIONARY PERSPECTIVE ON MALARIA: HELICOBACTER PYLORI-MEDIATED PROTECTION AGAINST EXPERIMENTAL CEREBRAL MALARIA

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Introduction: In malaria endemic areas the risk for cerebral malaria (CM) is highest in young children, but even in this group most cases are uncomplicated. It is still not well understood what makes certain individuals vulnerable to severe malaria. In recent years, accumulating evidence has pointed to the gut microbiota impacting a range of diseases through modulation of the immune responses, including the severity of malaria. The bacterium Helicobacter pylori is an ancient inhabitant of the human stomach with potent immune regulatory properties. There is a striking overlap in the geographical distribution of infections with H. pylori and Plasmodium parasites. In this study we investigated whether early-life H. pylori infection affect the risk for experimental CM.

Methods: Mice were orally gavaged with H. pylori or media control twice in their first week of life. At age 5-6 weeks, all mice were infected with P. berghei ANKA by injection. To investigate CM, mice were blindly scored according to a published coma and behavior scale and killed if reaching one of the predefined humane endpoints.

Results: Our results indicate that mice infected with H. pylori are significantly less likely to suffer from experimental CM than the control group. I will additionally present preliminary data on the effect of H. pylori on immune profiles.

Conclusions: This study indicates that early-life infection by H. pylori protects against experimental CM. If this finding is translational to humans, H. pylori status could, together with other prognostic markers, potentially in the future be used to tailor individual patient care. I am searching for clinical collaborators with access to serum samples from children suffering from cerebral malaria to investigate this.

Keywords: Immune regulation, Experimental cerebral malaria, Evolutionary medicine, Plasmodium berghei ANKA

P099 / #21

Topic: *AS02.1 Malaria*

THERAPEUTIC EFFICACY OF ARTEMETHER-LUMEFANTRINE (COARTEM®) FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN AFRICA: A SYSTEMATIC REVIEW

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Introduction: Early diagnosis and effective treatment is the cornerstone of malaria control. In this review, the efficacy of AL, the recommended first-line treatment for uncomplicated falciparum malaria in most African countries, was evaluated.

Methods: Articles published between January 2015 and July 2019 on the efficacy of AL for the treatment of uncomplicated falciparum malaria in Africa were systematically searched using comprehensive search strings from PubMed/Medline, SCOPUS, and grey literature from Google Scholar. Interventional studies that followed patients for at least 28 days were included. While computing the efficacy of AL, polymerase chain reaction (PCR)–corrected cure rate (adequate clinical and parasitological response, ACPR) at day 28 was considered as the main endpoint.

Results: In this review, 39 articles that reported the treatment outcome of 8, 320 patients were included. After 28 days of follow-up, the pooled PCR uncorrected and corrected APCR was at 87% (95%CI: 85-90%) and 97.0% (95%CI: 96-98%), respectively. The proportion of early treatment failure (ETF) was almost 0% and most of the included articles reported <8% late treatment failures. The reinfection and recrudescence rate was less than 10% and 2.6%, respectively within 28 days. We noted rapid fever and parasite clearance in which greater than 93% and 94% of patients were parasite and fever-free at day three following AL treatment.

Conclusions: Coartem® remains effective and thus could continue to be the drug of choice for the treatment of uncomplicated falciparum malaria for all age groups in Africa. However, the risk of new emerging resistance for this combination warrants regular monitoring of its efficacy across the continent.

Keywords: uncomplicated falciparum malaria, Africa, Therapeutic efficacy, Artemether–lumefantrine (AL)

P100 / #1260

Topic: *AS02.1 Malaria*

METHYLENE BLUE TREATMENT REVEALS BIOMARKERS ASSOCIATED WITH CEREBRAL MALARIA IN COATNEYI-INFECTED MACAQUE MODEL

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Introduction: Plasmodium falciparum remains a major threat to the public health with its most common severe form of complication, cerebral malaria, which is fatal within 24 to 72 hours. P. berghei ANKA infection in CB57BL/6 mice has been widely used as the murine model for human cerebral malaria, but its relevance has been questioned due to their dissimilarity in histopathology. Hence, P. coatneyi – the malaria parasites of non-human primates – shares similar pathophysiological features with P. falciparum infection, and has been sporadically used as a model for severe malaria.

Methods: With the emergence of drug resistance malaria, methylene blue has shown to be effective against chloroquine-resistant P. falciparum. Furthermore, methylene blue treatment has improved survival and ameliorated experimental cerebral malaria in murine model. Here, we compared the gene expression profiling in different organs (brain, heart, kidney and liver) of uninfected, untreated and methylene blue-treated Rhesus macaques infected with P. coatneyi.

Results: We were able to cluster the infected samples from uninfected and treated samples in brain stem based on their genetic profile. Differential gene expression analysis revealed the effectiveness of methylene blue treatment as it reversed the effect of infection on the brain tissues. By comparing the differential expressed genes in three datasets (human infected peripheral blood, Macaca mulata infected brain stem and Macaca mulata infected blood), we have successfully identified several genes that are associated with cerebral malaria.

Conclusions: These biomarkers would accelerate the prediction and diagnosis for cerebral malaria or other complicated infections by P. falciparum.

Keywords: Plasmodium coatneyi, Cerebral malaria, Macaque, Biomarkers, Methylene blue

P101 / #969

Topic: *AS02.1 Malaria*

STRONG FINE-SCALE SPATIAL AND TEMPORAL STRUCTURE OF RESIDUAL PLASMODIUM FALCIPARUM IN ZANZIBAR DETECTED THROUGH MULTIPLEXED AMPLICON SEQUENCING

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Introduction: Over the past 15 years, Zanzibar has made great strides towards malaria elimination; yet progress has stalled despite access to efficacious antimalarials and good vector control measurements. Parasite genotyping can help to understand sources of circulating parasites and identify residual transmission.

Methods: We sequenced 518 dried blood spot (DBS) Plasmodium falciparum samples at 35 loci using a novel, highly multiplexed droplet digital PCR (ddPCR)-based high-throughput amplicon deep sequencing method.

Results: Genotyping data was obtained for >80% of loci in 80% of samples at densities of ≥5 parasites/µL. The parasite population in Zanzibar was highly diverse (average heterozygosity = 0.73) and 70% of infections were polyclonal. Cases imported from mainland Tanzania (based on self-reported travel history) could not be distinguished from local cases. Strong fine-scale spatial and temporal structure in local parasite populations was observed, with two clearly separated clusters on the more remote Pemba Island. Relatedness analysis by identity-by-descent (IBD) revealed multiple near-clonal clusters in villages, linked to clinical cases. Travel history combined with genomic data revealed spread of these infections over the archipelago. No kelch13 mutations were identified, but high prevalence of known resistance-associated mutations in dhfr, dhps, mdr1, and mdr2 genes. No hrp2/hrp3 deletions were found.

Conclusions: High-resolution P. falciparum genotyping identified pronounced population structure in Zanzibar, but also highly related parasites across the archipelago, indicative of gene flow and migration. Isolated parasite populations could be controlled through targeted interventions.

Keywords: Plasmodium falciparum, amplicon sequencing, population genetics, Zanzibar, multiplex

P102 / #1220

Topic: *AS02.1 Malaria*

A SCALABLE CRISPR/CAS9 SYSTEM USING SHORT LENGTH HOMOLOGY REPAIR (HR) TEMPLATE FOR GENETIC SCREENS IN PLASMODIUM BERGHEI

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Introduction: Malaria parasite blood-stage proliferation is closely linked to clinical symptoms. Thus, understanding parasite invasion, multiplication, and egress mechanism are essential to develop better interventions. However, genetic modification of the malaria parasites is not straightforward. The blood-stage parasite is haploid and approximately 45 % of the genes are essential for blood-stage growth, which means blood-stage essential genes cannot be studied by straight knockout (KO) approaches. Highly efficient conditional knockdown (cKD) methods requiring minimal target-specific cloning will be required to overcome these problems.

Methods: To optimize gene editing efficiency we generated an improved P. berghei CRISPR/Cas9 system and examined the minimal requirements for efficient gene editing. We assayed the efficacy of repair using different types and lengths of homology repair (HR) templates.

Results: An improved CRISPR/Cas9 system for Plasmodium berghei using linear minimal length HR template was successfully developed. Conventionally, a 500 bp HR template is used for CRISPR/Cas9 editing of malaria parasites, we show a linear 50 bp HR template was sufficient for complete gene editing in P. berghei.

Conclusions: The advantages of a short linear HR template are 1) reduced cloning effort, 2) donor template (HR with intended edit) can be ordered at less cost. Moreover, we combined our optimized CRISPR/Cas9 system with a modular cKD approach to generate conditional loss-of-function mutants, which can be applied to analyze essential parasite genes in the blood stage. This improved strategy presents a significant step towards enabling large-scale analysis of essential gene function in malaria parasites.

Keywords: methodology, Plasmodium berghei, CRISPR/Cas9, conditional KD

P103 / #566

Topic: *AS02.1 Malaria*

CLINICAL TESTING OF AUTOMATED HEMATOLOGY ANALYZER XN-31 PROTOTYPE FOR MALARIA DIAGNOSIS

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Introduction: The automated hematology analyzer XN-31 prototype (XN-31p) is a new flowcytometry-based device developed by Sysmex Corporation to measure the number (MI-RBC#) and the ratio (MI-RBC%) of erythrocytes infected with malaria parasites. The XN-31p can provide those results in about 1 minute and also can simultaneously provide information on the malaria parasite species. In this study, we performed the clinical testing of the XN-31p.

Methods: Blood samples were collected from 80 patients who visited NCGM hospital for malaria diagnosis from through 2017 to 2019. The test results by the XN-31p were compared with those by microscopic observation, RDTs and the nested PCR.

Results: Thirty-three patients were diagnosed by the nested PCR as being malaria positive (28 Plasmodium falciparum, 2 P. vivax, 1 P. knowlesi, 1 mixed infection of P. falciparum and P. malariae, and 1 mixed infection of P. falciparum and P. ovale), and the other 47 were negative. The XN-31p detected 32 patients as “MI-RBC positive”, which almost matched the results by the nested PCR. Regarding the parasite species discrimination between P. falciparum and others, the XN-31p showed a positive coincidence rate of 0.848 with the nested PCR in discriminating P. falciparum from the other species. The MI-RBC(%) determined by the XN-31p showed a high correlation coefficient of more than 0.99 with the parasitemia obtained by microscopy.

Conclusions: The information on the MI-RBC#, MI-RBC(%) and the malaria parasite species could be obtained accurately and very rapidly with the XN-31p that will be useful for clinical diagnosis of malaria.

Disclosure: This study was funded from Sysmex Corporation under a joint research contract signed by NCGM and Sysmex Corporation. The authors interpreted the results objectively. The quality of the data was not influenced by the manufacturer.

Keywords: Flowcytometry, XN-31p, diagnosis, Malaria, Automated hematology analyzer

P104 / #651

Topic: *AS02.1 Malaria*

IN-VITRO ANTIPLASMODIAL ACTIVITY OF BIOSYNTHESIZED SILVER NANOPARTICLES USING PANDANUS CANARANUS AGAINST MALARIA PARASITE, PLASMODIUM FALCIPARUM

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Introduction: The utilization of various plant resources for the biosynthesis of metallic nanoparticles is called green nanotechnology, and it does not utilize any harmful chemical protocols. The present study reports the plant mediated synthesis of silver nanoparticles using the leaf extract of Pandanus canaranus, which acts as a reducing and capping agent. The aim of the present study was to assess the anti-plasmodial activity of synthesized AgNPs against malaria parasite, Plasmodium falciparum.

Methods: The obtained nanoparticles were characterized using UV-visible spectroscopy; EDX (energy-dispersive X-ray), SEM (Scanning electron microscope), XRD (X-ray diffraction) and Fourier transform infrared (FTIR) analysis. The efficacy of green synthesized AgNPs at different concentrations (25, 50, 75 and 100µg/mL) were tested on P. falciparum.

Results: Synthesized AgNPs particles were confirmed by analysing the excitation of surface plasmon resonance (SPR) using UV–vis spectrophotometer at 420 nm. The scanning electron micrograph showed structures of spherical, cubic shape, and the size range was found to be 40–60 nm. The EDX spectra showed the purity of the material and the complete chemical composition of the synthesized AgNPs. The synthesized AgNPs showed significant anti-plasmodial activity when compared to aqueous leaf extract of P. canaranus. The maximum efficacy was observed in synthesized AgNPs against P. canaranus (IC_{50}=100 µg/ml; 100%), respectively.

Conclusions: This method is considered as a new approach to control the malaria parasite, P. flaciparam. Therefore, this study provides report on the anti-plasmodial activity of synthesized AgNPs using P. canaranus against P. falciparum.

Keywords: Pandanus canaranus, Plasmodium falciparum, AgNPs, SEM, XRD

P105 / #1207

Topic: *AS02.1 Malaria*

HETEROGENEITY OF TOTAL IGG RESPONSES TO MULTIPLE MALARIA ANTIGENS IN TWO BIO-ECOLOGICAL ENVIRONMENTS IN THE HOHOE MUNICIPALITY OF GHANA

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Introduction: Background and Aims: Malaria vaccination in African populations calls for the evaluation of human immune responses in different bio-ecological environments. Though transmission in an area may be similar, factors such as sanitation, and behaviour within communities could cause differences in infections. This may affect immune responses to parasite antigens in the different communities. This study evaluated immune responses to three malaria antigens in an area with a similar transmission but different settlements in the Hohoe municipality of Ghana.

Methods: This cross-sectional study involved 327 asymptomatic children aged 1-12 years in both Rural (196) and Urban (131) settlements in the Hohoe municipality. Total IgG responses specific for three malaria antigens (PfCSP, MSP2-Fc27, MSP-3D7) was determined using indirect ELISA. IgG levels and other covariates were compared between the two settlements.

Results: Total IgG levels to the three antigens were higher in the rural settlement ($p>0.05$) with high seroprevalence except for MSP-Fc27. Bed net usage was similar between the two settlements. Parasite density in the rural versus urban settlement was (9.7% vs 3.8). Also, sub-microscopic parasitaemia was higher in the rural settlement compared to the urban community (17.3% vs 7.6%). In a multiple regression model, adjusting for confounders, to determine if higher IgG responses were associated with settlement, higher antibody responses were associated with residing in the rural compared to urban setting.

Conclusions: Conclusion: The results suggest that though the area is categorized as having medium malaria transmission, environmental and behavioural differences within settlements could influence antibody responses.

Keywords: transmission, Antigen, residence, Malaria

P106 / #1084

Topic: *AS02.1 Malaria*

THE EFFECT OF HAEMOGLOBINOPATHIES ON PLASMODIUM FALCIPARUM INFECTION RISK AMONG CHILDREN IN NORTHERN REGION, GHANA

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Introduction: Background: The effect of human erythrocyte variants encoding for most haemoglobinopathies has been reported only on a few variants. In this study, we investigated the prevalence of Hb A, S, C, and F variants and alpha thalassaemia on the risk of Plasmodium falciparum infection among children in Ghana.

Methods: A cross-sectional study was conducted among 1,045 children (1-14 years) in 13 malaria-endemic communities in the Northern Region of Ghana. P. falciparum infection and Hb phenotypes were diagnosed by malaria rapid diagnostic (RDT) and SickleSCAN tests, respectively, and retrospectively confirmed with PCR. Total IgG levels against malaria antigens (CSP, GLURP, MSP3, Pfs230, and two PfEMP1 proteins) and crude asexual blood-stage antigens were measured by ELISA.

Results: Wild type Hb (HbAA) was most frequent (70.2%), followed by HbAC (17.8%) and HbAS (8.5%). Other phenotypes (HbCC and HbSS) were less frequent (< 1%). Overall, 29% were heterozygous and 5.6% were homozygous mutants for alpha-thalassaemia. HbAC and HbAS were co-inherited with alpha-thalassaemia. P. falciparum infection risk was about three times higher among homozygous alpha thalassaemia individuals carrying HbAC (OR=2.97, p=0.09) and heterozygous carriers with HbAS variants (OR=2.86, p=0.09). HbAS individuals had significantly lower anti-PfEMP1 and higher anti-CSP antibodies.

Conclusions: Co-inheritance of haemoglobinopathies observed among the children increased their risk of P. falciparum infections in HbAC and HbAS carriers, suggesting an epistatic mechanism. Antibody responses against non-PfEMP1 antigens were higher among homozygous carriers, an indication of exposure to parasites.

Keywords: Haemoglobinopathies, Ghana, Plasmodium falciparum malaria

P107 / #673

Topic: *AS02.1 Malaria*

MOSQUITO BITE EXPOSURE AND P. VIVAX PVS25 DETECTION IN DRIED BLOOD SAMPLES

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Introduction: Background and aims: Recent studies suggest that Plasmodium gametocyte carriers are more attractive to mosquitoes, increasing the possibility of transmission to the vector. An. albimanus is one of the main malaria vectors in Colombia. This study aims to measure the association between exposure to mosquito bites and gametocyte carriage.

Methods: RNA was extracted from FTA cards from 45 malaria patients with P. vivax infection residing in Norte de Santander (Colombia) in 2019. The Pvs25 gene was detected through RT-PCR and IgG antibodies against Anopheles albimanus salivary peptides were detected through indirect ELISA.

Results: The study sample had a mean age of 33.7 years old (1 – 67 years old) and the presence of gametocytes was detected in 41 samples (91.1%). Our results indicate a positive correlation between parasite count and expression of Pvs25 (r^2= 0.3860 1, p= 0.0116). However, we also found a negative correlation between Pvs25 expression and IgG antibodies against An. albimanus PEROXI-P1 (r^2= -0.3754p= 0.0061), PEROXI-P2 (r^2= -0.363 1, p= 0.008) and PEROXI-P3 (r^2= -0.29 87, p= 0.03 15) peptides. This correlation was not observed when comparing Pvs25 to whole salivary gland extract from An. albimanus.

Conclusions: Discussion: Antibodies against salivary proteins are a reliable method to quantify exposure to mosquito bites. Contrary to our hypothesis, we found a negative correlation between IgG anti-salivary antibody levels and gametocytes suggesting a reduced expression of gametocyte antigens in people highly exposed to mosquito bites. However, more studies are needed to stablish the relevance of salivary peptides to evaluate the exposure of gametocyte carriers to mosquito bites.

Keywords: Mosquito bites, Pvs25, Gametocytes, Anopheles albimanus

P108 / #1215

Topic: *AS02.1 Malaria*

DIAGNOSTIC TEST ACCURACY OF THE SD BIOSENSOR STANDARDTM FOR IDENTIFYING GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) ACTIVITY: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Introduction: The World Health Organization recommends testing malaria patients for G6PD activity before receiving a radical cure with 8-aminoquinolines. Several point-of-care tests for detecting this activity are currently available, but the pooled diagnostic test accuracy (DTA) of SD Biosensor Standard™ G6PD (SD Biosensor) rapid test is unknown.

Methods: We performed a systematic review and DTA meta-analysis. Our protocol was submitted previously to the PROSPERO database. We searched MEDLINE, EMBASE, and SciELO databases up to January 15, 2022 . We included studies that reported G6PD activity using SD Biosensor (index test) and spectrophotometry (reference test) in patients with suspicion of G6PD deficient or intermediate (30-70%) activity. We assessed the risk of bias (RoB) with the QUADAS-2 tool and performed a random-effect bivariate meta-analysis to estimate the pooled sensitivity and specificity for critical G6PD activity thresholds (30%, 70%, 80%). We evaluated the heterogeneity graphically using crosshair and confidence regions on the receiver operating curve space plots.

Results: We screened 2,184 papers and included three studies conducted in Brazil, Bangladesh, the United States, and Thailand. Of these, two studies had a high, and one had a low RoB. The pooled sensitivity was 99.1% (95%CI 96.9-99.7%), 94.4%(91.1-96.6%), and 87.8% (71.0-95.5%); and the pooled specificity 97.4% (96.0-98.4%), 92.9% (86.2-96.4%) and 89.5% (77.6-95.4%); for the 30%, 70% and 80% activity of G6PD thresholds, respectively.

Conclusions: SD Biosensor test has very high DTA operative characteristics for identifying deficient activity using 30% thresholds, and good-to-acceptable DTA for 70% and 80% normal activity thresholds.

Disclosure: Funded by Global Health Strategies as part of the project: "Cost-effectiveness of tafenoquine in the radical cure of Plasmodium vivax malaria”

Keywords: Meta-analysis, diagnostic test accuracy, SD Biosensor, Glucose-6-phosphate dehydrogenase, G6PD activity

P109 / #404

Topic: *AS02.1 Malaria*

TRANSIENT INHIBITION OF IL-27 PROMOTES THE DEVELOPMENT OF PLASMODIUM-SPECIFIC CD4+ T CELL MEMORY

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Introduction: Immunity to malaria is known to develop after repeated infections but decline with loss of exposure. Here we studied the underlying mechanisms of antimalarial immunity, and investigated the role of cytokine IL-27 in the development of immunological memory to malaria, with a focus on $CD4^+$ T cell immunity during blood-stage malaria infection.

Methods: Using a chronic Plasmodium chabaudi chabaudi AS (Pcc) infection mouse model, we characterized malaria-specific $CD4^+$ T cell responses through adoptive transfer of Plasmodium-specific T-cell receptor transgenic PbT-II cells to wild-type C57BL/6 (WT) or $Il27^{-/-}$ mice.

Results: We found that PbT-II cells were maintained at higher levels during the memory phase in $Il27^{-/-}$ mice, and also in WT mice treated early on with anti-IL-27 Ab (αIL-27), suggesting that transient IL-27 inhibition promotes the generation of memory precursor $CD4^+$ T cells. Moreover, maintained PbT-II cells in αIL-27 mice had $CD127^+CXCR6^+Tbet^+$ Th1 memory-like and $CD127^-KLRG1^+$ effector-like $CD4^+$ T cell subsets, while IgG-treated mice had mostly $CD127^-KLRG1^-$ PbT-II, and similar trends were observed in polyclonal $CD4^+$ T cells. αIL-27 mice also showed enhanced T cell memory and antibody responses upon secondary infection.

Conclusions: Our results suggest the involvement of IL-27 in suppressing overall proliferation and Th1 fate commitment of PbT-II cells, without adversely affecting $TCF1^+$ precursors and Tfh memory subsets. Early IL-27 inhibition also contributed to an enhanced protective recall immunity. These findings provide important insights for malaria immunological memory, suggesting potential applications in vaccine development and other strategic interventions.

Keywords: malaria immunology, CD4+ T cell memory, IL-27, immunological memory

P110 / #91

Topic: *AS02.1 Malaria*

UNRAVELLING THE RIFIN EXPORT CODE: ONE STEP AT A TIME

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Introduction: Plasmodium falciparum extensively modifies the surface of the infected red blood cells (IRBC) during its intra-erythrocytic stages of infection facilitating interactions with the host via rosetting and cytoadherence, both associated with increase in virulence. Variants of the Repetitive Interspersed Family of proteins (RIFIN), particularly the subgroup-A, are expressed on the RBC surface, and have recently been shown to preferentially interact with the blood group A antigen on uninfected RBCs to form rosettes. In contrast, the subgroup-B of the RIFIN family shows an exclusively intra-parasitic localization. Apart from the presence of a 25 amino acid insert in subgroup-A (absent in subgroup-B), the subgroups do not show a very conspicuous difference in their protein sequences. Both the subgroups contain sequences corresponding to the host-targeting (HT)/Plasmodium Exported element (PEXEL) as well. Therefore, contributions from additional sequences are likely play roles in their trafficking. In this study, we attempt to dissect each of the sequence motifs associated with the targeting of these proteins and hope to shed light unto the complex protein trafficking pathways of Plasmodium falciparum.

Methods: GFP-tagged full legnth and truncated versions of A/B-RIFIN overexressing parasite transgenic lines were generated to look into the trafficking information present inherantly in their protein sequences.

Results: We observe that the N-termini of A and B-RIFINs show differences in their abilities to act as signal sequences.

Conclusions: The divergence in the trafficking pathways of A and B-RIFINs leading to their varied localisition starts right at the beginning of the protein's lifetime.

Keywords: plasmodium, rosetting, RBC, proteintrafficking

P111 / #1560

Topic: *AS02.1 Malaria*

CHARACTERISTICS AND PRESENTATION OF MALARIA IN ACUTELY ILL HIV-INFECTED CHILDREN

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Introduction: HIV infected children are susceptible to infections and HIV and malaria have overlapping characteristics and are associated with worse outcome. However, the etiology of febrile illness is under-explored in malaria endemic areas and there is limited information on the characteristics of malaria in HIV-infected children in endemic areas.

Methods: HIV-infected children aged 1 to 13 years presenting with an acute febrile illness were recruited at the Korle Bu Hospital, Ghana. Blood film for malaria parasitaemia, blood/urine cultures and comprehensive clinical investigations were conducted. A cohort of HIV uninfected children presenting with acute febrile illness was also recruited.

Results: Among the HIV-infected children, malaria parasitaemia was detected in 21 (n=105), while a positive blood culture was detected in 12 (n = 105). A positive blood culture and malaria parasitaemia was detected in three children. Malaria parasitaemia was detected in 58 (n=144) of the HIV-uninfected children. Among those with malaria parasitaemia, admission temperature and platelet counts were higher, but geometric parasite density was significantly lower, in the HIV-infected children compared with the non-HIV infected children. The most predominant presenting complaints were fever, together with vomiting, and diarrhoea, in both the HIV-infected (n= 7, 33.3%) and HIV-uninfected children (n=37, 63.8%). However, fever was likely to occur alone in the HIV-infected children with malaria.

Conclusions: Malaria and bacteremia, including co-infections, were detected in HIV-infected children presenting with a febrile illness. Both malaria and bacteraemia presented with a constellation of symptoms, but malaria was more likely to present with fever alone in HIV-infected children.

Keywords: Malaria, haematology, HIV

P112 / #1367

Topic: *AS02.1 Malaria*

CHARACTERIZATION OF EXTRACELLULAR VESICLES SECRETED BY HUMAN BRAIN ENDOTHELIAL CELLS DURING PLASMODIUM FALCIPARUM INFECTION.

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Introduction: After infection with Plasmodium falciparum, the most lethal species of malaria, infected erythrocytes (IE) cytoadhere to the microvasculature of the brain. Cytoadhesion has been pointed out as the main source of life-threatening outcomes, however, recent literature suggests it might not be the only cause of severe malaria. Not only are malaria parasites able to evade and affect the immune system, but also the normal functioning of endothelial cells (ECs) within the blood brain barrier (BBB). Dysfunction of brain ECs might predispose IE to cytoadhere, and thus promote BBB damage. Indirect cell signaling is mediated through secretion of extracellular vesicles (EVs) from different cells. EVs carry different types of genetic material and proteins and are taken up by a target cell changing its fate. Likewise, P. falciparum IE has been show to release EVs.

Methods: In this study we set up experiments for EV isolation from human brain ECs during co-incubation with P. falciparum. Different centrifugation, ultracentrifugation, sucrose cushion and size selection ultracentrifugation are used. For characterization we use electron microscopy, nano particle tracking, FACS analysis and miRNA sequencing.

Results: Previously, EVs secreted from P. falciparum-IEs and non-infected erythrocytes were compared. We found that IEs secrete EVs that significantly express 10 microRNA candidates higher than the ones secreted by non-infected erythrocytes. In this project EVs secreted from brain ECs are characterized.

Conclusions: We hypothesised that indirect communication among IEs and brain ECs through EVs mediates immunomodulatory and inflammatory effects. EVs role in pathogenesis of P. falciparum infection is still superficial, therefore, further analysis is essential.

Keywords: Plasmodium falciparum, Brain endothelial cells, extracellular vesicles

P113 / #115

Topic: *AS02.1 Malaria*

EVALUATING THE PERFORMANCE OF LAMP TECHNIQUE IN DIAGNOSIS TYPE OF PLASMODIUM SPECIES IN ANOPHELES MOSQUITOES, IN SOUTHEASTERN IRAN

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Introduction: BACKGROUND: Malaria is one of the main parasitic diseases and a major health issue in some countries. This study aims to determine the rate and type of infections of Anopheles mosquitoes with malaria parasites using the molecular LAMP method in the Southeastern Iran.

Methods: METHODS: In this study, 400 Anopheles mosquitoes were collected by the Zahedan Medical Insecticide Center in Nikshahr City, a high-risk area of malaria transmission in Sistan-Baluchestan Province. The mosquitoes were caught manually (by hand) in domestic (humans and animals), natural, and artificial outdoor places (Shelter pits). After DNA extraction, the LAMP method was used, which was compared with Multiplex Nested- PCR as a standard method.

Results: RESULTS: Out of 400 samples collected from Nikshahr City, 6 samples (1.5%) were infected with Plasmodium vivax. No Plasmodium falciparum or a mix (Plasmodium vivax and Plasmodium falciparum) was detected in this study.

Conclusions: CONCLUSIONS: The results of this study indicate that in places with transmission of both species, i.e. Plasmodium vivax and Plasmodium falciparum, detection of malaria parasites by the LAMP method could be very useful in spotting infections in the field. Thus, molecular epidemiological studies could be conducted annually to monitor malaria in endemic regions. The results of this research show that contamination with mosquito malaria vectors is increasing in Nikshahr City, and it seems that more studies will be required to eliminate malaria until 2026.

Keywords: Malaria, Nested-PCR, LAMP, Anopheles Mosquitoes

P114 / #116

Topic: *AS02.1 Malaria*

MALARIA DIAGNOSIS BY MULTIPLEX/NESTED PCR AND LAMP (LOOP-MEDIATED ISOTHERMAL AMPLIFICATION) IN SOUTHEASTERN IRAN

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Introduction: Abstract Malaria is one of the most serious health problems in many countries, including Iran. Accurate diagnosis is important regardless of the elimination status of a country. A cross-sectional study was performed on 105 people who were suspected to be positive for malaria infection in Sistan and Baluchistan, Iran.

Methods: Blood smears (thin and thick films) were stained with 10% Giemsa. DNA was extracted from the prepared thin and thick films for molecular methods. Multiplex/nested PCR (mn-PCR), loop-mediated isothermal amplification (LAMP), and light microscopy (LM) were compared with nested PCR as a gold standard.

Results: Of 105 subjects, 52 (49.5%), 58 (55.2%), 58 (55.2%), and 63 (60%) were positive for malaria by LM, nPCR, mn-PCR PCR and LAMP, respectively. The sensitivity, specificity and Kappa were 92.1%, 100%, and 0.9 for LAMP and 100%, 100%, and 1 for multiplex/nested PCR, respectively. Eight cases of co-infection (Plasmodium vivax and Plasmodium falciparum) that were not detected by the LM method were diagnosed by multiplex/nested PCR and LAMP.

Conclusions: The Multiplex/nested PCR (mn-PCR), loop-mediated isothermal amplification (LAMP) method and Light Microscopy (LM) were compared with Nested PCR as a gold standard.Out of 105 cases, 52 (49.5%), 58 (55.2%), 58 (55.2%) and 63 (60%) samples were were positive for malaria by LM, nPCR, mn-PCR PCR and LAMP methods respectively.The sensitivity, specificity and Kappa were 92.06%, 100%, and 0.903 for LAMP and 100%, 100%, and 1, for Multiplex/nested PCR, respectively. Eight cases of co-infection (Plasmodium vivax and Plasmodium falciparum) that were not detected by the LM method were diagnosed by multiplex/nested PCR and LAMP methods.

Keywords: Parasitology, Malaria, Polymerase chain reaction

P115 / #464

Topic: *AS02.1 Malaria*

SINGLE-CELL TRANSCRIPTOMICS TO CHART PLASMODIUM FALCIPARUM STAGE-TRANSITION IN THE MOSQUITO MIDGUT

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Introduction: Malaria is the deadliest among the vector-borne diseases. The dramatic bottleneck of parasite numbers that occurs in the gut of the obligatory vector mosquito provides a possible target for novel control strategies. The human-to-mosquito transmission phase is one of the major bottlenecks in the parasite lifecycle, in part due to the limited number of transmissible parasites taken up by the mosquito, but also due to immune factors from the human blood.

Methods:

- In this study, we use scRNA-seq to explore the P. falciparum developmental dynamics from unfertilized female gametes, through the zygote and the ookinete stages. All parasite cells were carefully isolated by micromanipulation from infected Anopheles gambiae mosquitoes.

Results: Using scRNA-seq, our data defines the genetics underlying the differentiation of a female gamete to an ookinete. We characterized five transcriptionally unique P. falciparum cell states as it develops in the An. gambiae mosquito. We also defined three distinct molecular signatures that orchestrate cell type transitions in the mosquito midgut. Genes related to DNA replication and metabolic processes were identified in early zygotes; reproduction, localization, and motility in intermediate stages, while early to mid-ookinetes express genes related to entry into its host and to down-regulation of metabolic processes

Conclusions: We provide insights into the timing of expression of genes connected to essential biological processes. Computational analyses identified the timing of expression for members of the ApiAP2 family of transcription factors. Moreover, we identify highly expressed genes with non-annotated function predicted to be intrinsically disordered proteins (IDPs) as novel candidates for antibody-based therapies.

Keywords: Single-cell RNA-seq, Plasmodium falciparum, Mosquito, Zygote, Malaria

P116 / #514

Topic: *AS02.1 Malaria*

OSTEOPONTIN AND MALARIA

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Introduction: Osteopontin (OPN) is a protein that participates in immune regulation and has been suggested to be involved in the immune response against malaria. It is found both intra- and extracellularly and is expressed by macrophages as well as by B- and T-cells. It is unknown if there is a difference in OPN expression during acute malaria in malaria naïve individuals compared to semi-immune individuals, and whether there is a direct correlation between OPN levels and parasitemia.

Methods: Blood samples were collected from malaria positive travelers from Sweden, and malaria positive individuals living in a highly malaria endemic region in Uganda. We then measured plasma concentrations of OPN by ELISA (Quantikine Human Osteopontin Immunoassay, R&D Systems, Abingdon, UK).

Results: There was no significant difference in median OPN plasma concentration between Plasmodium falciparum positive malaria naïve individuals compared to semi-immune individuals, but we found correlations between levels of OPN and parasitemia and differences according to age.

Conclusions: OPN could be of importance in formation of immunity against malaria.

Keywords: Osteopontin, immunity, P. falciparum, Malaria

P117 / #1708

Topic: *AS02.1 Malaria*

REVERSIBLE HOST CELL SURFACE REMODELING LIMITS IMMUNE RECOGNITION AND MAXIMIZES TRANSMISSION OF PLASMODIUM FALCIPARUM

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Introduction: P. falciparum extensively modifies its host RBC, by expressing parasite antigens on the infected RBC (iRBCs) surface and altering membrane lipid asymetry, leading to phosphatidylserine (PS) surface exposure on host cells. In asexual parasites these modifications enable the iRBCs to sequester and evade splenic clearance. Young transmissible forms of the parasite, gametocytes stage I-IV sequester and develop in the bone marrow, and reenter peripheral circulation at maturation (stage V). We have previously shown that acquired antibodies that allow immune recognition and clearance of iRBC specifically target immature but not mature gametocytes. We hypothesize that these host cell modifications (antigen expression and surface PS exposure) contribute to gametocyte survival in vivo.

Methods: We used flow cytometry, live imaging to quantify serum and antibody reactivity to intact iRBC surface for surface antigen expression, and annexin V binding for PS surface exposure. Transgenic parasites and inhibitors were used to perturb these host cell remodelling processes.

Results: Both expression of surface antigens and PS surface exposure showed a specific dynamic during gametocyte maturation, on in immature gametocytes and off in mature ones, independent of parasite strain and serum or antibodies used. By differentially perturbing each process we show that these modifications occur independently and are regulated by different unknown factors.

Conclusions: Our data suggest that these host cell modifications allow gametocytes to evade immune clearance, and thus contribute to mature gametocyte survival *in vivo* and onward transmission to mosquitoes. These findings have important implications for developing novel transmission blocking interventions.

Keywords: Gametocyte immune evasion, Host-parasite interactions, Transmission blocking

P118 / #1394

Topic: *AS02.1 Malaria*

MULTIPLEX PCR ASSAY FOR THE DIAGNOSIS OF MALARIA AND MOLECULAR DETECTION OF CHLOROQUINE RESISTANCE IN PLASMODIUM VIVAX

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Introduction: Malaria remains one of the major infectious diseases that affects most parts of the world. Currently, diagnosis of malaria is based on microscopy or rapid tests. Molecular assays offer an advantage of higher sensitivity and accurate identification of species even in mixed infections. Studies on drug resistance with respect to P. vivax are not as extensive as those on P.falciparum. Molecular detection of drug resistance help to detect upcoming novel mutations. Present study aimed to standardize molecular assays to detect human malaria causing Plasmodium species prevalent in India and to demonstrate the frequency of mutations in the gene Pvmdr 1 that is responsible for chloroquine resistance.

Methods: A multiplex PCR assay for detection of P. vivax, P. falciparum, P. ovale, P. malariae and P. knowlesi was standardized by using multiple permutations and combinations of PCR cycling conditions and set of published primers. All the samples (n=75)confirmed to be P. vivax mono infection were subjected to a PCR assay targeting the Pvmdr 1 gene. Amplified products were subjected to sequencing (n=39)to detect mutations in Pvmdr 1 gene.

Results: Multiplex PCR for detection of Plasmodium species was successfully standardised with a sensitivity of 90.9% and specificity of 85%. 35 out of 39 (89.7%) carried mutant alleles. Y976F and F1076L were the most common mutations detected. Other mutations that were detected in our study included Y976F (8%) and M980V (3.4%)

Conclusions: Detection of mutations may not always be a confirmatory marker of clinical resistance but their presence may be a sign of resistance accumulating at a genetic level that might manifest in future. Hence further studies are needed to keep track on the previously discovered mutations and discover novel mutations as well.

Keywords: Malaria, Multiplex PCR, P. vivax, Chloroquine, drug resistance

P119 / #1587

Topic: *AS02.1 Malaria*

MALARIA EPIDEMIOLOGY AMONG CHILDREN AND PREGNANT WOMEN, WESTERN EQUATORIA STATE, SOUTH SUDAN

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Introduction: This operational research is part of a project implemented by the NGO - CUAMM and funded by the Italian Agency for Development Cooperation and the Global Fund. The study has been approved by South Sudan Ministry of Health and aims at narrowing gaps in malaria epidemiology knowledge and diagnosis skills through large-scale molecular investigations and capacity building at primary health care centers (PHCC) in 3 Counties

Methods: The study is conducted at 3 PHCCs (Mundri, Lakamadi, Mvolo), targeting children under 5 years and pregnant women. Recruitment, on a volunteer basis, started in November 2021 and will last for 6 months, reaching 2000 participants. For each participant, malaria diagnosis is performed by Rapid Diagnostic Test, microscopy observation of blood slides and Next Generation Sequencing (NGS) of DNA extracted from Dried Blood Spot (DBS)

Results: Laboratories at the 3 PHCC have been equipped with instruments and reagents needed for malaria diagnosis. UNIPI has conducted intensive training at each PHCC on malaria clinical, epidemiology and diagnostic aspects as well as on study SOPs, followed by on-job supervision during recruitment. Despite staff strong motivation, malaria microscopy could not be implemented at PHCC level due to excessive workload and this activity will be centralized at Lui Hospital as referral site

Conclusions: Results from different methods will be compared to investigate causes of discordance. Malaria prevalence will be compared among seasons, PHCC and population groups. NGS will be used for *P. falciparum* genotyping of HRP2/3 deletion as well as of mutations associated with resistance to antimalarials. The generated data are expected to inform National Malaria Control Program planning and monitoring of malaria control interventions

Keywords: Malaria, Next Generation Sequencing, Epidemiology

P120 / #1617

Topic: *AS02.1 Malaria*

DEVELOPMENT OF A SENSITIVE MOLECULAR ASSAY FOR PLASMODIUM SPP FOR LOW PARASITAEMIC SAMPLES

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Introduction: Recently, droplet digital PCR (ddPCR) has been reported as promising quantitative and sensitive technique for the molecular detection of *Plasmodium*. The purpose of our study was to implement a ddPCR method able to detect the DNA of *Plasmodium* species in samples of blood with low parasitaemia. Moreover, considering our previous experience in the detection of *P. falciparum* DNA in serum samples when blood is not available, we tested the method in banked serum samples from patients with suspect of Hyperreactive Malarial Splenomegaly (HMS), in order to evaluate its application in this type of patients in which the diagnosis is particularly critical

Methods: We designed a “Pan-Plasmodium” set of primer and probe on a 18S rRNA gene region common to all the 5 human *Plasmodium* species. We set up the reaction and determined the limit of detection (LOD) and the limit of quantification (LOQ) of ddPCR assays. The same assays were then evaluated on a set of 56 low-parasitaemia blood samples, which showed discordant results at microscopy, Quantitative Buffy Coat (QBC) and RDT and on a set of 27 sera from patients with clinical suspect of HMS

Results: ddPCR assays for *Plasmodium* spp. showed good reproducibility and a linearity without non-specific signals until 0.1 trophozoites/µl. The assay was able to detect the *Plasmodium* signal in the low parasitaemia blood samples and the copy number was determined by ddPCR. In HMS sera samples we detected 37% of positive samples. Among patients without a microscopic/QBC confirmed we found 7% of positive sera samples

Conclusions: The results indicate that this ddPCR pan-plasmodium test can be used as a reliable assays when sensitivity is fundamental, as for example in case of clinical suspect of HMS or when the blood samples are not available and serum must be used

Keywords: Quantitative Buffy Coat (QBC), DNA, plasmodium, digital droplet PCR, Microscopy

P121 / #756

Topic: *AS02.1 Malaria*

PROTECTIVE T CELL TARGETS OF IMMUNITY TO MALARIA IDENTIFIED BY SYSTEMS-BASED APPROACHES

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Introduction: Vaccines against many diseases caused by complex pathogens are not available despite extensive research. The genome and proteome of these pathogens provide a foundation to identify effective targets for intervention.

Methods: Using malaria as a model, we have developed and applied proteome-wide screening strategies to identify and prioritize from the complete P. falciparum proteome the subset of antigens targeted by T cell or antibody responses from individuals with immunity to malaria. We also developed innovative computational approaches to analyze the large metric datasets. These unique omics-scale datasets of T-cell and antibody reactivity to P. falciparum provide novel insights into host-parasite responses and the foundation for rational vaccine discovery.

Results: Antigens recognized by T cells or antibodies were broadly distributed across the proteome. Unexpectedly, only 30% of the parasite proteome was targeted by antibody or T cell responses. Also, individual immune profiles comprised either high reactivity to a low number of antigens, or low reactivity to a high of antigens. Integrating our T cell and antibody datasets revealed that antigens preferentially recognized by T cells are distinct from antibody targets, with important implications for vaccine design. A subset of antigens recognized as priority targets for T cell responses were evaluated for immunogenicity and capacity to protect against homologous or heterologous cross-species sporozoite challenge in mice.

Conclusions: These studies establish T cell antigens identified by proteome-wide screening approaches as excellent targets for intervention. A rationally-designed genome-based vaccine based on these antigens could protect against all strains and all species of malaria.

Keywords: vaccines, T cells, antigen discovery, systems biology, Malaria

P122 / #817

Topic: *AS02.1 Malaria*

ANALYSIS OF ALLELIC CROSS-REACTIVITY OF VAR2CSA-SPECIFIC MONOCLONAL IGG ANTIBODIES BY A MULTIPLEXED REVERSE FLUOROSPOT ASSAY

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Introduction: Antibody cross-reactivity is a central characteristic of protective immunity against pathogens including the malaria parasite Plasmodium falciparum. Serological analysis of the naturally acquired immunity against key parasite polymorphic antigens, often does not allow the distinction between true cross-reactivity (one antibody recognizing multiple antigen variants) and apparent cross-reactivity (presence of multiple variant-specific antibodies).

Methods: To address this paucity in our understanding of how protective immunity is achieved in malaria, we adapted a reverse multiplexed FluoroSpot assay that allows the direct analysis of monoclonal antibody cross-reactivity against several antigen variants. We used three different domains (ID1-ID2, DBL3X, and DBL5ε) part of the VAR2CSA-type PfEMP1 – a notoriously polymorphic antigen involved in the pathogenesis of placental malaria – as a model.

Results: We demonstrated the assay's robustness in detecting variant-specific and cross-reactive responses at the single-cell level, both in previously characterized VAR2CSA-specific cell lines and in circulating memory B cells isolated from a small set of naturally exposed individuals. In the latter, most of the reacting single-cells were variant-specific indicating targeting of epitopes not conserved among the tested allelic variants.

Conclusions: We have developed an assay that is suitable for the interrogation of the degree of allelic cross-reactivity of monoclonal antibodies targeting VAR2CSA. The assay is adaptable to the analysis of other polymorphic antigens, rendering it a powerful tool in studies of immunity to malaria and many other diseases.

Keywords: antibody, FluoroSpot, VAR2CSA, cross-reactivity, placental malaria

P123 / #396

Topic: *AS02.1 Malaria*

NEW INSIGHTS INTO THE ROLE OF NUCLEAR ACTIN IN PLASMODIUM FALCIPARUM

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Introduction: Actin is a protein that plays an essential role in a wide variety of cellular events such as vesicular traffic, locomotion, and maintenance of cell shape, yet its functions are not limited to the cytoplasm; in the nucleus, actin is implicated in many nuclear processes such as transcription, gene repositioning, DNA repair and DNA replication. The Apicomplexa's actin is diverse from conventional actins, particularly in P. falciparum, where two actins, PfActin1 and PfActin2, exist. Currently, it is known that PfActin1 in the nucleus of P. falciparum participates in the activation of var genes.

Methods: To assess what other functions actin performs in the nucleus of P. falciparum during the intraerythrocytic cycle, we used transgenic parasites that contain an exogenous copy of PfActin1 labeled with three HA flags.

Results: We found that PfActin1 is located mainly in the nucleus of rings, followed by trophozoites and in lesser proportion in schizonts. Using co-IP assays, it was found that at each stage of the intraerythrocytic cycle, PfActin1 is part of several protein complexes involved in DNA replication, DNA repair, cell cycle, and transcriptional protein complexes that participate in chromatin modification and remodeling, pre-mRNA splicing and spliceosome assembly. In addition, 2-DE showed that there are differences in the isoelectric point of nuclear and cytoplasmic actin, suggesting that there are differential post-translational modifications that could participate in the shuffling nucleus-cytoplasm of actin of P. falciparum.

Conclusions: All those data suggest that PfActin1 in the nucleus of P. falciparum is a dynamic protein implicated in the transcription and replication of these parasites, key processes for gene regulation and malaria transmission.

Keywords: Nuclear Actin, Gene Regulation, Malaria

P124 / #1477

Topic: *AS02.1 Malaria*

ANALYSIS OF PLASMODIUM FALCIPARUM HRP2/3 GENE DELETION OR MUTATION IN ALL P. FALCIPARUM POSITIVE BLOOD SAMPLES AND CLINICAL CORRELATION

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Introduction: Despite efforts, malaria continues to be a major public health problem. Rapid diagnostic card tests (RDTs) enable timely and appropriate diagnosis especially in remote areas.Pfhrp2 is the most targeted antigen for the detection of Plasmodium falciparum infections. Genetic mutations and gene deletions are important emerging factors for false-negative RDTs which may delay the provision of life-saving treatment for the patients.

Methods: During the 2 years period, a total of 63 samples positive for malaria by any one of the methods (Peripheral smear/Quantitative buffy coat/PARASIGHT F/RDT) were included in the study. Polymerase chain reaction was done to confirm the infecting species of Plasmodium. Among these, microscopically confirmed P. falciparum but RDT negative samples were assessed for the presence of pfhrp2, pfhrp3, and their flanking genes using PCR. Sequencing was done to confirm. Follow up of the clinical outcomes were done.

Results: By PCR, 69.3% were P.vivax and only 20.6 % were P.falciparum among the 63 samples collected. Among these P.falciparum samples, 4 were found to be RDT negative but microscopically positive.Pfhrp2 &Pfhrp3 gene and their flanking genes were amplified for these 4 samples assuming that remaining 9 RDT positive samples carried Pfhrp2 gene. All 4 samples were found to be negative for both genes and exon 1-2 region of Pfhrp3 gene whereas 1/4 showed deletion of the flanking genes of Pfhrp2&Pfhrp3. Sequencing results also showed the same.

Conclusions: This is a first study on Pfhrp2/hrp3 gene deletion or mutation in P. falciparum to be done in South India to the best of our knowledge and provides molecular evidence for the existence of its deletion in a tertiary care centre in South India warranting periodic evaluation of Pfhrp2 based RDT use.

Keywords: PFHRP2/3 deletions, Rapid diagnostic cards, Malaria, Plasmodium falciparum

P125 / #1534

Topic: *AS02.1 Malaria*

PROFILE OF MUTATIONS IN PVDHFR, PVDHFS AND PVMDR1 AND PVK12 DRUG RESISTANCE GENES IN PLASMODIUM VIVAX CASES IN SOUTH INDIA

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Introduction: In malaria, 53% of the *Plasmodium vivax* burden is in the WHO SEAR with the majority being in India(47%). Anti-malarial resistance is a major concern and the identified molecular markers will aid us to monitor the drug resistance in endemic areas. Hence, presence of mutations in *pvdhfr, pvdhfs* and *pvmdr1* and *pvk12* was analysed in the *Plasmodium vivax* isolates.

Methods: A total of 80 samples collected during the period of 2 years in south India, positive for malaria by any one of the methods were included in the study. Conventional nested speciation PCR targeting 18SrRNA were done to confirm the infecting species of Plasmodium. Among these, microscopically confirmed *P. vivax* were assessed for the mutations in *pvdhfr*, *pvdhfs* and *pvmdr1* and *pvk12* drug resistance genes using conventional PCR and confirmed with sequencing. Follow up of the clinical outcomes were also done for these patients.

Results: Of the 80 positive samples collected (71 /80) 88.75% were *P.vivax* and (9/80) others by PCR. Among these *P.vivax* positive samples, they were assessed for the mutations in *pvdhfr*, *pvdhfs* and *pvmdr1* and *pvk12* drug resistance genes. For *P. vivax* isolates, an amplification rate of 40% was found for the pvmdr1 gene. The results of the study are in agreement with the high amplification rates for pfmdr1 gene evidenced in the Southeast Asia. Other genes are found at a higher frequency compared to west India and other Asian countries.

Conclusions: This study provides molecular evidence for the existence of high profile mutations in *pvdhfr, pvdhfs* and *pvmdr1* and *pvk12* drug resistance genes in a in south India warranting periodic evaluation of drug resistance in India, a south Asian country. This study supplements the current baseline data of the drug resistance and pressure in the country.

Keywords: Plasmodium vivax, pvdhfr, pvk12, drug resistance genes, Malaria

P126 / #455

Topic: *AS02.1 Malaria*

MALARIA AND AUTOANTIBODIES AGAINST ERYTHROCYTES

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Introduction: Immunity against Plasmodium falciparum malaria is slow to develop and requires several rounds of infection. Anemia is a common cause of death in severe malaria and many non-infected red blood cells (RBC) are lost, but the reason for this is not clear. Autoantibodies against RBC are known to occur but exactly what they are directed against or how common they are in malaria endemic areas has not been very well studied. In this study, we have investigated autoantibodies against RBC in a malaria endemic area in Uganda and we have characterized the specificity of several autoantibodies.

Methods: Routine hemagglutination techniques were used: To detect antibodies bound to RBC in vivo, we used the direct antiglobulin test. Gel cards and Indirect antiglobulin tests were used to identify autoantibodies circulating in plasma. For further identification of the specificity of the antibodies, we used RBC with known genotypes, particularly RBC lacking certain antigens that could be of interest in malaria research.

Results: We found antibodies against RBC in more than half of the Ugandan individuals. For comparison, we also used Swedish control samples where autoantibodies were found in only 5% of the samples. Specific antibodies directed against several RBC antigens that have also before been described to be of importance in merozoite invasion were identified.

Conclusions: Autoantibodies directed against RBC are prevalent in a malaria-endemic area, and we have determined the specificity of some of these antibodies.

Keywords: Red blood cells, Autoantibodies, Plasmodium falciparum, Malaria

P127 / #526

Topic: *AS02.1 Malaria*

PLASMODIUM FALCIPARUM INFECTION AND NATURAL ACQUIRED IMMUNITY TO MALARIA AMONG CHILDREN IN COMMUNITIES WITH SEASONAL MALARIA CHEMOPREVENTION IN NORTHERN GHANA

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Introduction: In recent years, Ghana has implemented the Seasonal Malaria Chemoprevention (SMC) as a targeted malaria control strategy against P. falciparum malaria among children <5 years. This study provides the malaria situation report among children in three Municipalities in Northern Ghana, where the SMC is being implemented by Ghana Health Service (GHS).

Methods: Two independent cross-sectional studies were carried out in 13 rural communities in the Tolon, Kumbugu, and Nanton Municipalities. The first was a household survey to document malaria cases among children and malaria control and treatment seeking behaviours of households. In the second, 1002 children (1-17 years) were screened for P. falciparum infection with RDT and PCR. Total IgG levels against malaria antigens (CSP, GLURP, MSP3, and Pfs230) and crude asexual blood stage antigens were also tested.

Results: Infection rate by PCR was higher in Tolon (27%) than in Nanton (19%) and Kumbugu (16%). Parasite-carriage was significantly higher among children >5 years, with three times higher infection risk in those 8-10 years compared to <5 years. Nevertheless, reported malaria cases were higher among younger than older children (50% vs. 39%). In a pattern that reflects the differential infection levels across the Municipalities, higher IgG levels against all the antigens were observed in Tolon.

Conclusions: The higher prevalence of malaria among younger children despite the relative lower parasite-carriage among them compared to older children, suggests that younger children are still prone to malaria despite the implementation of SMC in the communities. Thus, to effectively control malaria among younger children, other malaria control measures are needed in combination with SMC in communities.

Keywords: Seasonal Malaria Chemoprevention (SMC), Rapid Diagnosis Test (RDT), Plasmodium falciparum (P. falciparum), Polymerase Chain Reaction (PCR)

P128 / #1012

Topic: *AS02.1 Malaria*

PFEMP1 PROTEINS ARE TARGETS OF FUNCTIONAL ANTIBODIES ACQUIRED DURING SEVERE CHILDHOOD MALARIA

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Introduction: Plasmodium falciparum (P.f) malaria is an infectious disease where children less than 5 years of age are disproportionately affected. A particular virulence factor of P.f malaria is clonally variant PfEMP1 (P.f erythrocyte membrane protein) proteins, found on the surface of infected erythrocytes (IEs). PfEMP1-mediated vascular adhesion and sequestration cause severe malaria including cerebral malaria (CM) in children. We have linked expression of group A PfEMP1s to the development of severe malaria and CM. A particular ICAM-1-binding group A PfEMP1 (PDF1235w DBLb3_D4) is the target of naturally acquired immunity and its associations with exposure and immunity in children could be a useful information as a biomarker for vaccine development.

Methods: We used indirect ELISA to detect antibodies (Abs) to PDF1235w DBLb3_D4 in plasma from Beninese children less than 5 years of age and with different clinical presentation. In addition, Abs recognizing native PfEMP1 variants expressed on late-stage IEs were detected and quantified by flow cytometry. Inhibition properties as a function of the plasma were also determined using competitve ELISA.

Results: There was an increased IgG recognition of PDF1235w DBLb3_D4, and plasma from children with uncomplicated malaria inhibited the binding of the domain to ICAM-1. Also, there was an increased IgG recognition of plasma samples from children with severe malaria to native PFD1235w PfEMP1 expressed by selected 3D7-IEs compared to non-PFD1235w expressing 3D7-IEs.

Conclusions: In conclusion, Abs from participants included in the study were able to react to the domain, and that the IgG Abs have functional activity as they elicited inhibitory effects on the ICAM-1-binding to DBLβ domain encoded by dual-receptor binding PfEMP1.

Keywords: Malaria, Severe, pfemp1, Childhood, ICAM-1

P129 / #946

Topic: *AS02.1 Malaria*

PLASMODIUM FALCIPARUM TRANSMISSION MEASUREMENT BASED ON GENE DIVERSITY AND ANTIBODY RESPONSES IN IBADAN, NIGERIA

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Introduction: Nigeria is responsible for about 27% of the world's deaths due to P. falciparum malaria and there have been several international and national strategies to eradicate malaria in the country. This work was carried out to gain insights into the current transmission intensity in Oyo state, Nigeria, to be able to judge what the next steps should be in reducing spread of malaria in the area.

Methods: A cohort of 150 individuals was selected for inclusion in this study. Malaria infection was diagnosed using a rapid diagnostic test (RDT). DNA was extracted and the MSP1 and MSP2 genes were genotyped by using a nested PCR method. Levels of antibodies against P. falciparum was determined by ELISA.

Results: Approximately 1/5 of the participants were found to be infected with P. falciparum. MSP1 and MSP2 allelic families were detected in almost all of the isolates. MAD20 was the most prevalent MSP1 allelic family and K1 the least prevalent. For MSP2, both allelic families 3D7 and FC27 were common. Multiplicity of infection (MOI) was low for both MSP1 and MSP2. IgG antibody levels correlated positively with age but were similar for both infected and non-infected individuals.

Conclusions: The low MOI indicates low transmission intensity in the study area. However, levels of P. falciparum-specific antibodies point towards a high endemicity.

Keywords: Plasmodium falciparum, MSP1, MSP2, antibody

P130 / #1292

Topic: *AS02.1 Malaria*

FOCUS ON MALARIA ELIMINATION: THE POSSIBLE ROLE OF IVERMECTIN

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Introduction: Elimination of malaria may be possible focusing on the elimination of the parasite vector, Anopheles mosquitoes. The possible effect of the drug Ivermectin on Anopheles mosquitoes may make this safe drug an invaluable tool in the fight against the malaria parasites. This study was designed to find out the effect of Ivermectin on female naive Anopheles gambiae mosquitoes fed on the blood of rabbits treated with the drug Ivermectin.

Methods: The rabbits were placed in 4 separate cages according to their weights. Ivermectin treatment was administered orally according to weights of the rabbits with the exception of one clearly marked rabbit which served as control. Anopheles gambiae mosquitoes were reared in the laboratory. The female Anopheles mosquitoes were separated and placed in labelled and netted cages (50 mosquitoes per cage). The treated and untreated rabbits were introduced into the labelled netted mosquito cages respectively for 30 minutes so that the mosquitoes can feed on the rabbits, after which the rabbits were returned to their cages. Mortality rate of the mosquitoes were observed every 24hrs for 5days. Dead mosquitoes were removed using the aspirator and subsequently counted.

Results: Findings from the study showed 38%, 56% and 48% mosquitoes mortalities 24hours post feeding on the treated rabbits and 96%, 92% and 94% mortalities 48 hours post feeding. The mosquitoes that fed on untreated rabbits had 0% mortality 24hours post feeding and 4% mortality 48hours post feeding.

Conclusions: This study is on-going but there are strong indications that ivermectin may yet be an invaluable tool for malaria control in the future.

Keywords: Ivermectin, Malaria elimination, Anopheles Mosquitoes

P131 / #1745

Topic: *AS02.1 Malaria*

ABSENCE OF THE ST2/IL-33 PATHWAY IMPLIES INCREASED PULMONARY INFLAMMATION AND CONSEQUENTIAL EARLY DEATH DURING EXPERIMENTAL PLASMODIUM BERGHEI NK65 INFECTION

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Introduction: IL-33, which are produced predominantly by epithelial cells, can induce production of cytokines such as IL-4, IL-5 and IL-13 by various types of cells. It is known that this Th2-type cytokines contribute to host defense against malaria parasite infection, however, the roles this immunologic pathway in malaria parasite infection remain unclear. Thus, the objective of this work was to investigate the participation of the ST2/IL-33 pathway in the experimental severe pulmonary malaria model by *P. berghei* (*P. berghei*) NK65.

Methods: Wild-Type (WT) BALB/c background and genetically deficient mice for the ST2 receptor (ST2-/-), from seven to eight weeks of age, were inoculated with 10^4*P. berghei* NK65-infected erythrocytes intraperitoneally. On the fifth- and tenth-days post infection lung samples were collected for immunological (dosage of Th1, Th2 Th17 and Treg cytokines of tissue homogenates) and pathophysiological (HE histopathology and pulmonary mechanics) analyses.

Results: The most important findings revealed that ST2-/- mice showed early mortality, and hemorrhage and increased inflammatory infiltrate in the lung tissue, especially in late times of infection. Nonetheless, these phenomena do not seem to imply pulmonary dysfunction. However, the immune response in ST2-/- mice during *P. berghei* NK65 infection was skewed to Th1/Th17 type than Th2, associated with increased parasitic burden, tissue macrophages infiltration and activity.

Conclusions: Collectively, our results demonstrate that the Th2 immune response triggered by IL-33/ST2 pathway mediates susceptibility of *P. berghei* infection related with increased lung inflammation which consequently contributes early mortality.

Keywords: ST2/IL-33, lung inflammation, P. berghei NK65

P132 / #899

Topic: *AS02.1 Malaria*

CHARACTERISING PROTECTIVE MONOCLONAL ANTIBODIES TO PLASMODIUM FALCIPARUM ANTIGEN PFMSRP5

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Introduction: Developing a vaccine against Plasmodium falciparum malaria has been challenging primarily due to high levels of polymorphism and a complex parasite lifecycle. Consequently, a second-generation malaria vaccine will need to include multiple antigens that elicit synergistic antibodies (Abs). Antisera against P. falciparum merozoite antigen PfMSRP5 has been shown to inhibit P. falciparum in vitro. Furthermore, these Abs have synergistic interactions associated with lower malaria risk when combined with Abs to several merozoite antigens. PfMSRP5 is therefore a promising vaccine candidate, however, monoclonal (m) Abs to PfMSRP5 have yet to be defined.

Methods: In this study we produce anti-PfMSRP5 mAbs from naturally immune Ghanaian donors using EBV immortalisation of B cells and from immunised mice using hybridoma technology. Growth inhibition activity assays are performed to determine individual inhibition as well as synergistic inhibition utilising mAbs that target a range of merozoite antigens.

Results: To date, polyclonal sera from immunised mice has been shown to inhibit P. falciparum in vitro. Additionally, anti-PfMSRP5 mouse mAbs have been produced and anti-PfMSRP5 human mAb has been isolated and characterisation of all mAbs is currently underway.

Conclusions: Characterising mAbs against antigens that operate at distinct steps in the same pathway will lead to a better understanding of protective epitopes which may inform preventative vaccine strategies.

Keywords: Merozoite, Malaria, antibody

P133 / #541

Topic: *AS02.2 Schistosomiasis*

EFFICACY AND SAFETY OF PRAZIQUANTEL/MEBENDAZOLE IN THE TREATMENT OF HELMINTHIASIS IN SCHOOL AGE CHILDREN IN UGANDA

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Introduction: To monitor antihelminthic drug efficacy and safety of Praziquantel and Mebendazole for helminthiasis Control in School-age-children, Uganda.

Methods: The study enrolled 676 SAC aged 8-14years from Mpigi and Bundibugyo in Uganda. All treated at pre-baseline with Praziquantel and Mebendazole. Egg-reduction-rates calculated as decrement in intensity of S.mansoni/STH as pre-treatment proportion. The safety determined three-week post-treatment.

Results: The mean-age was 10.8years. Girls represented 53.8%, 50.7% aged between 10-14 years. S.mansoni significantly reduced (70.5%, 95%CI: 58.4-85.1) pre-treatment to (21.5%, 95%CI: 16.5-28.4) post-treatment. (29.9%, 95%CI:26.4-33.3) were positive at pre-treatment, (4%, 95%CI: 2.5-5.5) Post-treatment. Overall pre-and post-treatment prevalence for S.mansoni was 29.9%(95%CI: 26.4-33.3) and 4.0%(95%CI:2.5-5.5) respectively. 85.6%(95% CI: 80.8-90.5)PZQ cure-rate didn't defer with age/Sex whereas significant on S.mansoni intensities. The study showed 20.9% S.mansoni and 3.1%Hookworms had heavy intensities, 28.7% S.mansoni and 3.1%Hookworms had moderate intensities. Majority had light infections; 60.4%S.mansoni, 93.8%Hookworms,1.5%Ascaris,1.5%T.trichuira respectively. Significant egg- intensity reduction-rate of S.mansoni and STH with arithmetic mean 187.8 (95%CI:129.9-245.6) pre-treatment to 21.5% (95%CI: 16.5-28.4) post-treatment. Significant reductions in arithmetic mean for hookworm from 376.5(95%CI: 60.8-692.2) pre-treatment to 8.3(95%CI: 8.6-25.1)post-treatment and Ascaris from 366(95%CI:80.5-651.5) pre-treatment to 8.3% (95% CI: 8.6-25.1) post-treatment.

Conclusions: The study showed Praziquantel/Mebendazole were well tolerated. Research funding from WHO.

Keywords: Efficacy/safety,, Helminthiasis,, Uganda, Praziquantel,, Mebendazole,

P134 / #271

Topic: *AS02.2 Schistosomiasis*

COMPARING PRAZIQUANTEL-SUSCEPTIBLE AND PRAZIQUANTEL RESISTANT SCHISTOSOMA MANSONI USING A PROTEIOMIC ANALYSIS.

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Introduction: Praziquantel (PZQ) is currently the only drug used for treatment and control of schistosomiasis. Extensive use of this drug has brought concern about the emergence of PZQ-resistance/tolerance. In this in vitro study, we compared the proteomes of an Schistosoma mansoni strain stably resistant to PZQ and isogenic to its susceptible parentalcounterpart, identifying proteins from male and female adult parasites of both strains, exposed or not to PZQ.

Methods: S. mansoni adult parasite proteins were extracted and two-dimensional electrophoresis (2-DE) gels was performed. Protein spots selected for each group were manually excised and digested in-gel with trypsin for mass spectrometry identification. Digested peptides were analyzed in an Easy-nLC II nanoflow liquid chromatography system in tandem with an LTQ-Orbitrap Velos mass spectrometer. The list of peptide and fragment mass values generated by the mass spectrometer for each spot were submitted to an MS/MS ion search using the Mascot® 2.0 software against the WormBase ParaSite database.

Results: Were identified 60 different proteins on S. mansoni proteome, that correspond to 0.42% of the full parasite proteome. All those proteins were present in adult parasites not exposed to PZQ. However, some of these proteins were not detected when these adult parasites were exposed to the drug. This result could suggest the involvement of PZQ exposure on those protein expressions in resistant and susceptible strains.

Conclusions: This study allowed us to identify proteins that in our proteome analyses differ between PZQ-susceptible and PZQ-resistant parasites, however, their relationship or contribution towards the mechanism of resistance remains unclear and strongly needs further clarification.

Keywords: schistosomiasis, Praziquantel, PROTEIOMIC ANALYSIS, Schistosoma mansoni

P135 / #1051

Topic: *AS02.2 Schistosomiasis*

IN SEARCH OF LEAD COMPOUNDS FROM PHYTOCHEMICALS WITH SCHISTOSOMICIDAL POTENTIAL TARGETING THE AQUAPORIN 1 PROTEIN OF SCHISTOSOMA HAEMATOBIUM

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Introduction: Schistosomiasis is a neglected tropical disease affecting people living in rural areas. The search for an alternative drug to praziquantel, the mainstay for the treatment of schistosomiasis rests with medicinal plants use in Nigerian communities.

Methods: An ethnobotanical survey was done in Eggua and Alaapa communities in southwestern Nigeria, and the plants used were documented. Of these, three (Jatropha curcas, Mormodica charantia, Calotropis procera) were selected for the identification of lead compounds.These extracts were assayed in vitro for anti-schistosomal activity against Schistosoma haematobium eggs in terms of motility and death of eggs. The phytochemical profile of these methanolic extracts was determined by GC-MS. Identified phytochemicals from Jatropha curcas were subjected to in silico docking against Schistomiasis haematobium aquaporin 1 using Swissdock server. The active site of the parasite aquaporin1 protein was further investigated using Dogsitescorer.

Results: Only Jatropha curcas extract exhibited a strong potency against haematobium eggs in vitro, at minimum effective concentration of 100mM after 1hr. Nine compounds were identified, including 1,2-cyclopentanedione, 4H-pyran-4-one, 2-cycohexen1-one, Phytol, 9, 12,15-Octadecatrienoic acid, Octadecanoic acid and Hexadecanedioic acid. Hexadecanoic acid gave the best docking score -2085.54 for full fitness. There were thirteen binding pockets in Schistosoma haematobium aquaporin 1, each of which had a high affinity to the phytochemicals docked with it.

Conclusions: Hexadecanoic acid can be further investigated as a potential lead compound for the development of new schistosomicidal drugs. In vivo studies need to be carried out in the laboratory to validate this finding.

Keywords: treatment, lead compounds, phytochemicals, Schistosoma haematobium, aquaporins

P136 / #225

Topic: *AS02.2 Schistosomiasis*

MOLECULAR MALACOLOGY AND XENOMONITORING SCHISTOSOMIASIS: IMPLICATION OF BULINUS AFRICANUS AS AN INTERMEDIATE HOST OF SCHISTOSOMA HAEMATOBIUM IN LAKE MALAWI.

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Introduction: The life-cycle of Schistosoma haematobium, a trematode parasite, involves both humans and Bulinus snail species. Recent reports have identified Bulinus spp. novel to Lake Malawi, but their role in Schistosoma epidemiology is unknown. Due to the emergence of hybrids of S. haematobium and Schistosoma bovis south of Lake Malawi, this study sought to investigate the intermediate hosts of these Schistosoma spp., to identify parasite transmission foci, and determine potential areas for hybridization.

Methods: Infection screening was conducted on 106 snails collected in 2017 from Lake Malawi shoreline, Magochi Disrict, using both conventional and quantitative PCR xenomonitoring methods. Snails were selected for species identification (n=10) by inspecting a 644bp fragment of the cox1, which was later aligned to entries on GenBank. The distribution of Bulinus spp. and Schistosoma spp. was mapped onto Mangochi district.

Results: Four snails were matched to sequences of Bulinus africanus and another identified as a Bulinus angolensis-like specimen. Although no snails were infected with S. bovis, the qPCR cycle threshold values indicated that individuals from both snail species were developing prepatent infections with S. haematobium across the shoreline, including some Mangochi tourist beaches.

Conclusions: This study builds on recent surveys implicating the newly reported B. africanus and B. angolensis-like snails in the transmission of S. haematobium in Lake Malawi for the first time. There is a risk for introduction of S. bovis and subsequent hybridisation with the endemic S. haematobium, as B. africanus is a competent host of both parasites. The finding of snails infected with S. haematobium on tourist beaches poses a risk for its translocation to non-endemic areas.

Keywords: Schistosoma haematobium, Bulinus, Intermediate host, Xenomonitoring

P137 / #1731

Topic: *AS02.2 Schistosomiasis*

ASSESSING THE MOTIVATIONAL FACTORS OF CITIZEN SCIENCE PARTICIPANTS OF A SNAIL MONITORING NETWORK IN SOUTH-WEST UGANDA.

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Introduction: The ATRAP project employs a novel snail monitoring approach that is executed by non-specialists: an approach also called citizen science (CS). Here, members of the general public actively contribute to scientific work (monitoring **freshwater snails** that spread **schistosomiasis**). This way, large volumes of the much-needed data on snail distribution and ecology are generated. However, since CS is a fairly new concept in Uganda, information on the reasons why members of the public contribute their time, energy and skills is scarce. This study, therefore, investigates the factors driving participants of the ATRAP project by drawing inspiration from two psychological theories: the volunteer functions inventory (VFI) and the theory of planned behaviour (TPB).

Methods: A questionnaire that included the VFI and TPB components, as well as demographic information, was administered to the CS participants (n = 23), and a control group that consisted of candidate citizen scientists (n= 30). Data were collected using two different techniques (group and individual interviews).

Results: Compared to other CS networks, participants in this study are younger, have middle education and low-income status. Also, both active and control group participants are highly motivated regardless of the interview method. Contrary to our expectations, the social factor as a key driver had a low score in both groups.

Conclusions: The high average scores obtained in both interview techniques suggest consistency and thus no socially desirable answering. The general low score of the social factor across all the groups as a motivational factor indicates that the findings from the volunteering literature in the Global South, where social interaction stood out as key, cannot be extrapolated to CS projects.

Keywords: citizen science, snail monitoring, Vector-Borne Diseases, schistosomiasis, motivation

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Topic: *AS02.2 Schistosomiasis*

DISCOVERY OF SERUM BIOMARKERS FOR EARLY SCHISTOSOMA MEKONGI INFECTION WITH METABOLOMICS

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Introduction: Schistosomiasis is a parasitic disease affects million people worldwide, especially in African countries. *Schistosoma mekongi* is one of the species causing schistosomiasis in the southeast Asia. In chronic cases, patients may develop many life-threatening illnesses, for example, liver abscesses. Diagnosis relies on the detection of parasite eggs from patients' feces, which lacks sensitivity and timeliness. To cope with the problem, this study aims to identify serum biomarkers of early *S. mekongi* infection in mouse model using metabolomics.

Methods: Blood samples were collected from mice before and two weeks after infection, which was earlier than classical parasitological detection. Serum samples were extracted for metabolites and analyzed with a mass spectrometer. Metabolomic data analysis was performed using the MS-DIAL platform and MetaboAnalyst 5.0.

Results: From 19,582 total features identified, there were 187 features whose their levels were differentially changed following the infection. Multivariate analysis revealed separation between samples before and after infection. Focusing on significantly altered metabolites, Onopordopicrin and L-(+)-Lysine were increased and decreased metabolites from positive mode with the lowest *p*-value, respectively. On the other hand, Picrotin and unidentified feature (M/Z = 225.1859) were increased and decreased metabolites from negative mode with the lowest p-value, respectively. The 4 metabolites were highlighted as the interesting targets for biomarkers discovery in early schistosomiasis.

Conclusions: Findings of this study would be benefit for development of markers for detection of *S. mekongi* infection at early stage, which can help reduce number of patients and deaths from this disease.

Keywords: schistosomiasis, Schistosoma mekongi, metabolomics, Early biomarkers

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Topic: *AS02.2 Schistosomiasis*

SCHISTOSOMA HAEMATOBIUM URINARY TRACT COMPLICATIONS IN AFRICAN MIGRANTS CONSULTING IN PRIMARY CARE FACILITIES IN FRANCE: A RETROSPECTIVE COHORT OF 133 PATIENTS (2004-2020)

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Introduction: This study aimed to describe the urinary tract complications related to urogenital schistosomiasis (UGS) detected among African migrants who consulted in outpatient clinics in Paris.

Methods: A retrospective cohort study included all UGS diagnosed from 2004 to 2020. Cases were defined by the presence of typical Schistosoma haematobium eggs at urine microscopy. Ultrasonography (U-S) of the urinary tract was prescribed to each case. Demographic, clinical, biological and imaging data were collected and U-S outcomes classified according WHO Guidelines.

Results: First-line imaging was performed in 112/133 cases. All 112 patients were treated by praziquantel. Sex ratio (F/M) was 2/110, mean age 24.5 years, mean time interval between arrival in Europe and medical imaging 21.3 months, all patients came from West-Africa (Mali: 73 %). Among those with interpretable U-S outcomes, 33/107 (31 %) had abnormalities related to UGS, localized at the bladder in 32. No sociodemographic, clinical, or biological factors were found to be associated with U-S abnormalities. Six patients had a major abnormality, the most serious cases being a hydroureter and a pseudo-polyp of the bladder with squamous metaplasia. Post-cure imaging control was performed in 20/33 patients, of whom 12 (60 %) had a reversal of their abnormalities.

Conclusions: Urinary tract complications were predominantly at the bladder and moderate in most of the cases, although of unknown evolution in 5 patients. Praziquantel and U-S should be prescribed to any patient with positive urine microscopy and be considered in African migrants recently arrived in Europe, if urine microscopy stays repeatedly negative. Of utmost importance is the monitoring of abnormalities persisting despite 2 or 3 rounds of praziquantel.

Keywords: Urogenital schistosomiasis, Primary care, West-African migrants, Urinary tract complication, Ultrasonography

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Topic: *AS02.2 Schistosomiasis*

SCHISTOSOMIASIS IN THE PEOPLE'S REPUBLIC OF CHINA – DOWN BUT NOT OUT

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Introduction: Schistosomiasis has been subjected to extensive control efforts in the People's Republic of China (China) which aims to eliminate the disease by 2030. We describe baseline results of a longitudinal cohort study undertaken in the Dongting and Poyang lakes areas of central China designed to determine the prevalence of Schistosoma japonicum in humans, animals (goats and bovines) and Oncomelania snails utilizing molecular diagnostics procedures. Data from the Chinese National Schistosomiasis Control Programme (CNSCP) were compared with the molecular results obtained.

Methods: The baseline of a longitudinal cohort study was carried out across the 16 villages identified in Hunan and Jiangxi provinces. Snail surveys were performed with LAMP used to deterine if snails were infected. Human infection was assessed using Kato-Katz and real-time PCR (qPCR). Animal infection was determined using qPCR and digital droplet PCR.

Results: The prevalence of schistosomiasis in humans was 1.8% in Jiangxi and 8.0% in Hunan determined by real-time polymerase chain reaction (PCR), while 18.3% of animals were positive by digital droplet PCR. The CNSCP data indicated that all villages harboured S. japonicum-infected individuals, detected serologically by indirect haemagglutination assay (IHA), but very few, if any, of these were subsequently positive by Kato-Katz (KK).

Conclusions: Based on the outcome of the IHA and KK results, the CNSCP incorporates targeted human praziquantel chemotherapy but this approach can miss some infections as evidenced by the results reported here. Sensitive molecular diagnostics can play a key role in the elimination of schistosomiasis in China and inform control measures allowing for a more systematic approach to treatment.

Keywords: China, Schistosoma japonicum, LAMP, Real-time PCR, Kato-Katz

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Topic: *AS02.2 Schistosomiasis*

NOVEL CHEMOTHERAPY-BASED STRATEGIES FOR TREATMENT OF HELMINTHIASES AND ASSOCIATED CANCERS

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Introduction: Infection with some helminths is carcinogenic. It is key to understand parasite-host interactions and mechanisms associated with carcinogenesis to design control strategies. Helminths may produce metabolites that might trigger and/or contribute to infection-associated carcinogenesis. We have investigated novel combined chemotherapy-based approaches aiming, ultimately, to block the initiation of chemical carcinogenesis induced by the infection and to enhance the host immune response.

Methods: The effect of drugs such as Praziquantel (PZQ) and Artesunate (AS) alone or combined with antioxidants N-acetylcysteine and resveratrol was evaluated against *S. mansoni* newly transformed schistosomula and adult worms.[1] Additionally, we have studied the ability of these drugs and antioxidants to inhibit the formation in vitro of the putative initiators of helminth-induced carcinogenesis.[2]

Results: Combining the antioxidants with AS and PZQ enhanced the anthelmintic action.[1] Also, these combinations lead to almost a complete inhibition of parasitic metabolites tentatively involved in the initiation of helminth-associated carcinogenesis.[2]

Conclusions: These findings indicate that therapeutic approaches that combine drugs with antioxidants have great potential to treat carcinogenic-associated helminth infections. The efficacy of chemotherapy often depends on the host immune response, and accordingly, combined chemotherapy and immunotherapy using helminth recombinant antigens could be advantageous. **Acknowledgments:** MJG thanks ICETA for the Post-Doc fellowship. JMC thanks FCT for UIDB/00211/2020 and Strategic Project UI211. [1]Gouveia et al., Parasite and Vectors (2019) 12:309 [2]Gouveia et al., Molecules (2019), 24, 3842

Keywords: IMMUNOTHERAPY, antioxidants, Helminths, carcinogenesis, chemotherapy

P142 / #478

Topic: *AS02.2 Schistosomiasis*

NATURAL PRODUCTS IN ANTISCHISTOSOMAL DRUG DISCOVERY – WHAT CAN WE LEARN FROM INSECTS?

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Introduction: Insects represent the most species-rich class of animals on earth and hold a wide spectrum of biologically active molecules. However, insect-derived products have been largely neglected in drug discovery of novel antiparasitic compounds to date, with a few exceptions such as bee propolis.

Methods: We systematically explored insects as source for antischistosomals by testing insect molecules in whole-organism screenings in vitro and by applying molecular docking-based in silico screening of insect molecules against a known druggable target, thioredoxin glutathione reductase of Schistosoma mansoni (SmTGR).

Results: Using a new bioinformatics pipeline, we created a virtual library of over 1000 insect molecules and found several potential inhibitors of SmTGR by molecular docking. For one compound, buprestin H from jewel beetles, we tested and confirmed activity against S. mansoni in vitro [1]. By in-vitro screenings we additionally found antischistosomal activity for venom from an assassin bug and for a ladybird-derived alkaloid called harmonine at low micromolar concentrations [2; 3], while antimicrobial peptides from various insect groups had only mild effects. Venom and harmonine had pleiotropic effects on cells and tissues essential for survival and reproduction of schistosomes, which included an arrest of stem-cell proliferation. For harmonine, we additionally obtained first evidence for acetylcholinesterase as one potential molecular target.

Conclusions: Our studies highlight the potential of exploiting insects as a source for the discovery of antischistosomal compounds. [1] Gallinger, T.L. et al. 2022. Pharmaceuticals 15(2):119. [2] Tonk, M. et al. 2020. Antibiotics 9:664. [3] Kellershohn, J. et al. 2019. PLoS Negl Trop Dis 13:e0007240.

Keywords: Natural products, Anthelminthics, Schistosoma mansoni, insects, thioredoxin glutathione reductase

P143 / #416

Topic: *AS02.2 Schistosomiasis*

DEVELOPMENT OF A NOVEL RAPID POINT-OF-CARE PCR TEST FOR SCHISTOSOMIASIS

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Introduction: The WHO has targeted Schistosomiasis for elimination as a public health concern in this decade. However, current diagnostics for schistosomiasis lack the sensitivity and specificity required for achieving these goals. We are developing a novel point-of-care PCR test on a prototype platform produced by a local Munich startup, with the aim of having a sensitive, specific and fast PCR assay for field deployment to assist with elimination of the disease.

Methods: The novel PCR platform utilizes a new class of PCR based on magnetic bead technology, allowing for accurate detection of genetic elements within 10 minutes. We have developed primer-probe assays based on the cox1 gene of Schistosoma haematobium and mansoni, as well as the repetitive genetic elements Sm1.7 of S. mansoni and DraI of S. haematobium. Additionally, we are conducting long-read transcriptomic sequencing of Schitosoma mansoni to identify splice variants associated with the different life stages, with the aim being to identify novel targets for PCR assays that can be life-stage, and so disease-stage, specific; as well as targets that are altered in response to PZQ treatment – allowing us to monitor drug resistance patterns.

Results: Assays developed were tested on purified Schisotosoma DNA on both qPCR and novel PCR formats with sensitivity down to 0.0001ng/µl and specificity to distinguish between S. mansoni and S. haematobium.

Conclusions: We have developed a PCR assay with high sensitivity and specificity on a platform that offers rapid, point-of-care testing capacity. Further work will be conducted to assess the capacity for different sample inputs such as environmental samples, and multiplexing possibilities with other parasitic or female genital diseases.

Keywords: PCR, point-of-care, diagnostics, rapid test, schistosomiasis

P144 / #910

Topic: *AS02.2 Schistosomiasis*

OMEGA-1-LIKE ORTHOLOGUE OF THE SCHISTOSOMA HAEMATOBIUM EGG: CRISPR/CAS12A KNOCKOUT OF TRANSCRIPTION, NUCLEASE ACTIVITY, AND ALTERNATIVE ACTIVATION OF MACROPHAGES

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Introduction: Proteins secreted by schistosome eggs participate in pathogenesis and disease transmission. A ribonuclease of Schistosoma mansoni termed omega-1 provokes granuloma formation and the circumoval granuloma chaperones the egg during transit through the gut wall. Infection with S. haematobium is a principal risk factor for bladder cancer in regions endemic for this schistosome. We investigated an orthologue of omega-1 in eggs of S. haematobium.

Methods: Phylogenetic and bioinformatics tools were used to locate and characterize an orthologue of omega-1 in the S. haematobium genome, design gene-specific guide RNAs and analyze efficiency of programmed CRISPR gene knockout. Eggs of S. haematobium were obtained from livers of hamsters. Ribonucleoprotein complexes of Cas12a nuclease and guide RNAs were delivered to cultured eggs by electroporation. DNA, RNAs, and soluble proteins were recovered from these eggs. Amplicon libraries spanning targeted sites were sequenced. Other eggs were cultured in a Bowden-type chamber with macrophage, epithelial or bladder cancer cell lines. Western blots were performed using an antiserum raised against S. mansoni omega-1. Gene arrays representing human inflammatory cytokine and apoptosis pathways were investigated.

Results: At least two orthologues of omega-1 ribonuclease are expressed by the S. haematobium egg, and were susceptible to CRISPR/Cas12a catalyzed knockout. Non-homologous end joining based repair resulted in loss of gene transcription and gene deletions of up to 45 nt. S. haematobium omega appears to polarize a M2 macrophage and Th2 phenotype.

Conclusions: It is likely that S. haematobium omega-1 participates in immunopathogenesis of urogenital schistosomiasis. Further investigation is clearly warranted.

Keywords: Schistosoma haematobium, macrophage activation, omega-1, CRISPR/Cas12a, RNase activity

P145 / #1432

Topic: *AS02.2 Schistosomiasis*

SCHISTOSOMIASIS: LINKING DIET, MICROBIOME AND SNAIL COMPETENCE

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Introduction: Accruing evidence indicate that microbiomes mediate host-pathogen interactions. Moreover, food resources greatly influence organisms' microbiomes. Thus, variations in diet are expected to have significant impact on individual microbiota composition and, in turn, on the competence of vectors/hosts (their propensity to amplify pathogens at levels that are transmitted to other hosts). We tested this hypothesis on Bulinus truncatus snails that host Schistosoma haematobium the trematode responsible for urogenital schistosomiasis, a tropical neglected infectious disease. The purpose of this work is to characterize the diversity of diet and that of microbiomes in natural populations of Bulinus truncatus and to study their potential influence on the competence of B. truncatus toward S. haematobium.

Methods: We first conducted a malacological study at nine ecologically contrasted S. haematobium transmission sites in Senegal. The diet and the microbiomes' community were characterized on a total of 136 B. truncatus from the nine sampling sites using a metabarcoding approach. We also conducted an experimental infection of wild B. truncatus from four sites to quantify their competence at the population level.

Results: A total of 800 B. truncatus were sampled over the nine targeted sites. Natural prevalence of S. haematobium was low (2/800). The diet and microbiome communities are currently analyzed. The result of the experimental infection is due on end of March.

Conclusions: Together these results will document for the first time the diversity of microbiomes' communities and diet of B. truncatus from the field and their potential link with their competence.

Keywords: schistosomiasis, Vector competence, microbiota, Food resource

P146 / #976

Topic: *AS02.2 Schistosomiasis*

DEVELOPMENT OF A POINT OF CARE CIRCULATING ANODIC ANTIGEN TEST FOR URINE-BASED DIAGNOSIS OF ALL SCHISTOSOMA SPP.

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Introduction: Schistosomiasis is a debilitating disease affecting over 240 million people. Five main Schistosoma species infect humans. Detecting eggs in urine or stool is a standard diagnostic method but lacks sensitivity. The POC-CCA is an accurate test for S. mansoni (Sm) but limited for other species. Tests based on Circulating Anodic Antigen (CAA) detection target all Schistosoma spp. and are highly accurate but require laboratory facilities. The WHO recently published a Target Product Profile for schistosomiasis diagnostics and a urine-based, rapid, lateral flow (LF) POC-CAA test would be an ideal candidate.

Methods: LF components including antibody concentration, amount of conjugated gold nanoparticles, or membrane porosity were tested. Fresh negative donor urines were used for evaluating specificity, and a serial dilution of CAA-spiked negative urines allowed to estimate the Limit of Detection (LOD). Semiquantitative visual G-score readouts (G1-G10) were recorded after 15 and 30 minutes. Urine samples from a cohort of school-aged children (N=20) from a Sm endemic area in Uganda were used to assess clinical sensitivity and specificity, compared to Kato-Katz and POC-CCA tests.

Results: Initial test prototypes were highly specific and showed an LOD of 250 pg/mL of CAA in spiked samples. They detected high and moderate intensity endemic urines, but failed to identify low intensity infections and did not outperform the POC-CCA test. The optimised current prototype shows an LOD of 50 pg CAA/mL while maintaining specificity, and we will present the latest findings here.

Conclusions: A sensitive, non-invasive, easy-to-use, visual POC-CAA test is being developed and will enable the diagnosis of all types of human schistosomiasis, addressing WHO requirements.

Disclosure: The submitter is a PhD student whose project involves the collaboration with NG Biotech, a company that develops and manufactures lateral flow immunoassays. Elías has spent several months in NG Biotech but receives no financial revenue for publishing this

Keywords: Circulating Anodic Antigen, lateral flow immunoassay, non-invasive diagnostics, point-of-care

P147 / #1235

Topic: *AS02.2 Schistosomiasis*

MOLECULAR SYSTEMATICS OF THE LARVAL SCHISTOSOMES PARASITISING THE HORN SNAILS PIRENELLA CINGULATA (GMELIN) (CAENOGASTROPODA: POTAMIDIDAE) IN THE PERSIAN GULF

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Introduction: Avian schistosomes are known for their remarkable ability to adapt to various hosts and environments. Their cercariae causes cercarial dermatitis. Although many research have been done on freshwater avian schistosomes, less is known about their marine counterparts. This study intends to offer new data regarding schistosome diversity and marine-based host associations in the Persian Gulf.

Methods: During a large-scale survey assessing the larval trematode diversity in Pirenella cingulata along the northern Persian Gulf, a collection of avian schistosomes was characterised molecularly. Partial sequences of the mitochondrial cox1 and 28S rRNA genes were generated and used for species identification and infer phylogenetic relationships at family level.

Results: Molecular phylogenetic analyses confirmed two schistosome species, Ornithobilharzia canaliculata (Rudolphi, 1819) and a possible new species of Austrobilharzia Johnston, 1917. Matching new sequence data and published isolates of avian schistosomes elucidated the life-cycle of O. canaliculata. Phylogenetic inferences based on both molecular markers confirmed the sister-group relationship between Ornithobilharzia and Austrobilharzia, and their earlier diverging positions among the schistosomes.

Conclusions: Our study provides: (i) the first report of O. canaliculata; in the Persian Gulf; (ii) the first record of the species in a potamidid snail host; and (iii) the first elucidation of its life-cycle. Our results highlight the importance of molecular-based approach in the assessment of schistosome diversity and calls for further studies in order to a better understanding of their diversity, patterns of relationships, host associations, transmission strategies and distributional ranges.

Keywords: Austrobilharzia, Avian schistosomes, Ornithobilharzia canaliculata, Persian Gulf, Gulf of Oman

P148 / #1444

Topic: *AS02.2 Schistosomiasis*

PARAMETERS FOR THE REMOVAL OF SCHISTOSOME CERCARIAE FROM WATER USING SAND FILTERS

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Introduction: Schistosomiasis is a water borne parasitic disease affecting 240 million people. Schistosomes reproduce sexually in humans, releasing eggs in urine and faeces which hatch in freshwater and infect snails, where they reproduce asexually releasing hundreds of cercariae/day. These cercariae burrow directly into humans on contact with contaminated water, continuing the cycle. Mass drug administration has been the WHO recommended strategy for nearly 20 years, and whilst successful in some areas, there are hotspots across sub-Saharan Africa. Additional non-pharmaceutical interventions are needed to meet the WHO goal of eliminating schistosomiasis as a public health problem by 2030. The WHO roadmap states that improved access to safe water, sanitation and hygiene is needed. Water filtration could reduce reinfection rates by reducing exposure to cercariae infected water.

Methods: This study aims to provide WHO guidelines on the removal of schistosome cercariae from water using sand filtration to provide safe water for domestic use such as washing and bathing. A range of sand grain sizes (300 – 400 µm, 400 – 500 µm, 500 – 710 µm, and mixed) are being tested to determine the depth required to remove 99.9% of Schistosoma mansoni cercariae. The robustness of the filters will also tested by investigating different diameters (10cm to 50cm) and cercarial densities (1000/litre to 3000/litre) and the sand depths required to ensure full cercarial removal.

Results: Optimal designs will be presented. Results will provide recommendations for implementation in the field at household to school levels.

Conclusions: Filtration can succesfully remove cercariae, however locally sourced material and community preferences should be taken into account to improve uptake and sustainability.

Keywords: schistosomiasis, Sand filters, Cercariae, filtration, WASH

P149 / #919

Topic: *AS02.2 Schistosomiasis*

MALE-MEDIATED ROLES OF BIOGENIC AMINES IN THE REPRODUCTIVE DEVELOPMENT OF FEMALE SCHISTOSOMA MANSONI

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Introduction: Numerous eggs produced by mature females are responsible for the pathogenesis of schistosomiasis. Reproductive development of schistosome females rely on constant pairing with male partners, a process that is still not fully understood. Biogenic amines such as octopamine and dopamine together with their receptors are involved in neuromuscular signalling in Schistosoma mansoni. In invertebrates, biogenic amines also play roles in the regulation of copulation. In order to get more insight into the molecular mechanisms controlling the pairing-dependent female maturation, we studied the role of two S. mansoni genes annotated as tyrosine decarboxylase (Smtdc) and dopa decarboxylase (Smddc). The enzymes coded by both genes catalyse the biosynthesis of biogenic amines.

Methods: qRT-PCR; In situ hybridization; RNA interference; Morphological analysis; Phylogenetic analyses

Results: Structural and phylogenetic analyses substantiated the identities of Smtdc and Smddc. I confirmed a higher expression of Smtdc and Smddc in bM compared to unpaired males (sM) or females by qRT-PCR. WISH showed the localization of both genes in neuronal tissues near the gynecophoral canal of the male. RNAi led to a significant decrease in the egg production of first time-paired immature females (sF) upon single or double knockdowns of Smtdc and Smddc, respectively. Correspondingly, confocal laser scanning microscope exhibited a significant decrease in the number of mature oocytes of paired females upon RNAi.

Conclusions: Our findings provide first evidence for a pairing-dependent and male-dominated role of Smtdc and Smddc in the reproductive development of female S. mansoni. In addition, the data suggest a contribution of specialized neuronal cells and biogenic amines in this process.

Keywords: Biogenic amines, Gynecophoral canal, Neuronal cells, Reproductive development, Pairing-dependent

P150 / #1277

Topic: *AS02.2 Schistosomiasis*

SCHISTOSOMIASIS HOTSPOTS: A SYSTEMATIC REVIEW ON THE USE OF THIS TERM IN THE LIGHT OF NEW WHO DEFINITIONS

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Introduction: The World Health Organization (WHO) aims to eliminate schistosomiasis as a public health problem by 2030. However, this goal is hindered by persistent hotspots (PHSs). To understand what is driving PHSs, precise definitions are needed in scientific reporting. The WHO have released a provisional definition but also recognise this is an area requiring more investigation. We aimed to collate and compare all previously used definitions of schistosomiasis hotspots.

Methods: We carried out a systematic review of all published research following the PRISMA guidelines and a PICOS search strategy. Search terms were schisto* or bilharzia* and hotspot/s, hot-spot/s or hot spot/s. Inclusion criteria were to include the term hotspot and be focussed on schistosome parasites. We also collated expert opinions on what a hotspot definition is by interviewing key stakeholders including funders, researchers and control programme implementors.

Results: Preliminary findings showed that there are many definitions of schistosomiasis hotspots used in the literature, which can broadly be categorised into three groups: Biological, Operational and Morbidity. We also found that under the current WHO definition, many high prevalence regions could not be defined as a PHS due to not having adequate treatment coverage.

Conclusions: There are incongruencies in the use of ‘schistosomiasis hotspots’ across literature and amongst stakeholders. This continues to hinder progress towards WHO goals by missing significant PHS communities. Final results will be presented and recommendations provided for comprehensive definitions that recognise regions which are not reaching the coverage goals, to help aid the design of improved control strategies.

Keywords: schistosomiasis, Hotspot, Schistosoma

P151 / #749

Topic: *AS02.2 Schistosomiasis*

THE GASTROPOD HOSTS OF SCHISTOSOMES: PATTERNS, PROCESSES AND MECHANISMS

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Introduction: As one of the best known groups of parasites, the digenean family Schistosomatidae can offer unique insights with respect to processes underlying diversification of parasite lineages. Our aims are to gain a more detailed overview of gastropod lineages exploited by schistosomes, to infer what processes might lie behind the patterns observed, and to suggest underlying mechanisms amenable to testing.

Methods: Our own concerted search for schistosome infections among snails from multiple continents coupled with provision of sequence data for both schistosomes and infected gastropods along with examination of literature with comparable schistosome-gastropod sequence data provide the database from which our results were obtained.

Results: As far as known, all schistosome use either coenogastropod or heterobranch gastropod intermediate hosts. More basal gastropod lineages are not found infected with schistosomes. Marine, freshwater and amphibious life cycles are known. Experimental infection studies indicate relative specificity at the snail host level, yet paradoxically, the present-day record implies host switching has been pervasive, both within and between gastropod families.

Conclusions: Schistosomes have colonized several gastropod families but are conspicuously absent from others. Schistosome host switches may be facilitated by co-infections involving immunosuppressive parasites, altered temperature regimes or other conditions stressful to hosts, hybridization among diverging schistosomes, or new ecological circumstances placing schistosomes in constant contact with new host snail species, favoring rare infectious variants. Supported by NIH grant R37AI101438.

Keywords: schistosomes, gastropods, diversification

P152 / #631

Topic: *AS02.2 Schistosomiasis*

TRANSCRIPTIONAL RESPONSES OF THE VECTOR SNAIL BIOMPHALARIA GLABRATA TO THE TREMATODES SCHISTOSOMA MANSONI AND ECHINOSTOMA PARAENSEI AND TO THE NEMATODE DAUBAYLIA POTOMACA

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Introduction: Biomphalaria glabrata is an important Neotropical snail vector of human schistosomiasis. As part of an ongoing effort to characterize and eventually exploit the immune system of the snail for schistosomiasis control, we aimed to characterize the transcriptional response of B. glabrata when exposed to Schistosoma mansoni or to two other unrelated parasites, the fluke Echinostoma paraensei and the snail-infecting nematode Daubaylia potomaca. From this we can identify common or unique features in the snail response to different parasites.

Methods: B. glabrata M-line strain (susceptible to S. mansoni) snails were individually exposed to one of the three parasites, and at 2, 8 and 40 days post exposure (dpe), 7-8 snails/group were collected, as were unexposed control snails (matched at 2 and 40 dpe). cDNA libraries were paired-end sequenced on an Illumina NextSeq500. Bioinformatics tools were used for differential expression (DE) gene analysis.

Results: On average, 12 million raw reads/snail were sequenced. Each parasite provoked a distinctive overall pattern of responses at all time points, but the responses engendered by the two trematodes were more similar to each other (persistent patterns of overall down-regulation) than what was noted with the nematode (early down-regulation followed by dramatic late up-regulation).

Conclusions: The results are consistent with the need for trematodes to establish stable long-term infections in which progeny are continually produced, relative to the nematode which overwhelms the snails and is transmitted only when the snail is about to die. The snail genes responsive to all three parasites, and those expressed in a parasite-specific way will be discussed. This study was supported by NIH grants P20GM103452 and R37AI101438.

Keywords: Biomphalaria glabrata, Parasite, Transcriptomics, Vector biology, Comparative immunology

P153 / #187

Topic: *AS02.2 Schistosomiasis*

ASSOCIATIONS OF UROGENITAL SCHISTOSOMIASIS AND FEMALE GENITAL SCHISTOSOMIASIS: A CROSS-SECTIONAL STUDY AMONGST GIRLS AND WOMEN IN THE MATTA HEALTH AREA IN WEST CAMEROON.

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Introduction: Clinical pathology of Female Genital Schistosomiasis(FGS) has been described as resulting from the complex inflammatory response to antigens released by adult worms and viable eggs during urogenital schistosomiasis(UGS), which persits till after adult worms are destroyed. In Cameroon, UGS and FGS have been recorded in subpopulations but control strategies are seemingly not intergrated. This study set to find current risks and associations of both UGS and FGS, highlighting trends for better control.

Methods: In a cross-sectional study, a subgroup of 67 girls and women aged >13 were tested for both UGS (through urine filtration and microscopy), and FGS by gynecological exam. In bivariate analysis, Pearson's chi-squared tests were used to test the dependence of FGS or UGS on some reproductive health related independent variables.

Results: Age affected infection odds for both diseases, with young and older adults (aged 20-35; 36+) having increased chances (OR 6.14 (1.73, 26.10) ; and OR 6.43 (1.62, 30.35)) of contracting FGS. Contrarily, UGS was seen to diminish with age (OR 0.76, [5 0.21-2.53] and OR 0.42 [0.11-1.52]) as age groups increased from 20-35;36+. Women with FGS were more prone (OR 7.10 [2.49-22.10]) to suffer abnormal menstruation, though a significant difference was noted within UGS positive and negative women who reported this symptom (22 (56.4)/ 12(44.4).

Conclusions: To upscale UGS control inorder prevent FGS in later days, there is need in reconsidering and restructuring schistosomiasis intervention programs, drug availability protocols, and availing Praziquantel in health centers within endemic health areas for individual therapy, amplifying routine treatment of young girls and women.

Keywords: Reproductive health, Female Genital Schistosomiasis, Urogenital Genital Schistosmiasis, Associations

P154 / #87

Topic: *AS02.2 Schistosomiasis*

ABUNDANCE OF FRESHWATER SNAILS IN WATER BODIES-IMPLICATION FOR THE TRANSMISSION OF URINARY SCHISTOSOMIASIS IN RURAL COMMUNITIES OF SOUTHEAST NIGERIA.

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Introduction: Some species of freshwater snails are intermediate hosts of Schistosoma haematobium, and have been implicated in the transmission of urinary schistosomiasis in rural communities with inadequate water supply. Malacological studies are therefore important to identify these species.

Methods: Studies on freshwater snails and their possible role in the transmission of Urinary Schistosomiasis was conducted for four months (September-December, 2021) in rural areas of Ebonyi State, Southeast Nigeria, using scoop net snail sampling method and questionnaire survey method.

Results: A total number of 823 snails comprising of three species of Bulinus; Bulinus globosus, Bulinus tropicus and Bulinus truncatus were recorded. The most abundant was Bulinus globosus 324 (39 %), while Bulinus truncatus 229 (28 %) was the least in abundance. Chi-square test ($\alpha = 0.05$) shows that, there was no significance difference in the species of snails collected. Out of 823 snails collected, 92 (11 %) was found shedding cercariae with Bulinus globosus being the highest in cercariae shedding. The socio-demographic study in primary school pupils of Ezza North using of questionnaire, shows that, out of 1210 pupils interviewed, 451 (37 %) responded 'yes' to passage of blood in urine with higher response in males 246(20 %) than females 205(17 %).

Conclusions: Molluscicide should be used for snail control. Enhancement of the health agencies and focal persons' effort as well as provision of portable water supply can help to reduce transmission

Keywords: Parasite, Nigeria, schistosomiasis, water

P155 / #1599

Topic: *AS02.2 Schistosomiasis*

EVALUATION OF ANTISCHISTOSOMAL AND MOLLUSCIDAL POTENTIALS OF AZADIRACTHA INDICA, CALOTROPIS PROCERA, HYMENOCARDIA ACIDA, JATROPHA CURCAS, AND MORINGA OLEIFERA

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Introduction: Background and Aims: Schistosomiasis, caused by the parasite *Schistosoma,* had about 236.6 million people requiring preventive treatment with praziquantel (PZQ), the only available drug, in 2019. There is the urgent need to add to the grossly inadequate schistosomiasis chemotherapeutic armory. This study was therefore aimed at obtaining efficacious extracts of five medicinal plants and fractions/compounds thereof for development of medicines for schistosomiasis treatment.

Methods: Methods: Fourteen extracts and fractions of the plants under study were screened for schistosomicidal activity against *Schistosoma mansoni* in culture and LC_{50} and LC_{90} monitored over a 72-hour period. In vivo screening was done in mice infected with *S. mansoni* and antischistosomal effects assessed using adult worm count and oogram. Extracts were screened for molluscidal activity against *Biomphalaria alenxandrina.*

Results: Twelve extracts gave 100% mortality 72 hours post treatment at 100 µg/ml similar to PZQ. At 48 h, methanolic leaf extracts of C. procera and J. curcas gave 100% mortality while PZQ gave 90.91%. At 40 and 50 ppm, methanolic leaf extract of J. curcas gave 100% mortality after 48 h while that of C. procera at 30, 40 and 50 ppm gave 100% mortality after 24 h. The LC50 and LC90 were 12.9 and 32.9 for J. curcas and 10.1 and 12.9 for C. procera. Fractions of C. procera extract reduced worm load in infected mice by 44% and caused significant difference in the oogram pattern ($P < 0.05$). Ethylacetate leaf extract of J. curcas killed 100% of the snails at 80 and 50 ppm

Conclusions: Conclusion: Extracts and fractions of the studied plants contain schistomicidal and molluscidal compounds for development of medicines potentially more efficacious than PZQ and potent molluscides.

Keywords: Schisosomiasis, Schistosoma, Molluscidal, medicinal plants, Schistosomicidal

P156 / #1343

Topic: *AS02.2 Schistosomiasis*

AN ORIGINAL CAPTURE OF SCHISTOSOMA MANSONI MOTHER SPOROCYST TEGUMENTAL ULTRASTRUCTURE

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Introduction: Schistosoma mansoni tegument ultrastructure is well described regarding several stages of the parasite, especially the adult stage and the free stages of the trematode. On the contrary, the tegument changes of inner mollusk stages are poorly described using Scanning Electron Microscopy (SEM). This bibliographical imbalance could be explained by several factors. Some of the examples of the issues encountered can include technical requirements due to mother sporocysts cultivation after 24 hours comparing to the ease with which the other parasitic stages are collected. Our work aims to present several tegument modifications happening during miracidium to sporocyst transformation.

Methods: After several cultivation lengths in Chernin's Balanced Salt Solution (CBSS), Schistosoma mansoni sporocysts (NMRI strain) samples are collected and prepared for critical point drying, metallization and observation into a Hitachi S-4500 Scanning Electron Microscope, mounted with a tungsten cold source Field Emission Gun. Everhardt Thornley chamber detector was used (5kV).

Results: Sporocysts exhibit scars in early stages, progressively disappearing along the transformation. Sporocysts' length greatly increase across time and their body is early fully covered by microvilli.

Conclusions: This work brings new high quality SEM pictures of S. mansoni sporocysts transformation, allowing to publish updated pictures thanks to the technical innovations in the field of scanning electron microscopy made through these past twenty years.

Keywords: Schistosoma mansoni, Sporocyst, Ultrastructure, Scanning Electron Microscopy, Miracidium

P157 / #1680

Topic: *AS02.2 Schistosomiasis*

HOST-SPECIFIC SERUM FACTORS CONTROL THE DEVELOPMENT AND SURVIVAL OF SCHISTOSOMA MANSONI

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Introduction: Introduction: Schistosomiasis is caused by blood-dwelling flatworms which develop from skin-penetrating cercariae into adult worms. During experimental infection, only 20-30% of cercariae mature into adults. The reasons are unknown.

Methods: Materials and Methods: Using our recently developed novel serum- and cell-free *in vitro* culture system for newly transformed schistosomula (NTS), which supports long-term larval survival, we investigated the effects of mouse serum and its major soluble complement factors C1q, C3, C4 as well as sIgM *in vitro* and assessed worm development *in vivo* by infecting complement and sIgM-deficient animals.

Results: Results: In contrast to sera from humans and a broad variety of mammalian species, serum from mice, surprisingly, killed parasites already at skin stage *in vitro*. Interestingly, the most efficient killing component(s) were heat-labile but did not belong to the most abundant serum complement components or consisted of unspecific immunoglobulins. Infection of complement C1q and sIgM-deficient mice with *S. mansoni* as well as *in vitro* tests with sera from mice deficient for C3 and C4 revealed no major role for these soluble factors *in vivo* in regards to parasite maturation, fecundity and associated immunopathology. Rather, the reduction of parasite maturation from cercariae to adult worms was comparable to wild type mice.

Conclusions: Conclusion: This study reveals that not yet identified heat labile serum factors are major selective determinants to the host-specificity of schistosomiasis, by directly controlling schistosomal development and survival. Identification of these can lead to deepen our understanding of host-parasite interaction on the one hand side, and also lead to the identification of novel drug targets.

Keywords: schistosomiasis,, Host-parasite interaction, host serum factors, drug development

P158 / #1276

Topic: *AS02.2 Schistosomiasis*

IMPROVING FEMALE GENITAL SCHISTOSOMIASIS DIAGNOSIS WITHIN COMPREHENSIVE REPRODUCTIVE HEALTH SERVICES IN ZAMBIA

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Introduction: The World Health Organization (WHO) estimates that up to 56 million women and girls are living with female genital schistosomiasis (FGS) in sub-Saharan Africa. Identification of FGS is difficult due to confusion with symptoms of other genital abnormalities and gold standard diagnosis with colposcopy is not feasible or affordable in most health facilities. Our study aims to develop and pilot test a comprehensive algorithm that is feasible to implement in Zambian government clinics.

Methods: We recruited 538 women from a longitudinal cohort of HIV-negative female sex workers or single mothers ≥ 18 years in Lusaka and Ndola, Zambia. We used demographic, risk factor, and symptom data collected from standardized surveys, gynecological exams, and laboratory tests to develop a risk score for FGS among the derivation cohort (n = 340). After 10-fold internal cross validation, the algorithm was then validated in the external cohort (n = 198).

Results: The prevalence of FGS was 22.6% among the derivation cohort and 19.2% among external validation cohort. The risk algorithm had reasonable discrimination in the derivation cohort (AUC = 0.72, 95% CI: 0.66-0.79, p-value < 0.001). The internal cross validation had less reasonable discrimination (AUC = 0.64, 95% CI: 0.43, 0.83, p-value = 0.45). Using a score cut off of 3, the risk algorithm in the derivation cohort had 71% sensitivity, 60% specificity, 35% positive predictive value (PPV), and 88% negative predictive value (NPV).

Conclusions: This work is imperative to understanding the risk factors associated with FGS among a cohort of young adult women in Zambia and the opportunities, as well as challenges, for identifying an algorithm that is feasible to implement in Zambian government clinics.

Keywords: diagnostic algorithm, Zambia, Female Genital Schistosomiasis

P159 / #966

Topic: *AS02.2 Schistosomiasis*

LONG NON-CODING RNAS INVOLVED WITH REDUCED SENSITIVITY TO PRAZIQUANTEL IN SCHISTOSOMA MANSONI

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Introduction: Schistosomiasis is a very debilitating disease, estimated to affect more than 200 million people worldwide. Administration of praziquantel (PZQ) to infected individuals is the basis of current schistosomiasis therapy. PZQ is a safe, cheap and tolerable drug; however, the use of a single drug may contribute to resistance emergence. Long non-coding RNAs (lncRNAs) are RNAs longer than 200 nucleotides with low or no protein-coding potential that have been implicated in resistance to drug treatment in humans cancers. We have recently published for the first time a catalog of lncRNAs expressed in S. mansoni, allowing for their exploration at different parasite stages and conditions. The aim of the present work was to evaluate the involvement of lncRNAs in the development of reduced sensitivity to PZQ in S. mansoni.

Methods: We have reanalyzed public RNA-Seq datasets looking for lncRNAs differentially expressed (DE) in a laboratory strain of S. mansoni (PZQ-selected) whose susceptibility to PZQ was diminished across 9 passages through exposure to increasing sublethal doses of the drug. The raw reads were processed and DE genes, including lncRNAs, were identified.

Results: A read mapping rate of 60-80% was obtained. Differential expression analysis identified 1281 DE protein-coding genes along with 178 lncRNAs DE in the PZQ-selected strain compared with controls, including 119 intergenic, 47 antisense and 12 sense lncRNAs. lncRNA- protein coding genes co-expression analysis identified enrichment of pathways related to ion transport and vesicles.

Conclusions: This is the first step towards the functional characterization of lncRNAs possibly involved in PZQ resistance in S. mansoni. Selected lncRNAs will be used in RT-qPCR validations and phenotypic functional assays.

Keywords: resistance, Long non-coding RNAs, Schistosoma mansoni, Praziquantel

P160 / #1156

Topic: *AS02.2 Schistosomiasis*

A FIVE YEAR EVALUATION OF SCHISTOSOMIASIS DIAGNOSTICS IN A NON-ENDEMIC COUNTRY.

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Introduction: Schistosomiasis is one of the most prevalent neglected human parasitic infections in Africa, Middle East, Asia, South America and Caribbien. Schistosoma mansoni and Schistosoma haematobium are the most common species infecting travelers and migrants from endemic areas. Diagnosis of Schistosomiasis is by PCR, or microscopic detection of eggs in feces and urine. Sensitivity of microscopy is variable due to fluctuation of egg shedding. In 2017 we established a Schistosoma species PCR, improving our schistosomiasis diagnosis. However, in this study we point out the importance of performing parallel microscopy. The study aim was to verify if patients who were at risk of schistosomiasis had other intestinal parasitic infections.

Methods: All fecal samples analyzed by Schistosoma PCR from 2017- 2021 were included in a retrospective observational study. From 2017- 2019 microscopy was performed on PCR positive samples and on special request. Since 2020 microscopy has been performed on all samples, sent for Schistosoma PCR, only missing a few due to inadequate quantities.

Results: Of the 274 fecal samples analyzed, 42 cases of Schistosoma mansoni were PCR positive and 14 microscopy positive. In addition microscopy revealed 3 co-infections with Ascaris lumbricoides, 1 with hookworm species, and 1 with Entamoeba coli. In the PCR negative samples we detected 4 cases with Ascaris lumbricoides, 1 with Taenia species, 2 with Giardia lamblia, 5 with Entamoeba coli, 2 with hookworm, and 1 with Hymenolepis nana.

Conclusions: Although PCR is more sensitive than microscopy, you only find what the PCR detects. Different fecal parasitic infections have similar risk factors, and a combination of specific parasite PCR and microscopy improves diagnostic yield in non-endemic countries.

Keywords: Microscopy, PCR, schistosomiasis, co-infections

P161 / #506

Topic: *AS02.2 Schistosomiasis*

EFFECTS OF 24-NOR-URSODEOXYCHOLIC AND URSODEOXYCHOLIC ACID ON MITOCHONDRIAL DYNAMICS IN THE LIVER OF SCHISTOSOMA MANSONI INFECTED MICE

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Introduction: Background and aims: Hepatic fibrosis and granuloma formation around tissue entrapped eggs characterize the pathology of Schistosoma mansoni (S.m.) infection. S.m. infection affects mitochondrial biogenesis, mitochondrial dynamics (fusion/fission), and regulates innate and adaptive immune responses. We have already shown that 24-nor-ursodeoxycholic acid (norUDCA) has anti-inflammatory and anti-fibrotic effects in S.m. induced liver injury. The mechanism behind this is not yet fully understood. We therefore aimed to investigate whether norUDCA exerts its beneficial effects on liver fibrosis in murine schistosomiasis by compensating mitochondrial dysfunction.

Methods: NMRI mice were infected with 50 S.m. cercariae and after 12 weeks received either norUDCA- or ursodeoxycholic acid (UDCA)-enriched diet (0.5% wt/wt) for 4 weeks to evaluate liver pathology, as well as analyze mitochondrial dynamic genes expression level and respiration in isolated hepatocyte mitochondria using high-resolution respirometry.

Results: NorUDCA improved mitochondrial dynamics by reduction of mitochondrial fragmentation and enhancement of mitochondrial inner and outer membrane fusion. Moreover, norUDCA but not UDCA treatment of infected animals significantly improved OXPHOS capacity and ratio of respiration in the uncoupled state, and additionally increases the electron transport system capacity and cytochrome C oxidase function.

Conclusions: Conclusion: Our results demonstrate protective effects of norUDCA on hepatocyte mitochondria function which in turn contributes another piece to the puzzle of the broad effects of norUDCA on S.m. associated liver pathology.

Keywords: schistosomiasis, mitochondrial dysfunction, 24-nor-ursodeoxycholic acid, hepatic fibrosis

P162 / #1718

Topic: *AS02.2 Schistosomiasis*

BURDEN OF FEMALE GENITAL SCHISTOSOMIASIS AMONG WOMEN ATTENDING THE SAINT-LOUIS HOSPITAL IN THE NORTH OF SENEGAL.

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Introduction: Female genital schistosomiasis (FGS) is a public health problem in women living in endemic areas. The infection is associated with infertility, ectopic pregnancies, and miscarriage. In Senegal, there is a lack of epidemiological data on the burden of FGS. This study was done to describe the profile of cases.

Methods: A descriptive study was carried out to collect epidemiological data on women attending the hospital of Saint-Louis from 2019 to 2020. All study participants were examined under colposcopy. The signs and symptoms of FGS were identified using the pocket atlas for clinical health-care professionals developed by the WHO. Each image obtained was examined by two gynecologist and one mid-wife. Socio-demographics and clinical data were described.

Results: A total of 173 women have been included in the study. Signs of FGS have been identified in 45 patients (26 %). Among the patients positive, 46.7% of the patients have shown grainy sandy patch while 24.4% of them have presented rubbery papules. Lesions of homogenous sandy patch and abnormal blood vessels were respectively observed in 17.7% and 11.2% of the women. The Visual inspection with acetic acid has yielded 7 positive results (15%) among the FGS cases.

Conclusions: This study has shown the burden of FGS in the north of Senegal. Further studies are needed to propose the best strategies for the management of cases.

Keywords: Female Genital Schistosomiasis, Colposcopy, hospital setting

P163 / #533

Topic: *AS02.2 Schistosomiasis*

THE MARINE CYANOBACTERIAL METABOLITE GALLINAMIDE A AND ITS ANALOGUES ARE POTENT INHIBITORS OF SMCB1 DRUG TARGET AND EFFECTIVE ANTI-SCHISTOSOMALS

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Introduction: Schistosomiasis, a parasitic disease caused by blood flukes of the genus Schistosoma, is a global health problem with over 240 million people infected. Treatment relies on just one drug, and new therapies are needed. Schistosoma mansoni cathepsin B1 (SmCB1) is a critical cysteine protease for digestion of host blood proteins and a validated drug target. Gallinamide A is a natural peptidic metabolite from marine cyanobacteria, which inhibits several cysteine proteases.

Methods: Inhibitors were tested in a kinetic fluorescence assay against recombinant SmCB1 and ex vivo against cultured schistosomes. Crystal structure of the inhibitor-SmCB1 complex was determined by molecular replacement.

Results: We screened a library of 20 synthetic analogs of gallinamide A for inhibition of SmCB1 and identified inhibitors with low nanomolar potency. These compounds exhibited a strong suppression effect on live schistosomes and induced deleterious phenotypes. Furthermore, we solved the high-resolution 3D structure of SmCB1 in complex with gallinamide A and determined the binding mode of this covalent irreversible inhibitor in the SmCB1 active site.

Conclusions: Our study provides a new inhibitor template that can be exploited for the development of anti-schistosomal chemotherapeutics.

Keywords: SmCB1, Schistosoma, Cathepsins, Gallinamide A

P164 / #1110

Topic: *AS02.2 Schistosomiasis*

HEADING TOWARDS ELIMINATION: GPS-BASED PRECISION MAPPING SURVEYS FOR SCHISTOSOMIASIS ASSESSMENT

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Introduction: Precision mapping of schistosomiasis to guide micro-targeting of interventions will gain importance in elimination settings, where the heterogeneity of transmission is often pronounced. We provide a practical introduction and documentation of GPS-based household identification and participant recruitment, using Android-based applications for fine-scale schistosomiasis mapping at sub-district level in a remote area in Tanzania.

Methods: A household survey for urogenital schistosomiasis assessment was conducted in 20 communities in Pemba in 2021. For the survey, 1400 housing structures were prospectively and randomly selected from shapefile data. Enumerators searched for the houses' geolocation using the mobile applications Open Data Kit (ODK) and MAPS.ME. The number of inhabited structures, the median distance between the preselected and recorded locations, and the dropout rate was assessed.

Results: A total of 1396 (99.7%) housing structures were identified. The median distance between the preselected and recorded structures was 5.4 m. A total of 1098 (78.7%) houses were residential. Among them, 139 (12.6%) were dropped due to absence or refusal to participate. In 598 (62.4%) households, all members provided a urine sample of sufficient volume for testing.

Conclusions: The combination of ODK and an offline navigation application installed on mobile devices allows a very precise identification of housing structures. Dropouts due to non-residential housing structures, absence, non-participation and lack of urine need to be considered in survey design. Our findings can guide the planning and implementation of future precision mapping surveys and thus support schistosomiasis elimination efforts.

Keywords: fine-scale mapping, elimination, Urogenital schistosomiasis, interruption of transmission, Wayfinding

P165 / #1229

Topic: *AS02.2 Schistosomiasis*

NOVEL TOOLS AND STRATEGIES FOR BREAKING SCHISTOSOMIASIS TRANSMISSION IN PEMBA

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Introduction: Global elimination of schistosomiasis as public health problem is set as target in the WHO`s NTD Roadmap for 2030. Pemba has reached this goal since 2017. Our study will investigate novel tools and strategies to overcome challenges on the last mile towards interruption of transmission.

Methods: The primary endpoint of the 4-year intervention study is the sensitivity of a surveillance-response approach to detect and react to outbreaks of schistosomiasis in Pemba. In low-prevalence (LP) areas, surveillance-response consists of case detection, treatment of positives, and focal snail control. In high-transmission (HT) areas, preventive chemotherapy, snail control and behavioral interventions are implemented. Cross-sectional surveys in communities and schools serve to monitor the performance of the surveillance-response approach and impact of interventions.

Results: At baseline in 2020, the overall S. haematobium prevalence was 0.8% in community members and 1.2% in schoolchildren. Among the 20 implementation units, 15 were LP and 5 were HT areas. Individuals living <1 km from a water body with Bulinus globosus had higher odds of infection (OR: 18.0; 95% CI: 2.9-111.0) than individuals living >2 km away. At follow-up in 2021, the prevalence had decreased in HT areas from 2.1% to 1.4% in communities and from 2.8% to 1.2% in schools. In LP areas, the prevalence remained at 0.5% in communities and decreased from 0.5% to 0.4% in schools.

Conclusions: The comprehensive interventions resulted in a decrease in prevalence in HT areas and the surveillance-response activities maintained the very low prevalence in LP areas. Subsequent study years will shed more light on the performance of surveillance-response for interrupting schistosomiasis transmission.

Keywords: schistosomiasis, Surveillance-response, Test-and-Treat, Adaptive interventions, interruption of transmission

P166 / #1294

Topic: *AS02.2 Schistosomiasis*

IMPACT OF SEVEN YEARS OF MASS DRUG ADMINISTRATION AND RECRUDESCENCE OF SCHISTOSOMA HAEMATOBIUM INFECTIONS AFTER ONE YEAR OF TREATMENT GAP IN ZANZIBAR

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Introduction: Considerable progress towards the elimination of urogenital schistosomiasis was made by the Zanzibar Elimination of Schistosomiasis Transmission (ZEST) project from 2012 till 2016, when semi-annual praziquantel mass drug administration (MDA) alone or with additional snail control or behaviour change interventions were implemented. Annual MDA was continued in 2017, 2018, and 2020 but not in 2019, imposing a 16-month treatment gap. We monitored the Schistosoma haematobium prevalence from 2012 till 2021 and assessed recrudescence patterns with focus on 2020.

Methods: Repeated cross-sectional surveys were conducted at sub-district level from 2012 till 2021 in 90 communities and 90 schools in Zanzibar. Annually, around 4,500 adults and up to 20,000 children were surveyed. The S. haematobium prevalence was detected by urine filtration and reagent strips.

Results: In adults, the S. haematobium prevalence decreased from 3.9% in 2012 to 1.1% in 2021. In schoolchildren, the overall prevalence decreased from 6.6% to 2.5%, respectively, with vicissitudes over the years. Prominent recrudescence of infection from 2.8% in 2019 to 9.1% (+225%) in 2020 was observed in 29 schools with historically moderate prevalences (≥10%). Reinfection in 2020 was particularly striking in boys aged 9-16 years.

Conclusions: After 12 rounds of MDA over 7 years and a 16-month treatment gap, the urogenital schistosomiasis prevalence considerably rebounded in hotspot areas in 2020. Future elimination efforts in Zanzibar should focus on re-intensifying MDA plus additional interventions in hotspot areas. In low-prevalence areas, the strategy might be adapted from MDA to targeted surveillance-response.

Keywords: control, elimination, Hotspots, Recrudescence, schistosomiasis

P167 / #1184

Topic: *AS02.2 Schistosomiasis*

ALDOSE REDUCTASE - ENZYMATIC CHARACTERISATION OF A POTENTIAL TARGET PROTEIN IN SCHISTOSOMA MANSONI

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Introduction: Schistosomiasis, caused among others by the parasitic trematode species Schistosoma mansoni, leads to chronic inflammation and finally to liver fibrosis. If untreated, the disease can cause life-threatening complications. The current treatment of schistosomiasis is mainly based on a single drug, Praziquantel (PZQ). Due to the frequent use of PZQ, there is upcoming fear of emerging resistance. Therefore, it is necessary to find alternative drugs. Screening of potential drugs is currently mostly based on in vitro tests against different stages of the parasite. An attractive alternative is the establishment of enzyme assays with potential target proteins found in the parasite. With the use of such assays, large compound libraries can be tested in high-throughput screenings (HTS) without the need for animal experiments and in a time- and cost-efficient manner. A potential target protein for such HTS, based on its role in detoxification processes in other organisms, is an aldose reductase (AR) orthologue in S. mansoni (Smp_053220, SmAR1).

Methods: SmAR1 was recombinantly expressed in Escherichia coli and purified by immobilized metal ion affinity chromatography. The enzyme was characterised in more detail using a design-of-experiment approach to investigate its kinetic properties.

Results: We expressed SmAR1 successfully in the E. coli strain BL21(DE3). After purification, the enzyme was found to be active in vitro, and the pH-value and temperature for optimal activity and stability were investigated.

Conclusions: The findings enabled the establishment of an enzyme assay for SmAR1 for HTS.

Keywords: Aldose reductase, DoE, Schistosoma mansoni, Enzymatic characterisation

P168 / #1136

Topic: *AS02.2 Schistosomiasis*

THE SCHISTOSOME AND SNAIL RESOURCE (SSR) - SUPPORTING GLOBAL SCHISTOSOMIASIS RESEARCH

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Introduction: Schistosomiasis is a chronic tropical parasitic disease caused by schistosomes and transmitted by freshwater snails. It is a Neglected Tropical Disease of both humans and animals, with considerable health and economic impacts. While substantial advances have been made in the control of schistosomiasis, the diversity and complexity of Schistosoma species and their specific snail hosts warrants research requiring live material. Due to the complexity and costs of maintaining Schistosoma lifecycles currently, very few labs maintain the parasites and/or the snail hosts and current l cultures lack the natural genetic heterogeneity. Without the availability of diverse Schistosoma lifecycles, future research faces substantial obstacles.

Methods: The Schistosome and Snail Resource (SSR) is a Wellcome Trust funded biomedical resource run through a partnership of the Natural History Museum and the London School of Hygiene and Tropical Medicine in London. Its aim is to create and maintain live material (Schistosoma and snail host species) and lifecycles that are currently limited or that do not exist elsewhere.

Results: The SSR aims to provide access to: 1) the “standard” Schistosoma and snail species; 2) key African Schistosoma species/strains; 3) cultures of diverse snails, enhancing current and enabling new research. Our expertise in establishing and maintaining unique schistosome and snail collections, together with the state-of-the art snail and rodent facility will facilitate the development of the resource.

Conclusions: The SSR, is an open resource and will add considerable value by facilitating priority research needed to support schistosomiasis control and elimination.

Keywords: schistosomiasis, Biomphalaria, NTDs

P169 / #995

Topic: *AS02.2 Schistosomiasis*

IDENTIFICATION OF SCHISTOSOMA MANSONI ANTIGENS RELATED TO DRUG-INDUCED RESISTANCE IN HUMANS

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Introduction: Schistosomiasis is a very debilitating disease, afflicting more than 200 million people worldwide. Endemic area studies suggest different types of human resistance to Schistosoma infection. Praziquantel (PZQ) treatment of human populations is being associated with modifications in antibody levels and isotypes related to resistance, a phenomenon known as “drug-induced resistance” (DIR).

Methods: Here, we proposed to identify parasite’s targets related to DIR using a novel unbiased screening methodology, namely the use of a phage-display Schistosoma mansoni peptide library constructed by our group with synthetic oligonucleotides that encode the entire set of parasite proteins with known sequences. We used the library to screen serum samples from 5 patients susceptible to infection (SI) and 5 DIR patients from a cohort in the endemic area of Conde, Bahia, Brazil, plus 4 non-infected control individuals (CI). Samples were collected at 3 time points: day 0 (PZQ treatment), day 180, and day 540 post-PZQ treatment.

Results: The analysis showed a broad response of CI against 394 (72%) among the 544 peptides significantly enriched in all samples/timepoints. Among the remaining 150 peptides, 58 were conspicuously enriched in the DIR group, while SI patient samples specifically recognized 36 peptides. The latter peptides belong to proteins expressed across all life stages, while most proteinswhose peptides were exclusively DIR-enriched are expressed in schistosomula and were progressively captured by samples over the 3 experimental time points.

Conclusions: These findings indicate that DIR individuals developed a more specialized response against schistosomula antigens possibly protecting them from reinfection. Support: FAPESP/Agilent PITE, Fundação Butantan

Keywords: schistosomiasis, Phage-display, Human resistance, cDNA library

P170 / #1160

Topic: *AS02.2 Schistosomiasis*

RHESUS MACAQUES' IMMUNITY PAVING THE WAY TOWARDS THE IDENTIFICATION OF NEW PUTATIVE VACCINE CANDIDATES IN SCHISTOSOMA MANSONI

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Introduction: Rhesus macaques (Macaca mulatta) are an invaluable model for schistosomiasis vaccine development. Our previous work followed for 62 weeks (wk) the immune response and molecular markers involved in self-cure and long-term protection of 12 macaques infected and challenged with Schistosoma mansoni. The macaques' ability to shorten adult worms' longevity in a primary infection drives a robust resistance to challenge, possibly through antibody-mediated disruption of parasite homeostasis and nutrient uptake.

Methods: Here, we built a large-scale synthetic cDNA M13 phage-display library that encodes ~120,000 different 58-mer peptides, covering all ~12,000 known S. mansoni proteins (Smps), plus hundreds of in silico designed alternatively spliced isoforms of 39 Micro Exon Genes (MEGs). We screened sera collected at wk 0 (pre-infection), wk 8, 10, and 12 post-infection (pi), and wk 1 and 4 post-challenge (pc) against this non-biased library. Mimotopes recognized by each rhesus/timepoint were immunoprecipitated, their phages were deep-sequenced, and the peptides were identified.

Results: Wk 0 filtering step excluded 27 peptides. Enrichment statistical analysis showed 294 peptides captured at wk 8 pi and 1034 – 1217 peptides in the remaining weeks. Wk 8 had more peptides from possible MEG isoforms than from Smps, with 254 (86%) peptides of MEGs, followed by week 4 pc with 476 (43%). Phages containing a non-annotated 10-mer motif resulting from a putative frameshift of MEG 4.1 was highly captured (322 phages, 90% of all MEG 4.1).

Conclusions: This unbiased high throughput screening will help to identify a new set of vaccine candidates and to understand the mechanisms of rhesus macaques self-cure and resistance to infection and challenge. Support: FAPESP, Fundação Butantan

Keywords: Vaccine, schistosomiasis, cDNA library, Phage-display, Rhesus macaques

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Topic: *AS02.3 Intestinal parasitic diseases*

INTESTINAL BARRIER DEFECTS IN GIARDIASIS

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Introduction: Giardia duodenalis is an intestinal protozoan parasite commonly transmitted via contaminated drinking water. In addition to the usual intestinal distress symptoms (diarrhea, bloating, etc), giardiasis is associated with growth stunting in children under the age of two. Many factors could be contributing to growth faltering, such as intestinal morphological changes, enzymatic deficiencies, and barrier defects. While infection-mediated barrier defects have been observed in in vitro studies, in animal models, and in human studies, the mechanisms behind them are unclear. Several studies indicate that changes in tight junction proteins such as zona occludens-1 and occludin are related to these barrier defects, but parasite virulence factors which induce these changes and mechanisms by which the host senses the parasite have not been defined.

Methods: I will be focusing on the role of putative virulence factors secreted by the parasite, particularly various proteases, in initiating signaling on the intestine and resulting in tight junction defects. This hypothesis will be tested both in cell culture and organoid models, as well as in an animal model of infection-induced growth faltering.

Results: Addition of Giardia to Caco2 cells leads to tight junction alterations by immunofluorescence for ZO-1. Studies with protease inhibitors and other assays for barrier structure and function are underway.

Conclusions: Identification of the virulence factors responsible for barrier defects could inform pharmaceutical and vaccine development, in order to limit damage post infection and thus, reduce growth stunting in children.

Keywords: Giardia, barrier defects, infection

P172 / #722

Topic: *AS02.3 Intestinal parasitic diseases*

ISCA: NON-ESSENTIAL MITOSOMAL PROTEIN INVOLVED IN THE FORMATION OF CYTOSOLIC 4FE-4S CLUSTERS IN GIARDIA INTESTINALIS

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Introduction: Giardia intestinalis is an anaerobic human parasite causing more than 300 million diarrheal diseases worldwide a year. Due to its anaerobic lifestyle, G. intestinalis evolved a distinct cellular morphology and lacks functional mitochondria, which have been reduced to mitosomes. The only function of mitosomes appears to be the synthesis of iron-sulfur clusters by the ISC pathway. This pathway can be divided into an early (2Fe-2S) and a late pathway (4Fe-4S), which has long been thought to be absent in G. intestinalis. However, the components of the late pathway are encoded in the genome, opening the possibility of the formation of 4Fe-4S clusters in G. intestinalis. Here, we focus on one of them, the IscA protein, and the phenotypic characterization of its respective knockout strain.

Methods: CRISPR gene deletion, ^{55}Fe uptake, pyruvate:ferredoxin oxidoreductase activity assay

Results: Using our recently developed CRISPR-based knockout system, we were able to remove all four copies of the iscA gene from the genome of the G. intestinalis isolate WB. The deletion strain (ΔiscA) propagates stably in axenic culture, suggesting that G. intestinalis can at least partially overcome the loss of IscA by alternative means and that IscA itself is not essential. However, the ΔiscA strain suffers from several phenotypic defects and is unable to fully saturate the demand for 4Fe-4S clusters in some of its proteins, as shown, for instance, by reduced ^{55}Fe incorporation or decreased activity of cytosolic 4Fe-4S dependent pyruvate:ferredoxin oxidoreductase.

Conclusions: Our results confirm the involvement of IscA in the synthesis of 4Fe-4S clusters. However, unlike mitochondrial IscA proteins, mitosomal IscA influences the formation of cytosolic 4Fe-4S clusters.

Keywords: Giardia, iron-sulfur clusters, mitosome

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Topic: *AS02.3 Intestinal parasitic diseases*

SPECIES AND SUBTYPES IDENTIFIED THROUGH THE MICROBIOLOGICAL SURVEILLANCE PROGRAMME FOR CRYPTOSPORIDIUM IN SWEDEN 2018-2020

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Introduction: In 2018, the Public Health Agency of Sweden initiated a national microbiological surveillance programme for Cryptosporidium. The aim of the programme is to investigate what species and subtypes cause human domestic cryptosporidiosis in Sweden.

Methods: The primary diagnosis were made at local clinical laboratories using Ziehl-Neelsen staining and/or real-time PCR. Cryptosporidium positive samples were forwarded for further molecular typing of species (SSUrRNA) and subtypes (gp60) by Sanger sequencing.

Results: The incidence of cryptosporidiosis has increased and 2019 it peaked (10,5/ 100 000 inhabitants) due to several foodborne outbreaks. There are more domestic cases compared to infection acquired abroad. Majority of cases are due to infection with the zoonotic species C. parvum (81 % in total) Second most common species to cause infection is C. hominis (5 % in total). In 2020 no cases of C. hominis were detected, instead the second most common species to cause cryptosporidiosis was Cryptosporidium chipmunk genotype I. Infection with this species has been described as an emerging cause of cryptosporidiosis in Sweden and a small outbreak was identified. All Swedish cases have the subtype XIVaA20G2T1. Other uncommon species identified are C. erinacei, C. meleagridis, C. canis, C. cuniculus, C. ubiquitum, Cryptosporidium horse genotype and C. ditrichi.

Conclusions: Cryptosporidiosis is a zoonotic infection caused by C. parvum in Sweden. The majority of cases are domestic and not travel-related. 78 different C. parvum subtypes have been identified. Some are more common than others, but no specific subtype is dominating. Several foodborne and zoonotic clusters and outbreaks have been identified, all caused by infection with C. parvum.

Keywords: Cryptosporidiosis, Surveillance, Zoonosis

P174 / #781

Topic: *AS02.3 Intestinal parasitic diseases*

MOLECULAR DETECTION OF INTESTINAL MICROEUKARYOTES IN HIV+ PATIENTS ATTENDING A TERTIARY CARE UNIVERSITY HOSPITAL IN MADRID, SPAIN.

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Introduction: Opportunistic infections by microeukariotic enteroparasites (ME) are common in patients infected with the human immunodeficiency virus (HIV). This cross-sectional, prospective study determines the presence and molecular diversity of ME in HIV+ patients in a tertiary care hospital in Madrid, Spain.

Methods: Faecal samples (n=96) from HIV+ patients >18 years on antiretroviral therapy (CD4%: 771 cells/ µL) were collected during December 2020-June 2021. The identification and characterization of intestinal parasites were carried out by PCR and Sanger Sequencing.

Results: Entamoeba dispar was the most prevalent ME found (30%, 29/96), followed by Blastocystis sp. (20%, 19/96), Giardia duodenalis (17%, 16/96), and Cryptosporidium spp., E. bieneusi and E. histolytica (2%, 2/96 each). Co-infections were observed in 17% (16/96) of cases, being G. duodenalis + Blastocystis sp. the most common combination (31%, 5/16). Assemblages A (25%) and B (75%) were found within G. duodenalis (n=4), and ST1 (74%) and ST3 (26%) within Blastocystis sp. (n=19). Within Cryptosporidium, C. canis was the only species found (n= 1). Genotype A was observed within E. bieneusi (n=2).

Conclusions: Co-infections by two or more ME are common in the HIV+ patients. The presence of C. canis suggests a zoonotic transmission event. This is the first description of molecular diversity of E. bieneusi in HIV+ Spanish patients.

Keywords: intestinal microeukaryotes, HIV+, molecular

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Topic: *AS02.3 Intestinal parasitic diseases*

DETECTION AND GENOTYPING OF MICROEUKARYOTIC ENTEROPARASITES IN SYMPTOMATIC CHILDREN ATTENDING THREE PUBLIC HOSPITALS IN SPAIN

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Introduction: Gastrointestinal infections are a major reason for paediatric hospital admission globally. Besides viral and bacterial agents, microeukaryotic enteroparasites (ME) are also important contributors to the burden of diarrhoeal disease. This study determines the frequency and molecular diversity of ME in symptomatic children seeking medical attention at three public hospitals in Spain.

Methods: Individual stool samples (n= 1,114) from children (0–59 months) presenting with gastrointestinal symptoms were obtained. Detection, identification, and genotyping of ME were achieved by PCR and Sanger sequencing.

Results: Giardia duodenalis was the most prevalent ME found (9.8%, 109/1,114) followed by Cryptosporidium spp. (2%, 20/1,114), Blastocystis sp. (1%, 10/1,114), and Enterocytozoon bieneusi 0.2% (2/1,114). Sub-assemblages BIV (73%), AII (18%), and BIII (9%) were found within G. duodenalis (n=11), and sub-types ST4 (60%), ST1 (20%), and ST2 (10%) within Blastocystis sp. (n=10). Cryptosporidium-positive samples (n=20) belonged to C. parvum IIaA15G2R1 (45%), IIaA14G2R1 (5%), IIaA16G3R1 (5%), IIaA17G1R1 (5%), and IIdA17G2R1 (5%). A known (D) and a novel E. bieneusi genotypes were found (n=2). Entamoeba histolytica and Entamoeba dispar were undetected.

Conclusions: Giardia duodenalis and C. parvum were common findings in clinical paediatric patients. The apparent absence of C. hominis was surprising.

Keywords: microeukaryotic, enteroparasites, symptomatic children

P176 / #978

Topic: *AS02.3 Intestinal parasitic diseases*

FREQUENCY OF INTESTINAL MICROEUKARYOTES IN PATIENTS UNDERGOING SCREENING COLONOSCOPY FOR COLORECTAL CANCER

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Introduction: Blastocystis sp. is one of the most common parasites infecting the human gut. According to IARC, protist species are not carcinogenic to humans. However, Blastocystis sp. has been associated with colon affections including colorectal cancer (CRC). This ongoing study investigates potential associations among Blastocystis sp. and other intestinal protists and a higher risk of developing CRC.

Methods: Stool samples were collected from patient undergoing screening colonoscopy for CRC in two hospitals in Medellín, Colombia. The presence of microeukaryotic parasites was investigated by conventional (formalin-ether concentration technique, microscopic examination including Ziehl-Neelsen staining) and molecular (PCR and Sanger sequencing) methods.

Results: A total of 80 patients (47 with normal colonoscopy, 5 with polyps, and 28 with a CRC diagnosis) were included in the study. Blastocystis sp. was the most prevalent enteric protist found (30%, 24/80), followed by Giardia duodenalis (2.5%, 2/80) and commensal amoebas (6.3%, 5/80). Within Blastocystis, the sub-type ST3 allele 34 was the most frequently found in the surveyed clinical population (54%, 13/24). ST3 allele 34 was more prevalently found in CRC patients (n=7) compared to those with polyps (n=2) or normal colonoscopy (n=4).

Conclusions: Pathogenicity of Blastocystis remains disputable, but preliminary results from this study suggests that Blastocystis ST3 may enhance CRC development.

Keywords: microeukaryotes, colonoscopy, colorectal cancer, Intestinal Parasites

P177 / #1663

Topic: *AS02.3 Intestinal parasitic diseases*

PREVALENCE OF TOXOCARIASIS AND ITS RISK FACTORS IN PATIENTS WITH EOSINOPHILIA IN KOREA

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Introduction: Eosinophilia occurs commonly in many diseases including allergic diseases and helminthic infections. Toxocariasis has been suggested as one cause of eosinophilia. The present study was undertaken to examine the prevalence of toxocariasis in patients with eosinophilia and to identify the risk factors for toxocariasis.

Methods: This prospective cohort study recruited a total of 81 patients with eosinophilia (34 males and 47 females) who visited the outpatient clinic at Seoul National University Hospital from January 2017 to February 2018 and agreed to participate in this study. The prevalence of toxocariasis was examined by *T. canis*-specific ELISA, and the various risk factors for toxocariasis were evaluated by a questionnaire survey.

Results: Among 81 patients with eosinophilia, 18 were positive for anti-*T. canis* antibodies (22.2%); 88.9% were male (16/18) and 11.1% were female (2/18). Multivariate statistical analysis revealed that males (OR 21.876, 95% CI: 1.667–287.144) with a history of consuming the raw meat or livers of animals (OR 5.899, 95% CI: 1.004–34.669) and a heavy alcohol-drinking habit (OR 8.767, 95% CI: 1.018–75.497) were at higher risk of toxocariasis in patients with eosinophilia.

Conclusions: Toxocariasis should be considered a potential cause of eosinophilia when the patient has a history of eating the raw meat or livers of animals in Korea. A single course of albendazole is recommended to reduce the migration of *Toxocara* larvae in serologically positive cases with eosinophilia. (This work has been published in Korean Journal of Parasitology in 2020: doi: https://doi.org/10.3347/kjp.2020.58.4.413.)

Keywords: Toxocara canis, raw liver, Toxocariasis, eosinophilia, Risk factor

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Topic: *AS02.3 Intestinal parasitic diseases*

PRELIMINARY EVALUATION OF DIFFERENT METHODS TO DETECT AND QUANTIFY TAENIA EGGS IN WATER AND SLUDGE SAMPLES: SPIKING EXPERIMENT TO ASSESS RECOVERY RATES

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Introduction: An improved understanding of the environmental transmission of Taenia spp. is key to control of the parasite. Methods to detect and quantify Taenia eggs in different environmental matrices are generally not validated. This study aimed to assess the recovery rates of commonly used methods for detection of Taenia eggs in water and sludge samples.

Methods: Ten detection methods for Taenia spp. eggs were selected. Water and sludge samples were spiked with a high dose of Taenia saginata eggs, i.e. around 50 eggs/ml water and 200 eggs/g sludge, and were tested using five methods each. The two methods with the highest egg recovery were selected per matrix for assessment with a low spiking dose, i.e. 1 egg/ml and 4 eggs/g, respectively. Each time, five replicates were used. Recovery was defined as the proportion of the number of eggs recovered to the total number of eggs spiked.

Results: For the high spiking dose, all samples tested positive for all the methods. The mean egg recovery varied from 3% to 68% for water samples, and 4% to 69% for sludge samples. For the low spiking dose, all water samples tested positive using both methods. For sludge, one of the methods was able to detect all low dose replicates, whereas only one sample was positive using the other method.

Conclusions: Most methods performed inadequately in recovering Taenia eggs from water and sludge, with half of the methods having an average egg recovery below 10%. A more thorough validation is urgently needed.

Keywords: Environmental matrices, Taenia eggs, Recovery, Spiking experiment

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Topic: *AS02.3 Intestinal parasitic diseases*

THE PREVALENCE OF BLASTOCYSTIS SP. AND DIENTAMOEBA FRAGILIS IN CHILDREN: A CASE-CONTROL STUDY IN ANKARA, TURKEY

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Introduction: Blastocystis sp. and Dientamoeba fragilis are neglected intestinal protists with worldwide distributions. However, data on the occurrence of these parasites in children of differing immune status and symptomology are lacking. This study investigated the prevalence of Blastocystis sp. and D. fragilis and determined the subtypes (STs) of Blastocystis sp. in children from Turkey.

Methods: A total of 351 stool samples from children (1-18 years of age) (250 symptomatic and 101 asymptomatic) were analyzed using microscopic and molecular methods. DNA was extracted from all stool samples, and real-time PCR was used to detect the prevalence of Blastocystis sp. and D. fragilis. Next-generation amplicon sequencing was used for STs analysis of Blastocystis sp.

Results: Blastocystis sp. and D. fragilis were present in 13.4% (47/351) and 7.4% (26/351) of the samples, respectively. In the healthy controls, the presence of both protists was higher than the patient group (15.8% and 12.9%; 12.9% and 5.2%, respectively). For D. fragilis this difference was statistically significant ($p=0.01$). Only age was statistically related to the prevalence of both protists and was highest in the age group 7 to 13 years ($p<0.05$). Three subtypes of Blastocystis sp. were detected, 21.3% ST1 (10/47), 14.9% ST2 (7/47), 51.1% ST3 (24/47), and 4.3% ST1/ST3 (2/47). Four Blastocystis sp. isolates were not characterized. No statistically significant differences were observed between subtypes and study groups.

Conclusions: These findings highlighted the active circulation of these two protist in both groups of children and interestingly, suggest that their presence may be considered characteristic of a healthy intestinal microbiome.

Keywords: Blastocystis sp., Dientamoeba fragilis, Children, diagnosis

P180 / #635

Topic: *AS02.3 Intestinal parasitic diseases*

DETECTION OF BLASTOCYSTIS SP. AND DIENTAMOEBA FRAGILIS USING CONVENTIONAL AND MOLECULAR METHODS IN PATIENTS WITH CELIAC DISEASE

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Introduction: Blastocystis sp. and Dientamoeba fragilis (D. fragilis) are intestinal protists, which are common worldwide, but the pathogenic role of these organisms in gastrointestinal diseases is still controversial. Celiac disease (CD) is an autoimmune chronic enteropathy of the small intestine. Although some studies have shown a relationship between various parasites and immunological diseases, the relationship between Blastocystis sp., D. fragilis and CD is unknown. This study aimed to investigate the frequency of Blastocystis sp. and D. fragilis in stool samples from adult patients with CD by using conventional and molecular methods.

Methods: A total of 75 patients with CD and 75 healthy individuals were included in the study. Fresh stool specimens collected from each individual were analyzed by conventional and molecular methods (real-time PCR) for Blastocystis sp. and D. fragilis.

Results: Blastocystis sp. and D. fragilis were detected in 23 (30.7%) and 10 (13.3%) of the patients with CD, 29 (38.7%) and four (5.3%) of the healthy control group, respectively. In addition, co-infection was determined in eight (10.7%) of the patient group and six (18%) of the control group. There was no statistically significant difference in the prevalence of Blastocystis sp. and D. fragilis between CD patients and healthy individuals.

Conclusions: To the best our knowledge, this is the first study to investigate the presence of Blastocystis sp. and D. fragilis in CD patients using different methods in Turkey. When the patient and control groups were compared in terms of the presence of both protists with at least one method, no statistically significant difference was found. Further studies are needed to better understand the relationship between CD disease and parasitic infections.

Keywords: Blastocystis sp., Dientamoeba fragilis, Celiac disease, diagnosis

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Topic: *AS02.3 Intestinal parasitic diseases*

THE PREVALENCE OF BLASTOCYSTIS SP. AND DIENTAMOEBA FRAGILIS IN SYMPTOMATIC AND ASYMPTOMATIC ADULTS IN ANKARA, TURKEY

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Introduction: Dientamoeba fragilis and Blastocystis sp. are protists frequently found in the human gut worldwide. The role of Blastocystis sp. and D. fragilis in patients with gastrointestinal symptoms is still under debate.

Methods: A total of 244 stool samples from adults (19-84 years of age) (160 symptomatic and 84 asymptomatic) were analyzed using microscopic and molecular methods to detect D. fragilis and Blastocystis sp. Real-time PCR was used to detect the prevalence of Blastocystis sp. and D. fragilis. Next generation amplicon sequencing was used to determine subtype (ST) of Blastocystis sp.

Results: Blastocystis sp. and D. fragilis were present in 24.6% (60/244) and 11.94% (29/244) of the samples, respectively. In the asymptomatic individuals, the presence of both protists was higher than the individuals with symptomatology (25% and 24.4%; 14.3% and 10.6%, respectively). Those differences were not statistically significant. Three subtypes of Blastocystis sp. were detected, ST2 (6.7%; 4/60), ST3 (51.7%; 31/60), ST1/ST3 (25%; 15/60), ST2/ST3 (8.3%; 5/60) and ST1/ST2/ST3 (1.7%;1/60). ST1 was observed only in mixed STs infections. It was determined that more than one-third of Blastocystis positive infections were mixed STs infections. ST3 was detected in all mixed infections. Four Blastocystis sp. isolates were not characterized. No statistically significant differences were observed on subtypes identified in symptomatic and asymptomatic groups.

Conclusions: There was no association between presence of D. fragilis and Blastocystis sp. and gastrointestinal symptomatology. Interestingly, this suggests that the presence of these protists may be considered characteristic of a healthy intestinal microbiome.

Keywords: Blastocystis sp., Dientamoeba fragilis, diagnosis, Adults

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Topic: *AS02.3 Intestinal parasitic diseases*

GENOTYPIC DIVERSITY OF BLASTOCYSTIS ISOLATED FROM A COHORT OF PATIENTS IN EASTERN SAUDI ARABIA

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Introduction: Blastocystis, a protozoan parasite, is a common enteric human unicellular eukaryote worldwide; and might be a cause of human enteric diseases. To date, a study of genetic diversity of Blastocystis spp. In Saudi Arabia has not been performed. This cross-sectional study aims to detect the prevalence and genetic diversity of Blastocystis in university hospital, the Eastern Saudi Arabia. Also, to assess the association between Blastocystis colonization and patients' data and stool tests results.

Methods: Stool specimens and related data were collected from patients attending the King Fahad Hospital of the University, Eastern Province of Saudi Arabia. Stool specimens which were submitted to the microbiology lab, were examined microscopically for Blastocystis cysts and other parasites. Stool was cultured for Blastocystis. Blastocystis DNA was amplified using PCR assays and PCR products were sequenced to determine Blastocystis subtypes (STs).

Results: Among 1138 specimens cultured, Blastocystis was detected in almost 10% of them. Blastocystis DNA from all positive stool cultures, sequencing, and phylogeny in progress.

Conclusions: Assessing genetic diversity and phylogenetic relationship will provide a better understanding of the Blastocystis taxonomy, accurate parasite epidemiology, and transmission dynamics of the parasite; thus, facilitating the implementation of control strategies and informing drug design and vaccines.

Keywords: genotypic diversity,, Blastocystis, Saudi Arabia, Phylogenic analysis, Genotyping

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Topic: *AS02.3 Intestinal parasitic diseases*

MULTILOCUS GENOTYPING OF GIARDIA DUODENALIS IN EGYPTIAN CHILDREN: ASSEMBLAGE B PREDOMINANCE

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Introduction: Giardia duodenalis (G. duodenalis) is a common human and animal enteric protozoan parasite with high diversity. There is very little information available on the diversity of Giardia sub-assemblages and multi-locus genotypes infecting people in Egypt. Due to allelic sequence heterogenicity and single nucleotide polymorphism found in Giardia spp, multi-locus genotyping is highly recommended. This study aimed to identify the assemblages and sub-assemblages of G. duodenalis isolated from the stool of Egyptian children

Methods: using PCR based on 3 genetic loci: β-giardin (bg), glutamate dehydrogenase (gdh), and triose phosphate isomerase (tpi)]. Further sub-genotyping phylogenic analysis of DNA sequences was done at these loci using PCR-based sequencing and the phylogenetic trees were constructed using the neighbor-joining method. Related sociodemographic and clinical features of patients infected with G. duodenalis were also analysed.

Results: Two assemblages, A and B, were identified in isolates from the stool of Egyptian children with a significant predominance of assemblage B. Sequence analysis showed that assemblage B (BIII/BIV) isolates have a higher genetic polymorphism than assemblage A (AII) isolates. BIV was the most prevalent genetic variant of G. duodenalis found in the stool of the population studied. Among the studied variables, only flatulence was significantly associated with Giardia infection and assemblage.

Conclusions: The obtained results may support the anthroponotic transmission of Giardia and the occurrence of genetic exchange within assemblages in studied individuals.

Keywords: Multilocus, Genotyping, Phylogenic analysis, Giardia, Genotypic diversity

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Topic: *AS02.3 Intestinal parasitic diseases*

AN IN SILICO METHODOLOGY TO DESIGN A VACCINE AGAINST ASCARIS.

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Introduction: Ascariasis is the most prevalent zoonotic helminth disease affecting both humans and pigs. With rising concerns about the development of anthelmintic resistance in *Ascaris*, there is an increased interest in exploring vaccination as an alternative control method. We aimed at using an *in silico* approach to design a multi-epitope vaccine against ascariasis.

Methods: Novel proteins were selected from the three available *Ascaris* proteomes along with the previously identified vaccination targets As14, As16 and As37. The proteins were chosen based on the presence of T-cell and B-cell epitopes in extracellular domains and were antigenic, non-allergenic and non-toxic. The predicted epitopes and TLR4 adjuvant were combined using appropriate linkers. The designed vaccine's physicochemical and immunological characteristics were tested *in silico* to assess its usefulness.

Results: The multi-epitope vaccine was designed by combining two T-cell epitopes and two B-cell epitopes from seven distinct *Ascaris* antigens. The designed vaccine was predicted to be antigenic, non-allergenic and non-toxic, while also being stable and soluble. Immune simulations and molecular docking predicted its ability to promote the development of B-cell and memory T-helper cells, IgG and IFN-γ, and the binding to both TLR2 and TLR4.

Conclusions: Using an *in silico* methodology, we were able to design a multi-epitope vaccine predicted to be a useful candidate against ascariasis. The designed vaccine should now be tested experimentally to compare its effectiveness to other developed vaccines.

Keywords: Vaccine, Ascaris, zoonoses, Bioinformatics

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Topic: *AS02.3 Intestinal parasitic diseases*

DEVELOPMENT AND EVALUATION OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) TECHNIQUE FOR RAPID, ACCURATE, AND SPECIFIC DETECTION OF BLASTOCYSTIS SPP. IN AIDS PATIENTS

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Introduction: Blastocystis spp. is one of the most prevalent intestinal parasites with worldwide distribution. Various diagnostic methods with different sensitivities and specificities have been used to detect Blastocystis in clinical samples. The present study aims to develop and evaluate a LAMP assay to detect Blastocystis spp. in HIV/AIDS patients for the first time.

Methods: In this cross-sectional study, ninety-eight HIV/AIDS patients with an average CD4+ T lymphocyte count lower than 150 cells/mm^3 participated in the study. The presence of Blastocystis spp. in the stool samples collected from HIV/AIDS patients was examined by parasitology (direct wet mount and concentration assays) and molecular (PCR and LAMP) methods. The 18 SSU rRNA genomic target was used to design the specific primers for the PCR and LAMP assays. The specificity of designed primers for the LAMP assay was evaluated using the sequencing of a conventional PCR product by the external LAMP primers.

Results: Out of 98 stool samples from patients with AIDS, 9 (9.18%), 13 (13.26%), and 15 (15.30%) samples were detected positive for Blastocystis spp. by parasitology, PCR, and LAMP technique respectively. PCR amplification and subsequent sequencing of the product sequences revealed that the obtained partial sequences were identical to the corresponding 18 SSU rRNA sequences reported in GenBank.

Conclusions: The higher positivity rate for Blastocystis spp. among studied AIDS patients by LAMP technique showed the higher potential and effectiveness of this relatively new described molecular assay for detection of Blastocystis spp. in AIDS patients. The results obtained for the first time showed that the sensitivity and accuracy of the LAMP technique in the diagnosis of Blastocystis spp. in AIDS patients is very high.

Keywords: Accurate, AIDS patients, Blastocystis spp., LAMP, Rapid detection

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Topic: *AS02.3 Intestinal parasitic diseases*

EUKARYOTIC METATAXONOMIC ANALYSIS OF FAECES FOR THE IDENTIFICATION OF BLASTOCYSTIS AND OTHER INTESTINAL PARASITES

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Introduction: Blastocystis is an intestinal protist with a broad host range and high prevalence in human population worldwide. Next-generation sequencing (NGS) and metataxonomic based studies of microorganisms of public health significance has grown considerably in recent years, particularly for prokaryotes. However, a few studies have been reported for eukaryotes like Blastocystis. The aim of this study was to evaluate the presence of Blastocystis in fecal samples from Colombian children through a 18S ribosomal gene metataxonomic approach.

Methods: Twenty-eight stool samples from children <5 years from daycare centers from Medellin (Colombia), and with a microscopic diagnosis for Blastocystis were included in the study. DNA was extracted from fecal samples and the V3-V4 hypervariable regions of the 18S rRNA gene was amplified. Sequences were obtained on the Illumina MiSeq platform. Depurated reads were grouped into OTUs and the relative abundance and taxonomic rank for eukaryotes were calculated.

Results: Metataxonomic experiment detected Blastocystis in the 28 samples evaluated. Additionally, a taxonomic coverage for other intestinal parasites was achieved, including Cryptosporidium, Giardia, Dientamoeba fragilis, and ascaridida nematodes.

Conclusions: NGS-based approach enabled intestinal parasites detection and has the potential to evaluate the genetic diversity of eukaryotes in clinical samples. Acknowledgements. This work was supported by Convocatoria Programática 2019-2020: Área de Ciencias de la Salud, CODI (Project 2020-33903); and Convocatoria interna para financiar proyectos de investigación en la Escuela de Microbiología–sede central (Project 2021-40850). Universidad de Antioquia.

Keywords: Metataxonomic, NGS, Blastocystis, Intestinal Parasites

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Topic: *AS02.3 Intestinal parasitic diseases*

ENTAMOEBA MOSHKOVSKII – NEW UPCOMING ENTAMOEBA INFECTION IN INDIA

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Introduction: Nowadays importance of E. moshkovskii is increasing in study of amoebiasis along with Entamoeba histolytica as it is reported in human over the years in some cases. In this study, we aimed to determine the epidemiology and molecular characterization of Entamoeba moshkovskii to investigate genotypic variations among the local isolates around Kolkata.

Methods: Target population for hospital based systemic surveillance study is the diarrhoea patients admitted to ID Hospital and B C Roy Children Hospital, Kolkata. All microscopy positive samples were first subjected to Genus specific PCR targeting18SrDNA locus and then by species specific nested PCR assay for identification of E. moshkovskii. Positive amplified products were sequenced for characterization. Finally the evolutionary history of the isolate was inferred.

Results: 6.76% of the samples were positive for Entamoeba spp. The prevalence of E. moshkovskii was 3.42% and prevalence of others Entamoeba species was 3.34%. Distribution of Entamoeba moshkovskii were significantly associated with different age groups. A large number of positive samples were solely infected with E. moshkovskii and the sole infection with E. moshkovskii significantly associated with diarrheal incidence. A specific seasonal distribution was also observed. A large number the sequences obtained in this study were distinct from previously reported genotypes and considered as novel Entamoeba ribosomal lineages. All of the 68 sequences were deposited in NCBI GenBank.

Conclusions: The present study establishes E. moshkovskii to be one of the causative agents for acute diarrhoea in humans. Our finding plausibly raises alarm for urgent planning and implementation of prevention and control strategies against the disease.

Keywords: Entamoeba, Moshkovskii, intestinal parasite

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Topic: *AS02.3 Intestinal parasitic diseases*

EVALUATION OF RELATIVE FREQUENCY OF BLASTOCYSTIS GENOTYPES IN HEALTHY PEOPLE AND GASTROINTESTINAL SYMPTOMS IN ISFAHAN

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Introduction: Introduction: *Blastocystis* is an extracellular and immobilized protozoa that is currently the most common protozoan gastrointestinal tract in the human and a wide range of Animal. *Blastocystis* is divided into 17 subunits (STs) based on the small ribosomal subunit gene. ST1-ST9 found in human, and ST3 being the most common type of *Blastocystis* in humans. Its pathogenicity has not yet been established. The aim of the present study was to evaluate *Blastocystis* subtypes in asymptomatic and gastrointestinal symptoms in Isfahan..

Methods: Methods: A total of 160 asymptomatic patients and 328 individuals with gastrointestinal symptoms were sampled. The specimens were examined microscopically and all were cultured. DNA was extracted from positive cultures and then PCR was performed. 69 PCR products were purified and sent for sequencing.

Results: Results: From 160 (asymptomatic) 33 cases and from 328 (gastrointestinal symptoms) 40 cases were positive for *Blastocystis* PCR. Sequencing results showed that *Blastocystis* genotype in subgroups asymptomatic and with gastrointestinal symptoms had subtypes 1, 2 and 3. Most of the contamination is related to subtype 2 and then 3. In the gastrointestinal asymptomatic group, a subtype 7 case was also reported. There was no significant difference between the subtypes in the two groups. But there was a significant difference in ST2 and ST3 between the two groups.

Conclusions: Conclusion: *Blastocystis* infection was higher in asymptomatic group than in gastrointestinal group. According to the number of subjects in both groups, subtype 3 was more in asymptomatic subjects than in those with gastrointestinal symptoms. Interestingly, unlike other studies in Iran and other countries, subtype 2 is common in Isfahan.

Keywords: Blastocystis,, genotype, Isfahan, Iran

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Topic: *AS02.3 Intestinal parasitic diseases*

SPINY-HEADED WORMS (ACANTHOCEPHALA) AS HUMAN PARASITES: A GLOBAL UPDATE

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Introduction: Human acanthocephalosis is a rare food-borne disease caused by spiny-headed worms (Acanthocephala). The number of cases has historically been considered low, with very few reports in Africa, Asia and North America.

Methods: This update is based on a critical review of all available primary data.

Results: We found 74 records reporting more than 600 cases of human acanthocephalosis from 1857 to 2021. Our list includes 11 named and 4 unidentified species of acanthocephalans infecting humans throughout the world. The most common causative agent of human infections is Macracanthorhynchus hirudinaceus with more than 500 cases diagnosed in China, but some also known from other countries. Humans become infected after eating beetles or their larvae. The second most frequently found species is Moniliformis moniliformis with 38 cases reported mostly from Africa and Asia. Beetles and cockroaches serve as a source of human infection. Sixteen cases were also caused by marine acanthocephalans (species of Bolbosoma and Corynosoma) mainly in Japan. The source of infection are marine fishes and squids.

Conclusions: Acanthocephalans are rare intestinal parasites of humans, but the number of human cases has been highly underestimated. Molecular data are missing for almost all clinical cases, and thus health specialists are encouraged to fix worms found in ethanol for their molecular identification.
Acknowledgments: Institute of Parasitology, BC CAS (RVO: 60077344).

Keywords: food-borne disease, Macracanthorhynchus hirudinaceus, Moniliformis moniliformis, Bolbosoma, Corynosoma

P190 / #1410

Topic: *AS02.3 Intestinal parasitic diseases*

HOSPITALIZATIONS ASSOCIATED WITH STRONGYLOIDIASIS IN THE UNITED STATES, 2003-2018

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Introduction: Strongyloides stercoralis is considered to be historically endemic in Appalachia and the American South, but recent surveillance data, especially data evaluating strongyloidiasis associated with hospitalization, are lacking in most parts of the US.

Methods: We performed a population-based retrospective analysis on strongyloidiasis using the National Inpatient Sample from 2003-2018. Geographic distribution of strongyloidiasis associated hospitalization was assessed. Logistic regression was used to identify risk factors associated with strongyloidiasis.

Results: We identified 6931 hospitalizations associated with strongyloidiasis during the study period (11.8 per million hospitalizations). The rate of strongyloidiasis was highest in the US Northeast region including the Middle Atlantic division (47.1 cases per million population), and the East South Central division (27.5 cases per million population, adjusted odds ratio). Older age, male sex, non-white race/ethnicity, non-private insurance, and residence in neighborhoods with low median income were also associated with strongyloidiasis. Immunocompromising conditions, particularly human immunodeficiency virus infection, were present in 41.3% of hospitalizations with strongyloidiasis. In-hospital death was seen in 7.8% of cases with strongyloidiasis-associated hospitalization.

Conclusions: Strongyloidiasis-associated hospitalization is rare in the U.S. but can be associated with mortality. It occurs more frequently in poor and marginalized populations. Immunocompromised conditions were common among hospitalized patients with strongyloidiasis. Enhanced surveillance efforts are needed to inform health policies for improving the health of at-risk populations.

Keywords: Epidemiology, population-based study, strongyloidiasis

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Topic: *AS02.3 Intestinal parasitic diseases*

ENTEROBIASIS IN BULGARIA: A NEGLECTED INFECTION WITH INCREASING FREQUENCY

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Introduction: In Bulgaria,enterobiasis is the most common parasitosis, especially among children and adolescents who may have close contact. Although not subject to mandatory registration, the prevalence of enterobiasis is determined annually from data of parasitological structures in the country. Epidemiological surveillance is performed in accordance with the regulation of the Ministry of Health (MoH) for the diagnosis, prevention and control of indigenous parasitic diseases. Aim of this study was to conduct a retrospective epidemiological analyis of the prevalence of enterobiasis among Bulgarian population over the period 2010-2019.

Methods: Collected data on the reported cases in the country were used. Statistical indicators as prevalence, mean and confidence interval were determined.

Results: Data from the parasitological study of 4,886,389 individuals during the period 2010-2019 showed the presence of this parasite in 47,793 (0.98%) of them. Of the total positive results, children and adolescents were 50.5%. The largest number of infected was in the age group of 6-7 year olds (72%). The studied period is characterized by a gradual increase in the relative part of infected population from 0.74% in 2010 to 1.66% in 2019. A retrospective study shows that enterobiasis is registered in all 28 districts of Bulgaria.

Conclusions: Our results show the need to improve the health culture of the population and take action to reduce the spread by optimizing measures for surveillance and control of enterobiasis.
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Keywords: Enterobiasis,, Prevalence, retrospective analysis

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Topic: *AS02.3 Intestinal parasitic diseases*

A GENERAL FRAMEWORK TO SUPPORT COST-EFFICIENT SURVEY DESIGN CHOICES FOR THE CONTROL OF SOIL-TRANSMITTED HELMINTHS WHEN DEPLOYING KATO-KATZ THICK SMEAR

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Introduction: To monitor and evaluate soil-transmitted helminths (STH) control programs, the World Health Organization (WHO) recommends screening a single stool sample from 250 children across 5 schools (50 subjects per school) by deploying a single Kato-Katz thick smear (KK). However, whether these recommendations are sufficient to make adequate decision-making in low-intensity settings remains unclear.

Methods: We developed a general framework that allows for varying sources of variation in egg counts across schools, between individuals, within individuals (day-to-day variation), and between repeated smears, for each of the 3 STH species (*Ascaris, Trichuris,* and *Hookworm*). Then we determined the survey designs (number of schools, subjects per school, samples per subject, and smears per stool sample) that allowed for adequate decision-making using a lot quality assurance sampling approach around the 2% prevalence of any or moderate-to-heavy intensity (MHI) infections. Finally, we estimated the total operational cost for each of the different survey designs

Results: The required survey design to make adequate decision-making varies across STH species, and the total sample size decreases as a function of increased number of samples and smears per sample. Deploying duplicate KK and sampling 5 schools (50 and 64 subjects per school for 2% prevalence of any and MHI infections, respectively) was the most cost-efficient survey design for monitoring and evaluation of STH in low-intensity infections

Conclusions: We confirm that KK remains valuable for the STH program's endgame, though it is recommended to sample at least 64 subjects per school and deploy a duplicate KK on a single stool sample.

Keywords: moderate-to-heavy intensity, lot quality assurance sampling, survey design, Soil-transmitted helminths, Kato-Katz thick smear

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Topic: *AS02.3 Intestinal parasitic diseases*

FUNCTIONAL CURE OF EXPERIMENTAL DIGESTIVE CHAGAS DISEASE WITH TRYPANOCIDAL BENZNIDAZOLE CHEMOTHERAPY

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Introduction: Digestive Chagas disease (DCD) is caused by infection with Trypanosoma cruzi which results in damage to the enteric nervous system and gastrointestinal (GI) dysmotility. The lack of a robust, predictive animal model has held back research. The aim of this study was to establish DCD models to investigate of neuropathological changes, including functional impairment of GI transit. We also tested the hypothesis that benznidazole-mediated cure of infection translates into alleviation of DCD pathology.

Methods: Using the carmine red dye tracer, we determined total gut transit time in concert with real-time infection imaging of bioluminescent parasites in C3H/HeN mice. We then used immunofluorescence assays to analyse T. cruzi infection induced neuro-glial pathological alterations in the ENS.

Results: We established C3H mice infected with the TcI-JR strain as a robust model of chronic digestive transit dysfunction, which shows a significant delay in GI transit time. This model also exhibited significant faecal retention and recurrent bioluminescent foci in the colon, corroborating the colon as a specific GI region of parasite persistence. The colon was also a site of dramatic loss of myenteric neurons. Sterilisation of infection by early treatment resulted in sustained and complete reversal of GI transit delay, accompanied by enteric neuronal repair. However, late treatment only led to partial reversal of the DCD phenotype.

Conclusions: The experimental tractability of C3H:TcI-JR model together with pathological aspects represent an innovative platform to study DCD pathogenesis. Our data prove that pathogenesis is sustained by T. cruzi infection and can be interrupted by curative anti-parasitic chemotherapy with an early therapeutic intervention.

Keywords: Trypanosoma cruzi, Enteric nervous system, host-pathogen interactions, Mouse model, Digestive Chagas disease

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Topic: *AS02.3 Intestinal parasitic diseases*

PREVALENCE OF PARASITIC INFECTIONS AMONG PEOPLE HAVING CLOSE CONTACT WITH ANIMALS OF RURAL AREAS OF DISTRICT FAISALABAD, PAKISTAN

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Introduction: In poor and middle-income nations, intestinal and zoonotic parasitc diseases are still a public health issue.

Methods: A total of 360 samples were obtained from persons who had intimate contact with animals (shepherds, butchers, dairy farmers, pet cat and dog owners). 180 stool and 180 blood samples were collected from the same individuals from July 2020 to July 2021. These samples were examined by direct saline, iodine wet mount, flotation, sedimentation methods and ELISA.

Results: Out of 360 samples 57 (16%) individuals were positive and 303 (84%) were negative for parasitic infections. 43 (24%) were found infected with one or more than one intestinal protozoans from stool samples, while 14 (9%) were infected from toxoplasmosis from blood samples. 9 (19%) of the participants were infected with single parasite and 34 (81%) were infected with multiple infections. The prevalence of Entameoba histolytica (19.3%), Ascaris lumbricoides (17.5%), Giardia lamblia (18.4%), Crytospordium parum (11.4%), Strongloides stercoralis (4%), Blastocystis hominis (11.4%), Trichuris trichiura (3.5%), Ancylostoma duodenale (11%) and Echinococcus granulosus (3.5%) were detected. The adults were marginally more parasitized than children. The males were more infected than females. Significant association was found among the all groups for parasitic infections. However, shepherds were more infected than butchers and dairy farmers

Conclusions: Due to comparative based approach in different people having contact to animals the present study is of particular importance and interest. Improvement of personal hygiene and awareness leads to reduce the parasitic infection among population of rural areas.

Keywords: Shepherds, infection, ELISA, Parasites

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Topic: *AS02.3 Intestinal parasitic diseases*

RISK FACTORS ASSOCIATED WITH INTESTINAL PATHOGENIC PARASITES AMONG SCHOOL CHILDREN IN LAHORE, PAKISTAN

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Introduction: Intestinal parasites are the main cause for morbidity and deaths all over the world especially in the third world. Lack of education, poverty, drinking water, poor sanitation, unclean, hot and moist environment are the conditions responsible for these parasitic illnesses. Poor personal hygiene among children is measured an effective reason of parasitic invasion

Methods: A total of 150 faecal samples were collected from the children of 3 to 15 years of age belonging to Government and Private Schools. Various techniques like Sedimentation technique, Direct smear method, Formalin ether concentration technique, and McMaster were used to identify diverse stages of intestinal parasites. Different stages of parasites were recognized by these techniques.

Results: The parasites found were Giardia lamblia (4.65%), Ascaris lumbricoides (4.66%), Entamoeba histolytica (3.3%), Taenia saginata (4%), Hymenolepis nana (2%), Enterobius vermicularis (4%) and Trichuris trichura (2.66%). A. lumbricoides was found to the most frequent of all parasites.

Conclusions: The prevalence was found to be more among the individuals, with poor hygiene, having lack of education especially of mothers because they play a big role in child's upbringing and maintaining his good health. Educating cleanness alertness on parasitic diseases and request of helpful strategies for parents to raise socioeconomic circumstances may decline the load of infection.

Keywords: Intestinal Parasites, associated risks, personal hygiene

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IMPACT OF ANTHELMINTIC TREATMENT ON THE BURDEN OF HELMINTH INFECTIONS IN PRIMARY SCHOOL CHILDREN IN BIYELA HEALTH ZONE IN KINSHASA, DEMOCRATIC REPUBLIC OF THE CONGO

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Introduction: The study evaluated the impact of anthelminthic, given as non-investigational drugs, on helminths infection, anemia, and hemoglobin (Hb) level within the clinical trial whether antimalarial drugs were also evaluated as an intermittent preventive treatment strategy in schoolchildren in the Democratic Republic of Congo (DRC).

Methods: In a nested cohort study, 616 asymptomatic children were enrolled and follow-up from November 2012 to November 2013. They received 1 dose of PZQ and ALB at baseline and then 2 doses of ALB at 4 months intervals. During the 12 months of follow-up, stool and urine samples were collected for helminth infections diagnosis and finger prick blood for Hb level determination. Paired test were used to compare the status before and after treatment, and confounding variables for Hb level were tested by multiple linear regression analysis.

Results: At baseline, the prevalence of helminth infections and anemia were 39.2% (95%CI: 34.7-43.7), and 41.8% (95%CI: 37.3-46.3), respectively. Mean Hb level was 11.6g/dl±1.3. After 12 months post-anthelminthic treatment, helminth infections reduced to 7.2% ($p<0.0001$). There was no change in Hb level and anemia in the control which received only the anthelminthic drug ($p=1.00$ and $p=0.26$, respectively) at 12 months, compared to those who received active antimalarial Sulfadoxine Pyrimethamine (SP) ($p=0.02$ and $p=0.09$, respectively) and SP+Piperaquine (PQ) ($p=0.01$ and $p<0.0001$, respectively). Similarly, no difference in Hb level was observed among the infected and uninfected schoolchildren at 12 months after anthelminthic treatment.

Conclusions: These findings suggest that anthelminthic treatment reduces significantly the prevalence of helminth infections. But there was any impact on anemia and Hb level.

Keywords: helminth infections, anthelminthic, Hb level, Schoolchildren, Democratic Republic of the Congo

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ACCURACY OF A RECOMBINANT-ANTIGEN IMMUNOCHROMATOGRAPHIC TEST FOR DETECTION OF STRONGYLOIDES STERCORALIS INFECTION IN MIGRANTS FROM SUB-SAHARAN AFRICA

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Introduction: Strongyloides stercoralis infection can cause relevant morbidity and lead to death immunocompromised individuals. Its diagnosis is challenging due to the absence of a diagnostic gold standard. The infection is highly prevalent in migrants from endemic countries in tropical and subtropical areas, and a rapid diagnostic test would be helpful for screening activities. Aim of this study was to estimate the accuracy of a novel immunochromatographic test (ICT) based on the recombinant antigen NIE for the diagnosis of S. stercoralis infection.

Methods: We tested the ICT in a cohort of well-characterized frozen sera available in the biobank of a referral hospital for parasitic diseases in Italy. We included sera from migrants from sub-Saharan Africa, that had matching results for agar plate culture (APC) and/or PCR for S. stercoralis, plus results of a commercial ELISA and an in-house immunofluorescence test (IFAT) for strongyloidiasis. Two blinded readers independently read the ICT, and a third one was involved in case of discrepant results. The accuracy of the ICT was assessed both against the panel of fecal test results and with latent class analysis (LCA).

Results: Agreement between readers was excellent (Cohen's kappa=92.7%, 95%CI 88.3%-97.1%). When assessed against the results of the fecal tests, the sensitivity and specificity of the ICT were 82.4% (95%CI 75.7%-89.0%) and 73.8% (95%CI 66.8%-80.9%), respectively. With LCA, sensitivity and specificity were 86.3% (95%CI 80.1%-92.5%) and 73.9% (95%CI 67.0%-80.8%), respectively.

Conclusions: The results of the ICT demonstrated easy to be interpreted. Accuracy proved good, though sensitivity might be further improved for screening purposes.

Keywords: rapid diagnostic test, immunochromatographic test, recombinant antigen, strongyloides, strongyloidiasis

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THE ROLE OF SIRTUINS IN THE ENTAMOEBA DEVELOPMENT

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Introduction: The protozoan parasite *Entamoeba histolytica* the causative agent of amoebiasis, infects about 50 million people annually. This parasite has a life cycle that alternates between two stages: a cyst which is the infective stage and trophozoite, the invasive stage. Encystation is an important process in the biology of the parasite, however, the molecular mechanisms, particularly epigenetic mechanisms are not completely understood. Sirtuins are class III histone deacetylases (HDACs) proteins and have been implicated in key cellular processes, including cell survival, autophagy, apoptosis, gene transcription, DNA repair, stress response, and genome stability. Our aim is to determine the participation of sirtuins in the *Entamoeba* development and stress response.

Methods: Bioinformatic search using sirtuin sequences from human as a query was performed. Expression of *E. invadens* sirtuins under different stress conditions was determined by RT-PCR. Sirtuins genes that showed higher expression were selected for the over-expression by make Myc-tagged construct in an *Entamoeba* expression vector and generate stable parasite transfectants. The effect of overexpression of these genes will be evaluated by assaying encystation efficiency and phenotypes of altered cyst maturation and cyst morphology.

Results: We identified from the genome sequence of *E. invadens* 6 genes that contain a conserved catalytic domain of sirtuins. RT-PCR experiments showed a differential expression between trophozoites and cysts for 3 genes called EiSir2a, EiSir2c, and EiSir2f. Currently, the role of overexpression is being determined.

Conclusions: *E. invadens* possess 3 sirtuins genes differentially expressed during encystation and can play an essential role as a regulator of this process.

Keywords: Entamoeba, Sirtuins, Encystation, Epigenetic regulation

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CAN WE DISTINGUISH PATHOGENIC FROM NONPATHOGENIC ENTAMOEBA?

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Introduction: Amebiasis is a significant cause of diarrhea in low- and middle-income countries, in travelers and increasingly is recognized as a sexually transmitted disease. Identification of Entamoeba species is typically achieved by microscopic examination of stained slides. However, microscopic examination cannot distinguish between Entamoeba histolytica, which is pathogenic, and Entamoeba dispar, which has been considered nonpathogenic.

Methods: In order to differentiate the two species, we developed and validated real-time PCR assays. We utilized the assay to detect Entamoeba in patients experiencing diarrheal illness for whom amebiasis had either been diagnosed or was suspected.

Results: Surprisingly over a 5-year period 92% of specimens that tested positive for intestinal amoeba contained E. dispar and not E. histolytica. We also expanded our molecular testing to include E. hartmanni, E. coli as well as Blastocystis. Comparing results from molecular detection to microscopy identification based on morphology we find that reported characteristics, particularly expected size of the parasite, do not always match the reported ranges.

Conclusions: Thus, we find molecular detection is not only more sensitive and more specific than microscopy but also may reveal important information about pathogenicity.

Keywords: Microscopy, Entamoeba, Molecular assays, Clinical parasitology

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COMPARISON OF MICROSCOPIC METHODS AND QPCR FOR QUANTITATION OF HOOKWORM INFECTIONS IN AN ENDEMIC COMMUNITY IN SOUTHERN INDIA

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Introduction: Detection of soil transmitted helminths (STH) is mostly based on microscopic methods. However in low intensity infections or infections with zoonotic species and due to variable egg excretion rates, microscopy-based methods are sub-optimal. Here we compare microscopy-based methods with qPCR and its ability to quantify infection intensity.

Methods: Stool samples (n=52) from a community survey that were hookworm positive by microscopy, were tested by Kato-Katz, McMaster and a multi-parallel qPCR targeting non-coding repetitive sequences of N. americanus and A. duodenale.

Results: Eggs per gram (EPG) detected by Kato Katz and McMaster methods showed a good correlation (Lin's concordance correlation coefficient 0.98, 95% CI 0.97-0.99). A good linear correlation of EPG by both methods with DNA concentration (based on Ct values) was seen (Spearman's rho (ρ)=0.81, p<0.001 and 0.78, p<0.001 for Kato Katz and Mcmaster respectively). Comparison of Ct values and EPG between light and moderate to heavy intensity (MHI) infections was not possible due to the small number of MHI infections. When samples were divided into two groups with EPG <500 and >500, the range of Ct values was higher for samples with EPG <500 (n=36) (mean Ct 21.6, SD 4.1; median 20.1, range 17.8-37.5) when compared to samples with EPG >500 (n=16) (mean Ct 17.3, SD 1.2; median 17.3, range 15.3-19.7) with some overlap (Independent t test, p <0.001).

Conclusions: Quantitation of hookworm infection intensity by qPCR was comparable to Kato-Katz and McMaster methods but further optimization taking into account the developmental stages present in stool samples, DNA yield, and copy number of the gene target is required prior to deployment in community based surveys.

Keywords: Soil Transmitted Helminths, McMaster, Kato Katz, qPCR

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INHIBITION OF ENTAMOEBA HISTOLYTICA UDP-GLUCOSE 4-EPIMERASE (GALE) - THE BRIDGE BETWEEN GLYCAN BIOSYNTHESIS AND ENERGY METABOLISM

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Introduction: In the life of the anaerobic Entamoeba histolytica (E.h.), which causes amoebic dysentery and liver abscess, simple sugars and complex glycans play a major role in attachment or generation of energy. The UDP-glucose 4-epimerase (GalE) serves as a link between the galactose (Gal) and glucose (Glc) worlds. Thus, the aims of our work were to characterise this enzyme´s kinetics, and to study the effect of ebselen, ethacrynic acid and diethylstilbestrol, which are GalE inhibitors in Trypanosoma brucei.

Methods: Recombinant E.h. GalE was expressed, purified and its activity was measured with a spectrophotometer. Substrate specificity and inhibition of GalE was studied using reverse-phase HPLC. The effect of the three inhibitors against the trophozoites was measured in a 96-well plate under anaerobic conditions.

Results: We determined the kinetic constants of E.h. GalE and characterised its temperature and pH dependency. GalE was able to epimerise UDP-Glc to UDP-Gal, and in addition UDP-GlcNAc to UDP-GalNAc. All inhibitors suppressed the growth of E.h. trophozoites; however, only ebselen inhibited the recombinant E.h. GalE.

Conclusions: Our work underlines the importance of GalE in the sugar metabolism of E.h.. The ability to epimerise also UDP-GalNAc to UDP-GlcNAc could help the amoebae to generate chitin for the cyst wall. Furthermore, UDP-Gal can be used for the biosynthesis of glycans. Ebselen inhibits GalE and growth of the trophozoites, thus constitutes a potential candidate for anti-amoeba chemotherapy.

Keywords: Entamoeba histolytica, galactose metabolism, UDP-glucose 4-epimerase, Ebselen, anti-amoeba chemotherapy

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CHARACTERIZATION OF ENTAMOEBA HISTOLYTICA KERP2 AND ITS POTENTIAL APPLICATION AS A CELL-PENETRATING PEPTIDE IN HUMAN CELLS

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Introduction: A unique protein family in Entamoeba histolytica, lysine and glutamic-acid rich proteins (KERPs) family, were identified due to their interaction with the brush border of the human intestinal epithelium cells. Among three KERPs, KERP1, has been proven critical in amoebic virulence by improving the formation of liver abscesses. However, the functions of the rest of KERPs, which may also play some vital roles in amoebic infection, remain unknown. Our research focuses on KERP2, which has an internalization behavior in mammalian epithelium cells and macrophages, and aims to utilize it as a cell-penetrating peptide for the drug delivery system.

Methods: The methods in this study include protein structure modeling, recombinant protein synthesis and purification, cDNA preparation, polymerase chain reaction, quantitative real-time PCR, growth-kinetics analysis, indirect fluorescent assay, live imaging, and flow cytometry assay.

Results: Protein modeling revealed a structurally conserved SAP domain in KERP2, suggesting its potential capacity for DNA binding. Our data revealed that KERP2 located in E. histolytica nucleus could be secreted upon interaction with Caco-2 cells and finally be internalized in Caco-2 cells. Indeed, the KERP2 fragment close to the N-terminus was demonstrated to be involved in parasite-host interaction, while C-terminus is related to E. histolytica nucleus translocation.

Conclusions: In our study, KERP2 and its penetrating peptide have been shown to interact with mammalian cells. However, the physiological function and traffic mechanism of KERP2 and its downstream effect on human cells are still unclear. Further investigation will mainly focus on the physiological roles of KERP2 that may benefit E. histolytica in the host intestine.

Keywords: Parasite-host interaction, Cell penetrating peptides, intercellular communication, Gene regulator

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GENOTYPE CHARACTERISTICS OF GIARDIA DUODENALIS IN PATIENTS USING HIGH RESOLUTION MELTING ANALYSIS TECHNIQUE IN KHORRAMABAD, IRAN

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Introduction: We aimed at genotyping and evaluating the predominance of G. duodenalis assemblages isolated from patients referred to medical laboratories in Khorramabad, Iran from Nov 2015 to Sep 2016. Hence, the development of a cost-effective HRM approach to determine genotypes of G. duodenalis based on the triosephosphate isomerase (tpi) gene was examined and the genotyping results with and without diarrhea was compared.

Methods: Seventy G. duodenalis positive fecal samples were collected. A microscopic confirmation for the presence of Giardia spp. was performed, cysts of 70 Giardia spp. positive specimens were concentrated using sucrose flotation technique and sucrose solution PCR amplification was performed on 69 of 70 (98.5%) samples, and High Resolution Melting (HRM) analysis was performed using a software.

Results: The results showed two distinct genotypes (assemblages A and B) of G. duodenalis but infections with mixture of both assemblages were not detected. The genotypes of G. duodenalis showed that the sub assemblage AI, BIII and BIV were present in a proportion of 68.1%, 20.3% and 11.6% respectively in samples. Assemblage AI was significantly ($P<0.05$) more frequently found in patients with diarrhea.

Conclusions: The sub-assemblage AI, BIII, and BIV are more zoonotic potential. According to the comparison of the results of this study with the results of previous studies in this area and around of it, as well as the way people live and keep pets. This pattern established in Khorramabad city. HRM can be an ideal technique to detect and genotyping of G. duodenalis in clinical samples

Keywords: Triosephosphate isomerase gene, High resolution melting, Giardia duodenalis, Genotyping

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Topic: *AS02.3 Intestinal parasitic diseases*

CLINICAL ASPECTS OF BEEF TAPEWORM INFECTION

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Introduction: In Bulgaria sporadic cases of Taenia saginata /beef tapeworm/ infection are observed in humans in areas with developed cattle breeding. The timely diagnosis and effective treatment of patients, health education and sanitary control of beef are essential to decrease its incidence. The aim of the study was to analyze the clinical manifestations of beef tapeworm infection and the effectiveness of treatment with anthelmintics niclosamide and praziquantel.

Methods: A total of 52 patients with T. saginata infection (18-72 years old) were included in the study for a 15-year period. A half of them took praziquantel 600 mg/once, the others took niclosamide 2 g/once. All of the patients took magnesium sulfuricum 25 g as a purgative 8 hours after the intake of these drugs. The follow up lasted 6 months.

Results: Patients sought medical help due to observations of tapeworms proglottides in the feces and underwear, and also complaints of abdominal discomfort. Laboratory tests showed mild anemia in 5% of the patients, allergic symptoms (itchy rash) in 18%, eosinophilia in 32%. Both niclosamide and praziquantel were effective in all cases. After taking a purgative, the whole strobilae of tapeworms were expelled. No recurrences were registered.

Conclusions: The symptoms like anemia, allergies and abdominal discomfort are transient but passage of active segments is a constant. The treatment is maximally effective when magnesium sulfuricum is taken after anthelmintics.

Keywords: anthelmintics, Cestodes, beef tapeworm

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Topic: *AS02.3 Intestinal parasitic diseases*

RELATION BETWEEN LOA LOA MICROFILAREMIA AND CLINICAL MANIFESTATIONS IN FIVE ONCHOCERCHIASIS COMMUNITIES IN SOUTH OF GABON

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Introduction: Loiasis is a chronic disease caused by Loa loa filarial species prevalent in central and West Africa. To estimate the risk of occurrence of serious side effects in case of mass treatment with ivermectin, relationships between microfilaremia and clinical symptom were analysed in onchocerciasis

Methods: Prospective study was conducted between january and febrary 2020 in five communities in south of Gabon. Volunteer's blood and stool were collected to isolate microfilariae and intestinal parasites with direct microscopic examination. Onchocerciasis IgG4 were detected using rapid Ov16 tests. Data were recorded in an Excel spreadsheet and analyzed using the Statview 5.0 software. Statistical significance was set at $p<0.05$ for all analysis.

Results: Of the 471 participants examined, 338 (71.8%) were infected by at least one parasite. L. loa microfilaria was identified in 16.8% (n=79) of individuals. The median L. loa density was 800 [interquartile range 200-5200]. Pruritus was the most common (60.5%, n=285). Adult worm in eye and Calabar swelling were found in 24.2% (n=114) and 23.1% (n=109) of participants respectively ($p<0.01$). Prevalences of pruritus and adult worm in eye were more frequent in patients with L. loa microfilaremiae ($p<0.01$).

Conclusions: Prevalences of L loa in the present study and antecedent of worm migration in eye were below the threshold at risk of occurrence of serious side effects after mass treatment with ivermectin as defined by RAPLOA. To reduce the burden of parasitic infection in rural area mass drog administration should be considered.

Keywords: Loa loa, STH, Pruritus, Adult worm in eye, Onchocerciasis

P206 / #108

Topic: *AS02.3 Intestinal parasitic diseases*

THE METHYLTRANSFERASES EHPRMTA, EHPRMT5 AND EHPKMT2 PARTICIPATE IN THE STRESS RESPONSE AND IN VITRO VIRULENCE OF ENTAMOEBA HISTOLYTICA

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Introduction: Protein methylation is a posttranslational modification (PTM) involved in the stability, localization and interaction of cellular proteins with their binding partners. Arginine and lysine methylation regulate epigenetics and several cellular signaling pathways. Entamoeba histolytica, the etiologic agent of human amebiasis, has five PRMTs and four PKMTs, whose recombinant proteins transfer methyl groups to commercial histones, suggesting that they perform arginine and lysine methylation in the parasite chromatin, which may participate in epigenetics. In addition, EhPRMTs and EhPKMTs are also found in the cytoplasm of trophozoites, suggesting that they might regulate other cellular processes. However, the role of these proteins in the parasite biology remains unknown until now.

Methods: The expresion and localiztion of EhPRMTA, EhPRMT5 and EhPKMT2 in trophozoites under different conditions was analyzed by Western blot and immunofluorescence. In addition, we investigated the effect of their knockdown by RNA antisense on the viability of trophozoites subjectd to these enviroments and on their in vitro virulence.

Results: The expression level and localization of the analyzed enzymes changed in trophozoites subjected to thermal, oxidative and nutritional stress, as well as during phagocytosis, a virulence-related property of E. histolytica. Furthermore, knockdown of EhPRMTA and EhPKMT2 affected rate growth, survivel under stress conditions and the in vitro virulence of trophozoites.

Conclusions: Results indicate that arginine and lysine methylation by EhPRMTA, EhPRMT5 and EhPKMT2 may control epigenetics and/or signaling pathways that affect the survival and virulence of E. histolytica, making them attractive drug targets against amebiasis.

Keywords: Entamoeba histolytica, Arginine Methyltransferases, Lysine Methyltransferases, stress response, in vitro virulence

P207 / #1653

Topic: *AS02.3 Intestinal parasitic diseases*

FIBRILLIN IS A CRYPTOSPORIDIUM PARVUM OOCYST WALL PROTEIN: TO THE PERIPHERY AND BEYOND

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Introduction: *Cryptosporidium* is a major diarrhoeal parasite in young children without access to clean drinking water. Transmission occurs by ingestion of environmentally resilient oocysts, which can persist in the environment for months. Oocysts can survive chlorination-based water disinfection, protected by their double-layered oocyst wall. Despite the key role in transmission, the proteins which make up the oocyst wall are poorly characterised.

Methods: Our work describes a new oocyst wall protein, named fibrillin. A proteome of the wall identified fibrillin as a highly enriched wall protein. We hypothesise fibrillin is a structural oocyst wall protein, required for correct formation of the oocyst wall. Using CRISPR/Cas9 we generated fibrillin-tagged transgenic parasite strains and observed oocyst development in intestinal organoids by immunofluorescence and immune-electron microscopy.

Results: Fibrillin was confirmed as an inner oocyst wall protein. Expression was restricted to female parasites (which develop into oocysts) and oocysts. During oocyst development fibrillin localised within the wall forming bodies (WFBs) which aligned to the periphery and secreted to form the wall after zygote formation. Essentiality of fibrillin was investigated by attempting a CRISPR/Cas9 directed knockout; preliminary data suggests loss of fibrillin does not affect parasite survival.

Conclusions: We confirm that fibrillin is an excellent marker of oocyst wall formation and will further our understanding of wall structure and synthesis.

Keywords: Cryptosporidium, Oocyst, transmission, organoids, CRISPR

P208 / #1011

Topic: *AS02.3 Intestinal parasitic diseases*

A POSSIBLE ROLE OF EXOSOMAL HYDROLASE RECEPTORS IN THE PATHOGENESIS OF THE HUMAN INTESTINAL PARASITE ENTAMOEBA HISTOLYTICA

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Introduction: Exosomes are 30 to 100 nm vesicles derived from endocytic compartments that are secreted extracellularly. Pathogenic organisms release exosomes mainly for intercellular communication, immune modulation, and virulence.

Methods: Here we describe isolation, morphological and functional characterization of the exosomes and two membrane-spanning proteins from the human intestinal parasite Entamoeba histolytica.

Results: Negative staining transmission electron microscopy analysis of the extracellular vesicles purified from culture supernatant by sucrose gradient fractionation, verified the presence of exosomes of about 100 nm diameter. The proteome analysis of exosome-enriched fraction revealed several membrane-spanning proteins including the cell surface metalloprotease GP63-2 and two members of the cysteine protease binding protein family (CPBF1 and CPBF8) to name a few. Amoebic CPBFs are hydrolase receptors that are functionally similar to sortilins. The detection of CPBF1 and 8 in exosomes is intriguing as these proteins are reported to be receptors of degradative enzymes (cysteine proteases, amylases, b-hexosaminidase and lysozymes) that allow their targeting to the lysosomes. Expression of HA-tagged CPBF1 and CPBF8 confirmed the presence of these membrane proteins in crude extracellular vesicle fractions. We also confirmed by blue-native PAGE analysis that CPBF1 forms a ~200 kDa complex, suggesting it may form dimers similar to what has been reported in exosomal sortilins in mammals.

Conclusions: We are currently determining potential secondary ligands of the exosomal CPBFs in order to shed light on the role of CPBF-containing exosomes in the pathogenicity of E. histolytica.

Keywords: exosomes, Pathogenesis, Entamoeba histolytica, extracellular vesicles, membrane proteins

P209 / #488

Topic: *AS02.3 Intestinal parasitic diseases*

GIARDIA DUODENALIS RESHAPES INTESTINAL MUCOSAL IMMUNITY TO PREVENT TISSUE DAMAGE AND ATTENUATE INFLAMMATORY-DRIVEN PROCESSES

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Introduction: Enteric infections that cause diarrheal disease are the second-leading cause of death in children worldwide. Recent epidemiological studies identified a decreased incidence and severity of life-threatening diarrhea in those children co-infected with the intestinal protozoan parasite Giardia duodenalis.

Methods: To determine how Giardia infection ameliorates tissue damage and reshapes mucosal immunity to confer a host protective effect, and demonstrate that this “protist” can function as a potent anti-inflammatory agent, we induced a Th1-driven/IFN-gamma-mediated lethal ileitis by co-infecting Giardia-infected mice with Toxoplasma gondii, an intestinal protozoan parasite that causes a Crohn’s disease-like enteritis.

Results: We show that Giardia induces a robust type-2 associated cytokine response, an antigen specific Th2 immune response within the lamina propria, and causes a precipitous expansion of IL-10-producing CD4 T cells, that protects the host from tissue damage. We found that the presence of Giardia significantly reduced Toxoplasma-mediated inflammation in the small intestine, by downregulating the frequency of $Tbet^{+}IFN\text{-}y^{+}Foxp3^{+}IL\text{-}10^{+}$ Th1 cells. Moreover, Giardia induced the expansion of IL-10 producing$ST2^{+}$CD4+ T cells expressing GATA-3, which downregulated Toxoplasma-driven IFN-y. Recombinant IL-10 injection only reduced the frequency of Th1 cells but was not sufficient to completely revert the histopathology induced during Toxoplasma infection, suggesting the importance of a cellular component.

Conclusions: Since Giardia induced a protective Th2 response, we are currently examining if Giardia colonization can confer protection to other inflammatory driven processes associated with, for example, DSS-induced colitis.

Keywords: Giardia duodenalis, mucosal immunity, inflammatory bowel disorders, Type 2 inflammation, Toxoplasma gondii

P210 / #502

Topic: *AS02.3 Intestinal parasitic diseases*

FAECAL MICROBIOTA IN BALB/C INTERFERON-GAMMA KNOCK-OUT MICE BEFORE AND AFTER PERORAL INOCULATION WITH TOXOPLASMA GONDII OOCYSTS

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Introduction: Little is known regarding the potential interplay between Toxoplasma gondii infection and faecal microbiota.

Methods: We collected 58 faecal samples from seven BALB/c interferon-gamma knock-out mice before and after peroral inoculation with Toxoplasma gondii oocysts, and from one mouse before and after peroral inoculation with Hammondia hammondi oocysts. The samples, 5−8 per mouse, were collected every other day, starting three days before the inoculations and until up to eleven days after inoculation. DNA was extracted and submitted to metabarcoding of small subunit ribosomal DNA for detection and differentiation of bacteria, fungi and parasites.

Results: Faecal samples from the mouse inoculated with H. hammondi showed a higher abundance of Bacteroidetes reads compared with the samples from mice inoculated with T. gondii throughout the study. All eight mice had a relatively stable faecal microbiota at the phylum level across the samples; however, in mice inoculated with T. gondii, the Firmicutes:Bacteroidota ratio changed from 1.6 to 2.6. No DNA of T. gondii nor of H. hammondi was detected in the faecal samples.

Conclusions: The observations from this study add to the knowledge on T. gondii infection and microbiota of the gastrointestinal tract.

Keywords: Toxoplasma gondii, faecal microbiota, Hammondia hammondi

P211 / #668

Topic: *AS02.3 Intestinal parasitic diseases*

THE TRANSCRIPTOME OF HUMAN INTESTINAL EPITHELIAL CELL LINE (CACO-2) EXPOSED TO THE EXOSOMES AND WHOLE LARVAE OF ANISAKIS SIMPLEX S. S.

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Introduction: Anisakis simplex is a parasitic nematode of marine organisms. Humans may be an accidental host for this species. The finding that parasitic nematodes can release exosomes was the breakthrough discovery. The secretion of exosomes as signaling molecules by parasitic nematodes has been poorly studied. This prompted us to characterize the transcriptome of host cells exposed to A. simplex exosomes as well as whole larvae.

Methods: Using pair-end RNA sequencing on an Illumina Novaseq™ 6000, the transcriptome of the human intestinal epithelial cell line CACO-2 was characterized, and differences were found between the transcriptomes of CACO-2 cultured with the exosomes of A. simplex (isolated by ultracentrifugation) and those cultured directly with whole larvae.

Results: An average of 67,000,000 reads were obtained from the generated cDNA libraries for each CACO-2 sample and assembled into ~280,000 transcripts. Using bioinformatics analyses, 157 upregulated and 160 downregulated differentially expressed genes (DEGs) were identified between CACO-2 cultured with and without whole larvae. Furthermore, 1,603 DEGs were identified between CACO-2 cultured with and without A. simplex exosomes. In addition, long noncoding RNAs were identified and the interactions between mRNAs and lncRNAs were characterised. It was also shown that CACO-2 cells responded to exosomes and whole larvae with differential changes in cytokine secretion ($p < 0.05$).

Conclusions: The obtained results will expand the existing knowledge about the role of exosomes in the host-parasite communication. Funding source: This work was funded by National Science Centre of Poland, grant no. 2019/33/N/NZ6/01353. R. S. is also a recipient of a scholarship from the UE, grant no. POWR.03.05.00-00-Z310/17.

Keywords: Transcriptomics, Anisakis simplex, CACO-2 cell line, exosomes

P212 / #68

Topic: *AS02.3 Intestinal parasitic diseases*

RILUZOL ANTIAMEBIC ACTIVITY AGAINST ENTAMOEBA HISTOLYTICA TROPHOZOITES.

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Introduction: Amebiasis is caused by the parasite Entamoeba histolytica (E. histolytica); approximately 50 million people may suffer from the disease. Metronidazole and its derivatives are the most effective drugs against this protozoan. However, it has been reported that compounds derived from nitroimidazoles cause genotoxic, teratogenic, mutagenic, and carcinogenic effects in animals, in addition to the resistance of trophozoites of E. histolytica to this drug. It has been shown that compounds derived from benzothiazoles (riluzole (6-(trifluoromethoxy) benzothiazol-2-amine), exhibit activity against some protozoal parasites. The aim of this study, is to evaluate for the first time, the in vitro antiamoebic activity of Riluzole on E. histolytica trophozoites.

Methods: Trophozoites were treated with different concentrations of Riluzole after 5 h, time viability was determined using the WST-1 cell proliferation reagent. The 50% inhibitory concentration (IC_{50}) was obtained by linear regression between the concentration of the compound and the percentage of inhibition. The cytotoxicity of benzothiazole in the cell line Vero was also evaluated.

Results: A decrease in amoebic viability was observed from the concentration of 30 µM to 480 µM, the latter being the one that presented a greater antiamoebic activity, observing around 51% of dead amoebae. The IC_{50} was 319.5 µM at 5 h. For the cytotoxicity assays, benzothiazole did not show any cytotoxic effect on the Vero cell line.

Conclusions: Our results suggest that riluzole could be an alternative treatment against E. histolytica.

Disclosure: This work was supported by SIP-IPN 20201063.

Keywords: Riluzole, Antiamebic activity, Entamoeba histolytica, benzothiazoles

P213 / #559

Topic: *AS02.3 Intestinal parasitic diseases*

GENETIC DIFFERENCES AMONG HUMAN TRICHURIS POPULATIONS WITH DIFFERING RESPONSES TO ALBENDAZOLE-IVERMECTIN COMBINATION TREATMENT.

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Introduction: Trichuris trichiura, one of the most important Soil Transmitted Helminth (STH) species, infects over 500 million people globally. Mass drug administration treatment programs use either albendazole or mebendazole but these benzimidazole drugs have very low efficacy against T. trichiura, with egg reduction rates (ERR) typically <50%. Combination albendazole-ivermectin treatment has shown considerable improvement in efficacy.

Methods: An albendazole-ivermectin combination treatment trial was performed in three regions – Laos, Tanzania, and Cote d'Ivoire, revealing high efficacy with ERR above 98% in Tanzania and Laos but much lower efficacy in Cote d'Ivoire with ERR below 70%. To explore whether this difference in efficacy could be due to genetic differences in the parasite populations, we performed Illumina short-read deep amplicon sequencing of multiple mitochondrial and ribosomal DNA loci on T. trichiura PCR positive fecal samples from the three regions.

Results: Primers targeting the mitochondrial nad1, nad4, cox-1, and the major β-tubulin gene generated haplotypes mapping to the appropriate reference sequences from all the samples from Tanzania and Laos, but not from Cote d'Ivoire. Phylogenetic analysis of the ribosomal ITS-1 and ITS-2 loci revealed that haplotypes from the samples in Cote d'Ivoire clustered separately from those found in Tanzania and Laos populations in a clade containing Trichuris sequences from non-human primates and Trichuris suis.

Conclusions: This study demonstrates that the Trichuris population in Cote d'Ivoire is genetically divergent to those from Tanzania and Laos, likely a cryptic species, and has a lower sensitivity to albendazole-ivermectin combination treatment.

Keywords: Trichuris, combination treatment efficacy, genetic diversity, amplicon sequencing

P214 / #1028

Topic: *AS02.3 Intestinal parasitic diseases*

MOLECULAR IDENTIFICATION OF INTESTINAL PROTOZOA IN HUMAN AND DOG IN BARRANQUILLA, COLOMBIA

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Introduction: Intestinal protozoans are an important health problem of worldwide distribution. Some protozoan parasites have zoonotic potential. Prevalence data and discrimination of genotypes for some protozoan parasites are scarce. This work aims to identify genotypes and species of two protozoan parasites in a population of Barranquilla, Colombia.

Methods: People who inhabit the neighborhood, Las Florez, in Barranquilla city participate in this work. This location is near to Magdalena river. People who gave informed consent were included in this study. All participants collect a fecal sample in a container delivered by investigators. Dogs samples were collected by participants with pets; also, investigators collect fecal samples in some streets of the neighborhood. First, we obtained a fecal sediment; then, rupture cyst with enzymatic, mechanical, and thermal shock was done. Sediment was used for DNA extraction. For genotyping of Giardia intestinalis, amplification of 41E-HP was done. For identification of amebas (E. histolytica; E. dispar; E. moshkovskii), we uses a multiplex PCR method.

Results: A total of 79 fecal samples were collected. According to the source: 75% were human and 25% were dogs samples. We could analyze 44 samples (24 humans and 20 dogs) for molecular identification. From humans, 45,8% were positive for Giardia intestinalis (6 for assemblage A and 5 for assemblage B). In relation with dogs, samples were positive in 15% (2 assemblage B and 1 assemblage A). On the other hand, amebas were identified in 6,81% of samples (2 for E. histolytica and 1 for E. dispar)

Conclusions: Molecular tools are important to the identification of parasites, especially protozoa. Protozoan parasites are present in humans and dogs and its important for zoonotic potential.

Keywords: Molecular identification, Entamoeba spp., protozoans, Genotyping, Giardia

P215 / #1122

Topic: *AS02.3 Intestinal parasitic diseases*

DIVERSIFICATION AND ISOTYPE-SPECIFIC FUNCTIONS OF VPS35-1 AND VPS35-2 OF THE RETROMER COMPLEX IN THE PARASITIC PROTOZOAN ENTAMOEBA HISTOLYTICA

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Introduction: Entamoeba histolytica is the unicellular parasite responsible for human amoebiasis and remains as one of the top three parasitic causes of mortality worldwide. Host cell attachment, phagocytosis/trogocytosis, vesicular traffic and secretion of cytolytic factors are mainly involved in amoebic pathogenesis. The retromer complex is involved in the retrograde transport of a variety of carriers/receptors from endosomes to the Golgi or the plasma membrane. The retromer is composed of the core complex including Vps26, 29, and 35. Unlike other organisms, the genome of E. histolytica encodes a large panel of multiple isotypes for Vps26 and Vps35 possesses several retromer complexes which include different isotypes of core components (6 x Vps26 and 5 x Vps35 in E. histolytica; one each in S. cerevisiae; 2 x Vps26 in H. sapiens; 2 x Vps26 and 3 x Vps35 in A. thaliana). Although it has been shown that two human Vps26a/b isotypes specifically bind to different proteins, the specific role of each isotype remains unclear. In this poster, we focused on characterization of two Vps35s, Vps35-1 and Vps35-2, because Vps35 is important for cargo selection and they showed high robust mRNA expression.

Methods: Intracellular and secreted protease activity and endocytosis efficiency of HA-Vps35-1 and HA-Vps35-2 overexpressing strains were quantified.

Results: the intracellular and secreted protease activity was increased in HA-Vps35-2 expressing, but not HA-Vps35-1 expressing, strains. On the contrary, endocytosis of the fluid-phase marker was decreased in both HA-Vps35-1 and HA-Vps35-2 expressing strains.

Conclusions: These data clearly demonstrated that two Vps35 isotypes have different roles in the oligomeric complex formation of the retromer, endocytosis, and secretion.

Keywords: membrane traffic, retromer, Entamoeba histolytica

P216 / #1651

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

INVESTIGATING THE EFFECT OF THE NITRIC OXIDE DONOR L-ARGININE ON ALBENDAZOLE EFFICACY IN TRICHINELLA SPIRALIS-INDUCED MYOSITIS AND MYOCARDITIS IN MICE

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Introduction: *Trichinella spiralis* is a unique nematode that occupies muscle cells transforming them into nurse cells, stimulating an immune response with mixed cytokine state and nitric oxide (NO) production. Arginine, a precursor of nitric oxide, is a widely used nutritional supplement with suggested immune-enhancing and wound-healing properties. The current study has been designed to assess the effect of L-arginine, on the muscular phase of experimental trichinellosis in mice with and without albendazole (ALB) administration.

Methods: The study included five groups; negative non-infected control group (G1); positive infected non-treated control group (G2); ALB-treated group (G3); L-arginine-treated group (G4); and combined ALB/L-arginine regimen group (G5). Study parameters included larval count, histopathological changes, serum NO, NO synthase (iNOS) immunohistochemical expression and TNF-α and IFN-γ gene expression in infected muscle tissue.

Results: Compared to G2, larval count was reduced in G3 and G5, by 95.5% and 69,9%, respectively, while increased in G4 by 51.8%. Histopathological examination of G5 demonstrated partial larval degeneration with mononuclear inflammatory cells infiltration of the capsule, and structural improvement of muscle architecture compared to G3. Serum NO and tissue iNOS, TNF-α and INF-γ were higher in G5 in comparison to G3.

Conclusions: The regenerative effect of L-arginine on muscle fibers and its possible cardioprotective effect advocates this amino acid as a beneficial supplement in chronic *Trichinella*-induced myositis and myocarditis, however, its effect on the parasite is questionable. The fact that it increases the muscle parasite load warrants further investigation to clarify its relative importance.

Disclosure: This abstract is fully based on original article accepted for publication in the Parasitologists United Journal (Online ISSN: 2090-2646), Vol. 15, No. 1, April, 2022 (Acceptance date 28 March 2022).

Keywords: Trichinosis, Nitric oxide, L-arginine, Albendazole, inflammation

P217 / #370

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THYMUS DAENENSIS CELAK. AND THYMUS VULGARIS L. ESSENTIAL OILS: IN VITRO EFFECTS ON PROTOSCOLICES OF ECHINOCOCCUS GRANULOSUS

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Introduction: Access to natural drugs that can have a significant effect on human hydatidosis has made it possible to use ultrasound and CT scans to empty and inject the scolex killer and re-drain through the skin or PAIR hydatid cyst pharmacotherapy has grown a lot in the world today. In this study, the lethal effect of essential oil of daenensis thyme (Thymus daenensis Celak) and garden thyme (Thymus vulgaris L.) on hydatid cyst protoscolices were performed in vitro.

Methods: In this experimental study, at first, infected sheep livers were collected from Yasuj slaughterhouse. Thymus daenensis Celak. and Thymus vulgaris L. EO was obtained and processed in the Medicinal Plants Research Center of Yasuj University of Medical Sciences laboratory. Also, the major compounds of plant EO were determined by GC-Mass spectrometry. Then, the in vitro protoscolicidal percentage of EO of them at concentrations 10, 5, 2.5, 1.25, and 0.625 mg/ml in durations of 3, 5, 10, 15, 30, and 60 Min was determined. The collected data was analyzed using SPSS software version 21 through descriptive and inferential statistics with a 95% confidence level.

Results: The number of constituents in Thymus daenensis Celak. EO is 20 compounds, the highest percentage of active ingredients is related to thymol (61.91%). Thymus vulgaris L. EO was 16 compounds, the highest percentage of active ingredients was thymol (53.08). Porotoscolicidal effect for Thymus daenensis Celak. and Thymus vulgaris L. was 2.5 mg/ml which caused 100% death of protoscoleces in 3 Min and 1 Min, respectively.

Conclusions: The data suggest that Thymus daenensis Celak. (daenensis thyme) and Thymus vulgaris L. (garden thyme) EO have an excellent potential scolicide in vitro; however, further investigations are required to determine its efficacy in vivo.

Keywords: Thymus daenensis Celak., Thymus vulgaris L., scolicidal activity, hydatid cyst

P218 / #1422

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

LEISHMANICIDAL EFFECT OF ESSENTIAL OIL OF MEXICAN OREGANO LIPPIA BERLANDIERI

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Introduction: Leishmaniasis is a neglected vector-borne disease. In Mexico, cutaneous leishmaniasis caused by *Leishmania mexicana* is common. Currently, the drugs available for the treatment of leishmaniasis are toxic, expensive, and often ineffective, making it necessary to find new therapeutic alternatives. Extracts and essential oils (EO) of medicinal and aromatic plants have been shown antimicrobial activity such as the EO of *Lippia berlandieri*, known as Mexican oregano. The aim was to characterize *in vitro* the effect induced by the Mexican oregano *L. berlandieri* EO and its major components on *L. mexicana* promastigotes.

Methods: The effect of *L. berlandieri* EO, thymol, and carvacrol, was determined on *L. mexicana* promastigotes in stationary phase. Metabolic inhibition and cytotoxicity were determined by the Alamar blue method. To determine the mechanism of induced death, cytomorphological changes, mitochondrial membrane potential, membrane integrity, and DNA fragmentation were evaluated by flow cytometry.

Results: Mexican oregano EO, thymol, and carvacrol showed a leishmanicidal effect. Thymol was the most active compound. Both compounds and EO induced cell death by apoptosis, as evidenced by loss of mitochondrial membrane potential, exposure of phosphatidylserine, DNA fragmentation, and volume reduction, while preserving plasma membrane integrity.

Conclusions: Mexican oregano EO has a leishmanicidal effect and is an accessible and affordable alternative that can be further explored.

Keywords: Leishmania, oregano, medicinal plants, essential oil, Leishmania mexicana

P219 / #645

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

COMPUTER-AIDED DRUG REPURPOSING FOR LEISHMANIASIS

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Introduction: Leishmaniasis is a neglected tropical disease reported in 98 countries worldwide. Available drugs are insufficient due to inefficacy, side effects and high cost. Hence, searching for new treatments has been a need for years. In the present work drug repurposing and chemo-informatics were combined to identify new potential antileishmanial compounds.

Methods: An ensemble of three models to predict in vitro activity against promastigotes was developed by using linear discriminant analysis and a database of 196144 compounds tested against L. major promastigotes, which is available in PubChem Bioassay. The ensemble was used to classify the potential of active pharmaceutical ingredients of drugs in current use for indications other than leishmaniasis. To validate the models, compounds either classified as active (20) or as inactive (16) were tested against promastigotes. Active compounds were further tested against intracellular amastigotes and in a mouse model of cutaneous leishmaniasis.

Results: The accuracy of the models, their specificity and sensibility were over 90 %. Moreover, none of the compounds classified as inactive by the models were active at concentration under 250 µg/mL. On the contrary, 60 % of those classified as active were parasiticide under 50 µg/mL. Thioridazine, an antipsychotic drug, was among the most active compounds identified, with IC50 in promastigotes of L. major, L. mexicana and L. amazonensis of 0.7-3.8 µM; IC50 against intracellular amastigotes of 1.3-6.1 µM and a 50 % efficacy dose by oral route of 17.4 ± 1.2 mg/kg.

Conclusions: Results support the validity of the approach and the potential of drug repurposing as a source of new antileishmanial agents.

Keywords: QSAR, drug repurposing, Leishmania

P220 / #1045

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

PP2C OF LEISHMANIA MEXICANA AS A VIRULENCE FACTOR IN MURINE LEISHMANIASIS

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Introduction: Several molecules appear to play a role in the pathogenesis of Leishmania. These virulence factors enable the parasite to invade and establish infection in the mammalian host. Phosphatase protein 2C (PP2C) requires metal cation Mg+2 or Mn+2 to exhibit its activity. PP2C has been localized in the flagellar pocket and the flagellum of L. mexicana and also in promastigotes and amastigotes in culture media. Hence, the identification of PP2C as a virulence factor during the progression of Leishmaniasis may establish an important target for altering the evolution of the disorder.

Methods: Three groups of BALB/c mice were inoculated in the right hind footpad (n=7). The first and second group with promastigotes of L. mexicana from the TAB3 strain, the second group with the LAC strain, and the control with PBS. The TAB3 employed presently was isolated from patients with diffuse cutaneous leishmania (DCL) and the LAC from patients with local cutaneous Leishmaniasis (LCL). The infected tissue was evaluated for the phosphatase activity and Western blot. The presence of PP2C in infected tissue was examined by immunohistochemistry.

Results: The activity and the lesions proved to be higher in the LAC versus TAB3. In the Western blot of the control and TAB3 mice, PP2C was not expressed. In the lesions infected with LAC, contrarily, PP2C was expressed in the latter week. PP2C was identified by immunohistochemistry in the amastigotes of mice infected.

Conclusions: This PP2C phosphatase could possibly be considered as a virulence factor in the development of Leishmania and a marker for the evolution of disease. Acknowledgments MMAG is grateful to the SEP-CONACyT Mexico [grant # 284018 and 212422] for financing this work and to the DGAPA-PAPIIT IN212422 for partial sponsorship.

Keywords: Leishmania, virulence, PP2C phosphatase

P221 / #1537

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

CHARACTERIZATION OF DIFFERENTIALLY ABUNDANT PROTEINS AMONG LEISHMANIA (VIANNIA) BRAZILIENSIS STRAINS ISOLATED FROM ATYPICAL OR TYPICAL LESIONS

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Introduction: Leishmania (Viannia) braziliensis is the main etiological agent of cutaneous and mucocutaneous leishmaniasis in Latin America. Non-ulcerated atypical tegumentary leishmaniasis cases caused by L. braziliensis have been reported in several regions of the American continent, including the Xacriabá indigenous reserve in São João das Missões/Minas Gerais, Brazil. Parasites isolated from these atypical clinical lesions are resistant to antimony-based therapeutics.

Methods: In the present study, proteins displaying differential abundance in two strains of L. braziliensis isolated from patients with atypical lesions compared with four strains isolated from patients with typical lesions were identified using a quantitative proteomics approach based on tandem mass tag labeling (TMT) and mass spectrometry.

Results: A total of 532 ($P<0.05$) differentially abundant proteins were identified (298 upregulated and 234 downregulated) in strains from atypical lesions compared to strains from typical lesions. Prominent positively regulated proteins in atypical strains included those that may confer greater survival inside macrophages, proteins related to antimony resistance, and proteins associated with higher peroxidase activity. Additionally, we identified proteins showing potential as new drug and vaccine targets.

Conclusions: Our findings contribute to the characterization of these intriguing L. braziliensis strains and provide a novel perspective on Atypical Cutaneous Leishmaniasis (ACL) cases that have been associated with therapeutic failures.

Keywords: TMT, antimony resistance, Leishmania braziliensis, Atypical wounds, proteome

P222 / #583

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

MITOCHONDRIALLY TARGETED IRON CHELATORS AND THEIR DERIVATIVES SELECTIVELY ELIMINATE KINETOPLASTID PARASITES

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Introduction: Unicellular parasites compete with their hosts for many vital nutrients including iron. Therefore iron-chelation therapy is one of the promising approaches against serious human parasites, such as Plasmodium, Trypanosoma, or Leishmania. Antiparasitic effect of FDA approved iron chelators Deferoxamine and deferasirox was previously studied, however, with limited success so far. Our approach is based on chemical modification of the abovementioned iron chelators via introduction of specific triphenylphosphonium vector (TPP). TPP vector ensures preferential uptake of new compounds into parasitic mitochondria, where the iron is heavily utilized. The fact that kinetoplastid parasites possess a single mitochondrion implies that modulation or impairment of mitochondrial function will affect these microorganisms more than human cells, providing a wider therapeutic window.

Methods: Assessing EC_{50} values allowed to select the most potent compounds and further experiments focused on assays such as cellular respiration, membrane potential, and mitochondrial intactness. Chelators pre-treated with iron were used to assess to what extent the iron-chelating properties affect the parasite.

Results: This work shows that mitochondrially-targeted chelators inhibit the propagation of parasites in vitro and have less of an impact on human fibroblast cell line. Consecutive experiments showed that the compounds effectively decrease the mitochondrial potential of parasites, affect ROS levels, in high concentrations decrease cellular respiration, and permeabilize mitochondria.

Conclusions: Mitochondrially targeted iron chelators are effective against kinetoplastid parasites and present a potential method of therapeutic intervention.

Keywords: Iron chelator, Mitochondria, Trypanosoma, Leishmania

P223 / #1542

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

HOST-PARASITE INTERACTION: DECIPHERING THE TAENIA SOLIUM EXCRETORY SECRETORY PROTEINS INDUCED METABOLIC CONSTRAINTS IN THE MACROPHAGES

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Introduction: Neurocysticercosis (NCC), a NTD is caused by the larvae of helminth *Taenia solium* and is a major cause of “acquired epilepsy”. The metabolic state of immune cells determines the fate of immune response. Helminth excretory/secretory proteins (ESPs) suppress the immune response and their effect on metabolic status of cells is unknown for *T. solium*. To bridge the gap in our understanding of NCC, we studied the exometabolome of macrophages in NCC pathogenesis.

Methods: ESPs were derived by culturing cysts in RPMI 1640 for 24H. The macrophages were derived from U937 cell line. The M0 macrophages were differentiated into M1 and M2 cell line using LPS and IL4. The differentiated cells were stimulated with and without ESP for 24H. The supernatant was collected after 24H, quenched and processed for H1NMR. The samples were acquired on Bruker 800MHz NMR spectrometer followed by spectral assignment. Statistical analysis was carried on pattern recognition approach performed online using MetaboAnalyst.

Results: The study groups were analysed pairwise i.e M0 vs M0+ESP, M1 vs M1+ESP, M2 vs M2+ESP.They appeared as distinct groups on a PLS-DA plot. The M0+ESP group had a heterogenous fingerprint of metabolites, with succinate having highest VIP score. It had high phenylalanine to tyrosine ratio, an indicator of oxidative stress. A significant metabolic shift in the M1+ESP phenotype was observed with 22 significantly upregulated metabolites. M1+ESP and M2+ESP states did not separate as distinct groups on PLS-DA plots. Succinate is marker for M2 macrophages and was found to be significantly upregulated in all treatment states.

Conclusions: The snapshot of differentially regulated macrophage activation state in response to *T. solium* ESPs suggest immune suppressive potential of ESPs.

Keywords: NTDs, neurocysticercosis, Excretory/Secretory Proteins, Immune modulation, metabolites

P224 / #1107

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

IN VITRO AND IN VIVO EFFECTS OF QUINONE DERIVATIVES ON TRYPANOSOMA CRUZI

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Introduction: Trypanosoma cruzi, is a protozoan that causes Chagas Disease in Central and South America. Due to there is no effective chemotherapy against this parasite, an effective therapeutic agent without side effects is urgently needed. Previously, we found that komaroviquinone had a trypanocidal activity and the quinone moiety was critical to show inhibitory effects.

Methods: To search for the drug against T. cruzi, we analyzed the effects of five novel quinone derivatives A to E on the Tulahuen (TcVI) and Y (TcII) strains of T. cruzi. Both strains were infected to HT1080 and Hela cells as hosts in vitro, respectively. After incubation for 3 to 5 days, the infection rate and the number of amastigotes per cell were determined after Diff-Quik staining. In vivo, C57/B6 mice were infected with T. cruzi and the bloodstream trypomastigotes were detected.

Results: In vitro, B to E showed the typanocidal activity on trypomastigotes and inhibited the amastigotes growth on both strains. The IC50 value of D for the number of amastigotes per infected cell against Tulahuen strain was 0.16 uM, which was markedly more effective than benznidazol. Besides, D did not show cytotoxicity in the host cells and the selectivity index for the amastigotes growth of Tulahuen strain was more than 600. The mice treated with D showed a significant reduction of blood stream trypomastigotes compared to the untreated mice.

Conclusions: Our findings indicate that D as a promising lead compound for Chagas disease.

Keywords: Tryapnosoma cruzi, quinone derivatives, amastigotes growth

P225 / #1217

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

ENRICHMENT OF LEISHMANIA LIPIDS WITH ARACHIDONIC OR DOCOSAHEXAENOIC ACIDS INCREASES THE PRODUCTION OF LIPID MEDIATORS AND PARASITE INFECTIVITY

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Introduction: Leishmania parasite are the causative agent of visceral leishmaniasis (VL), mucocutaneous leishmaniasis (MCL) or cutaneous leishmaniasis (CL) in humans, and canine leishmaniasis. Leishmania is an intracellular parasite who infected macrophages of mammalian host. The Leishmania life cycle is divided in two phases, the promastigote stage on the insect vector and the amastigote stage inside the host's macrophages. Lipids are important in biology of the parasite because they provide an interaction platform between the parasite and the host during the infection. In this work, we focused on the role of 2 fatty acids in the infectivity of Leishmania.

Methods: Here we carried out fatty acid supplementation with AA or DHA on two L. infantum strains, a visceral (MON-1) and a cutaneous (MON-24), to evaluate the role of these fatty acids in parasite/macrophage interactions.

Results: Supplementation with AA and DHA has been associated with a tendency to increase parasite infectivity in both studied strains. ROS production was significantly increased in macrophages infected with AA supplemented promastigote in MON-24. Few effect were observed with DHA supplementation. An increase and a production of oxygenated metabolites derivate of the 2 fatty acids was observed in both strains. Enriched promastigotes with DHA showed increased concentration of 17-HDoHE and 14- HDoHE. In the case of promastigote enriched with AA some metabolites were produced, particularly 8-HETE, 12- HETE, 14,15- HETE.

Conclusions: Our data indicate that Leishmania infectivity may be modulated by exogenous fatty acids. We propose that AA or DHA enrichment of promastigote lipids promote the production of inflammatory regulators that modulate parasite infectivity

Keywords: Leishmania infantum, macrophages, arachidonic acid, docosahexaenoic acid, Reactive Oxygen Species

P226 / #1258

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

DEVELOPMENT OF AN ORAL NANOVACCINE FOR DOGS AGAINST ECHINOCOCCUS GRANULOSUS

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Introduction: Dogs are the main source of animal and human cystic echinococcosis caused by the Cestode parasite Echinococcus granulosus. Dog vaccination seems to be a good strategy to control this parasitic disease.

Methods: Here we present the development of a polymeric nanoparticle-based oral vaccine for dogs against Echinococcus granulosus delivered in enteric-coated capsules. To achieve our target, we encapsulated two recombinant antigens into biodegradable polymeric nanoparticles in the presence of Monophosphoryl lipid A as an adjuvant to ensure efficient delivery and activation of a protective mucosal immune response.

Results: The formulated delivery system showed a nanoparticle size less than 200 nm with more than 80% antigen encapsulation efficiency and conserved integrity and immunogenicity. The nanoparticle surface was coated with chitosan to enhance adhesion to the gut mucosa and a subsequent antigen delivery. Chitosan-coated nanoparticles showed a higher cell internalization in murine macrophages and dendritic cells as well as a higher penetration into Caco-2 cells in vitro. To ensure the best storage and to facilitate administration, antigen-loaded nanoparticles were freeze-dried and enteric-coated capsules were filled with the obtained powder.

Conclusions: The successful encapsulation of the E. granulosus antigens and the definitive formulation of the described NPs allowed the successful activating effect on canine immunological cells. The addition of chitosan increased the internalization of nanoparticles and promoted their adhesion to the mucosa. A preliminary in vivo test on dogs gave a good protection rate and experiments will be performed in the future to confirm the protective effect of our vaccine.

Keywords: Dog, Polymeric nanoparticles, Chitosan, Echinococcus granulosus, Oral vaccine

P227 / #489

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

PEOPLE AS PREY: HUMAN DNA IN CHILEAN SYLVATIC TRIATOMINES' DIET DETECTED BY NEXT GENERATION SEQUENCING.

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Introduction: Triatomines are insects, vectors of Trypanosoma cruzi, protozoan that causes Chagas disease in humans. The transmission entails feeding on a mammal host by an infected triatomine, release of infective dejections, and the infection of mucous membranes, skin abrasions or the biting site by the parasite. Therefore, transmission is related to the triatomine-human contact rate.

Methods: In this cross-sectional study, we evaluated if human was present in the diet of Chilean sylvatic triatomine species from 33 sites, encompassing 1100 km, with an overall frequency of T. cruzi-infection of 51.2%. First, we amplified the vertebrate cytochrome b gene (Cytb) from DNA samples obtained from triatomine intestinal contents. We sequenced Cytb positive PCR products in pools of 10 triatomines each, grouped by site and T. cruzi infection status. The filtered sequences were grouped into operational taxonomic units (OTU) using 97% identity and retained if they were composed of at least 100 reads; OTUs were identified through BLAST with the NCBI nucleotide database, choosing the match with best score.

Results: Human was part of the triatomine diet in 19 sites, representing 14.1% of the sequences. There were no significant differences in the detection of human DNA by infection status. Human was present in the diet of the triatomine species Mepraia parapatrica, M. spinolai and Triatoma infestans.

Conclusions: The human-triatomine contact rate is noteworthy, so education must be enforced for local inhabitants, workers and tourists that arrive to endemic areas, to avoid the risk of exposure to Chagas disease vectors. Funding: VID-UChile ENL01/21; FONDECYT 1221045, 1190392, 1180940, 1180119; ANID Programa Becas – Doctorado Becas Chile 2019 72200391, 72200094; U. Viña del Mar FIIUVM-CTC-2211.

Keywords: vector, chagas disease, bloodmeal, bite, Triatominae

P228 / #561

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THE EVOLUTION OF BLOOD FEEDING IN TRIATOMINES: A MULTINATIONAL INITIATIVE TO OBTAIN NEW KISSING BUG GENOMES AND UNDERTAKE THE COMPARATIVE GENOMICS OF CHAGAS DISEASE VECTORS.

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Introduction: Chagas disease is caused by Trypanosoma cruzi, with a complex cycle involving vertebrates and vectors, killing around 10,000 people yearly. Chagas disease vectors are hematophagous insects (Hemiptera: Reduviidae: Triatominae), with 152 extant species. Genomics research on these vectors is lacking, despite their importance for human health. Whole genome assemblies from Rhodnius prolixus, Triatoma rubrofasciata and T. infestans are available, with different degrees of completion, providing just a glimpse of the genomic particularities of these vectors. In our project, we aim to fill the whole genome gap in Triatominae and contribute to the understanding of triatomine biology, with a particular interest in the evolution of hematophagy and their adaptation to the built environment.

Methods: Using long and short read techniques, we aim to sequence, de novo assemble and annotate nine whole genomes from triatomine species belonging to six different genera.

Results: To date, we obtained short read sequences of seven triatomine species, and long reads of six species, and have de novo assembled three species: Rhodnius ecuadoriensis, Panstrongylus geniculatus and Mepraia spinolai. We are working on long read transcriptome sequencing to complement the annotation, and we have also sequenced a predatory reduviid to perform comparative genomics.

Conclusions: Whole genome sequencing can provide information regarding speciation, hybridisation, expansions and contractions in gene families, mechanisms of resistance to insecticides, and signals of adaptation in triatomines, among others. Funding: ANID Programa Becas/Doctorado

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Keywords: Triatominae, vector, genomics, hematophagy, chagas disease

P229 / #810

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

TOWARDS A WHOLE GENOME SEQUENCE OF THE DIURNAL CHAGAS DISEASE VECTOR, MEPRAIA SPINOLAI (HEMIPTERA: REDUVIIDAE: TRIATOMINAE).

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Introduction: Chagas disease is caused by the parasite Trypanosoma cruzi, transmitted to mammals mainly by triatomine bugs. Mepraia spinolai is a triatomine widespread in arid, semiarid and Mediterranean ecosystems of North Central Chile. This triatomine is described as sylvatic, but it is found in high numbers within peridomestic structures, and adults frequently invade houses, with some reports of colonization. This vector displays diurnal behaviour and nymph camouflage. Females are wingless and males display a variety of alary phenotypes. Our aim was to sequence the M. spinolai genome to better understand its biology.

Methods: The thorax of an F2 female was homogenized, digested overnight with proteinase K and extracted using a commercial kit, measuring its quality and quantity. A total of 50 fmol were sequenced in a portable single molecule platform for genomic DNA. After basecalling the long reads using Guppy 5.1.12, they were assembled using Flye 2.8.3, and the quality of the first draft assembly was assessed with assembly-stats 1.0.1 and BUSCO 5.2.2 against the Hemiptera database.

Results: We obtained a 903.1 Mbp assembly of 5,081 contigs, with a N50 of 377,249 bp. The complete busco genes corresponded to 2148 (85.5% of the 2510 genes in the hemiptera_odb10), with 84.5% single copy, and 1.0% duplicated; 2.4% were fragmented, and 12.1% were missing.

Conclusions: Future steps include short read sequencing to improve the noisy long read assembly, and transcriptome-aided annotation of the genome. This is encouraging for future studies on vectors of this neglected disease providing new insights into their biology and potential targets for control.
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Keywords: genomics, chagas disease, Triatominae, adaptation, vector

P230 / #85

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

ANTI-TOXOPLASMA ANTIBODIES ATTACH TO HUMAN BREAST CANCER TISSUE BUT NOT TO NORMAL BREAST TISSUE

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Introduction: Background: Anti-cancer effects of Toxoplasma gondii has been shown in different publications. However, the mechanism of action has not been clarified yet. In this work reaction of rabbit anti-Toxoplasma antibodies with the surface of human breast cancer and normal tissues has been investigated.

Methods: . Methods: To produce antibodies, Toxoplasma gondii tachyzoites lysate was injected subcutaneously into a rabbit, and the antibodies were then purified. The reaction of sections of human breast cancer and normal tissues with anti-Toxoplasma or normal rabbit antibodies was investigated using the immunohistochemistry technique.

Results: A strong reaction between breast cancer tissue sections and anti-Toxoplasma antibodies was observed. However, no reaction or very faint reaction was seen following treatment of breast cancer tissue sections with normal rabbit antibodies.

Conclusions: Conclusion: Anti-Toxoplasma antibodies attach selectively to breast cancer sections and not to normal breast sections.

Keywords: antibody, cancer, breast tissue, toxoplasma

P231 / #826

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

BIO-MEMBRANE SELEX AS A NEW APPROACH FOR SELECTING SS-DNA APTAMERS THAT BIND TO THE HYDATID CYST LAMINATED LAYER

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Introduction: Hydatid cyst (HC) is the larval stage of the canine intestinal tapeworm (cestode), Echinococcus granulosus. In addition to the high global economic cost to livestock farming, the infection can lead to dangerous problems for human health. Therefore, research into new diagnosis and treatment approaches is valuable. This study is set out to explore aptamers that bind to HC antigens.

Methods: The similarity between HC genotype in sheep and humans, sheep HCs were collected and were used as a biological membrane for aptamer selection. Four Bio-Membrane SELEX rounds were conducted and ssDNA aptamers were selected. Selected aptamers' affinity and specificity to the laminated layer antigens were evaluated using membrane staining by fluorescein primer as a probe. Biotinylated primer was used as a probe for aptahistochemistry and dot blot techniques. Subsequently, cloning and plasmid extraction was conducted. The affinity and specificity of sequenced aptamers were examined with the dot blot method.

Results: Selected aptamers reacted with HC wall in aptahistochemistry, aptahistofluorescent, and dot blot experiments. Following cloning and sequencing, 20 sequences were achieved. A strong reaction between HC total antigens and sequenced aptamers has emerged in the dot blot method.

Conclusions: In this investigation, we propose a novel method to determine specific aptamers. Bio-Membrane SELEX, could be assumed as a practical and sensitive method for aptamer selection. Selected aptamers in this study possibly may be used for specific HC antigens detection.

Keywords: Parasites, Helminthes, Aptamer, SELEX, Echinococcus granulosus

P232 / #1581

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

STRUCTURAL AND FUNCTIONAL CHARACTERISATION OF PROLYL OLIGOPEPTIDASE TC80 FROM TRYPANOSOMA CRUZI

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Introduction: Chagas disease is a global-health concern being one of the major neglected tropical diseases, killing 12,000 people per year and infecting 6 million people worldwide, with 70 million people estimated to be at risk. The disease is caused by the parasite *Trypanosoma cruzi*, endemic to South America but spreading fast around the world, heavily due to human migration. *Trypanosoma cruzi* 80kDa prolyl oligopeptidase (Tc80) is used by the parasite to degrade components of the extracellular matrix, such as collagen and fibronectin. Efforts to develop specific Tc80 inhibitors have been hampered by the high sequence homology between the catalytic site of Tc80 and the catalytic sites of human peptidases. However, it has been recently demonstrated that anti-Tc80 polyclonal antibodies are able to protect mice from a lethal dose of *T. cruzi*, suggesting that invasion-blocking antibody/antibodies can be induced, although they have not been isolated nor characterized from sera.

Methods: Tc80 was expressed recombinantly and purificed to produce a panel of monoclonal antibodies (mAbs) in mice, which have been tested in vitro for their binding affinity to Tc80 and their effects on enzyme activity.

Results: Anti-Tc80 mAbs bind Tc80 and one of them reduces its enzymatic activity in vitro. Crystallisation screens of Tc80 apo- have been performed and will be followed in presence of the Fab fragments from the different mAbs to highlight key epitopes.

Conclusions: We are in the process of determining the crystal structures of Tc80 apo- and in complex with the best anti-Tc80 mAb/mAbs, which may lead in the long-term to the identification of the molecular determinants of Tc80 immunogenicity. Monoclonal antibodies will also be tested for their diagnostic and therapeutic potential.

Keywords: Immunogen design, structural biology, Trypanosoma cruzi, Vaccine development

P233 / #1205

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

STRUCTURAL CHARACTERIZATION OF TOXOPLASMA GONDII BRAIN CYSTS IN IMMUNOSUPPRESSED MICE USING IMAGEJ SOFTWARE

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Introduction: Toxoplasma gondii is an obligate intracellular parasite with a complex life cycle. Conversion of tachyzoites into encysted bradyzoites is essential for parasite persistence in the host, while rupture of tissue cysts results in reactivated toxoplasmosis (RT) in the setting of a weakened immune system. Our previous results suggested the potential of computational image analysis in describing the complexity of T. gondii brain cysts. Here we present results on structural alterations of cysts after drug-induced immunosuppression.

Methods: Mice chronically infected with type II T. gondii strain were treated using cyclophosphamide and hydrocortisone (experimental group-EG) or left untreated as infection controls (IC). Treated mice were distinguished as having reactivated (PCR+) or not (PCR-) by peripheral blood qPCR-based detection of T. gondii DNA. A total of 55 images of T. gondii brain cysts, 27 from IC and 28 from EG (21 PCR- and 7 PCR+) were analyzed for diameter (D), circularity, packing density (PD), fractal dimension (FD), and lacunarity using ImageJ software.

Results: We found that FD and lacunarity did not vary significantly among groups. However, circularity was significantly higher in the PCR+ mice, while a negative correlation between D and PD was observed only in IC.

Conclusions: Fractal analysis revealed a highly uniform structure of T. gondii brain cysts in mice regardless of their immune status, yet the absence of a negative correlation between D and PD in EG indicates changes in cyst occupancy in immunosuppressed mice. Along with the observed increase in cyst circularity in PCR+ mice, these results further the perspectives for the use of image analysis in experimental models of toxoplasmosis.

Keywords: Toxoplasma gondii, immunosuppression, cysts, image analysis

P234 / #1369

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

SCOPEPLOT: A SINGLE MOLECULE FLUORESCENT IN SITU HYBRIDISATION (SMFISH) ANALYSIS TOOL FOR THE AUTOMATED DETECTION OF TRANSCRIPTIONAL ACTIVITY IN TISSUES

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Introduction: Spatial transcriptomic tools, such as single molecule fluorescent in situ hybridisation (smFISH) provide a means to image transcriptional factors within tissues. However, resulting datasets are large and tools for quantifying these data are lacking. The aim of this project was to generate a tool for the automated detection of in situ hybridisation signals.

Methods: We developed a bioinformatic pipeline, using Qupath to quantify cells and RNA transcripts in smFISH data. We further developed a purpose-built, automated, Python-based analysis tool for processing large datasets, compatible with Qupath outputs.

Results: We have developed a robust analytical pipeline to count cells positive for multiple targets with >75% accuracy. This software enables quantification of single sub-cellular “transcriptional spots”, representing individual RNA transcripts, as well clustering behaviour in host cells. Additionally, it enables an automatic partition of cells based on RNA co-expression and specific RNA targets. Using a previously obtained dataset of mice experimentally infected with trypanosomes, we demonstrate that our pipeline accurately and significantly detects spatially-resolved co-expression of Il10 in $Cd79^{+}$ B cells in the brain of infected mice compared to naïve controls.

Conclusions: Our pipeline offers semi-automated data processing for multi-dimensional, multi-target smFISH datasets and can be applied to a variety of biological questions. Integration of smFISH with this pipeline facilitates the investigation of molecular interactions between cell populations in tissues during health and disease.

Keywords: Software Development, Trypanosomiasis, Spatial Transcriptomics, Bioinformatics

P235 / #1372

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

ORGANIZATION OF MITOCHONDRIAL GENE EXPRESSION IN TRYPANOSOMA BRUCEI

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Introduction: Trypanosoma brucei (T. brucei) has a unique mitochondrial DNA (mtDNA) network called the kinetoplast DNA (kDNA), which contains about 5000 minicircles and 25 maxicircles. The latter are equivalent to other eukaryotic mtDNAs, encoding subunits of the respiratory chain, ribosomal proteins and mitochondrial rRNAs. Many of these genes are cryptogenes, which require extensive RNA editing (U insertion and deletion).
In T. brucei, many replicative enzymes have been tagged and mapped to the APS flanking the kDNA. However, resolution of epifluorescence microscopy images has been limiting. The development of new immunofluorescent imaging techniques such as ultrastructure expansion microscopy (U-ExM) have helped to drastically increase spatial resolution and the identification of enzymes in potential subdomains now becomes possible.

Methods: To get a first overview of proteins located at the antipodal sites, I have performed a bioinformatic analysis using the publicly available TrypTag database. As expected, many replicative enzymes mapped to the antipodal sites. Additionally, enzymes involved in RNA editing and translation locate to the antipodal sites. I used ultrastructure expansion microscopy (U-ExM) in combination with epitope tagging to localize the four gene expression processes in T. brucei mitochondria.

Results: Besides replication, editing and translation, I tagged and localized the mitochondrial RNA polymerase (mtRNAP) in order to determine the site of kDNA transcription, which was not known in T. brucei mitochondria.

Conclusions: Ultrastructure expansion microscopy (U-ExM) in combination with epitope tagging allowed to localize the four gene expression processes in T. brucei mitochondria.

Keywords: Trypanosoma, Mitochondrial Gene Expression, kDNA

P236 / #1262

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

LEISHMANIASIS IN SWEDEN 2017-2021, A NATIONWIDE OBSERVATIONAL STUDY INCLUDING LABORATORY AND CLINICAL DATA

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Introduction: Leishmaniasis is a rare imported parasitic disease in Sweden and diagnosis requires a high clinical suspicion. A nationwide study was conducted to describe patient characteristics, clinical presentation, country of infection, laboratory diagnosis, treatment and outcome.

Methods: Patients with laboratory-confirmed leishmaniasis were prospectively included from 2017 through 2021. Laboratory testing was performed at The Public Health Agency of Sweden. Both clinical and laboratory data were collected for each patient.

Results: Of the 93 laboratory-confirmed cases, 60% were male; median age 34 years (range: 1-82). Most cases were clinically diagnosed either in a centre for dermatology or infectious diseases. A third of cases were infected in the Syrian Arab Republic, 20% in Spain and 13% in Afghanistan. Cutaneous leishmaniasis was most common (86%) and half of these were caused by Leishmania tropica, 18% L. major and 15% L. infantum. Visceral leishmaniasis occurred in nine patients (eight infected in Spain with L. infantum, one in Ethiopia with L. donovani) and three patients had mucocutaneous leishmaniasis (two L. infantum, one L.V. braziliensis).

Conclusions: Despite the number of leishmaniasis cases in Sweden being halved after the start of the pandemic, patients from the Syrian Arab Republic are still presenting with CL infections. Of concern is the patient group with medically-induced immunosuppression that acquired leishmaniasis in the Mediterranean region. Awareness among Swedish health professionals is important to ensure correct and timely diagnosis, and treatment based on the parasite species.

Keywords: clinical, molecular, Leishmaniasis, Epidemiology

P237 / #472

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

ANTIKINETOPLASTID ACTIVITY OF SESQUITERPENES ISOLATED FROM THE ZOANTHID PALYTHOA AFF. CLAVATA

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Introduction: Leishmaniasis and Chagas disease are neglected tropical diseases that cause problems in developing countries. Current treatments for these diseases are not very effective and highly toxic since they require very prolonged treatments. In this study, the antikinetoplastid activity of 13 sesquiterpene lactones obtained from Palythoa aff. clavata was screened against L. amazonensis, L. donovani and T. cruzi.

Methods: Polyps of Palythoa aff. clavata were collected from Lanzarote, the 13 extracts obtained were tested against the extracellular forms of T. cruzi and Leishmania spp. and against the amastigote form of L. amazonensis using an alamarblue-based colorimetric assay. To determine the selectivity, the toxicity of these compounds was studied with the same method, using alamarblue against murine macrophages. The most selective compounds were subjected to mechanism of action studies to determine the type of cell death produced.

Results: The results revealed that the sesquiterpene lactones anhydroartemorin (2), cis,trans-costunolide-14-acetate (3) and 4-hydroxyarbusculin A (11) were the most selective against the kinetoplastid species studied. Alterations in the mechanisms of action against these three more selective compounds were observed.

Conclusions: The best selectivity indexes obtained were cis,trans-costuno lide-14-acetate (3) against promastigotes of L. donovani and anhydroartemorin (2) against T. cruzi. In the case of amastigotes of L. amazonensis, the most selective products were anhydroartemorin (2) and 4β-hydroxyarbusculin A (11). Lactones 2, 3 and 11 seem to induce an apoptotic-like death or programmed cell death in the kinetoplastids, and therefore could contribute to eliminate the inflammation problems caused by a necrotic process.

Disclosure: BIOALGRI (PID2019-109476RB-C21); PI18/01380; RICET (RD16/0027/0001); CIBER (CB21/13/00100); Cabildo de Tenerife, MEDI y FDCAN; MICINN, Agencia Estatal de Investigación (AEI) and Fondo Europeo de Desarrollo Regional (ERDF) (21/0587). ACIISI by FEDER (TESIS

Keywords: chemotherapy, PCD, kinetoplastids, Palythoa, sesquiterpenes

P238 / #751

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

PEDIATRIC CYSTIC ECHINOCOCCOSIS: GENETIC DIVERSITY AND HAPLOTYPES OF ECHINOCOCCUS SPECIES IN ALGERIAN CHILDREN

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Introduction: Echinococcosis is a hyperendemic disease in the Mediterranean region. Notably Algeria, pediatric echinococcosis remains a public health concern. This study aimed to report the clinical manifestations, genotypes of Echinococcus species infecting children in eastern Algeria.

Methods: The present study was conducted with samples from children ages 3 to 14 years old who received surgical treatment for hydatid cysts. Records of the patients include demographic data, clinical manifestations of the disease, type of cysts, and other related factors. The molecular analysis realized the characteristics of cysts' genotypes and subsequently analyzed by comparing the PCR-amplified DNA sequences of target mitochondrial cytochrome c oxidase subunit 1 (cox1).

Results: 25 pediatric patients, twelve (48%) female and thirteen (52%) male, were evaluated. All the patients showed one of the symptoms (dry cough, lower thoracic pain, right hypochondrium pain, vomiting). The most common localizations of cases were determined in liver (n=11, 44%) and lung (n=11, 44%). The co-occurrence of liver and lung was seen in 1 case (4%), and two cases were from a rare location (kidney and mediastinal) was reported. The cysts were fertile and viable in 13 cases (52%), and 12 were sterile. The molecular analysis showed that all samples were identified as E. granulosus sensu stricto (G1 and G3). Also, it indicated the presence of the common E. granulosus haplotype described from other areas in the region.

Conclusions: Pediatric cystic echinococcosis should be kept in mind when pain is encountered anywhere in the body, particularly in patients living in the endemic regions. In addition, these findings may provide more information on molecular characteristics of E. granulosus s.s. from Algeria.

Keywords: Algeria, Cystic echinococcosis, Echinococcus, Pediatric

P239 / #911

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

WHY ARE AFFORDABLE DIAGNOSTIC TESTS, SUCH AS LEISHMANIN SKIN TEST FOR CUTANEOUS LEISHMANIASIS, NOT USED IN DEVELOPING REGIONS OF THE WORLD?

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Introduction: Leishmanin skin test(LST) is an immunological diagnostic test used in field studies and diagnosis of cutaneous leishmaniasis(CL).It is a reaction based on the delayed-type hypersensitivity and exhibits high predictive value,being positive in more than 90% of CL cases.Leishmania phenol-killed promastigotes are inoculated through an intradermal injection.CL is a neglected disease(ND),endemic in developing countries(DC),which depend on low-cost techniques for the diagnosis,like parasitological and immunological exams(LST).Parasitological exams show the disadvantage of subjectivity, a large variability in sensitivity, and contamination.Furthermore, some endemic regions for CL do not have the equipment to support PCR.By this abstract, we address the importance of production and use of the LST in DC.

Methods: Based on publications from Braz LMA,2018(/10.1590/S1678-9946201961017)and Carstens-Kass et al,2021(PLoS Negl Trop Dis.10.1371/journal.pntd.0009531), we evaluated the use of LST in DC.

Results: By the TDR, the World Health Organization began wide distribution of the antigen LST produced in Iran but ceased the distribution, and the Iranian leishmanin is no longer available nowadays.In 2017, the Brazilian Health Regulatory Agency (ANVISA) determined the interruption of LST antigen production by the local provider (CPPI)..

Conclusions: Therefore, it is necessary to consider affordable diagnostic tests such as LST for ND, because they offer a favorable clinical applicability at a low cost, and was inexplicably suspended in CL endemic countries.ND are again uncared for by governments and it is necessary to draw attention to this situation, at least among the scientific community. Acknowledgements: FAPESP 2019/19375-0

Keywords: affordable diagnostic tests, Leishmanin skin test, developing countries, Cutaneous leishmaniasis

P240 / #1746

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

GENE SILENCING IN TAENIA CRASSICEPS

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Introduction: Cysticercosis is a parasitic tissue infection caused by larval cysts of the tapeworm Taenia solium. Neurocysticercosis (NC) is one of the most frequent parasitic diseases of the central nervous system. Cysticidal drugs, albendazole and praziquantel, are generally effective when parasites localize in the parenchyma. In contrast, parasites lodged in the subarachnoid basal cisterns are less responsive to treatment. Therefore, novel therapies are urgently needed for NC. Gene silencing by siRNA is a powerful tool that has been widely used to identify drug targets in parasites.

Methods: Mathods: A gene silencing procedure on cysticerci of the taeniid cestode Taenia crassiceps is described in this work

Results: Genome database searches were performed in order to find out if relevant genes involved in gene silencing and non-coding RNA processing, Argonaute and Dicer (AGO and Dcr) are present in T. crassiceps. We found three AGO and two Dcr orthologues that were designed TcAGO1, Tc2 and Tc3, as well as TcDcr1 and TcDcr2. In order to elucidate the evolutionary relationships of T. crassiceps TcAGO and TcDcr genes, separate phylogenetic analyses were carried out for each, including AGO and Dcr orthologues of other 20 platyhelminthes. Our findings showed a close phylogenetic relationship of TcAGO and TcDcr with those previously described for Echinococcus spp. Our RT-PCR studies demonstrated expression of all TcAGO and TcDcr orthologues. Our results show that the gene silencing machinery in T. crassiceps is functionally active by inducing silencing of Tc Enolase A (~90%).

Conclusions: Our results demonstrate that gene silencing is functional in T. crassiceps, and suggest that siRNAs can be used as a molecular methodology to identify novel targets for drug development in Taenids.

Keywords: neurocysticercosis, siRNA, gene silencing, Taenia crassiceps

P241 / #318

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THE APPROACH OF AMPLICON-BASED NEXT-GENERATION SEQUENCING SHOWS THE SIMULTANEOUS PRESENCE OF SEVERAL LEISHMANIA SPECIES IN VISCERAL LEISHMANIASIS CASES.

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Introduction: Visceral leishmaniasis is a neglected tropical disease, caused by parasitic species of the mammalian protozoan: Leishmania donovani complex. In the Americas, is primarily caused by Leishmania infantum and propagated by female Phlebotomine sandflies through bites. Interestingly, in recent years Visceral Leishmaniasis cases have been associated with other Leishmania species such as L. amazonensis and L. colombiensis, both human and canine cases.

Methods: We implemented the use of an amplicon-based next-generation sequencing approach used in the Cutaneous Leishmaniasis context previously. Herein, using this high-depth sequencing for targeting a region on the HSP70 gene and identifying Visceral Leishmaniasis etiologic agents. In this approach, six samples from five patients with Visceral Leishmaniasis diagnoses were selected and analyzed for identifying the DNA of Leishmania spp. and other Trypanosomatides.

Results: As was expected, all samples harbored DNA of L. infantum, just one was mono-infected and the other five were found to be co-infected with other Leishmania species or with Trypanosoma cruzi. Additionally, we correlated the molecular results with the data from the clinical history of each patient, which allowed us to better understand the context of the acquisition of the infection and the evolution of the disease in each case.

Conclusions: Our study demonstrates the usefulness of the amplicon-based NGS to identify trypanosomatid coinfections in clinical samples, being pioneer research in the study of coinfections by different species of Leishmania in cases of visceral leishmaniasis in the Americas, which represents an interesting study panorama considering their biological, clinical and epidemiological implications.

Keywords: Visceral Leishmaniasis, HSP70, amplicon-based NGS, co-infection

P242 / #609

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THE BURDEN OF HUMAN CYSTIC ECHINOCOCCOSIS IN EUROPE DURING 2000-2021: A SYSTEMATIC REVIEW ON NATIONAL-BASED DOCUMENTED CASES

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Introduction: The neglected zoonosis cystic echinococcosis (CE) affects worldwide poor pastoral and rural communities but also those of medium-high income countries, including Europe, where it should be managed as an orphan and rare disease. Even if human CE is a notifiable infectious disease in some European countries, in practice it is largely underreported.

Methods: Data on the burden of CE in Europe was extracted by means of systematic review (SR) approach from both scientific and grey literature accounting for the period 2000-2021. Different grade of evidence was collected from different datasets. Data source providing the utmost quantitative effect of the burden at country level was selected with no data overlapping in time and space.

Results: During 2016-2019, a yearly average of 413 confirmed human cases of CE were reported in Europe by EFSA/ECDC in the “EU One Health 2019 Zoonoses Report”. Irrespectively of the previous picture, data extraction from this SR identified around 53,000 human CE infections in Europe reported from 38 countries during last 20 years. Bulgaria, Italy and Spain accounted for 75% of the total numerical burden. Hospital discharge records available only from 5 European countries recorded around 68,000 hospitalizations. Extended ultrasound population-based surveys estimated around 45,000 infections only in endemic areas of Bulgaria and Romania.

Conclusions: Collection of accurate epidemiological and clinical data is needed to give a reliable picture of the burden of this disease in Europe, providing a statistically supported case series for future evaluation of efficacy and effectiveness of interventions. This research was funded by MEME project from the EU’s Horizon 2020 (773830), One Health EJP. https://onehealthejp.eu/jrp-meme/

Keywords: Cystic echinococcosis, Europe, Echinococcus granulosus sensu lato, burden of disease

P243 / #457

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

IN VITRO ACTIVITY OF OXASQUALENOIDS ISOLATED FROM LAURENCIA VIRIDIS AGAINST NAEGLERIA FOWLERI STRAINS

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Introduction: Naegleria fowleri is the causative pathogen of primary amoebic meningoencephalitis. This central nervous system affecting disease causes the death of more than 95% of people who suffer from it. Today, few drugs are able to act effectively on the parasite with minimal cytotoxicity to the patient. This, together with the rapid evolution of the disease, makes the search for new drugs necessary. Therefore, in this work, the activity of nine molecules derived from Laurencia viridis against trophozoites of two strains of N. fowleri was studied, focusing the study on those that presented a better selectivity index.

Methods: The in vitro activity against trophozoites of two strains of Naegleria fowleri (ATCC® 30808™ and ATCC® 30215™) and the in vitro cytotoxicity in a murine macrophage cell line were evaluated, both using a colorimetric assay based on the reagent alamarBlue®. To determine the type of cell death, some of characteristic cell death processes were evaluated and made visible using a fluorescence microscope and the corresponding reagents.

Results: Saiyacenol X and Yucatecone showed great activity against both strains of N. fowleri, focusing the study on the second molecule due to its low cytotoxicity. The inhibitory concentrations 50 values of Yucatecone were 28.53 ± 5.04 µM and 16.25 ± 1.23 µM in ATCC® 30808™ and ATCC® 30215™, respectively. For this reason, the analysis of the study focused on this molecule, which favored the presence of different metabolic events, observing the induction of characteristic apoptotic processes in undergoing cells.

Conclusions: The mechanisms of action studied indicate that the compound Yucatecone induces programmed cell death in Naegleria fowleri, being considered a future alternative in chemotherapy against this pathogen.

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Keywords: Naegleria fowleri, Laurencia viridis, Yucatecone, Programmed cell death

P244 / #703

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

SPECIES CHECKLIST AND SPATIOTEMPORAL HABITAT SUITABILITY ANALYSIS FOR THE PHLEBOTOMINE SAND FLIES IN CYPRUS

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Introduction: Visceral and cutaneous leishmaniasis are described as important public health problems in Cyprus. The disease was prevalent before 1945, and was nearly eradicated by 1996. The recent increase in its frequency and geographical spread is largely attributed to environmental changes; thus, it is necessary to examine the drivers of its vector, the Phlebotomine sand flies.

Methods: The present study compiles a sand fly presence and distribution database for Cyprus through an in-depth literature review and the joint monitoring effort of three research teams across the island. Land type suitability maps were created for four *Phlebotomus* species of medical importance, *i.e. P. papatasi, P. tobbi, P. ariasi*, and *P. perniciosus*, based on expert opinion. Furthermore, a high-resolution seasonal habitat suitability map was generated for *P. papatasi* by incorporating some of the most important environmental drivers of sand fly population dynamics in a predictive mathematical model.

Results: We found that Cyprus hosts a rich Phlebotomine fauna with 18 species. We identified areas of high relevance for the four *Phlebotomus* species, and found that *P. papatasi* is widespread, especially in areas characterised by high urbanisation. Although vector abundance uniformly peaks on the island during the end of summer, distinct suitability profiles and potential hotspots were identified before and after that peak.

Conclusions: The rich sand fly fauna exhibiting different patterns of distribution throughout the island and the habitat suitability analysis presented herein could facilitate public health planning and disease prevention.

Keywords: population dynamics, Environmental drivers, Leishmaniasis, Vector- borne Disease, Phlebotomine sand flies distribution

P245 / #1324

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

EXPRESSION OF MEIOSIS/HOMOLOGOUS-RECOMBINATION RELATED GENES DURING THE WHOLE TRYPANOSOMA CRUZI LIFE CYCLE.

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Introduction: Trypanosoma cruzi has a complex life cycle with 4 morphological different biological stages, transit between nutritional and oxidative stress. Some authors suggest T. cruzi follows clonal reproduction; however, recent genomic and transcriptomic studies have evidenced an unorthodox capacity of recombination.

Methods: We decided to evaluate the gene expression of 10 meiosis/homologous-recombination-related genes during the T. cruzi life cycle and two points of oxidative stress induction. We conducted RNA extraction, designed primers, standardized the RT-qPCR, and sequenced the products. We made a relative quantification of genes expression.

Results: Our results show a basal expression of all genes during the life cycle that could indicate their participation in various cellular processes. We found three profiles of gene expression increased: i) associated with RAD51/MRE11/NBS1 in amastigotes after 72 hours of cell-infection, ii) HAP2/RPA/RAD50 increasing during metacyclogenesis with the highest levels in metacyclic trypomastigotes (MT) and iii) MND1/BRCA2 that had the same expression between MT and cell-derived trypomastigotes. We do not find expression changed to oxidative induction.

Conclusions: Recently studies in other trypanosomatids have remarked the influence of HAP2 and RPA in recombination. If T. cruzi uses the same repertoire of genes, our findings could suggest MT as a stage of recombination. More studies are required to corroborate our results and understand the recombination in this parasite.

Keywords: Trypanosoma cruzi, life cycle, Meiosis, Homologous recombination, Genes expression

P246 / #1303

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

TRANSCRIPTIONAL CHANGES DURING METACYCLOGENESIS OF TRYPANOSOMA CRUZI ISOLATED FROM COLOMBIA

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Introduction: During its life cycle, T. cruzi changes from epimastigotes to metacyclic trypomastigotes, a process known as metacyclogenesis. This differentiation stage is essential because the parasite acquires the form to infect humans. In this work, the transcriptome of metacyclic trypomastigotes and epimastigotes was analyzed to identify differentially expressed genes that may be involved in metacyclogenesis.

Methods: in vitro induction of metacyclogenesis was performed to obtain metacyclic trypomastigotes from a culture of epimastigotes. RNA-seq in triplicate from metacyclic trypomastigotes and epimastigotes was performed. We implemented a genome reference-based approach using the Dm28c strain to assemble the transcriptome, and differential gene expression analysis was done using DESEq2. Gene ontology analysis was performed using Tritrypdb.

Results: According to the RNA-seq results, we identified 17,120 total genes. 513 genes were differentially expressed in metacyclic trypomastigotes; 221 were up-regulated and 292 down-regulated. These genes are related to relevant biological processes in metacyclogenesis, such as infectivity, differentiation, metabolism, cell division, DNA replication, and response to oxidative stress.

Conclusions: The results obtained in this work generate new knowledge about the biology of T. cruzi, applied to the understanding of virulence, infection processes, differentiation, parasite-host interaction, and in the long term to know more about the pathology of Chagas disease.

Keywords: Trypanosoma cruzi, Metacyclogenesis, Transcriptomics

P247 / #1448

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

MOROCCAN PRIMARY LEISHMANIA MAJOR STRAINS DIFFERENTLY MODULATE INFECTION PROFILE, INOS AND IL-1B GENE EXPRESSION IN MICE

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Introduction: In Morocco, zoonotic cutaneous leishmaniasis (ZCL) caused by Leishmania major is a public health issue characterized by polymorphic clinical lesions. This clinical polymorphism could depend on the vector, parasite genetic background, and host immune response. The overall objective of our work was to determine whether genetic L. major diversity impacted the host immunopathology. Five L. major strains were isolated from CL patients' lesions during a field mission in 2 ZCL endemic provinces: MHOM/MA/2017/T18, MHOM/MA/2017/T15, MHOM/MA/2017/T13, and MHOM/MA/2017/Z41, MHOM/MA/2017/Z04.

Methods: Swiss mice were injected with metacyclic promastigotes into the hind footpads to initiate infection. After 3 and 13 post-Infection (pi) mice were sacrificed, draining lymphatic nodes (DLNs), spleens, and livers were collected to detect the parasite kinetoplast DNA (kDNA) by PCR and to analyze inducible Nitric Oxide Synthase (iNOS) and IL1-β genes expression by RT-qPCR.

Results: Swiss mice infected with L. major strains showed infection patterns similar to resistant phenotype. They exhibited different infection profiles through differences in lesions' development and stabilization durations, which were longer with MHOM/MA/17/Z04 strain. Leishmania kDNA was detected in 100% spleens and livers, and 90% DLNs for mice infected with MHOM/MA/17/Z04 strain. RT-qPCR analysis revealed a decreased iNOS and IL-1β genes expression in DLNs and spleens of mice infected with the same strain at 3 and 13 weeks pi.

Conclusions: Swiss mice infected with Moroccan L. major strains showed different infection and cytokines expression profiles, suggesting that these responses were related to Leishmania strains.

Keywords: Leishmania Major, clinical polymorphism, IL-1 β, kDNA, iNOS

P248 / #436

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

ROLE OF TRYPANOTHIONE METABOLISM IN ANTIMONY RESISTANCE OF LEISHMANIA TROPICA CLINICAL ISOLATES

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Introduction: Trypanothione metabolism, the main form of thiol, has shown to play a central role in antimony resistance of laboratory-generated resistant Leishmania spp. but the mechanism of antimony resistance in the clinical isolates of L. tropica causing anthroponotic cutaneous leishmaniasis (ACL) is less studied.

Methods: L. tropica isolates were collected from patients who were either treatment-responsive (MAS=S1 to S5) or unresponsive (MAR=R1 to R4) to Glucantime® (meglumine antimoniate=MA). Isolates were tested for sensitivity to trivalent antimony (SbIII) in promastigotes and to pentavalent antimony (SbV) in intracellular amastigotes stages. Intracellular thiol levels were assayed and trypanothione reductase (TR) and tryparedoxin peroxidase I (TryP) were analysed at protein level and enzymatic activity in isolates.

Results: The MAR isolates had an approximate two fold increase in the levels of intracellular thiols ($P< 0.05$) accompanied by an average 5-10 fold increase in in vitro resistance to antimony. TryP was amplified at the protein level in all MAR strains as compared to the MAS strains (range: 2.8-5.6 fold). All MAR isolates metabolized H_2O_2 at higher rates than MAS isolates (8.55±0.75 nmol/min/mg vs. 3.14±0.36 nmol/min/mg) ($P< 0.05$). Levels of TryR protein were also markedly elevated in 3 out of 4 MAR isolates (range: 2.2-4.1 fold). This was accompanied by overexpressed TryR activity (mean level of 46.83±2.43 for extracts of MAR vs. 20.98±3.02 for MAS strains) ($P< 0.05$).

Conclusions: TryP, active enzyme in peroxide detoxification, and TryR activities were overexpressed on average in extracts of MAR strains. Enhanced anti-oxidant defenses through thiol metabolism may play a significant role in clinical resistance of ACL patients to Glucantime.

Keywords: Leishmania tropica, drug resistance, anthroponotic cutaneous leishmaniasis, thiol, antimonials

P249 / #476

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

ANTI- AND PRO-INFLAMMATORY CYTOKINES IN HUMAN ANTHROPONOTIC (ACL) AND ZOONOTIC CUTANEOUS LEISHMANIASIS (ZCL)

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Introduction: Anthroponotic cutaneous leishmaniasis (ACL) due to Leishmania tropica and zoonotic CL (ZCL) due to L. major have different clinical and epidemiological features. This experiment aimed to determine whether pro- and anti-inflammatory cytokines are involved in diverse pathogenicity of Leishmania species causing CL.

Methods: Blood samples were obtained from 6 patients with active ACL and active ZCL and 5 healthy controls (HC) without history of CL. Peripheral blood mononuclear cells (PBMCs) and monocyte-derived macrophages (MDMs) were cultured in the presence of either phytohemaglutinin (PHA), soluble Leishmania antigen (SLA), live Leishmania or without stimulation. After 24 and 48 hours for PBMCs and 4, 10 and 24 hours for MDMs, RNAs were extracted. Levels of gene expressions were analysed for IFN-γ, MCP-1 (CCL2), TGF-B and IL-8 (CXCL8) and RANTES (CCL5) in PBMCs and MDMs by real-time RT-PCR technique.

Results: PBMCs from both ZCL and ACL cases expressed significantly higher IFN- γ ($P < .001$). PBMCs from ACL patients expressed significantly higher IL-8 compared with ZCL cases and HC when stimulated with live L. major or L .tropica promastigotes ($P < .001$). Upregulation of TGF-B was seen in ACL cases in comparison with ZCL and HCs ($P < .05$). After 4 and 10 hours, L. major infected MDMs expressed higher IFN-γ ($P < .05$), and after 10 hours, L. tropica infected MDMs expressed significantly higher IFN-γ and IL-8 and RANTES (CCL5) compared with noninfected cells ($P < .05$).

Conclusions: This study shows differential parasite-mediated stimulations of the inflammatory response with L. major vs L. tropica ex vivo which might be associated with different clinical features of ACL and ZCL.

Keywords: pro-inflammatory cytokines, Cutaneous leishmaniasis, Leishmania Major, anti-inflammatory cytokines, Leishmania tropica

P250 / #1034

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

RESPONSE TO INFECTION BY TRYPANOSOMA CRUZI IN A MURINE MODEL

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Introduction: Cardiopathy is a common, irreversible manifestation of the chronic phase of Chagas disease; however, there is controversy as to how the causes for progression from the acute to the chronic phase are defined. In this work, the presence of the parasite is correlated with the occurrence of cell infiltration and fibrosis in cardiac tissues, as well as IgG detection and disease progression in a murine model.

Methods: 50 CD1 mice were infected intraperitoneally with T. cruzi and 30 mice with saline solution as a control group, parasitaemia was quantified and heart sections and HE and Masson staining were performed. IgG titers were determined by ELISA test.

Results: A increase in parasitemia levels was observed after 15 days post-infection (dpi), 4.1 × 10^6 parasites on 33 dpi, amastigote nests were observed on 15-62 dpi. The lymphocytic infiltration and fibrotic lesions from 8 dpi until the end of the study. Plasma cells were present at 40-60 dpi, accompanied by seropositivity to ELISA on 40-100 dpi, was regarded as the hallmark of the transition phase. The chronic phase, characterized by the absence of amastigotes, presence of cell infiltration, fibrotic lesions, and seropositivity, started on 62 dpi. A strong correlation between parasitemia and the presence of amastigote nests was found ($r^2 = 0.930$), and that between fibrosis and lymphocyte infiltration on 100 dpi was strong ($r^2 = 0.899$).

Conclusions: The murine model is suitable to study Chagas disease. The acute phase was determined to occur on 1-60 dpi, while the chronic phase starts on 62 dpi, and fibrotic damage is a consequence of the continuous inflammatory infiltration; on the other hand, fibrosis was determined to start on the acute phase, being more apparent in the chronic phase, when Chagas disease-related cardiopathy is induced.

Keywords: chagas disease, lymphocyte infiltration, ELISA, chronic and acute phases, Trypanosoma cruzi

P251 / #1660

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

EXPLORING THE ASSOCIATION BETWEEN PRECIPITATION AND POPULATION CASES OF OCULAR TOXOPLASMOSIS IN COLOMBIA

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Introduction: A relationship between precipitation and ocular toxoplasmosis (OT) reactivation and congenital toxoplasmosis has been proposed. This study aimed to investigate the relationship between precipitation and the frequency of new cases of OT in Colombia from 2015 to 2019.

Methods: Retrospective cohort study using a claims-based database from the Colombian Ministry of Health and national registries of precipitation from the Institute of Hydrology, Meteorology, and Environmental Studies. To estimate the daily number of cases of OT, interpolating data from the average number of annual cases. Then, exposures (mean daily precipitation) in the case period in which the events (interpolated OT new cases) were compared by a quasi-Poisson regression, combined with a non-linear distributed delay model to estimate the non-linear and delay-response curve.

Results: There were 1,741 new records of OT. OT new cases between all departments were significantly different ($p < 0.01$). The cumulative exposure-response curve was decreasing for most departments. Nevertheless, Chocó, Bogotá, Cesar, Cauca, and Guajira have a pattern contrary to that observed in the rest of the country, showing that with a certain amount of precipitation accumulates, the RR increases. The response curves to the one-day delay showed that precipitation influence the RR; however, the trends vary by department.

Conclusions: Precipitation influence the RR for new cases of OT. However, there are inconstant trends between geographical regions (departments), probably due to additional variables that could influence the RR.

Keywords: Ocular toxoplasmosis, Toxoplasmosis, Rainfall, Epidemiology, Colombia

P252 / #548

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

VPS36 DYSFUNCTION HAS SEVERE CONSEQUENCES ON LEISHMANIA MAJOR VESICLE RELEASE AND INFECTIVITY

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Introduction: Leishmania is a protozoan parasite that infects mammalian macrophage cells by evading innate and adaptive immune responses. Our lab has shown that small extracellular vesicles (sEVs) released by Leishmania within the sandfly increase infection severity. To assess the impact of inhibiting Leishmania sEV production, the gene Vps36, a protein key to sEV production in eukaryotes, was disrupted in L. major and sEV production and infectivity were assessed.

Methods: Vps36 was disrupted using CRISPR/Cas9 and restored using plasmid recombination, generating VPs36null and Vps36addback L. major strains. sEVs were collected from cultured L. major using differential ultracentrifugation and analyzed using nanoparticle tracking analysis (NTA), Transmission Electron Microscopy (TEM), RNAseq, and LC-MS/MS proteomic analysis. Balb/C mice footpads were injected with WT and Vps36null L. major parasite either alone or supplemented with purified WT L. major sEVs and lesions were measured.

Results: TEM showed Vps36null L. major still produced sEVs in culture and after temperature shock, but NTA results suggest they produce significantly less sEVs compared to WT. Vps36addback restored sEV production. Vps36null L. major sEVs have a unique protein profile compared to WT L. major sEVs whereas there is no significant difference between the whole parasites. Vps36null L. major failed to induce a high level of infection in susceptible Balb/C mice even when co-injected with WT L. major sEVs compared to severe infection caused by WT L. major.

Conclusions: Our results suggest Vps36 plays a key role in sEV production by L. major and interferes with vesicle packaging, resulting in a severe reduction in infectious capability. This effect is specific to Vps36 disruption as verified by Vps36addback.

Keywords: Leishmania, CRISPR, Infectious disease, Vps36, extracellular vesicles

P253 / #1464

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

LEISHMANIA IN SAND FLIES FROM AL-MADINAH AL-MUNAWARAH, SAUDI ARABIA

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Introduction: Cutaneous leishmaniasis (CL) is caused by flagellated protozoan parasite of the genus Leishmania. CL is a major health problem in Saudi Arabia including Al-madinah Al-munawarah province. We aimed to identify Leishmania species isolated from sand fly using molecular analysis.

Methods: Sand fly were collected from the province of Al-madinah Al-munawarah. Female sand flies collected were subjected to DNA extraction followed by molecular analysis using 3 step PCR protocol. Positive sample by direct ITS PCR or ITS1 nested PCR (nPCR) was considered true positive. Leishmania speciation was done by PCR/nPCR-restriction fragment length polymorphism (RFLP) technique.

Results: Both anthroponotic, L.tropica and the zoonotic species, L. major were identified. Detailed results will be presented.

Conclusions: For accurate molecular diagnosis and speciation of Leishmania, we recommend using ITS1 nPCR for negative cases by ITS1 PCR. Further, exploration of Leishmania transmission dynamics in vectors and reservoir animals is essential for designing effective preventative measures.

Keywords: sand flies, Al-madinah Al-munawarah, Leishmania

P254 / #1748

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

TAENIA ASIATICA: LATEST UPDATE

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Introduction: Taenia asiatica was first described by Eom and Rim in 1993. The life cycle of T. asiatica differs from T. saginata in its intermediate host(pig) as well as in the infected organs (liver). Geographically it is known that T. saginata endemic areas are now shared by T. asiatica in Asian region-Korea, Taiwan, the Philippines, China, Thailand, Indonesia, Vietnam, Japan, Lao PDR, Nepal and India with some more suspected countries-and in some areas it's rather the most common species (Advances in Parasitology, Vol. 108: 133-173, 2020).

Methods: The molecular tools employed for T. asiatica used so far are nucleotide sequence of mitochondrial genes, nuclear ribosomal genes and nuclear genes that lead to development of the subsequent molec- ular techniques, such as PCR-RFLP, PCR-RAPD, BESST-base, LAMP and qPCR.

Results: The mitochondrial genomes of T. asiatica comprise 13,703bp and contain 36 genes including 12 protein-coding genes, 2 ribosomal RNAs (rRNAs, a small and a large sub- unit), and 22 transfer RNAs (tRNAs). Sequence differences in the full genome of T. asiatica and T. saginata mitochondria is 4.6%, while T. solium differs by 11%. Hox gene orthology in T. asiatica was established by comparative analysis with Platyhelminthes Hox genes. T. asiatica Hox revealed six Hox orthologs including two lab/Hox1, two Hox3, one Dfd/Hox4 and one Lox/Lox4.

Conclusions: Hybridization between T. asiatica and T. saginata was definitely observed in these species which are sympatrically endemic in the regions of Korea, Thailand, China and Lao PDR. Comparative analyses of T. asiatica, T. saginata and T. solium genomes were also reported with genome features.

Keywords: cestode, tapeworm, Taenia, asiatica, update

P255 / #954

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THERAPEUTIC EFFECT OF TOPICAL MILTEFOSINE HYDROGELS IN BALB/C MICE INFECTED WITH LEISHMANIA VIANNIA SPECIES

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Introduction: A topical miltefosine treatment could be a therapeutic alternative for human cutaneous leishmaniasis (CL). The aim was to evaluate the dose-dependent response for the antileishmanial effect of MTF in CL-infected mice.

Methods: Miltefosine-hydrogels were prepared at 0 %, 0.06%, 0.17%, 0.5% y 1.5% w/v of MTF. Physicochemical and stability properties were determined using standard methodologies. BALB/c mice were intradermal infected with L. (V.) braziliensis and L. (V.) panamensis. Treatment (MTF and vehicle) was applied topically for 25 days. At 60 days' post-treatment skin lesion was removed for histopathological analysis and parasite-loads by qPCR. Oral MTF at 30 mg/Kg/28 was used as control.

Results: MTF-hydrogels were transparent, homogeneous and stables. Treatments using 1.5% and 0.5% MTF were effective with a lesion reduction of 100% and 87.9% in L. (V.) braziliensis and 100% in L. (V.) panamensis infected mice. A large amount of dermal fibrin without leukocyte infiltrate and amastigotes were microscopically observed. A mean of 0.0 and 385.7 parasites in L. (V.) braziliensis and 2299.6 and 0.0 parasites per lesion in L. (V.) panamensis were detected. Parasite-loads of non-treated mice were $249.9x10^3$ and $153.1x10^3$. The effective concentrations were EC_{50}: 0.22 %; EC_{90}: 0.47% MTF for L. (V.) braziliensis and EC_{50} 0.29; EC_{90} 0.34 %MTF for. L. (V.) panamensis. MTF-hydrogels did not induce irritation signs.

Conclusions: The topical 0.5 and 1.5% miltefosine treatments were efficacious and non-toxic in CL-infected mice. Acknowledgements
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Keywords: topical treatments, BALB/c mice, Cutaneous leishmaniasis, miltefosine

P256 / #793

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

CURRENT STATUS OF LYMPHATIC FILARIASIS IN THREE SELECTED COMMUNITIES IN SAGBAMA LOCAL GOVERNMENT AREA OF BAYELSA STATE, NIGERIA

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Introduction: Background and Aim: Lymphatic Filariasis (LF) is one of the Neglected Tropical Diseases (NTD) that poses major public health concerns around the world. The aim of this study was to ascertain the current status of lymphatic filariasis in three selected communities in Sagbama LGA Bayelsa State, Nigeria

Methods: Blood samples were collected at night through vein puncture and examined using Giemsa staining and Knott concentration techniques. Indoor resting mosquitoes were collected using the pyrethrum spray technique and dissected for filarial worms.

Results: Out of 274 participants 37(13.5%) were infected with LF, Adagbabiri had the highest prevalence of 21(21.4%) followed by Sagbama 11(12.6%) and 5(5.6%) in Tungbabiri. The females were more infected than males, 22(59.5%) and 15(40.5%) respectively, there was no significant difference (P>0.05) between sex. The highest prevalence of 19.4% was recorded in ages 40-49yrs and the least prevalence of 5.3% in 10-19yrs. Low microfilaria density was observed among the age groups and sex across the 3 communities. Participants' knowledge of LF was very low but a good perception of socio-economic consequences of LF was recorded across the three communities. No filarial larvae were recovered from the mosquitoes dissected.

Conclusions: Conclusion: Although low prevalence was observed in the study, there is a need for control programs and surveillance of LF in Sagbama Local Government Area.

Keywords: Lymphatic Filariasis, Microfilaria, Prevalence, Surveillance

P257 / #1567

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

VIRULENCE OF ISOLATES FROM TRIATOMA PHYLLOSOMA (HEMIPTERA: REDUVIIDAE), AN ENDEMIC MEXICAN VECTOR OF CHAGAS DISEASE

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Introduction: Chagas disease is caused by *Trypanosoma cruzi*, transmitted by hematophagous hemiptera. In Mexico, in the state of Oaxaca, a prevalence of 17.7% and 672,947 infected individuals have been estimated. The aim of this study was to measure the parasitaemia and survival of three isolates of *T. cruzi* obtained from *Meccus phyllosoma*.

Methods: The isolates used were obtained from *Meccus phyllosoma* species infected with *T. cruzi* from three locations in Oaxaca, Mexico, Tehuantitla, Vixhana and Guichivere. To the parasitaemia mice were inoculated intraperitoneally with one million parasites. The parasitaemia was quantified on Neubauers chamber and survival were record during a period of 30 days.

Results: The isolate from the Tehuantitla locality presented 2.3 X 10^7 parasites/mL; the Vixhana isolate presented 1.6 x 10^7 and Guichivere 1.2 x 10^7, the latter being the one with the lowest parasitemia ($p=0.001$). Survival for the three isolates was 0% (<0.001) on different days.

Conclusions: The behavior of the isolates was different even coming from the same species of triatomine. The isolate from Tehuantitla presented higher parasitaemia, compared to the isolates from Vixhana and Guichivere. Regarding survival, all the mice died, showed high mortality in three isolates but in different days.

Keywords: Trypanosoma cruzi, Triatoma phyllosoma, parasitaemia, survival

P258 / #272

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THE EFFECT OF SILYBUM MARIANUM AND ITS COMBINATION WITH ALBENDAZOLE IN THE PREVENTION AND TREATMENT OF HYDATID CYST

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Introduction: Background: Cystic echinococcosis (CE), is a parasitic zoonosis caused by Echinococcus granulosus. Surgical intervention remains the mainstay of CE treatment, together with scolicidal agents to inactivate live protoscolices. This study evaluated the scolicidal effects of Silybum marianum ethanolic extract and its combination with albendazole in vitro and in vivo.

Methods: Method: S. marianum ethanolic extract was prepared and analysed by HPLC. Cytotoxicity was measured in mouse macrophage cells (J774A.1 cell line) using MTT method. The in vitro scolicidal activity of the extract alone and combined with albendazole was tested in triplicate at different concentrations (1.95-500 µg/ml). Mice received prophylaxis for 4 weeks before infection or were treated 2 months after infection with 62.5 mg/kg three times a week.

Results: HPLC analysis showed that the S. marianum extract contained mostly silydianin (14.41%).The cytotoxicity against the macrophages for S. marianum (500 µg/ml) after 24 h was only 12%. In vitro, the highest scolicidal activity (75%) was seen with 500 µg/ml after 60 min in combination with albendazole. The best result in vivo occurred in the preventive group using albendazole + Silybum marianum 62.5 µg/mL with cyst size 0.7± 0.28 mm in comparison to 7.8±2.82 mm in the control group. The cyst size for preventive and treated of Silybum marianum were 0.85±0.21 and 1.05± 0.21 respectively.

Conclusions: Conclusion: These data show the potential scolicidal effects of this extract in vitro and in-vivo. As the best results were seen in the preventive group, Silybum marianum could be added to albendazole treatment before surgery to prevent secondary hydatidosis. However, more research is needed to determine its safety and effectiveness in humans.

Keywords: Prevention, treatment, Echinococcus granulosus, Silybum marianum, Albendazole

P259 / #299

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

EVALUATION OF THE EFFECT OF IMIQUIMOD AND ITS COMBINATION WITH ALBENDAZOLE ON PROTOSCOLICES OF ECHINOCOCCUS GRANULOSUS AND IN INFECTED BALB/C MICE

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Introduction: Cystic echinococcosis (CE) is an important zoonotic disease. The available therapies (benzimidazoles) are of limited effectiveness in many cases. Additionally, secondary CE is a continuing problem despite the use of scolicidal agents pre- and post-surgery. We therefore conducted a study to evaluate the effect of imiquimod, an immunomodulatory compound with demonstrated anti-parasitic activity, and its combination with albendazole on Echinococcus granulosus protoscolices (PSCs) and in infected mice.

Methods: Imiquimod at concentrations of 0.19 - 100 µg/ml and albendazole at concentrations of 1.95-500 µg/ml were used in vitro against PSCs. Cytotoxicity was measured in mouse macrophage cells (J774A.1 cell line) using MTT method. Scolicidal activity of imiquimod alone and combined with albendazole were tested in triplicate with incubation for 5, 10, 20, 30, and 60 min. Mice received prophylaxis for 4 weeks before infection or were treated 2 months after infection with 6.25 mg/kg twice a week intraperitoneally.

Results: The drug concentrations used had only minimal cytotoxic effects at the highest concentrations used. The greatest reduction in viability (75%) at 60 min was seen with imiquimod (100 µg/ml), followed by albendazole 500 µg/ml (69%) and imiquimod plus albendazole 275 µg/ml (72%) ($P < 0.05$) whilst the cytotoxicity of imiquimod (100 µg/ml) against macrophages for after 24 h was only 15%. The mean cyst sizes for prevention and treatment in the imiquimod and combined groups were 1.7±0.84, 2.1±0.84, 1.75±1 and 2.15±1 mm respectively compared to 7.8±2.8 mm in the control group.

Conclusions: These data suggest that imiquimod is potentially scolicidal in vitro and in vivo, both alone and in combination with albendazole.

Keywords: Echinococcus granulosus, Albendazole, In vitro, In vivo, Imiquimod

P260 / #390

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

INDUCTION OF APOPTOSIS BY MORPHINE ON INFECTED MACROPHAGES WITH TOXOPLASMA GONDII IN VITRO

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Introduction: The use of opioids such as morphine has been applied as a therapeutic modality. Morphine can effect ordinary functions of immune cells including, macrophages, which produce a variety of factors necessary for a functional immune response in humans and experimental animals.

Methods: Infected macrophages were cultured in the presence of various concentrations of morphine and incubated for 24 h. Cells not exposed to drug were used as the negative controls, while sulfadiazine plus pyrimethamine were considered as positive controls. After incubation the cells were washed in cold PBS and centrifuged at 1,000 g for 5min. The supernatant was then drained off and replaced with 500 µL binding buffer, followed by 5 µL of annexin V and 5 µL of propidium iodide (PI). Eventually, samples were analyzed by FACSCaliber flow cytometer (BD Biosciences) with FlowJo software.

Results: The percentage of apoptosis induced after 24 h in T. gondii infected macrophages were 5.35 %, 7.85 %, 9.26 %, 10.41 %, and 11.72 %, after being treated with 100, 10, 1, 0.1, and 0.01 µg/ml of morphine, respectively, while in the negative control group (macrophages) was 8.21 %. Maximum toxicity was related to low concentration of morphine. Additionally, the percentage of apoptosis in infected macrophages group treated with sulfadiazine, pyrimethamine and sulfadiazine plus pyrimethamine were 7.20 %, 15.08 % and 12.52 %, respectively.

Conclusions: Morphine was able to induce apoptosis in parasite-infected macrophages. In addition, necrosis was low in infected macrophages treated with morphine compared to healthy macrophages without treatment. Therefore, this drug may have beneficial effects against Toxoplasma gondii infection.

Keywords: Toxoplasma gondii, morphine, apoptosis, In vitro

P261 / #431

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

EVALUATION OF CHAGAS DISEASE SCREENING AND PREVALENCE IN AT-RISK PEOPLE WITH HIV

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Introduction: Chagas disease (CD) is caused by the American protozoa Trypanosoma cruzi. In the United States (US) >200,000 people (mostly Latin American immigrants) are estimated to be infected. CD can result in dangerous clinical complications; 20-30% develop cardiac or gastrointestinal sequelae, and the immunocompromised may develop T. cruzi reactivation (>70% mortality). The objective of our study is to measure CD prevalence in at-risk People with HIV (PWH).

Methods: This is an ongoing cross-sectional study at a large HIV clinic in Houston, TX where >5,000 PWH are seen annually (>30% Latinx). Any PWH ≥18yo with CD risk factors (e.g., birth in endemic country) is invited to enroll. At enrollment, we collect 1) demographic/clinical data and 2) blood samples for T. cruzi assays (three serologic tests and one PCR).

Results: As of March 2022, 204 are enrolled (165 men [81%], 39 women [19%]). Birth regions include North America (n=138; Mexico=65%, US=2%), Central America (n=58; 28%), and South America (n=8; 4%). Average CD4 count is 532 cells/mL, SD=324. Average HIV viral load is 5,378 copies/mL, SD=18,464. T. cruzi serologic results via Hemagen IgG ELISA are available for 101: 96 are negative (95%), 4 are positive (4%), and 1 is equivocal (1%). T. cruzi serologic results via Wiener Chagatest are available for 158: 100% are negative including all with positive or equivocal Hemagen tests. Confirmation via a third serologic test (InBios) and T. cruzi PCR results are pending.

Conclusions: Early results emphasize the importance of dual serologic screening in at-risk PWH. At the conclusion of this study, we will better understand the prevalence of CD in at-risk PWH in our community and the effectiveness of a systematic CD screening protocol for PWH with a wide range of CD4 counts and virologic control.

Keywords: chagas disease, HIV, Trypanosoma cruzi

P262 / #835

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THE COMPLETE MITOCHONDRIAL DNA OF TRYPANOSOMA CRUZI: MAXICIRCLES AND MINICIRCLES

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Introduction: The mitochondrial DNA or kinetoplast of Trypanosomatids consists of a few maxicircles and thousands of minicircles concatenated into a complex network. Maxicircles are equivalent to eukaryotic mitochondrial DNAs, while minicircles have guide RNAs involved in U-insertion/deletion editing processes for the maturation of the maxicircle-encoded transcripts. Therefore, this study aimed to sequence, assemble, annotate, and analyze the complete repertoire of maxicircle and minicircle sequences of different Trypanosoma cruzi strains, the parasite that cause the Chagas disease.

Methods: We sequenced, assembled and annotated the complete maxicircle sequence of the Y and Bug2148 strains. Bug2148 was sequenced using PacBio method and Y with Illumina. Minicircles of Y strain were also assembled, tested by PCR and analyzed by MUSCLE and WebLogo3.

Results: Maxicircle sequence of Bug2148 is the longest assembled to date, and is composed of 21 genes, most of them conserved among Trypanosomatids. In previous studies, T. cruzi minicircles showed a conserved structure around 1.4 Kb, with four highly conserved regions and other four hypervariable regions interspersed between them. However, our results suggest that the parasite minicircles display several sizes and numbers of conserved and hypervariable regions, contrary to those previous studies. Besides, this heterogeneity is also reflected in the three conserved sequence blocks of the conserved regions that play a key role in the minicircle replication.

Conclusions: Our results indicate that the different consensus sequences of the maxicircles and minicircles seem to be more complex than previously described with at least four different groups in T. cruzi minicircles.

Keywords: kinetoplast DNA, Trypanosoma cruzi, maxicircle, minicircle, strain

P263 / #2054

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

DUAL SINGLE-CELL SEQUENCING OF TOXOPLASMA GONDII INFECTED MOUSE BMDCS, REVEALS DISPARATE INFECTION DYNAMICS.

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Introduction: Toxoplasmosis has variable outcomes and is highly influenced by the host as well as parasite genetics. While *T. gondii* strains (type I - III) share the ability to interfere with host-cell immunity and disseminate into tissue regions to convert into tissue cysts, their virulence is known to differ greatly. Here, we investigate host-pathogen interactions between*T.gondii* and dendritic cells and monocytes at the transcriptional level in single cells.

Methods: We performed dual single-cell RNA sequencing in GM-CSF stimulated bone-marrow-derived dendritic mouse cells at 3, and 12h post *T. gondii* infection (type I and type II).

Results: Both investigated *T. gondii strains* infect at least two subpopulations of bone marrow-derived dendritic cells, (GM-Macs and GM-DCs) and evoke differential host responses at 12h p.i. in GM-Macs but not in GM-DCs. Further, we find two time-dependent *T. gondii* gene expression modules, with correlating host-gene expression showing clear differences in the modulation of immunity. The transcriptional host cell response to the presence of *T. gondii* parasites, particularly for the more virulent type II strain, is similar to stimulation with oligodeoxynucleotides (cpG-B).

Conclusions: Our results support the premise, that different *T. gondii* strains have varying effects on host cell expression of different cells. These observed differences are more pronounced in GM-Macs infected over a longer period of time (12h p.i.). We further discover potential novel *T. gondii* genes involved in host-pathogen interaction processes. We hope to contribute a compelling resource to understand transcriptional host-pathogen interactions, with an anticipated role to study and understand Toxoplasmosis and other apicomplexan parasite infections.

Keywords: scSeq, Dual, toxoplasma, Transcriptomics, Host-parasite interaction

P264 / #761

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

SERO-EPIDEMIOLOGY OF LATENT INFECTIONS IN HIV-INFECTED PATIENTS DURING THE LAST 10 YEARS IN NORTHEASTERN, IRAN (A RETROSPECTIVE STUDY)

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Introduction: Chronic and latent bacterial, viral and parasitic infections can lead to complications in patients with human immunodeficiency virus (HIV) and can result in death. The aim of this study was to investigate latent toxoplasmosis, hepatitis B and C, syphilis, tuberculosis, as well as cytomegalovirus, and human T-lymphotropic virus-type I (HTLV-I) infections in known HIV patients in the Khorasan-e-Razavi province of Iran.

Methods: This retrospective cross-sectional study was conducted from April 2009 to March 2018. Epidemiological data and mode of acquisition of HIV patients were collected from four infection and behavioral disease consultation centers in Mashhad city. All subjects underwent serological and screening tests to identify latent infections shortly after HIV diagnosis.

Results: This study included 255 HIV-infected individuals with a mean age of 41.30 ± 12.44 SD years, of whom 164 (64.3%) were male. 43.5% were infected with HIV through injection of drugs. The results of serological tests showed that 44.3% of HIV positive individuals tested positive for Toxoplasma, 7.5% for hepatitis B, 45.5% for hepatitis C, 78.4% for cytomegalovirus, 1.6% for syphilis, 4.7% for HTLV-I, and 32.6% for tuberculosis. The mean age of patients with positive test results for Toxoplasma, hepatitis C and tuberculosis was significantly higher than that of patients with negative test results ($P < 0.05$). Furthermore, HCV was significantly more common in men who used IV drugs ($p < 0.05$).

Conclusions: The results show that latent CMV, hepatitis C and Toxoplasma infection are more common among these patients . This signifies the importance of paying attention to prophylaxis that greatly reduce the mortality of people living with HIV.

Keywords: Sero-epidemiology, Latent infections, HIV-infected patients, Northeastern, Iran

P265 / #1373

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

UNRAVELING THE KDNA KINETOCHORE

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Introduction: Trypanosoma brucei is a unicellular protozoan parasite that belongs to the group of Kinetoplastida, which is characterized by the presence of a kinetoplast. The disk-shaped structure consists of a single genome that is packed into a big network of interlocked DNA molecules consisting of maxi- and minicircles, called kDNA. To correctly segregate the newly replicated DNA, the kDNA is connected to the basal body of the flagellum through a complex called tripartite attachment complex (TAC). Several proteins localized between the basal body and the kDNA have been described. However, the kDNA-binding proteins connecting kDNA to the TAC remain unknown. My hypothesis is that a set of proteins (kDNA kinetochore) interacts with a centromeric region on the kDNA, connecting it to the TAC. My aim is to identify and characterize these kDNA kinetochore components and characterize the centromeric sequence.

Methods: To find interacting proteins of my candidates in vivo, I chose to perform BioID experiments. BioID uses a biotin ligase which biotinylates neighboring proteins. Another method is ultrastructure expansion microscopy for visualization.

Results: Tb927.11.16120, a DNA-binding protein, previously described in our lab and TbKap6, an HMG-box containing protein (Wang et al, 2014) are essential for kinetoplast DNA replication and maintenance. Since both proteins are DNA binding proteins and both localize at the kDNA, they are potential mitochondrial kinetochore proteins. I will be showing results of the BioID experiments on these 2 proteins with additional microscopy images.

Conclusions: In this work, we hope to provide insights from an understudied eukaryotic group that might help us get a better understanding on the evolution of one of the defining organelles of eukaryotes

Keywords: Kinetoplast, kDNA, Kinetochore, Mitochondria

P266 / #1270

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THE ACHILLES' HEEL OF THE FOX TAPEWORM? - INVESTIGATION OF THE THREONINE METABOLISM OF ECHINOCOCCUS MULTILOCULARIS

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Introduction: Alveolar echinococcosis (AE) is a severe zoonotic disease caused by the metacestode (MC) of the fox tapeworm Echinococcus multilocularis. Novel treatment options are urgently needed. In vitro experiments showed strong scavenging of threonine by E. multilocularis MCs. We thus aim to elucidate the threonine metabolism of E. multilocularis with a specific focus on threonine dehydrogenase (TDH), an enzyme that is actively expressed in E. multilocularis MC. EmTDH is potentially an interesting future drug target against AE, as human TDH is a non-functional pseudogene. Quinazoline carboxamids (Qc) are potent inhibitors of mouse TDH and we aim to test them against E. multilocularis.

Methods: $^{13}C_4$ L-threonine and its metabolites were traced in in vitro cultured MC to give new insights into how threonine is metabolized in E. multilocularis. In addition, we currently study the effect of L-threonine on the growth of in vitro cultured MC and on the proliferation of E. multilocularis stem cells. Qcs will be tested in organism-based and target-based screening approaches to show whether they can inhibit the threonine metabolism in E. multilocularis.

Results: The flux analysis with $^{13}C_4$ L-threonine showed that E. multilocularis MC take up threonine and metabolize it to glycine. In addition, threonine may be used for the biosynthesis of glutathione. Growth assays showed significant increase in diameter of threonine-treated MC.

Conclusions: Flux analysis and growth assays provided evidence that threonine metabolism is important for E. multilocularis and inhibiting this metabolism could provide new treatment options. Our experiments with Qcs will show whether they might provide interesting candidates for future treatment.

Keywords: Echinococcus multilocularis, metacestode growth assay, threonine metabolism

P267 / #1305

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

IN VITRO DRUG SCREENING CASCADE FOR ECHINOCOCCUS GRANULOSUS

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Introduction: Cestodes of the genus Echinococcus cause the severe zoonotic diseases alveolar echinococcosis (AE) and cystic echinococcosis (CE). New treatment options against both diseases are urgently needed. An in vitro drug screening pipeline is established for the AE-causing stages (metacestodes) of E. multilocularis. In contrary, drug efficacy assessments against E. granulosus are mainly performed via the subjective eosin exclusion test on protoscoleces (PSCs), which are not the disease-causing stage for CE. Thus, there is the urgent need for objective drug tests against E. granulosus.

Methods: We applied an in vitro screening cascade established for E. multilocularis to E. granulosus to compare the efficacy of several standard drugs (niclosamide, nitazoxanide, albendazole, monepantel, mefloquine, buparvaquone and MMV665807). Efficacy against metacestodes was compared via a damage marker release assay and a vesicle viability assay. Efficacy against PSCs was compared via motility assay.

Results: Comparing drug efficacy against E. multilocularis and E. granulosus in vitro, the damage marker release assay and the metacestode viability assay showed similar drug responses. Stem cell assays are still ongoing and results will be presented at the conference. Interestingly, the drugs MMV665807, niclosamide and nitazoxanide showed a higher efficacy against E. granulosus PSCs compared to E. multilocularis PSCs.

Conclusions: Our results show that our established drug screening cascade to identify novel drugs against E. multilocularis metacestodes and PSCs can also be applied to E. granulosus. This will allow the medium throughput screening of drugs against E. granulosus as well.

Keywords: drug screening, Echinococcus multilocularis, Echinococcus granulosus

P268 / #298

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

TREATMENT FAILURE IN CUTANEOUS LEISHMANIASIS PATIENTS REFERRED TO THE SCHOOL OF PUBLIC HEALTH, TEHRAN UNIVERSITY OF MEDICAL SCIENCES DURING 2008-2017.

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Introduction: Cutaneous leishmaniasis (CL) is a vector borne disease predominantly found countries. It is an important infectious disease in the Eastern Mediterranean countries, including Iran. For more than 6 decades, pentavalent antimonials have been used successfully worldwide for the treatment of leishmaniasis, but over the past few years, clinical resistance to these medications has increased. In this study, we evaluated CL patients who did not show any desirable responses to the anti-leishmanial treatment within a 10-year period (2008 to 2017).

Methods: This is a retrospective study. All patients from different parts of Iran suspected of having cutaneous leishmaniasis, who were referred to the laboratory of leishmaniosis in the School of Public Health at Tehran University of Medical Sciences (TUMS) from 2008–2017 were parasitological examined.

Results: During this period, a total of 1480 suspected CL patients were referred to the laboratory of leishmaniosis. Samples from 655 patients (70.8%) suspected of having CL were positive microscopically. The failure rate in patients treated with anti-leishmaniasis medications for a minimum of three complete treatment periods was 1.83% (12 cases). There was no association between the number and size of skin lesions and patient characteristics. Also, the route of drug administration had no significant effect on the number and size of lesions.

Conclusions: In the present study, treatment failure was found in some confirmed CL patients treated with meglumine antimoniate. Over the past few years, it seems that had been increased in resistance to these medications. So, a review of the correct implementation of the treatment protocol and/or a combination therapy may be helpful in preventing an increase in the rate of treatment failure.

Keywords: Cutaneous leishmaniasis, Anti-Leishmania drug, Treatment failure, Iran

P269 / #974

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

IN SILICO SCREENING OF A POTENTIAL INHIBITOR TARGETING THE GLYCOPHOSPHATIDYLINOSITOL PATHWAY FOR TREATMENT OF LEISHMANIA DONOVANI.

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Introduction: Leishmaniasis is a neglected parasitic disease occurring due to Leishmania donavani infection. An effective treatment regime with fewer side effects has necessitated the exploration of newer potential molecular scaffolds. The Glycophosphatidylinositol (GPI) pathway responsible for cell signaling is crucial for parasitic survival. Hence, its disruption can prove lethal for the parasite. The enzyme, N-acetylglucosamine-phosphatidylinositol de-N-acetylase (NAGP-deacetylase) from GPI pathway catalyzes deacetylation of N-acetylglucosaminylphosphatidylinositol to glucosaminylphosphatidylinositol and is a prominent protein target for design of novel molecules.

Methods: An in silico approach comprising high throughput virtual screening with natural product diverse library set from Chembridge database, coupled with docking, molecular dynamics simulation, extensive MM-GBSA based energy calculations and drug-likeliness evaluation was employed to screen out compounds targeting NAGP-deacetylase.

Results: The study yielded two compounds that mimic the GlcNAc moiety, 14671 and 4610, with drug-like attributes and binding free energies of -50±4 and -54±4 kcal/mol respectively from the initial 12 docked hit molecules. Both compounds were positioned similarly in the binding pocket and exhibited stable protein-ligand interactions throughout MD simulations.

Conclusions: The two identified molecules interacted favorably with catalytic active site residues and displayed significant ligand binding efficacy towards the enzyme. These druggable molecules can be explored further as possible scaffolds for developing inhibitors as anti-leishmaniasis agents with enhanced potency against NAGP-deacetylase. (DOI:10.1080/07391102.2021.2025429)

Keywords: virtual screening, Molecular Dynamics, in silico, Leishmania donovani, drug design

P270 / #1267

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

QPCR QUANTIFICATION OF WOLBACHIA ENDOSYMBIONTS FROM FEW ONCHOCERCA VOLVULUS MICROFILARIAE FOR LONGITUDINAL MONITORING OF ANTIWOLBACHIAL CLINICAL DRUG TRIALS

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Introduction: Onchocerciasis (river blindness) is a neglected tropical disease targeted for elimination by the WHO; affecting 21 million people in Sub-Saharan Africa and Latin America. The disease is caused by the filarial nematode Onchocerca volvulus, which lives in symbiosis with intracellular Wolbachia Gram-negative bacteria. Targeting Wolbachia with antibiotics causes permanent sterilization and kills adult worms. Assessing treatment success of antiwolbachial therapy is essential for development of new regimens. However, the current diagnostic to assess Wolbachia depletion is histological examination of adult worms and faces two major obstacles. First, it cannot be used earlier than 6 months post treatment; the gold standard is 20-27 months post treatment. Second, nodules can only be used once, limiting the number of time points to monitor Wolbachia depletion.

Methods: We established a TaqMan qPCR amplifying the single-copy gene ftsZ to quantify Wolbachia from MF from skin snips (Schlabe S., etal 2022, DOI: 10.1007/s00436-021-07411-5). We implemented the qPCR on MF from skin snips collected from phase 2 antiwolbachial drug trials in Ghana (MoRion; ISRCTN43697583 and ASTAWOL; PACTR202009704006025).

Results: The qPCR copy number was calculated at different time points following treatment and used as a surrogate parameter for monitoring Wolbachia depletion in adult worms.

Conclusions: The analysis of the results is ongoing and will be presented.

Keywords: Onchocerca volvulus, Wolbachia, qPCR

P271 / #384

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THE ENIGMATIC FUNCTION OF SMALL TIMS IN THE HYDROGENOSOME OF TRICHOMONAS VAGINALIS

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Introduction: Small Tims in intermembrane space (IMS) of mitochondria mediate sorting of imported proteins to their final destination. In mitochondria, five small Tims have been recognized that form two functional pairs Tim9/10 and Tim8/13, and Tim12. The import of small Tims is dependent on the mitochondrial intermembrane space sorting signal that includes highly conserved twin Cx_3C motif. They are trapped within IMS via the oxidative folding by the mitochondrial import and assembly (MIA) pathway. Trichomonas vaginalis possess anaerobic type of mitochondria named hydrogenosome that contain two small Tims named TvTim9/10A and TvTim9/10B. TvTims possess only a single cysteine residue, and MIA pathway is absent. Thus, a function of hydrogenosomal small Tims remains enigmatic.

Methods: Small Tims were expressed in T. vaginalis with hemagglutinin tag, their localization studied using immunofluorescence microscopy and western blotting of cellular fractions. Immunoprecipitated protein complexes were analyzed by mass spectrometry. Combinations of Tims were co-expressed in E. coli to investigate their interactions. Selected small Tim genes were knocked out using CRISPR/Cas 9 system.

Results: In the genome of T. vaginalis five homologues of small Tims were identified. Fluorescence microscopy confirmed their associations with the hydrogenosomal membranes. Localization was supported by western blotting. Tim9/10A formed a complex with Tim9/10C, but not Tim9/10B. Analyses of proteomic data showed presence of ADP/ATP carrier. Knockout of Tim9/10A and Tim9/10B did not lead to shift in the hydrogenosomal proteome.

Conclusions: Five small Tims operate in the hydrogenosomal IMS. Tim9/10A and Tim9/10C form a complex. Their role in protein sorting needs further investigations.

Keywords: trichomonas, protein sorting, hydrogenosome

P272 / #650

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

IDENTIFYING ANTI-PROTISTAL COMPOUNDS FROM A NOVEL MICROBIAL METABOLITE LIBRARY

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Introduction: Microaerophilic protistan infections contribute significant global disease burdens, disproportionately impacting developing countries and their public health systems. Three human microaerophilic protists, Giardia lamblia, Trichomonas vaginalis and Entamoeba histolytica, infect ~400million people annually, causing gastrointestinal and urogenital tract infections and long-term, post-infectious sequalae. Treatments are limited to nitroheterocyclics; however, their reduced efficacy mean new drugs are urgently required. High-throughput screening (HTS) is key to identifying new candidates and compound classes with microaerophilic activity and new modes of action (MOA).

Methods: We used killing assays and performed HTS of a pure-compound microbial metabolite library containing a range of bioactive natural products (Lam et al., 2021).

Results: We found 44 “hits” against T. vaginalis, 48 against T. foetus (a significant veterinary pathogen) and 74 against G. lamblia at micromolar concentrations (<10µM). With parallel counter-screens in cell lines, we show substantial overlaps between anti-protistal and anti-tumour activity, suggesting compounds impacting high metabolic demands of protists may have similar MOA as observed in tumours. We verified the nanomolar activity of a polyene anti-angiogenic with an active epoxide moiety. We modelled this compound’s activity in silico, confirming the molecular interactions of the active epoxide against putative targets in T. vaginalis, G. lamblia and E. histolytica, defining a new rationale to refine epoxide-polyene candidates.

Conclusions: Overall, this contributes to the growing body of evidence which highlight the potential of microbial metabolites as novel anti-protistals.

Keywords: high-throughput screening, microbial metabolites, microaerophiles, anti-parasitics, drug-discovery

P273 / #1323

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

COMPARING THE HISTOPATHOLOGICAL PROFILE OF COLON TISSUE FROM FOUR EXPERIMENTAL CHAGAS DISEASE MODELS WITH VARYING SEVERITY OF GUT DYSFUNCTION.

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Introduction: The mechanisms underpinning the pathogenesis of digestive Chagas disease are poorly understood. The aim of this study was to compare mouse models with varying severity of gut dysfunction at a histopathological level.

Methods: Histological and immunohistological (IHC) approaches were used to compare chronic colon pathology in the colon caused by two T. cruzi strains (TcCLBR, TcJR) in two mouse strains (BALB/c, C3H). Parameters were focused on the smooth muscle and included inflammation, fibrosis, hyperplasia, neuronal damage and expression of immune response biomarkers.

Results: Of the four infection models, there was only a significant increase in cellular infiltrates within the C3H-TcJR model vs naïve control (62% p = 0.03). There was an increase in collagen content, indicative of fibrosis - 6% and 9%, respectively, in C3H-TcJR/TcCLBR models - and an increase of 4% and 6% in the BALB/c-TcJR/CL models. Inducible nitric oxide synthase (iNOS) expression also significantly increased in C3H-TcJR (p= 0.02), but not in any other model. There was an increase (37%) in oesophageal muscle thickness in the C3H-TcJR model, and structural disruption of a subset of myenteric ganglia.

Conclusions: The C3H-TcJR infection model exhibited a range of histopathological changes in the colonic tunica muscularis. Inflammation, fibrosis and iNOS expression were focal and more pronounced in the mesentery attachment region, but disruption of myenteric ganglion structure appeared more random. This model also displays more severe transit time delays, indicating there is an association between T. cruzi-induced immunopathology and functional abnormalities, which corroborates the translational value of this model for developing interventions that can prevent digestive Chagas disease.

Keywords: Pathogenesis, Enteric neuropathy, chagas disease

P274 / #766

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

RECOMBINANT VACCINIA VIRUS VACCINE EXPRESSING AMA1 OR MIC8 OF TOXOPLASMA GONDII INDUCES PROTECTION AGAINST TOXOPLASMOSIS

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Introduction: Toxoplasmosis is a parasitic infection caused by the intracellular protozoan Toxoplasma gondii. Although it is usually asymptomatic, but it can be fetal in the immunocompromised individuals, and the infection of T. gondii in a pregnant woman can cause severe diseases for the developing fetus. However, there is no effective vaccine available against toxoplasmosis.

Methods: In this study, recombinant vaccinia virus (pRB21) expressing AMA1 or MIC8 of T. gondii were generated and the vaccinia virus were characterized by western. Mice (BALB/c) were immunized with vaccinia virus twice, and T. gondi-specific IgG antibody responses in sera were determined by ELISA. At week 4 post boost, immunized mice were orally infected with T. gondii (ME49) and B cell responses in the spleen were analyzed by flow cytometry, and the protection was determined.

Results: AMA1 or MIC8 gene of T. gondii were successfully cloned into vaccinia virus vector pRB21 (pRB21-AMA1, pRB21-MIC8). AMA1 or MIC8 protein in vaccinia virus was found to be reactive to T. gondii-infected polyclonal antibodies. Compared to naïve mice, the vaccinated mice showed significantly higher levels of parasite-specific IgG antibody responses upon prime and boost. Upon challenged infection, total B cells in spleen were found to be significantly higher in vaccinia virus expressing MIC8. Vaccinia virus pRB21-AMA1 showed similar levels of reduction of brain cysts to vaccinia virus pRB21-MIC8 compared to non-immunized control.

Conclusions: Vaccinia virus pRB21-AMA1 showed similar level of protection to vaccinia virus pRB21-MIC8. Vaccinia virus could be a vaccine platform against toxoplasmosis. In the near future, vaccine efficacy induced by vaccinia virus expressing multi-antigen of T. gondii will be developed.

Keywords: Vaccine, Vaccinia virus, Toxoplasma gondii

P275 / #1162

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

TRICHOMONAS TENAX INDUCES EPITHELIAL CELL DEATH AND PRO-INFLAMMATORY RESPONSES IN MACROPHAGES.

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Introduction: Trichomonas tenax is a parasitic protozoan in the oral cavity, especially in people with poor oral hygiene and periodontal disease. However, the studies on T. tenax are limited, and the impact, pathogenicity, and host interactions are still largely unknown. In this study, we tried to explore the role of Trichomonas tenax in the host and related diseases.

Methods: To investigate the cytotoxicity of T. tenax and cytokine responses caused by T. tenax to host cells, we used different cell lines co-culture with T. tenax.

Results: From microscopic observations, we found that T. tenax adhered to the surrounding human lung epithelial cells, the epithelial cells atrophied, and had larger intercellular spaces. From the cytopathic effect test, we found that T. tenax caused cell damage to the epithelial cells and led to cell death. Next, we found that T. tenax induced the expression of pro-inflammatory cytokines in mouse macrophages Raw 264.7 and human differential THP-1 cells. The lysate and cell debris of T. tenax also induced the expression of pro-inflammatory cytokines, IL-1β and TNF-α, in Raw 264.7 macrophages. More importantly, the expression levels of pro-inflammatory cytokines induced by T. tenax lysate and cell debris are much higher than those induced by live T. tenax.

Conclusions: In summary, T. tenax can cause cell death and induce pro-inflammatory cytokines expression, but these inflammatory responses are non-typical compared to known. These findings may help us clarify the impact of T. tenax on the host and related diseases.

Keywords: Trichomonas tenax, cytotoxicity, inflammatory response

P276 / #1264

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

POPULATION GENOMIC STRUCTURE OF TRYPANOSOMA CRUZI

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Introduction: Trypanosoma cruzi is a kinetoplastid protozoan parasite that causes Chagas disease in Latin America. T. cruzi has a complex population structure, with six major genetic lineages in circulation, two of which are known to be hybrids strongly associated with human transmission in the Southern Cone of Latin America. Others circulate in complex transmission cycles though-out the continent involving multiple host and reservoir species, silvatic and domestic.

Methods: The frequency and mechanism of contemporary genetic exchange in T. cruzi, its adaptive significance, and role in driving population structure, has been slow to come to light.

Results: The first population genomics studies of T. cruzi, including ours, indicate that multiple mating systems (sexual, parasexual, clonal) may operate in parallel. The ecological and evolutionary drivers of such composite population structures are not clear.

Conclusions: I'll summarise the current status of our understanding of T. cruzi population structure, and new avenues for exploring the ecological genomics of this fascinating and deadly parasite.

Keywords: Trypanosoma cruzi, sex, genomics, Epidemiology, Ecuador

P277 / #469

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

DEHYDROTHYRSIFEROL AND LAURENCIA ALGAE DERIVATIVES: ANTI-KINETOPLASTID EFFECT, STUDY OF APOPTOSIS AND IN VIVO ACTIVITY

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Introduction: Available treatments for leishmaniasis and Chagas disease include drugs that produce toxicity in the patient, appearance of resistance, and without effectiveness in the chronic phase (Chagas disease). It highlights the need for new compounds with leishmanicidal and trypanocidal activity more effective and less toxic for the patients.

Methods: Natural compounds isolated from the alga Laurencia johnstonii and the semi-synthetic derivatives from Dehydrothyrsiferol (DHT), were evaluated in vitro against the promastigote and epimastigote forms of Leishmania spp. and Trypanosoma cruzi, using the alamarBlue method. Different assays were also performed, including the measurement of mitochondrial membrane potential, and chromatin condensation among other events related to cell death process, known in this type of organism as apoptosis-like. Groups of BALB/c mice were infected at the base of the tail using L. amazonensis. DHT was administered topically at two different doses, for 14 days. Effectiveness of the treatment was evaluated by weekly measurements of lesion size and by quantification of parasite loads in the infected tissues at the end of treatment by quantitative real-time PCR.

Results: The isolated and the semi-synthetic compounds showed a dose-dependent inhibition with IC50 values up to 3 µg/ml in vitro. DHT showed physiological changes in the different parasites corresponding to an apoptotic process.

Conclusions: DHT showed similar parasite load results (in skin, liver and spleen) to miltefosine, considering this terpene as a good source of compounds for future studies. Ack: I18/01380; RICET (RD16/0027/0001); CIBER (CB21/13/00100); Cabildo Tenerife, MEDI & FDCAN; MICINN, AEI (21/0587). ACIISI (RLRE, DSH, IAJ, ARL, CJBE, PPP), ESF & FEDER, Alumni ULL.

Keywords: Leishmania, Trypanosoma, apoptosis-like, In vitro, In vivo

P278 / #597

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

REPURPOSING OF MITOCHONDRIA-TARGETED TAMOXIFEN: NOVEL ANTI-CANCER DRUG EXHIBITS POTENT ACTIVITY AGAINST MAJOR PROTOZOAN AND FUNGAL PATHOGENS

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Introduction: Many currently available anti-parasitic and anti-fungal drugs have serious limitations, while the development of new therapeutics is a costly and lengthy process. Therefore, repurposing drugs with approved clinical properties might be the straightforward approach for novel treatment options. We tested a new investigational anti-cancer drug MitoTam, a mitochondria-targeted derivative of tamoxifen.

Methods: To assess the potency of MitoTam we used a wide range of distinct pathogens and calculated IC_{50}. We employed proteomic analysis and several advanced biochemical methods to quantify mitochondrial membrane potential, membrane integrity, ATP/ADP levels, respiration, and others using Trypanosoma brucei as a model parasite.

Results: We found that MitoTam is highly efficient against evolutionary distinct parasitic protists in vitro, including pathogenic fungi, Plasmodium falciparum, and species of trypanosomatid parasites. The treatment also significantly reduced parasitemia of Leishmania mexicana and Trypanosoma brucei in the mice model. MitoTam rapidly altered mitochondrial functions, affecting respiration, lowering ATP levels, and dissipating mitochondrial membrane potential. MitoTam mode of action involves disruption of the inner mitochondrial membrane leading to rapid organelle depolarization and cell death.

Conclusions: Altogether, MitoTam is an excellent candidate drug against many pathogens for which there are no modern and effective therapies and for which drug development is not a priority.

Keywords: drug repurposing, Mitochondria, Trypanosoma, plasmodium, Leishmania

P279 / #570

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

FUNCTIONALIZED GRAPHENE QUANTUM DOT APPENDED WITH AMPHOTERICIN B AS A NOVEL TREATMENT OPTION FOR VISCERAL LEISHMANIASIS.

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Introduction: Graphene-Quantum Dots (GQDs) are of ultra-small size (2-10nm), easy to synthesize, non-toxic, biocompatible and with excellent loading capacity due to π-π stacking interactions. In Visceral Leishmaniasis, the amastigotes of Leishmania donovani lodges inside the organs associated with the reticuloendothelial system i.e., macrophages in spleen, liver and bone marrow. GQD-AmB conjugate helps in the targeted treatment of affected spleen and liver and the intrinsic fluorescence properties of GQDs allow the simultaneous tracking of whole process of internalization.

Methods: GQD-AmB uptake kinetics in J774.A1 cells were measured using a flow cytometer and confirmed through microscopy. For testing the antileishmanial efficacy, the percentage of infected macrophages and IC50 values were determined. For the cytotoxity testing, drug-treated macrophages were analysed using MTT assay and CC50 was found out. A pharmacokinetic study was done to get an idea about the time required for the formulations to get cleared from the blood. The levels of inflammatory cytokines were measured upon treatment with GQDs-AmB.

Results: The GQDs-AmB readily entered into the macrophages and there was a significant reduction in the parasite burden with negligible toxicity in comparison to the conventional drug. A marked increase was seen in the level of IFN- γ and IL10 remain unaltered. The pharmacokinetic profile displayed a biphasic release pattern.

Conclusions: The toxicity and high cost of the conventional drugs necessitated the need for a novel, cost-effective treatment option. The IC50 value of functionalized GQD-AmB is substantially lower than the CC50 value, indicating that they are nontoxic to macrophages and suppress the intramacrophage parasites at very low doses.

Keywords: Drug discovery, NANOMEDICINE, Visceral Leishmaniasis, CARBON-BASED NANOPARTICLE

P280 / #242

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

SEROPREVALENCE OF HUMAN TAENIA SOLIUM CYSTICERCOSIS AND ASSOCIATED DEMOGRAPHIC FACTORS IN CENTRAL AND SOUTHERN, TANZANIA

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Introduction: Taenia solium taeniosis/ cysticercosis is emerging as a serious public health and economic problem in many developing countries. Tanzania is among the sub-Saharan African countries highly endemic for porcine cysticercosis, which increases the risk of human taeniosis and cysticercosis. Most studies have been carried out on porcine cysticercosis reporting endemicity in most regions of the country. This study was performed to estimate seroprevalence of human cysticercosis and associated demographic factors in Kongwa and songwe districts. .

Methods: This cross-sectional study included 42 villages (28 from Kongwa, 14 from Songwe) randomly selected with probability proportion to population size. Blood samples were collected from 1552 participants (1040 from Kongwa, and 512 from Songwe), one per household and tested using antigen-ELISA and Western blot IgG-assay

Results: 29 (1.9%)participants had circulating antigens for T.solium, while 32 (2.1%) had antibodies against T. solium cysticercosis. A total of 19 (1.2%) individuals tested positive for both tests. Males had significantly higher prevalence of T. solium cysticercosis than female based on antigen-ELISA (OR 4.17; 95% CI: 1.25- 13.85), Westernblot IgG assay (OR 4.67; 95% CI: 1.42-15.5) and both tests (OR=8.64, 95%C.I.15-64.86). Participants in the age of 15-25 years had higher prevalence of antigen-ELISA seropositivity than older persons (OR 3.98; 95% C1: 1.51-10.01). This study has estimated prevalence of active as well as exposure to the infection.

Conclusions: Appropriate interventions, including health education, should be implemented to control the infection in central and southern highlands of Tanzania.

Keywords: Human, Cysticercosis, Prevalence, Tanzania

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Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

COMPARATIVE PROTEOMIC ANALYSIS OF THE EFFECT OF BACTERIA AND MAMMALIAN CELLS ON NAEGLERIA FOWLERI AND ACANTHAMOEBA CASTELLANII

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Introduction: Worldwide distributed free-living amoebae Naegleria fowleri and Acanthamoeba castellanii are found in soil and aquatic environments where they feed on bacteria and other protists. On rare occasions, these organisms can cause deadly diseases in humans with high mortality. We aim to describe the interactions of both amoebas with bacterial and mammalian cells on a protein level.

Methods: To observe the short-term effect of the presence of bacteria and mammalian cells, we incubated N. fowleri and A. castellanii with Enterobacter aerogenes or fibrosarcoma cells for 6 hours. In addition, we cultivated amoebae for several passages with host cells and isolated N. fowleri from the brain of infected mice. All samples were subjected to comparative label-free proteomic analysis with axenic amoebae used as a control.

Results: Proteomic analysis unveiled 143 and 323 upregulated proteins in A. castellani incubated for 6 hours with bacteria, or host cells, respectively. 721 proteins were upregulated in A. castellani after long-term co-cultivation with host cells. In N. fowleri, only 6 and 20 proteins were upregulated after 6 hours of incubation with bacteria, or host cells, respectively. 113 proteins were upregulated in N. fowleri after long-term co-cultivation with host cells. 384 proteins were upregulated in N. fowleri isolated from the brain.

Conclusions: In conclusion, the two studied amoebae react differently to the presence of bacteria and host cells. A. castellanii generally shows pronounced proteome changes when co-cultured with bacteria or host cells. In contrast, significant changes in the proteome of N. fowleri were only observed when in contact with the host in vivo. Importantly, the data from our study will allow identifying possible virulence factors for both amoebae.

Keywords: Naegleria fowleri, Acanthamoeba castellanii, Comparative proteomics

P282 / #468

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

IN VITRO VALIDATION OF THE AMOEBICIDAL ACTIVITY OF COMMERCIAL EYE DROPS.

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Introduction: Acanthamoeba spp, an opportunistic protozoa belonging to the free-living amoeba (FLA), can cause severe pathologies in humans such as Acanthamoeba keratitis (KA). KA is more common in contact lens wearers, usually associated with poor hygiene habits and recurrent infections. In the present study, the amoebicidal activity of different commercial ophthalmic solution was evaluated in vitro against the trophozoite and cyst stages of four strains of Acanthamoeba spp. In order to evaluate the different compounds containing in commercial ophthalmic solutions, such as chemicals and active ingredients (tobramycin, timolol..), different assays were carried out to evaluate if the ophthalmic solutions induce in the parasites characteristics compatible with programmed cell death, such as chromatin condensation, membrane permeabilization or decreased ATP production levels.

Methods: The anti-amoebic activity of the evaluated eye drops solutions against the trophozoite and cyst stages of Acanthamoeba was determined using a fluorometric assay based on the alamarBlue™ Reagent Assay. The evaluation of the mechanisms of programmed cell death was carried out using commercial kits.

Results: All eye drops showed amoebicidal activity against trophozoite and cyst stages of all Acanthamoeba strains tested. Tobdradex^{MR}, Cusimolol®, Colicursi® and Matrix Ocular® demonstrated the lowest IC_{50} values. A. castellanii Neff was the most sensitive strain to these ophthalmic solutions.

Conclusions: In the present study, Matrix Ocular®, TobraDex^{MR} and Cusimolol® were actives against both stages of the Acanthamoeba castellanii Neff, A. polyphaga, A. griffin and A. quina, inducing apoptosis-like process through the mitochondrial pathway in these four pathogenic strains.

Disclosure: I18/01380; RICET (RD16/0027/0001); CIBER (CB21/13/00100); Cabildo de Tenerife, MEDI y FDCAN; MICINN, Agencia Estatal de Investigación (AEI) and Fondo Europeo de Desarrollo Regional (ERDF) (21/0587). ACIISI ARL, IAJ, CJBE, RLRE, DSNH, PPP), FSE y FEDER, Pr

Keywords: Programmed cell death, eye-drop, Acanthamoeba, amoebicidal

P283 / #1750

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

CHARACTERIZATION OF THE NATURAL TRANSMISSION OF TRYPANOSOMA CRUZI IN ALTO BENI, DEPARTMENT OF LA PAZ, BOLIVIA, AN EMERGING FOCUS OF CHAGAS DISEASE IN THE AMAZON REGION

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Introduction: The transmission of Chagas disease (CHD) in the Amazon region is usually between triatomines and wild mammal. The region of Alto Beni, La Paz, Bolivia was not considered endemic for the inexistence of *Triatoma infestans*, but in this region more than 20 years ago, a first case of autochthonous acute CHD was reported and also the adaptation process of *Rhodnius stali* (9.4% of dwelling infestation, colonization rate of 52.9%, 5.8% of the insects infected by *T. cruzi*). In the inhabitants the seroprevalence for CHD was 3% with some autochthonous cases. With the aim of characterizing the primary cycle of *Trypanosoma cruzi* transmission in this region, we carry out epidemiological, parasitological and entomological studies.

Methods: Between 2017 and 2018, we searched triatomines in 17 *Attalea phalerata* palms (in the wild area) using traps with animal bait. Blood samples from 18 dogs and capture wild mammals (using Tomahawk traps) were collected to identify natural infection by *T. cruzi,* additionally, tissues samples obtaining for aspiration from wild mammals were also analyzed. *T. cruzi* was characterized using PCR-multiplex.

Results: In the forest were found nymphs of *R. stali* in *A. phalerata* without infection by *T. cruzi*. Four *Didelphis marsupialis* (100%) and two dogs were identified infected with *T. cruzi* TcI.

Conclusions: These results demonstrate the primary cycle of *T. cruzi* transmission in the forest with *R. stali* living in *A. phalerata* as vector, and *D. marsupialis* as the main reservoir, with affectation of population due to domiciliation process of *R. stali* and its role as a secondary vector of CHD in this region related to Amazon. This results contribute to the knowledge of epidemiology of CHD in the Bolivian Amazon.

Keywords: chagas disease, Trypanosoma cruzi, Rhodnius stali, Amazon

P284 / #1751

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

ECHINOCOCCOSIS IN BOLIVIA: FROM HISTORICAL REVIEW TO MOLECULAR FINDINGS

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Introduction: Cystic echinococcosis (CE) and alveolar echinococcosis are zoonosis due to *Echinococcus* sp. Human CE is endemic in South America, however, until 2020, their real importance in Bolivia was unknown. Only *E. granulosus sensu stricto* (*s.s.*) was identified using molecular tools in two Bolivian patients (Kamenetzky et al, 2002; Jarovsky et al. 2020), and additionally a *Cuniculus paca* infected by *Echinococcus vogeli*, was reported in Santa Cruz (Gardner et al, 1988).

Methods: We developed a large and comprehensive review of documents published in Bolivia (Ali et al, 2021), and were analyzed the sequence of the cox1 gene in cysts from livestock and humans, and dogs-fecal samples (Ali et al, 2020). These data analyzed conjunctly provide evidences about the epidemiological situation of the echinococcosis in Bolivia.

Results: The first CE cases were reported in 1910 (animals), and 1913 (humans). Since then, have been reported 876 human cases. The highest human prevalence showed 4.1% of the population with signs of CE at ultrasound screening and 24% of dogs, were positive for coproantigens of *E. granulosus sensu lato* (*s.l.*) in the region of Tupiza (Potosí). The highest reports of animal CE were in cattle from Potosí (37.5%) and in llamas from Oruro 26.9%, with low rates in pigs and sheep from La Paz. *E. granulosus s.s.* (G1) was identified in humans (La Paz, Oruro, Potosi); cattle (La Paz, Cochabamba, Beni), and sheep (La Paz); *Echinococcus ortleppi* (G5) in cattle (La Paz, Cochabamba), and *Echinococcus intermedius* (G7) in pigs (Santa Cruz). Canids from La Paz, were identified infected by *E. granulosus s.s.* and *E. ortleppi*.

Conclusions: Bolivia is highly endemic in CE involving different parasite species and hosts, in 8 out of 9 departments.

Keywords: echinococcus ortleppi, Echinococcus intermedius, Cystic echinococcosis, Hydatid disease, Echinococcus granulosus

P285 / #513

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

ATORVASTATIN DECREASES PRO-INFLAMMATORY CYTOKINE SECRETION OF T. CRUZI-INFECTED MACROPHAGES THROUGH RHO KINASE INHIBITION, WHICH IMPEDES ENDOTHELIAL CELL ACTIVATION

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Introduction: Chronic Chagas Cardiopathy (CCC), caused by Trypanosoma cruzi (T. cruzi), is the most common cause of non-ischemic cardiomyopathy in Latin America. In patients with CCC, the pro-inflammatory environment generates endothelial disfunction and chronic myocarditis. Rho Kinase (ROCK) is involved in pro-inflammatory cytokine expression, however, the effect of T. cruzi on this pathway is still unknown. Atorvastatin inhibits HMG-CoA reductase, impeding the activation of RhoA/ROCK. Consequently, in this work, we evaluated the effect of T. cruzi and atorvastatin on the activation of ROCK and the cytokine secretion of macrophages and the subsequent endothelial cell activation.

Methods: Constitutively active ROCK-expressing human macrophages (PMA-induced U937 cells) were infected with T. cruzi and treated with atorvastatin to evaluate the ROCK pathway and cytokine secretion. In addition, the medium of the macrophages was co-cultured with human cardiac endothelial cells (HMVEC) for the evaluation of adhesion molecules expression and adhesion assay.

Results: The results showed that T. cruzi infection activate ROCK pathway in macrophages, inducing pro-inflammatory cytokines secretion. Atorvastatin decreased the pro-inflammatory phenotype in wild type but not in constitutively active ROCK-expressing macrophages. Secondly, atorvastatin-treated macrophages media decreases the endothelial activation and adhesion in HMVEC cells.

Conclusions: In conclusion, atorvastatin, by changing the cytokine profile of macrophages through inhibition of ROCK pathway could constitute a new therapy to prevent the chronic inflammation produced in CCC. Acknowledgments: FONDECYT N°1210359, Beca ANID N° 21170427.

Keywords: Atorvastatin, Trypanosoma cruzi, RHO Kinase

P286 / #83

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

EVALUATION OF IMMUNE RESPONSE OF DSPC NANOLIPOSOMES CONTAINING IMIQUIMOD ADJUVANT AGAINST TOXOPLASMOSIS

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Introduction: Toxoplasmosis is a common infection worldwide caused by Toxoplasma gondii, an intracellular parasite. In the present study, DSPC liposome with Imiquimod Adjuvant encapsulated in the liposome with tactile antigens of Toxoplasma gondii is used, and the immunity level obtained from vaccination and resistance to Toxoplasmosis is evaluated.

Methods: In the present research, a nano-liposomal vaccine containing soluble antigens (SA) was designed to evaluate the immunity and protective efficacy against T. gondii infection in BALB/c mice. Soluble antigens (SA) were achieved from tachyzoites, encapsulated in the liposome, and investigated via scanning electron microscope. Three times with 2-week intervals, BALB/c mice were immunized subcutaneously with different formulations. The level of protection against infection was assessed through the percent survival survey of BALB/c mice after challenge with tachyzoites of T. gondii RH strain; also, the type of generated immune response was determined by evaluating the generation of cytokine (IFN-γ, IL-4) and titration of IgG isotypes.

Results: The immunization with liposome DSPC+ SA and liposome DSPC+ Imiquimod + SA induced a substantial increase in anti-Toxoplasma IgG antibody as compared to the PBS group ($p < 0.05$). The IgG2a and IFN-γ secretion highest levels were seen with liposome DSPC+ Imiquimod + SA more than the control group ($p < 0.01$) and ($p < 0.0001$), respectively. After challenge with tachyzoites, less mortality was detected in the immunized mice by liposome DSPC + Imiquimod + SA that was meaningfully different ($p < 0.01$) in comparison to other groups.

Conclusions: Vaccination with liposome DSPC + Imiquimod + SA showed more survival rate and cellular immune reaction against T. gondii.

Keywords: Toxoplasmosis, Liposome DSPC, Imiquimod, Vaccine

P287 / #1480

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

LEISHMANIA GENOTYPING BASED ON HEAT-SHOCK PROTEIN 70 SEQUENCING

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Introduction: Leishmania is a parasite, transmitted by the bite of a female sandfly and can cause leishmaniasis. In Denmark all cases of leishmaniasis are associated with travel to endemic areas. The disease can either be visceral cutaneous or mucocutaneous, each with different clinical signs and treatments. Species differentiation can be a challenge depending on the molecular targets used for diagnosis and detection. The aim of this study was to implement a heat-shock protein (hsp) 70 gene-sequencing assay for improved identification of Leishmania species. Moreover, the applicability of the hsp70 assay was compared with an assay using the internal transcribed spacer (ITS) region of the ribosomal gene of Leishmania. Both are PCR-based methods but differ in the target region of the parasite.

Methods: We analysed 39 travel-associated real-time PCR-positive samples collected between January 2018 and December 2020 in Denmark.

Results: The hsp70 assay was able to accurately discriminate between Leishmania species based on single nucleotide polymorphisms at specific locations within the hsp70 gene. Most of the samples were positive for L. infantum and L. tropica, which cause cutaneous/visceral and cutaneous leishmaniasis, respectively. No cases of L. donovani were detected in the sample set. We also detected several cases of L. braziliensis and L. panamensis.

Conclusions: In summary, the hps70 assay offers a better resolution than the ITS assay. L. infantum was the most commonly observed parasite in our sample set.

Keywords: Leishmania, typing, PCR, Leishmaniasis

P288 / #1334

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

ECHINOCOCCUS MULTILOCULARIS IN RED FOX (VULPES VULPES), RACCOON DOGS (NYCTEREUTES PROCYONOIDES) AND DOGS IN ESTONIA

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Introduction: Echinococcus multilocularis is present in Estonian nature, both red fox and raccoon dog act as definitive host species for this parasite. The role of domestic dogs as possible link for human infection is also unknown, although few human cases of alveolar echinococcosis have been reported. The aim of the current study was to determine the prevalence of E. multilocularis in wild and domestic definitive hosts

Methods: In 2020/2021, we collected 42 legally hunted red fox and raccoon dog carcasses and 102 dog excrements to detect E. multilocularis in these host species. Segmental sedimentation and counting technique (SSCT) was used for identification of adult worms in hunted carcasses. Dog excrements were analysed by simple flotation technique to detect intestinal parasites. In addition, all dog excrement samples were analysed by coproPCR to determine possible E. multilocularis infection.

Results: E. multilocularis infects wild carnivores in Estonia and the prevalence of the parasite has been relatively constant.

Conclusions: The occurrence of this tapeworm in dogs still needs further study. This work was supported by funding from the European Union’s Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme (MEME project; https://onehealthejp.eu/jrp-meme/).

Keywords: raccoon dog, Dog, Estonia, Echinococcud multilocularis, red fox

P289 / #684

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

FORMULATION OF NEEM OIL-LOADED SOLID LIPID NANOPARTICLES AND EVALUATION OF ITS ANTI-TOXOPLASMA ACTIVITY

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Introduction: Toxoplasmosis is caused by an intracellular zoonotic protozoan, Toxoplasma gondii, which could be lethal in immunocompromised patients. This study aimed to synthesize Neem oil-loaded solid lipid nanoparticles (NeO-SLNs) and to evaluate the anti-Toxoplasma activity of this component

Methods: The NeO-SLNs were constructed, and their shape and size distribution were evaluated using transmission electron microscope (TEM) and dynamic light scattering (DLS), respectively. An MTT assay was employed to evaluate the cell toxicity of the component. The anti-Toxoplasma activity of NeO-SLNs was investigated using vital (trypan-blue) staining. Anti-intracellular Toxoplasma activity of NeO-SLNs was evaluated in infected Vero.

Results: The TEM analysis represented round shape NeO-SLNs with clear and stable margins. DLS analysis showed a mean particle size 74.44±57.33 nm for SLNs, and most of nanoparticles were in range 30 to 70 nm. The cell toxicity of NeO-SLN significantly reduced based on the concentration (P-value = 0.0013). The 50% cytotoxic concentration (CC_{50}) for NeO-SLN was > 10 mg/mL. NeO-SLN showed a statistically significant anti-Toxoplasma activity (P-value < 0.0001). NeO-SLN was also able to kill intracellular Toxoplasma in a concentration-depended manner (P-value = 0.0317).

Conclusions: Our findings demonstrated that the NeO-SLN was able to kill T. gondii tachyzoites in concentration 100 µg/mL with a cell toxicity lower than 20%. Our results suggest that nanoformulation of natural products could be an alternative method to increase efficiency and decrease toxicity of natural products.

Keywords: Toxoplasma gondii, Neem oil-loaded solid lipid nanoparticles, Anti-Toxoplasma activity

P290 / #847

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

AZATHIOPRINE DOWNREGULATES AUTOPHAGIC PATHWAYS, BUT NEUTRALIZED THE MTORC1 PHOSPHORYLATION DUE TO TOXOPLASMA GONDII

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Introduction: Autophagy process is an important defense mechanism against intracellular infection. The target of rapamycin (mTOR) is a potent inhibitor of autophagy. Manipulation of mTORC1 plays critical role in limiting the development of Toxoplasma gondii. On the other hand, Azathioprine (AZA), which is prescribed in inflammatory bowel diseases (IBD), downregulates mTORC1 to activate autophagy. Therefore, it was hypothesized that the presence of T. gondii may modulate autophagic process, AZA as a potential drug, be able to suppress autophagic pathway of Toxoplasma

Methods: PMA-activated THP-1 cell line was incubated with AZA, T. gondii tachyzoites, and AZA/T. gondii tachyzoites for 6 h. The expression of Atg5, Atg7, Atg12, and LC3b was evaluated using real-time PCR. In addition, phosphorylation of mTORC1 was investigated using western blot analysis

Results: The results revealed statistically significant downregulation of Atg12 for 1.43 folds (P-value=0.0062) after treatment with Azathioprine. The expression of Atg 12, Atg 7 revealed a statistically significant upregulation after treatment with Azathioprine/tachyzoite (1.51fold; P-value=0.0419), 1.52 (P-value=0.0224), respectively. Western blot analysis showed that Toxoplasma increases phosphorylation of mTORC1 to block autophagy, while AZA dephosphorylated mTORC1 and induced autophagy, even at the presence of Toxoplasma.

Conclusions: Our results showed that AZA neutralized the mTORC1 phosphorylation due to Toxoplasma. Therefore, it seems that tachyzoites of T. gondii do not interfere autophagy induced by AZA..

Keywords: Azathioprine, mTORC1 signaling, Autophagy, Toxoplasma gondii

P291 / #1629

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

GROWTH INHIBITORY STUDIES OF COMPOUNDS PURIFIED FROM EXTRACTS OF GUIERA SENEGALENSIS AGAINST BLOODSTREAM TRYPANOSOMA BRUCEI BRUCEI

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Introduction: Background and Aims: African trypanosomiasis is a neglected tropical disease of both humans and animals caused by parasitic protozoa of the genus Trypanosoma. An estimated 55 million people are at risk of infection. The disease is fatal if untreated and it militates against growth of livestock industry in sub-Saharan Africa. No vaccine is available and vector elimination remains a mirage. Available drugs are severely limited by unacceptable toxicity, difficulty crossing the blood-brain-barrier, lack of a drug to treat both stages and the two types of the disease, and resistance. The aim of this study was to obtain phytocompounds from which efficacious medicines that overcome the limitations of current medicines can be developed.

Methods: Methods: Bioactive compounds were purified from leaf extracts of *Guiera senegalensis* using chromatography techniques and brine shrimp lethality assay. The bioactive compounds were then screened for growth inhibition studies against bloodstream form of *Trypanosoma brucei.*

Results: Results: Growth inhibition assay revealed that the compounds A3, A4, A7, A8, and TA inhibited cell growth (100%) at 10 µg/ml concentration while A9, A10, TA2, and TA3 reduced cell growth by about 90%. All these compounds showed 100% growth inhibition at or above 40 µg/ml. Compounds T7 and T8 showed 100% growth inhibition even at 2.5 µg/ml and IC_{50} was 120 ng/ml. T3, T4 and TA showed 50% growth inhibition at 3.5, 2.5, 5.0 µg/ml, respectively.

Conclusions: Conclusions: Compounds T7 and T8 are remarkably potent and are vital hits that when progressed to optimized leads (drug development candidates) will lead to development of antitrypanosomal medicines that may overcome limitations of current therapies. This is also true of compounds T3, T4 and TA.

Keywords: Trypanosomiasis, Trypanosoma brucei, medicinal plants, Guiera senegalensis

P292 / #1351

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

SODIUM DIETHYLDITHIOCARBAMATE A POTENTIAL DRUG TOWARDS CHAGAS DISEASE: PHARMACOLOGICAL AND TOXICOLOGICAL CHARACTERIZATION AND ACT MECHANISM

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Introduction: Chagas disease is a zoonotic present principally in Latin America, however due to immigration process is present around all world and currently not present an efficient treatment against chronic stage of disease. Sodium Diethyldithiocarbamate (DETC) is a compound belong the carbamate class and due to its composition can act as metal chelator and can stimulate the production of reactive oxygen species. These mechanisms are efficient ways to lead the parasite Trypanosoma cruzi to death.

Methods: To evaluate the efficacy of DETC were performed an in vitro essay of antiparasitic activity of DETC against different strains of T. cruzi in different evolution forms and its cytotoxicity towards three different cell lines (macrophages, epithelial and fibroblast). Furthermore, was evaluated the mechanism of cell death induced determinate by markers in controlled death way and non-controlled, in addition was determinate the action of DETC in parasites mitochondria by cytometry. Lastly was visualized the action of DETC in parasite surface by scanning electron microscopy and produced a nanosystem with DETC and validated.

Results: DETC was more efficient than benznodale , the common drug used in Chagas Disease treatment, against all tested strains in different evolutive forms and moreover present low toxicity with CC_{50} > 500µM against all cell lines used. In addition scanning electron microscopy shown that DETC can induce the pore formation in parasite membrane, reduced almost all mitochondrial activity of parasite and provoke a non-controlled death by parasite. Lastly, the nanoformulations of DETC present a controlled release of drug that resulted in a lower toxicity towards cells, besides improve bioavailability.

Conclusions: DETC is a drug with capacity to eliminate T. cruzi.

Keywords: Nanosystem, chagas disease, Sodium Diethyldithiocarbamate, Antiparasitic activity

P293 / #1360

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THE DIAGNOSTIC VALUE, FEASIBILITY AND COST-EFFECTIVENESS OF TRANSITIONING TO OV16 RAPID DIAGNOSTIC TESTING IN ONCHOCERCIASIS SURVEILLANCE IN GHANA

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Introduction: Skin snip microscopy is increasingly becoming less sensitive as a diagnostic and surveillance tool for onchocerciasis programmes as we near elimination targets. The SD BIOLINE Onchocerciasis IgG4 (Ov16) rapid test, though sometimes used in surveillance activities requires further studies on standardization and field testing before it can replace skin snip microscopy. The aim of this study was to assess the feasibility of transitioning to Ov16 rapid testing in onchocerciasis surveillance activities in Ghana.

Methods: A cross-sectional study was conducted in 6 endemic communities in the Tain District and the Wenchi Municipality in the Bono Region of Ghana. The study included 483 individuals, aged 5 years upwards, who agreed to participate in Ov16 rapid testing, skin snip microscopy and/or nodule palpation. Exit interviews and costing analysis were also performed.

Results: The seropositivity rate among study participants was 25.5% of all those who participated in the Ov16 rapid testing. The Ov16 rapid test was 100% sensitive and 80% specific compared to 40% sensitivity and 91% specificity of skin snip microscopy. Most of the study technicians and community dwellers preferred 0v16 rapid test, citing its ease of use, simplicity, and quick turnaround time. In terms of the cost-analysis, the total cost per test was $14.94 and $74.33 for Ov16 RDT and skin snip microscopy, respectively.

Conclusions: The present study has demonstrated, for the first time in Ghana, that the Ov16 RDT is more sensitive, acceptable, cost-effective and feasible to implement in onchocerciasis control programmes even in low prevalence settings. This evidence, aside being crucial for onchocerciasis control programmes in Ghana, could also inform programmatic decisions in other countries.

Keywords: diagnostic value, feasibility, Onchocerciasis, skin snip microscopy, Ov16 rapid test

P294 / #540

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

AMPLICON-BASED NEXT-GENERATION SEQUENCING PROTOCOL TO IDENTIFY LEISHMANIA SPECIES AND OTHER TRYPANOSOMATIDS.

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Introduction: Leishmania and Trypanosoma are members of Trypanosomatidae family, responsible for important infections which are a threat to public health, affecting many low-income countries in the tropics. In this study we describe the application of an amplicon-based next-generation sequencing (NGS) assay to detect and identify trypanosomatid species in mammalian reservoirs, human patients, and sand fly vectors throughout regions of Leishmania endemicity.

Methods: The DNA of each sample was extracted to identify the presence of trypanosomatids through conventional PCR using heat shock protein 70 (HSP70) gene as the target. The PCR products underwent sequencing by Sanger sequencing and NGS. The trypanosomatid species were identified by using BLASTn against a reference database built from trypanosomatid-derived HSP70 sequences. Finally, the alpha and beta indexes indexes of amplicon sequence variants were calculated for each group.

Results: The results obtained revealed (i) the presence of mixed infections with more than two Leishmania species in 34% of CL samples analyzed (ii) the identification of Trypanosoma cruzi in samples from wild reservoirs and sand fly vectors and (iii) coinfection events with three different Leishmania species in domestic reservoirs.

Conclusions: These findings depose the traditional paradigm of leishmaniasis as being a single-species- driven infection and redraw the choreography of host-pathogen interaction in the context of multiparasitism. Further research is needed to decipher how coinfections may influence disease progression. This knowledge is key to developing an integrated approach for diagnosis and treatment

Keywords: Leishmania, NGS, amplicon-based NGS

P295 / #305

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

NP4NTD: DISCOVERY OF NEW ANTIPARASITIC DRUG CANDIDATES FROM MICROBIAL NATURAL PRODUCTS.

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Introduction: Leishmaniasis and Chagas disease are neglected tropical diseases (NTDs) and an emerging health problem in developed countries. New therapeutic solutions are required due to increasing resistance and side effects of existing treatments against Leishmania species and Trypanosoma cruzi. Natural products (NPs) are unique and prolific sources of chemical diversity and new bioactive compounds. The aim is the discovery of new NPs scaffolds with novel MoA against Leishmania and T. cruzi and the development of a new parasite painting approach to characterize the MoA of candidate hits.

Methods: A high throughput high content imaging screening (HTS/HCS) against intracellular L. donovani and T. cruzi will be run with 40K microbial extracts of MEDINA NPs collections. The most promising confirmed hits dereplicated against NPs libraries will be selected for bioassay-guided isolation and structures elucidation of actives molecules using HPLC/MS and NMR. Non-cytotoxic compounds will be profiled in the HCS innovative parasite painting assay.

Results: 20K microbial extracts were screened on HCS intracellular parasite models of T. cruzi and Leishmania. We identified 810 hits against both assays and 23 pure bioactive compounds against T. cruzi and L. donovani, were so far isolated. Cell Painting for both parasites are being developed using a high-content imaging system, targeting distinct cellular patterns. Reference compounds with different MoAs are used to train the model to enable to screen new compounds to predict MoA.

Conclusions: The project has a high potential of identifying new NPs hits that could become drug candidates for NTDs, taking advantage of the HCS expertise and innovative parasite painting approaches. La Caixa Foundation funding HR20-00584.

Keywords: Neglected Tropical Diseases, Leishmania donovani, Trypanosoma cruzi

P296 / #445

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

ISOLATION AND MOLECULAR IDENTIFICATION OF FREE-LIVING AMOEBAS IN WATER AND SOIL IN TENERIFE

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Introduction: Free-living amoebae (FLA) are widely distributed protozoa that contain some genera considered to be pathogenic microorganisms. They can cause skin or brain disorders. They are able to colonize different environments such as water, soil, dust and air, among others. Among the free-living amoeba, the genus Acanthamoeba is the most common isolated from soil samples. The aim of this work was to evaluate the presence of free-living amoebas in soil and water samples collected between 2021 and 2022 in Tenerife, Canary Islands.

Methods: The water samples were subjected to the membrane filtration technique, while soils were grown directly on plates of non-nutritious 2% agar (ANN). Both samples were incubated at room temperature and monitored daily for the presence of free-living amoebas. DNA was perfomed from the plates on which there was an abundant growth of FLA. Finally, PCR amplification of the 18S rRNA gene and sequencing of the DF3 region of Acanthamoeba 18S rDNA was performed.

Results: The analyzed samples were collected during the months of 2021 and 2022 in different points of Tenerife. Soil samples were obtained from school gardens and private farms, whereas water samples were collected from ornamental fountains located in parks and recreational areas, taps, fish tanks... FLA were detected in ANN in all soil samples, and mostly in water samples. The genus Acanthamoeba was the most isolated according to molecular analysis.

Conclusions: As a preliminary result of the samples analyzed, it has been determined that the predominant genus was Acanthamoeba spp. genotype T4. In addition, the presence of Acanthamoeba quina was described in the soil samples examined.

Disclosure: I18/01380; RICET (RD16/0027/0001); CIBER (CB21/13/00100); Cabildo de Tenerife, MEDI y FDCAN; MICINN, Agencia Estatal de Investigación (AEI) and Fondo Europeo de Desarrollo Regional (ERDF) (21/0587), ACIISI (PPP, RLRE, DSH, IAJ, ARL, CJBE), FSE y FEDER, Pr

Keywords: water, Acanthamoeba, free-living amoebae, Tenerife, soil

P297 / #1116

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

A FLOW CYTOMETRIC NONO ASSAY REVEALS LEISHMANIA INFANTUM INFECTION OF PRIMARY HUMAN MYELOID CELLS

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Introduction: Circulating phagocytic cells often serve as cellular targets for large number of pathogens such as Leishmania parasites that are transmitted through the bites of infected phlebotomine sandflies. Studying primary cells in an infectious context requires long-time procedures for cell isolation that may affect the analysis performed. Investigating short-life span cells as neutrophils constitutes another challenge. Herein, we assessed the extent of blood infection by Leishmania infantum focusing on phagocytic cells

Methods: We used a no-lyse and no-wash technique (the NoNo assay) to monitor by flow cytometry the infection of phagocytic cells in total blood with fluorescent parasites

Results: The NoNo assay allowed us to highlight the selective tropism of Leishmania infantum for myeloid cells. We demonstrated that among monocyte cell populations, L. infantum preferentially infects classical monocytes ($CD14^{+}CD16^{-}$) at early time point (4 hrs post-infection). Interestingly the non-classical ($CD14^{-}CD16^{+}$) and intermediate ($CD14^{+}CD16^{+}$) monocyte cell subsets were increasingly infected at later time points (24 hrs). Moreover, the flow cytometric NoNo assay allows the evaluation of infected neutrophils in a whole blood environment.

Conclusions: This new assay demonstrates that promastigote Leishmania parasites infect blood cells without any cell isolation or activation. Overall, the NoNo assay provides a novel methodology to better understand host-pathogen interactions.

Keywords: Leishmania, Flow cytometry, Monocyte, Neutrophil, Human

P298 / #924

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THE EMERGENCE OF VISCERAL LEISHMANIASIS IN SOUTHEASTERN BULGARIA

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Introduction: Bulgaria is potentially endemic for visceral leishmaniasis caused by Leishmania d. infantum. Sporadic indigenous cases have been diagnosed periodically over the last 3 decades after a long period without registration of clinical cases. The aim of the study was to present a brief description of visceral leishmaniasis in patients living in Southeastern Bulgaria.

Methods: A total of 18 patients (aged 9 months-59 years) were observed in the last 15 years. Five of them (28%) were children. The adults (72%) were farmers, workers, employees, businessmen. The used diagnostic methods were ELISA test, PCR, a bone marrow examination. The etiological treatment in different cases was with meglumine antimoniate, allopurinol, miltefosine.

Results: The patients were presented with irregular body temperature that cannot be reduced by antibiotics, fatigue and anemia. With the development of the disease severe splenomegaly, hepatomegaly, pancytopenia occured. The special diagnostic techniques proved L. d. infantum. The patients were treated with meglumine antimoniate combined with allopurinol. In 2 cases of relapse, probably from resistant strains, the effective therapy was with miltefosine.

Conclusions: With the emergence of indigenous cases of visceral leishmaniasis, Bulgaria enters the list of endemic countries. The early diagnosis and treatment of the patients and the reduction of a stray dog population (as a source of invasion) are essential for the control of this disease.

Keywords: Visceral Leishmaniasis, miltefosine, splenomegaly

P299 / #859

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

FURAN DERIVATIVE AFFECTS THE CELL DIVISION AND MITOCHONDRIAL INTEGRITY OF TACHYZOITES OF T. GONDII

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Introduction: Toxoplasma gondii is an obligate intracellular protozoan, the causative agent of toxoplasmosis. The current treatment against toxoplasmosis is based on the combination between sulfadiazine and pyrimethamine, being extremely toxic and unable to clear the infection. Due to the need for new active drugs against T. gondii, we described here the effects of the Furan derivatives on T. gondii in vitro. These furan derivatives already presented an activity against Leishmania amazonensis and Trypanosoma cruzi.

Methods: The anti-Toxoplasma effects of the furan derivatives was evaluated using tachyzoites of T. gondii from RH strain and as host cells the human foreskin fibroblast (HFF) and the epithelial lineage from Macaca mulatta LLC-MK2. High content screening and other microscopy techniques were employed for a detailed analysis of the infected cells after treatments.

Results: The most effective derivative was the 3h, presenting a 50% inhibitory concentration (IC50) of 4.3 µM after 48 h of treatment. The 50% cytotoxic concentration (CC50) to the host cells was 50 µM after a 72-hour treatment. The ultrastructural analysis showed that after treatment the parasites presented cytoplasmic emptying, mitochondrial swelling and cell division affected. It was revealed by TUNEL assay that the parasites suffered apoptosis as cell death.

Conclusions: These findings suggest that the furan derivative 3h could be a candidate for deeper studies to find alternative drugs to treat T. gondii infections.

Keywords: Toxoplasma gondii, tachyzoites, chemotherapy, Ultrastructure, Furan derivatives

P300 / #1685

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

HELMINTHIC DEHYDROGENASE DRIVES PGE2 AND IL-10 PRODUCTION IN MONOCYTES TO POTENTIATE TREG INDUCTION

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Introduction: Immunoregulation of inflammatory, infection-triggered processes in the brain constitutes a central mechanism to control devastating disease manifestations such as epilepsy. Observational studies implicate the viability of *Taenia solium* cysts as key factor determining severity of neurocysticercosis (NCC), the most common cause of epilepsy, especially in children, in Sub-Saharan Africa. Viable, in contrast to decaying, cysts mostly remain clinically silent by yet unknown mechanisms, potentially involving Tregs in controlling inflammation.

Methods: Peripheral and brain immune cells from mice and healthy volunteers were pulsed with viable (CLys, CSN), dead cyst (CVF) materials and rGDH of *T. solium*. Cell modulation and underlying mechanistic aspects were analyzed via FACS surrogate markers and LC/MS/MS profiling of eicosanoids and precursors and antagonists to PGE2/IL-10 receptors.

Results: Here, we show that the enzyme glutamate dehydrogenase from viable cysts instructs tolerogenic monocytes to release IL-10 and the lipid mediator PGE_2. These act in concert, converting naive $CD4^+$ T cells into $CD127^-CD25^{hi}FoxP3^+CTLA\text{-}4^+$ Tregs, through the G protein-coupled receptors EP2 and EP4 and IL-10 receptor. Moreover, while viable cyst products strongly upregulate IL-10 and PGE_2 transcription in microglia, intravesicular fluid, released during cyst decay, induces proinflammatory microglia and TGF-β as potential drivers of epilepsy. Inhibition of PGE_2 synthesis and IL-10 signaling prevents Treg induction by viable cyst products.

Conclusions: Harnessing PGE_2-IL-10 axis and targeting TGF-ß signaling may offer an important therapeutic strategy in inflammatory epilepsy and NCC. Identification of proteomic signatures may pave the way to novel biomarkers.

Keywords: neurocysticercosis, Taenia solium, dehydrogenases, immuneregulation, Treg induction

P301 / #1280

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THE METABOLISM OF ECHINOCOCCUS MULTILOCULARIS UNDER NEW DRUG TESTING

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Introduction: Echinococcus multilocularis causes the severe disease alveolar echinococcosis (AE) in humans and animals. Treatments for AE are limited and not always effective, and therefore, new treatment options are urgently needed. We test different drugs against E. multilocularis in vitro with a focus on inhibitors of mitochondrial energy generation. We are particularly interested in the malate dismutation (MD) pathway, a helminth-specific pathway not active in mammals.

Methods: For in vitro drug activity assessments, we apply in vitro cultures of E. multilocularis metacestodes and isolated stem cells. We have developed and refined a standardized screening cascade including microscopy, damage marker release, cell viability, stem cell proliferation, and differentiation, as well as effects on electron transfer chain and MD activity. These methods are complemented by in vivo testing in mouse models of AE. Based on these techniques, we assess the activity of compounds of interest and further identify their mode of action. We in silico identified all genes implicated in mitochondrial energy generation in E. multilocularis and assess their expression on the transcriptomic and proteomic level under different growth conditions and under the effects of drugs.

Results: We improved and standardized different methods for the screening of new drugs against E. multilocularis with a focus on energy generation. Expression analysis for energy generating pathways is ongoing and will be presented at the conference as well.

Conclusions: With this improved screening cascade, and the better knowledge of the E. multilocularis energy generating pathways, we will be able to identify new potential drugs to treat AE.

Keywords: Echinococcus multilocularis, Cestodes, drug testing, Metabolism

P302 / #447

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THE THERAPEUTIC POTENTIAL OF NOVEL ISOBENZOFURANONES AGAINST NAEGLERIA FOWLERI.

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Introduction: The lethal encephalitis known as Primary Amoebic Encephalitis is caused by parasite agent Naegleria fowleri. Affecting in most of the cases children and young adults, with mortality rates above 95% of register cases, the current therapeutics options are not fully effective and safety. Hence, there is an urgent need to develop novel therapeutic agents with good safety level for the patient. Phthalides, also denominate isobenzofuranones, are a small group of natural molecules, isolated from different plants and/or fungi used in the traditional medicine around the world, with reported antibacterial, antitumoral and antifungal activity. On the other hand, the antiparasitic activity have been reported in the protozoa Leishmania spp. The aim of this study was to evaluate the antiparasitic against N. fowleri of 14 isobenzofuranones derivatives and the induction of Programmed Cell Death in treated cells.

Methods: The anti-Naegleria activity was evaluated in N. fowleri strains ATCC® 30808™ and 30215™ using alamarBlue® reagent activity assay. The evaluation of PCD processes were assessed using commercial kits.

Results: The obtained result show that 3 compounds were able to eliminate N. fowleri and induce the activation of PCD process in treated cells.

Conclusions: The tested isobenzofuranones could be considered as potential therapeutic candidates for the future treatment options of PAM.

Keywords: PCD induction, Naegleria fowleri, phthalides, isobenzofuranones, primary amoebic encephalitis

P303 / #999

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

NOVEL APPROACHES FOR TOXOCAROSIS DIAGNOSIS: PRELIMINARY RESULTS ON ELISA WITH SYNTHETIC PEPTIDES

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Introduction: An ELISA was performed with several synthetic peptides from Tc-CTL-1 excretory-secretory protein from T. canis larva to evaluate their potential to improve toxocarosis diagnosis.

Methods: Epitope predictions were made with ABCPRED, BEPIPRED, BCEPRED, IgPRED and the physico-chemical scales of hydrophilicity and antigenicity via the Immune Epitope Database (http://tools.iedb.org/main/bcell) using the default threshold values. Selection criteria included sequences with 50% or more residues in common and predicted by two methods. SignalP 5.0 confirmed the presence of signal peptide, and selected peptides were mapped onto the Tc-CTL-1 3D structure modelled using I-TASSER and PyMol. Eight out of 54 predicted peptides were synthesized by automated Fmoc-solid phase peptide synthesis. IgG antibodies were obtained from BALB/c mice inoculated with T. canis, Strongyloides venezuelensis and Schistosoma mansoni. Indirect IgG ELISA was performed with different periods, temperatures, buffers, and dilutions.

Results: The peptides were not reactive to any of the samples tested, suggesting peptides might not be immunogenic to T. canis samples and do not share antigenic characteristics with the other helminths. The small molecular weight could interfere with peptide adsorption to the ELISA support.

Conclusions: This is the first study using synthetic peptides for the diagnosis of Toxocara in a murine model. The remaining predicted peptides are currently under evaluation.

Keywords: ELISA, diagnosis, toxocara, peptides, BALB/c

P304 / #444

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

ISOLATED COMPOUNDS FROM GONGOLARIA ABIES-MARINA INDUCE TUBULIN AND ACTIN CYTOSKELETON DISORGANIZATION AND PROGRAMMED CELL DEATH IN ACANTHAMOEBA SPP.

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Introduction: Acanthamoeba is a genus of ubiquitous opportunistic parasites within the group of FLA, which can cause severe eye pathologies such as AK, or nerve damage as GAE. The resistance to disinfectants or current therapies represent a public health concern. The species of brown algae Gongolaria abies-marina (GAM) has been shown to possess numerous interesting bioactivities. In this study we carried out a bioguided fractionation of the G. abies-marina extracts against Acanthamoeba spp.

Methods: A bioguided isolation from extracts of GAM was carried out to evaluate its amoebicidal activity against Acanthamoeba spp. Extracts were fractionated using Sephadex LH-20, Silica gel and Lobar LiChroprep Si 60 columns, and were purified by HPLC. The IC50 and the CC50 were determinated. A double-stain apoptosis kit, immunofluorescence staining of intracellular tubulin and actin, ATP levels, ROS generation and JC-1 kit were used.

Results: The isolated pure compounds were named as CB, D and P30. Showed amoebicidal activity against tree strains of Acanthamoeba tested and demonstrated no cytotoxicity. Also, induced chromatin condensation, mitochondrial malfunction, membrane permeability loss and ROS production. Furthermore, suggesting a disorganization of the surface actin, and showed a destruction or disorganization of the tubulin microtubules.

Conclusions: The isolated compounds CB, P30 and D demonstrated amoebicidal activity and induced features compatible with apoptosis in Acanthamoeba spp., including disorganization or destruction on surface trophozoite actin and intracellular tubulin microtubules. Hence, those molecules could represent a new horizon in the field of Acanthamoeba current therapies, advocating the development of compounds with natural origin.

Disclosure: I18/01380; RICET (RD16/0027/0001); CIBER (CB21/13/00100); Cabildo de Tenerife, MEDI y FDCAN; MICINN, Agencia Estatal de Investigación (AEI) and Fondo Europeo de Desarrollo Regional (ERDF) (21/0587)., ACIISI (RLRE, DSH, IAJ, ARL, CJBE, PPP), FSE y FEDER, P

Keywords: actin, Acanthamoeba, apoptosis-like, Gongolaria abies-marina, tubulin

P305 / #937

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

CURRENT STATUS OF ECHINOCOCCOSIS CONTROL IN THE FALKLAND ISLANDS -IS ERADICATION POSSIBLE?

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Introduction: An intensive control programme for Echinococcosis has been in operation in the Falkland Islands for over 50 years resulting in a reduction of infection in sheep from >50% to less than 1 %. The current study was designed to evaluate if transmission of E. granulosus between dogs and sheep was still occurring and what steps may be required to eradicate the parasite completely.

Methods: Infection in dogs was established by copro-antigen and copro-PCR testing. Infection in sheep was established from abattoir records (2006-2020) for all taeniid cestodes. Evaluation of farming practice relating to dog ownership and slaughter of sheep was conducted by questionnaires and farm visits in 50 out of 81 farms.

Results: Eight dogs (1.4%) tested positive in 2010, none in 2012, 6 in 2014 (1.%) and 4 (0.68%) in 2018. Abattoir data indicated that the prevalence of E. granulosus in sheep was very low (Mean = 0.007%). However T. hydatigena (Mean = 2.3%) and T. ovis (Mean = 0.08%) were higher. Analysis of yearly data showed peaks and troughs in prevalence indicating the sporadic presence of infected dogs may be responsible for maintaining transmission. Farm management risks were: unregulated local slaughter of sheep at cull sites with no disposal of carcases; free roaming dogs which could access sheep carcases; disposal of untreated offal in shallow coastal waters.

Conclusions: Results show that, even with 5 weekly dosing of dogs with praziquantel, some are infected with taeniid cestodes and are active in transmission to sheep. Elements of current farming practice provide opportunities for dogs to access sheep offal and these need to be rectified before eradication of E. granulosus can occur. In addition, the failure of the praziquantel dosing campaign to clear infection in all dogs needs to be investigated.

Keywords: echinococcosis, control programme, Taeniid

P306 / #1536

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

CONGENITAL TOXOPLASMOSIS: A HISTOPATHOLOGICAL STUDY OF AGE-RELATED BRAIN CHANGES.

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Introduction: Congenital toxoplasmosis is a widespread worldwide disease producing varying degrees of damage to the fetus including ocular and neurological impairment. However, the underlying mechanisms are not yet clear. Therefore, the current study aimed to investigate the progress of congenital cerebral toxoplasmosis in experimentally infected offspring animal model at different age groups till become adults.

Methods: To fulfill this aim, the offspring of Me49 *T. gondii* infected pregnant mice were divided into groups; embryo, infant, young and adult phases. Blood and brain samples were collected for further studies including hormonal, histopathological and immunohistochemical staining of glial fibrillary acidic protein (GFAP) and synaptophysin (SYN).

Results: Our results showed several encephalitic changes in the infected groups ranging from gliosis to reduced cortical cell number and fibrinoid degeneration of the brain. We showed increased expression of GFAP and SYN indicating activation of astrocytes and modification of the synaptic function, respectively. These changes started intrauterine following congenital infection and increased progressively afterward. Moreover, infected mice had elevated corticosterone levels.

Conclusions: In conclusion, the current study provided new evidences for the cellular changes especially in the infected embryo and highlighted the role of GFAP and SYN that may be used as indicators for *T. gondii*-related neuropathy.

Keywords: synaptophysin, congenital toxoplasmosis, GFAP, gliosis, corticosterone

P307 / #361

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

INVESTIGATION OF ANTIPARASITIC ACTIVITY OF IRANIAN GANODERMA LUCIDUM EXTRACT ON CUTANEOUS LEISHMANIASIS IN ANIMAL MODEL

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Introduction: Research has shown that Ganoderma lucidum contains 400 types of biologically active substances such as triglycerides, polysaccharides, nucleotides, sterols, steroids, fatty acids and Protein. The aim of this study was to investigate the anti-leishmaniasis effect of its hydro alcoholic extract in an animal model that we had previously tested in vitro phase.

Methods: After preparing the extract by maceration method, culturing the standard Leishmania major in NNN and RPMI media, injecting metacyclic form into the BALB/c and creating the leishmaniasis, the extract was applied in 5 concentrations (10,50,100,150, 200 mg/mL). Treatment with glucantime considered as positive controls. Mean wound diameter was recorded weekly. The spleen and liver parasitic load assessed after the end of treatment. Data were analyzed using ANOVA, Tukey and LSD follow-up test.

Results: A significant difference was obtained between the mean wound diameter before and after treatment in the groups of 150 and 200 (mg/mL) based on the Tuki and LSD follow-up tests ($P \leq 0.05$). There was no significant difference between the mean diameter of wounds before and after treatment in the untreated groups,70% alcohol, concentrations of 10, 50,100 (mg/mL) and Glucantime group. Parasitic burden was the lowest in group 200 (mg/mL) and glucantime.

Conclusions: The 200 (mg/mL) concentrations showed a significant effect on the size reducing of leishmaniasis which can be due to the proven anti-inflammatory, antimicrobial and immunomodulatory properties of this mushrooms because of the presence of compounds such as tannins, flavonoids, triterpenoids and polysaccharides and their effect on macrophages, NK cells, T and B lymphocytes to produce super oxides and cytokines and eradication of Leishmania parasite.

Keywords: Cutaneous leishmaniasis, hydro alcoholic extract, Ganoderma lucidum

P308 / #675

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

CHAGAS' DISEASE AMONG SCHOOL STUDENTS FROM CHIAPAS, MEXICO: TWO CASES OF CHAGASIC CARDIOMYOPATHY

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Introduction: Chagas disease is a vector-borne life-threatening illness originally confined to the Americas. Seroprevalence studies have been reported in the Mexican state of Chiapas; nevertheless, no clinical/cardiological studies have been conducted to detect underage cases.

Methods: A serological screening by ELISA was conducted on 1556 blood samples from school pupils; seropositivity was confirmed by indirect ELISA and indirect immunofluorescence. Seropositive cases were clinically assessed in a hospital, and electrocardiographic and echocardiographic studies were performed.

Results: Seropositivity was confirmed in three cases in the population under study (0.19%). Cardiological studies confirmed the presence of alterations associated with Chagasic cardiomyopathy in two of the three patients.

Conclusions: The conditions for active transmission of T. cruzi infection are met in the rural localities under study; additionally, the presence of Chagasic cardiomyopathy in underage patients highlights the relevance of early detection of cases to provide specific treatment at the onset of the infection and to implement epidemiological surveillance as suggested by PAHO/WHO.

Keywords: Chagas' disease, Chagasic cardiomyopathy, electrocardiography, echocardiography, Trypanosoma cruzi

P309 / #454

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

IN VITRO ACTIVITY EVALUATION OF GONGOLARIA ABIES-MARINA ISOLATED COMPOUNDS AGAINST LEISHMANIA SPP AND TRYPANOSOMA CRUZI.

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Introduction: Leishmaniasis and Chagas disease are diseases caused by the protozoa Leishmania spp. and Trypanosoma cruzi, respectively, affecting millions of people worldwide. The available treatment against these parasites is limited and has multiple drawbacks. The algae belong to the genus Gongolaria spp. contain a wide variety of compounds which have been shown to have different biological activities so it seems to be a promising source of interesting molecules for the development of new antiprotozoal drugs.

Methods: The seaweed G. abies-marina was collected in Spain. A dichloromethane/ethyl acetate extract was obtained and then, successive chromatographic fractionations were performed until finally isolated four pure compounds. To evaluate its leishmanicidal and trypanocidal activity and their cytotoxicity in vitro, a colorimetric assay based on the alamarBlue® reagent was performed. In order to investigate the mechanism of action for their antiparasitic effects, different assays were performed to study the induction of the apoptosis-like mechanism..

Results: The results showed that the extract, fractions and pure compounds isolated from G. abies-marina showed potent and promising leishmanicidal and trypanocidal activity similar to the reference active principles. Assays to determine the mechanisms of action at the cellular level of these compounds showed that the pure compounds appear to induce programmed cell death in the parasites.

Conclusions: Compounds isolated from G. abies-marina have a potent leishmanicidal and trypanocidal activity, appearing to be a promising source of molecules for the development of new antiprotozoal treatment. Projects PI18/01380, RD16/0027/0001(RICET), DSH, CJBE, RLRE, ARL, and IAJ all cofounded by ACIISI, FEDER. Alumni ULL

Keywords: chemotherapy, Leishmania, Trypanosoma cruzi, Gongolaria abies-marina

P310 / #1733

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

EVALUATION OF ANALYTIC AND DIAGNOSTIC PERFORMANCES OF FOUR COMMERCIAL KITS FOR SEROLOGICAL DIAGNOSIS OF CYSTIC ECHINOCOCCOSIS IN HUMAN SERA

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Introduction: Cystic echinococcosis (CE) is a disease caused by *Echinococcus granulosus* sensu lato (s.l.), a zoonotic agent affecting humans and animals with a worldwide distribution. Usually, human diagnosis of CE is performed by means of immunological methods in support to imagine techniques.

Methods: The purpose of our research was to evaluate the analytic and diagnostic performances of four commercial immunological assays, regularly used for the detection of IgG antibodies against *E. granulosus* and *E. multilocularis*. In particular, according to the quality management system, the following parameters were evaluated: operator skills, specificity, sensitivity, repeatability, reproducibility, accuracy, positive and negative predictive values. Subsequently, the parameters related to each test were compared respectively the other. For this aim a total of 259 sera, grouped in positive (n = 74) and negative (n = 185) were analysed by each assay.

Results: According to our findings, the parameters of all assays demonstrated to be from good to excellent, being immunoblotting (IB) the most reliable, followed by the immunochromatographic test (ICT) and finally the two enzyme linked immunosorbent assay (ELISAs).

Conclusions: In conclusion, the four commercial tests, given their excellent values, demonstrated to be reliable diagnostic tools and a valid support to the clinical evaluation and imagine techniques for the diagnosis of CE in human patients.

Keywords: Echinococcus granulosus sensu stricto, Cystic echinococcosis, Diagnostic techniques, serodiagnosis, Analytic and diagnostic performances

P311 / #64

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

CONGENITAL COINFECTION OF CHAGAS DISEASE AND TOXOPLASMOSIS.

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Introduction: Acute toxoplasmosis adquired during pregnancy presents 40% of global congenital (Tx) transmission. However, Chagas Disease (ChD) during chronic infection presents between 4-10% of congenital transmission. We report a case of congenital coinfection of Tx and ChD in a newborn.

Methods: Clincal case report.

Results: Full-term male newborn (40 weeks), normal examination at birth, son of bolivian mother (from Potosi) based in Buenos Aires City, Argentina. Pregnancy was not monitored and only had serology studies at 3rd trimester, with positive results for ChD and toxoplasmosis IgG and IgM. Treatment was not indicated at the time. At 3 weeks old, he presented jaundice and heart murmur. Serology studies were performed for Tx with positive IgM and IgG. Normal funduscopic examination, cerebral ultrasound and central nervous system CT scan. Echocardiogram with intraventricular communication, patent ductus arteriosus. Received treatment for Tx at one month old with sulfadiazine, pyrimethamine and leucovorine, until the age of 12 months. About ChD studies, presented negative parasitemia by microhematocrit and positive by PCR. Also positive serology tests at 8 months old, so treatment was indicated with benznidazole for 2 months. Good clinical development with normal cardiology examination at 10 months old assuming it non related to parasitic infections. Serological response showed negative Tx IgM at 11 months old and progressive chronic infection values of IgG. ChD tests presented negative PCR at the end of treatment and lower serologic results 4 months later.

Conclusions: Treatment for Tx did not affect ChD. PCR technique for ChD is not validated for diagnosis yet, it’s a useful method for early treatment response endorsed by descending serology results.

Keywords: congenital toxoplasmosis, congenital chagas disease, paediatric infections

P312 / #932

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

VERMAMOEBA VERMIFORMIS - A FREE-LIVING AMOEBA (FLA) WITH CLOSE CONTACT TO HUMANS

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Introduction: The presence of several facultative parasitic Free-living amoebae (FLA) in habitats related to human activities supports their public health relevance. The cyst-forming FLA has been detected within the human environment. While several FLA have proved to be facultative pathogenic microorganisms, the medical significance of V. vermiformis (resp. Hartmannella vermiformis) has been discussed for several years.

Methods: Two human cases involving V. vermiformis have been recently examined in Germany: The first case included the only non-keratitis case report so far - with an exclusive isolation of V. vermiformis from a human (Scheid et al. 2019). In the second case, V. vermiformis was detected within the contact lens cases of a bacterial keratitis patient. Although V. vermiformis seemed to be a contaminant without (significant) involvement in the pathogenesis, this cases report shows paradigmatically how easily humans may find themselves in close contact with these FLA.

Results: Additionally, a wide range of FLA is known as vectors of pathogenic microorganisms (endocytobionts), hereby emphasizing their environmental significance. Among those FLA serving as hosts for and vectors of (pathogenic) endocytobionts, there are also descriptions of V. vermiformis as a vehicle and a reservoir of those endocytobionts. These endocytobionts may also play a significant role in aggravating the infection or in enhancing inflammatory processes

Conclusions: The examples of close contact to humans, the involvement in animal and human health, the role as vector of pathogenic microorganisms and the pathogenicity in cell cultures led to the assumption that V. vermiformis should be considered relevant in terms of public health and environmental health.

Keywords: vermamoeba, Free-living amoeba, case reports, public health significance

P313 / #527

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

COULD TAPEWORM-STIMULATED MURINE IMMUNE RESPONSE FIGHT CANCER?

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Introduction: Several studies have shown that infection with helminths may suppress the cancer development, but the mechanisms are still widely speculative. Our work investigates the possible pathways leading to the different suppression of B16F10 melanomas in the peritoneal cavity or their complete elimination observed in different strains of mice infected with the tapeworms Mesocestoides corti and Taenia crassiceps. One of the possible ways cestodes could affect cancer is by stimulating an immune response that is unfavorable for cancer development.

Methods: Flow cytometry was used to determine selected immune cell populations associated with cancer in the blood, liver, lungs, and peritoneal cavity of ICR and C57BL/6J mice infected with tapeworm larvae which were subsequently challenged intraperitoneally with B16F10 melanoma cells.

Results: Infected mice show higher levels of granulocytes and macrophages, especially in the peritoneal cavity; changes in the myeloid populations have also been observed.

Conclusions: Due to the diverse levels of cancer growth suppression by the tapeworm infections in the ICR and C57BL/6J mice strains, we suspect the involvement of the immune response stimulated by M. corti and T. crassiceps infection. Especially eosinophils and peritoneal macrophages could contribute to the observed effect. Funding source: Czech Science Foundation (21-28946S), European Regional Development Fund and Ministry of Education, Youth and Sports of the Czech Republic (CZ.02.1.01/0.0/0.0/16_019/ 0000759): Centre for Research of Pathogenicity and Virulence of Parasites, Charles University institutional funding (PROGRES Q43, Cooperatio Biology, UNCE/SCI/012-204072/2018, SVV 260432/2018.

Keywords: immunity, tapeworm, cancer

P314 / #283

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

TELOMERE SHORTENING IN CANCER CELLS INDUCED BY TOXOPLASMA GONDII-DERIVED GRA16

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Introduction: Toxoplasma gondii is an Apicomplexan protozoal parasite that infects mammalians including humans. We newly demonstrated that GRA16, one of dense granule proteins in the cytoplasm of T. gondii, induces the shortening of telomere length via dephosphorylation of hTERT in human HCT116 cells.

Methods: The present study investigated the molecular mechanism of GRA16 for regulating hTERT activity and telomere shortening using GRA16-gene transferred HCT116 human colorectal cancer cells (GRA16-stable cells).

Results: GRA16-stable cells induced the dephosphorylation of hTERT and the shortening of telomere length with decreases in Shelterin complex and hTERT transcription factors. Moreover, GRA16 directly decreases hTERT expression by down-regulating the expression and phosphorylation of transcriptional factors of TERT (STAT3, E2F1 and c-Myc), and was followed in the cell cycle arrest and apoptosis. The dephosphorylation of hTERT was induced effectively by the signal pathway of HAUSP/PTEN/p-AKT(S473) not PP2A-B55/p-AKT(T308) in the results using inhibitors for PTEN and PP2A and siRNAs for them.

Conclusions: Accordingly, our results highlight that GRA16 is a new promising telomerase inhibitor resulted in shortening of telomere length via telomerase inactivation by inducing the activation of the tumor suppressor PTEN.

Keywords: GRA16, Telomerase, PTEN, Toxoplasma gondii, Telomere shortening

P315 / #1638

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

TREATMENT OF ACUTE AND CHRONIC TOXOPLASMOSIS IN MICE USING CURCUMIN NANOEMULSION

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Introduction: The aim of this study was to prepare curcumin nanoemulsion (CR-NE) and test its efficiency in treatment of acute and chronic toxoplasmosis in mice model.

Methods: CR-NE 1% was prepared using spontaneous emulsification by soybean as oil phase, Tween 80 and Tween 85, ethanol and distilled water. Particle size and zeta potential of NE were measured. Stability was assessed after storage for 2 months. In vivo experiments were carried out using 50 BALB/c mice inoculated with virulent RH strain (type I) and 50 with avirulent Tehran strain (type II) of Toxoplasma gondii and treated with CR-NE (1% w/v), CR suspension (CR-S, 1% w/v), and NE without CR (NE-no CR).

Results: The mean particle size and zeta potential of CR-NE included 215.66±16.8 nm and −29.46±2.65 mV, respectively. Particles size was stable after three freeze- thaw cycles. The survival time of mice infected with RH strain of T. gondii and treated with CR-NE extended from 8 to 10 days post infection. The differences were statistically significant between the survival time of mice in CR-NE-treated group compared with control group ($P<0.001$). Furthermore, CR-NE significantly decreased the mean counts of peritoneum tachyzoites from 5,962.5±666 in control group to 627.5±73 in CR-NE-treated mice ($P<0.001$). In mice inoculated with bradyzoites of T. gondii, Tehran strain (chronic phase) and treated with CR-NE, the average number and size of tissue cysts decreased significantly to 17.2±15.6 and 31.5±6.26 µm, in comparison with control group ($P<0.001$).

Conclusions: Results showed the potential of CR-S and CR-NE in treatment of acute and chronic toxoplasmosis in mice model. However, CR-NE was more efficient than CR-S, especially in those with latent bradyzoites in brain.

Keywords: Curcumin nanoemulsion, Toxoplasma gondii, RH strain, Tehran strain, Curcumin suspension

P316 / #569

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

DOMESTIC MAMMALS AS POTENTIAL RESERVOIR HOSTS FOR LEISHMANIA DONOVANI IN INDIA

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Introduction: Visceral Leishmaniasis on the Indian subcontinent is thought to have an anthroponotic transmission cycle. There is no direct evidence that a mammalian host other than humans can be infected with Leishmania donovani and transmit infection to the sand fly vector. Recent studies of the potential impact of sand fly feeding on other domestic species in the Indian subcontinent found evidence of sand fly feeding on dogs, cows, water buffaloes and goats, which begs the question: “Is there a possibility of non-human reservoirs?”.

Methods: We collected blood from these animals for qPCR and serology and performed xenodiagnosis using colonized Phlebotomus argentipes sand flies to feed on animals residing in villages with active Leishmania transmission based on current human cases. Xenodiagnoses on animals within the endemic area were performed and blood-fed flies were analyzed for the presence of Leishmania via qPCR 48hrs after feeding.

Results: We found positive evidence of Leishmania infection in these domestic mammals but they were not infectious to vector sand flies.

Conclusions: Monitoring infection in sand flies and non-human blood meal sources in endemic villages leads to scientific proof of exposure and parasitemia in resident animals. Lack of infectiousness of these domestic animals to vector sand flies indicates that they likely play no role, or a very limited role in Leishmania donovani transmission to people in Bihar. Continued surveillance of domestic animals in outbreak villages is necessary to ensure that a non-human reservoir is not established, including animals not present in this study, dogs.

Keywords: Domestic animals, Visceral Leishmaniasis, zoonoses, Phlebotomus argentipes, India

P317 / #2064

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

DIVERGENT AND ESSENTIAL COMPONENTS OF TOXOPLASMA GONDII MITOCHONDRIA

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Introduction: *Toxoplasma* mitochondria is an important source of new targets for drug discovery of apicomplexan diseases. By comparing *T. gondii* mitochondria with other (often more commonly studied) eukaryotes we could identify critical differences between parasite and host mitochondria.

Methods: Our group is focused on the study of the composition, structure and function of essential mitochondrial protein-complexes in *Toxoplasma*.

Results: We discovered novel components of these complexes that are conserved across apicomplexan and missing from the host. We are studying the functional implications of including these unique components into otherwise universally conserved complexes for parasite mitochondrial metabolism and for complex assembly and integrity.

Conclusions: Our work demonstrates that these new components are essential for mitochondria operation and parasite survival and reveal mechanistic insight into divergent functional features between parasite and host that may be used to inform intervention strategies in the future .

Keywords: Toxoplasma gondii, Mitochondria, protein-complexes

P318 / #391

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THE CENTRAL NERVOUS SYSTEM OF MICE AS A BATTLEFIELD FOR NEUROPATHOGENIC SCHISTOSOME TRICHOBILHARZIA REGENTI AND EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Introduction: With the incidence of multiple sclerosis (MS) increasing worldwide, there is a great need for new treatments. Some helminths were proved to alleviate the symptoms of both MS and experimental autoimmune encephalomyelitis (EAE), the laboratory model of MS. Nevertheless, the effect of neuropathogenic helminths has not been studied. Therefore, we investigated how the neuropathogenic schistosome Trichobilharzia regenti, which stimulates the tissue-repairing immune milieu, changes the course of EAE in mice.

Methods: Induction of EAE by s.c. injection of MOG peptide and i.p. injection of pertussis toxin followed by clinical scoring, low infection by T. regenti with the onset of EAE signs, flow cytometry of the CNS with focus on T cells and myeloid cells.

Results: Short-term infection by T. regenti (3 days) did not influence immune cell populations in the CNS. By 21 days post infection, T. regenti attracted monocytes/macrophages and eosinophils to the CNS and reduced the numbers of Th1 and Th17 lymphocytes. However, no significant changes in the clinical score were observed.

Conclusions: Low infection dose of T. regenti triggered the CNS infiltration by myeloid cells rather than inflammatory T cells, but it did not significantly change the outcome of EAE. Funding: Charles University Grant Agency (580120), Czech Science Foundation (18-11140S), ERDF and Ministry of Education, Youth and Sports of the Czech Republic (CZ.02.1.01/0.0/0.0/16_019/ 0000759: Centre for Research of Pathogenicity and Virulence of Parasites), Charles University institutional funding (PROGRES Q43, Cooperatio Biology, UNCE/SCI/012-204072/2018, SVV 260432/2018).

Keywords: Schistosome, Central nervous system, experimental autoimmune encephalomyelitis, immunity

P319 / #1203

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

MULTI-DOMAIN FLAVODIIRON PROTEINS FROM TRICHOMONAS VAGINALIS CYTOSOL CONTRIBUTE TO PROTECTION AGAINST OXIDATIVE AND NITROSATIVE STRESS DURING THE INFECTION

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Introduction: Trichomonas vaginalis is exposed to nitric oxide produced by host immune system during the infection of human urogenital tract. The ability to reduce NO by the parasite was previously described, however, the enzyme responsible for this activity was not detected so far. T. vaginalis genome encodes four paralogues of flavodiiron proteins (FDPs), a large family of proteins endowed with O_2 and/or NO reductase activity. Ferredoxin-dependent FDP consisting of only two core domains (a metallo-β-lactamase-like and a flavodoxin domain) was previously identified as a main oxygen scavenging enzyme in the hydrogenosomes, oxygen-sensitive organelles that participate in carbohydrate metabolism. Another three FDP paralogues encode putatively cytosolic proteins containing additional domains that allow for direct use of NAD(P)H as a reductant without the need for extra partner.

Methods: Spectrophotometric analysis of O_2 and NO reductase activity in the cell cytosol and of the recombinant proteins purified from trichomonad cells.

Results: We show that multi-domain FDPs possess both O_2 and NO reducing/detoxifying activity. This activity is very different between the trichomonad strains that vary in the virulence. Strains that were cultured for a long time in vitro showed significantly lower O_2 and NO reducing activity than the strains cultivated for only a short period after the isolation from patients. Two multi-domain FDP paralogues from virulent strains were isolated and characterized.

Conclusions: Differences in O_2 and NO reducing activity among T. vaginalis strains may relate to different virulence. Multi-domain FDPs present in trichomonad cytosol can detoxify O_2 and NO and thus contribute to protection against oxidative and nitrosative stress during the infection.

Keywords: trichomonas, oxidative/nitrosative stress, virulence

P320 / #1159

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

PLASMEPSIN IX/X ORTHOLOGUES IN BABESIA AND THEIR VALIDATION AS NOVEL DRUG TARGETS

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Introduction: Babesiosis is a tick-borne malaria-like disease caused by parasites from the apicomplexan genus Babesia. As in malaria-causing relatives, host cell invasion and egress are key processes for the propagation of Babesia intraerythrocytic stages. We identified two clade C Babesia divergens aspartyl proteases (BdASP3s), homologues of Toxoplasma gondii TgASP3 and Plasmodium falciparum PMIX/X, and evaluated their potential driving roles in proteolytic cascades and protein maturation associated with Babesia invasion and egress.

Methods: We produced recombinant BdASP3s in two different expression systems. We used them to generate specific antibodies for immunostaining and microscopy, and to evaluate their enzymatic activity and susceptibility to a hydroxyl-ethyl-amine-based scaffold compound 49c - the specific inhibitor of TgASP3 and PMX/IX.

Results: Here, we show the expression of native BdASP3s in intraerythrocytic stages. 49c inhibited the propagation of B. divergens (ex-vivo) and its specificity towards BdASP3s was evaluated in kinetic assays using recombinant BdASP3s and TgASP3-specific substrates. Trans-genera complementation approach with iKD-TgASP3 T. gondii strain showed both BdASP3s mimicking the secretory pathway of TgASP3 suggesting its involvement in protein maturation. However, BdASP3s did not subvert the iKD-TgASP3 deleterious phenotype indicating their species-specific function.

Conclusions: We demonstrate two BdASP3s as effectively druggable proteolytic targets for the development of the yet missing Babesia-specific chemotherapy.

Keywords: Babesia, aspartyl protease, drug target, Apicomplexa

P321 / #1121

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

GAMING4HEALTH – DEVELOPING SERIOUS GAMES TO CONTROL TAENIA SOLIUM

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Introduction: Taenia solium is considered the most important foodborne parasite due to its substantial economic and health impacts, particularly in low-income countries. Lack of knowledge and awareness are recognised risk factors, thus health education should form a core component of control efforts, to sustain externally imposed control strategies such as human and pig treatments. In several studies, serious games have shown the ability to engage players in completely different ways compared to other media, inside and outside schools, and are cost-effective. In the project 'Gaming4Health', the overall aim is to design, implement and evaluate the use of serious games to control and prevent T. solium and assess the potential of children as active agents of change.

Methods: Within the project two serious games – a board and digital game - will be developed and evaluated to educate school going children and related actors in their community, about T. solium cysticercosis and how to prevent it. The pork tapeworm will be used as a model to evaluate game play in view of knowledge uptake and behaviour change in a community of a highly endemic area in Zambia. Gaming impact will be measured and evaluated via a mixed methods approach in school going children, and also at the community level, linking the children to their families and surrounding community. Key indicators will be based on correlated parasite presence in pigs.

Results: Currently, the digital game is under development and the boardgame is being optimized based on a pilot study conducted in Belgium and a preliminary assessment in Zambia.

Conclusions: Based on the results, recommendations will be formulated and disseminated at all levels to advance the control of the pork tapeworm as part of the WHO goals for priority Neglected Tropical Diseases.

Keywords: serious games, multidisciplinary research, Games, Taenia solium cysticercosis/taeniosis, prevention/control

P322 / #619

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

SOIL CONTAMINATION BY ECHINOCOCCUS MULTILOCULARIS: SPATIO-TEMPORAL VARIATION IN THREE HIGH ENDEMIC FRENCH VILLAGES

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Introduction: Echinococcus multilocularis (Em) is the causative agent of alveolar echinococcosis, a severe zoonosis caused by ingestion of microscopic eggs expelled in the environment through feces of infected carnivores. Investigating the environmental contamination is essential because it is the source of indirect human infections (foodborne, hand to mouth…). Here, we assessed the extent of soil contamination by Em eggs in 3 French high endemic rural villages, as well as its temporal and spatial variations.

Methods: Between 150 to 200 soil samples were collected annually during three years in each village along the same paths of 14-26 km with samples taken every 100m. Soil sampled were subjected to a flotation technique to concentrate eggs and were then tested for Em DNA detection. In parallel, carnivore fecal samples were collected along the same paths every two months and tested for Em DNA to identify a possible correlation between the proportion of Em positive soil samples and the kilometric abundance index of Em positive fecal samples (KAI).

Results: The proportion of soil samples with Em eggs varied in function of year and village from 1.5% to 42% displaying high inter-annual and spatial variations within each village. It was relatively well correlated with the KAI at the village scale but not at the sampling point scale.

Conclusions: The high proportions of soil contaminated by Em eggs, observed here some years, highlight the strong Em eggs environmental contamination representing a zoonotic risk notably by foodborne contamination. Nevertheless, the spatiotemporal variations observed in each village argue for a global limited persistence of the eggs <1 year. The discrepancies between soil and feces data confirmed the need to consider the correlation at the global village scale.

Keywords: Echinococcus multilocularis, taeniid eggs, soil, environmental contamination, Real-time PCR

P323 / #1400

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

HAPLOTYPES OF ECHINOCOCCUS GRANULOSUS SENSU STRICTO IN CHILE AND THEIR COMPARISON USING COX 1 GENE SEQUENCES WITH HAPLOTYPES FROM OTHER CONTINENTS

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Introduction: Cystic echinococcosis is one of the zoonoses caused by the cestode Echinococcus granulosus sensu stricto (s.s). In Chile and in the world, E. granulosus is capable of surviving in varied and extreme climates.

Methods: We evaluated the genetic diversity of E. granulosus s.s. of 46 hydatid cysts isolated from sheep, goats, cattle and humans, in 3 regions of Chile, Coquimbo, La Araucanía and Magallanes, analyzing the mitochondrial cox1 gene and comparing it with 336 sequences reported for South America, Europe, Asia, Oceania and Africa.

Results: In Chile, 4 haplotypes were detected, EG01, EG1A, EG1D, EgCL16. The cox1 parsimony network showed a star typology, with EG01 in the center. The Tajima`s D and F de Fu`s neutrality indices were negative for the populations of Coquimbo (D= -0.93302; Fs= -0.003) and Magallanes (D= -0.17406; Fs= -0.121), indicating an excess of rare polymorphic sites and population expansion. The Fisher fixation index by pairs (Fst), with low and negative values between Coquimbo-La Araucanía (-0.08761). Highest and most significant Fst among the populations of La Araucanía-Magallanes (0.10703), indicating differences between these populations. In Europe, the Middle East, Asia, Africa and Australia, the haplotype networks show a stellate typology and the EG01 genotype in the center, presenting negative Fu`s F and Tajima`s D neutrality indices.

Conclusions: Our results show that the population genetic structure of E. granulosus s.s is complex. In Chile there are different haplotypes in the different regions, which possibly becomes even more complex if longer sequences are used, which allow studying the microdiversity of E. granulosus s.s. in the different hosts.

Keywords: cox1, Echinococcus granulosus, zoonoses

P324 / #1423

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

ANALYSIS OF THE CONTROL OF CYSTEINE SUPPLY FOR THE SYNTHESIS OF TRYPANOTHION IN TRYPANOSOMA CRUZI

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Introduction: Trypanosoma cruzi has trypanotion [$T(SH)_2$] as its main antioxidant defense. The Metabolic Control Analysis (MCA) were used to quantitatively determined the degree of control that each enzyme exerts on the flow of this metabolic pathway where it was observed that Gamma-glutamylcysteine synthetase (ɤECS) had a CJai of 0.69. On the other hand, in cultures of T. cruzi epimastigotes supplemented cysteine (Cys) there is an increase in the concentration of [$T(SH)_2$] of 4 times. These results allow us to hypothesize that the CJai determined for the ɤECS could be shared with the Cys transport (CysT).

Methods: The entry of [35S]-Cys in epimastigotes and trypomastigotes of T. cruzi. Also the effect of Cys was evaluated in epimastigotes after for 24 h of incubation at 100µM. At last by were exposed parasites at concentrations of PAG for 24 h and the CysT was determined as well as the concentration of thiol by HPLC.

Results: The CysT was kinetically characterized at 25°C, Vm values of 1226 ± 360 U/mg $prot^{-1}$ and Km Cys of 489µM; while at 37 °C the speed increased 2.2 times, but the Km didn't change, these last data were similar in trypomastigotes. It was determined that cys modify 2 times the rate of CysT. Subsequently, propargylglycine was used to evaluate whether CysT could increase the entry of this metabolite as a mode of compensation, this increased 2 times in trypomastigoes. The thiols in parasites exposed to PAG were determined, finding that at 10 µM the Cys concentration dropped ~ 60%, while that GSH decreased by 70 - 90% and T(SH)2 decreased by 20 - 70% at high PAG concentrations.

Conclusions: We can conclude that CysT the cysteine transporter possibly plays a more important role in the trypomastigote stage as it is increased when physiological conditions were used. conacyt donation 282663.

Keywords: Trypanosoma cruzi, trypanothion, Metabolism, cysteine transporter, Cysteine

P325 / #915

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

FEATURES OF THE TREATMENT OF LIVER ECHINOCOCCOSIS

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Introduction: Echinococcosis is a helminthiasis from the group of cestodes, which has a chronic course, in which echinococcal cysts are formed in the liver and other organs and tissues. The disease remains a serious medical problem in many countries around the world, where large endemic foci persist and an increasing number of patients.

Methods: We examined 53 patients with echinococcosis who were examined and treated at the Department of Medical Parasitology and Tropical Diseases in 2012-2018. Treatment of patients with echinococcosis was performed by us using complex therapy with albendazole at a dose of 10 mg / kg body weight per day for 28 days. Pathogenetic and symptomatic therapy was performed simultaneously with antiparasitic therapy.

Results: During the control clinical and laboratory examination of patients after complex anthelmintic therapy, it was found that the condition of the patients improved significantly, the symptoms of asthenia and intoxication disappeared more quickly, objective clinical, laboratory and instrumental characteristics normalized or markedly improved. Based on the principles of calculating the cost of comprehensive medical services, it was found that the treatment of echinococcosis of the liver on an outpatient basis is the least expensive (3-4 times) compared to conservative and surgical treatment in the hospital.

Conclusions: Conservative therapy is an effective method of treating echinococcosis in primary lesions and recurrent cases.
In the situation of choosing the method of treatment of echinococcosis of the liver should take into account not only the medical aspect of the problem as a priority, but also economic, because the cost of medical centers for surgical treatment of the disease is 20 times higher than the cost of outpatient treatment.

Keywords: echinococcosis, THE LIVER, treatment, patients

P326 / #359

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

APOPTOTIC EFFECTS OF IMIQUIMOD IN COMPARISON TO PYRIMETHAMINE AND SULFADIAZINE ON TACHYZOITES OF TOXOPLASMA GONDII IN VITRO

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Introduction: Imiquimod is a member of non-nucleoside isocyclic imidazoquinoline amines, which have been shown to have potent activity against multiple microbial pathogens including Toxoplasma gondii infection. Accordingly, the current study was planned to evaluate the apoptotic effects of imiquimod in comparison to pyrimethamine and sulfadiazine on the tachyzoites of Toxoplasma gondii in vitro.

Methods: Tachyzoites were cultured in the presence of various concentrations of drugs and incubated for 6 h. Wells without drug and sulfadiazine plus pyrimethamine were used as negative and positive controls, respectively. The parasites were transferred to 1.5 ml microtubes and centrifuged at 3000 rpm for 5 min. The supernatant was then drained off and replaced with 500 µL binding buffer. Afterwards, 5 µL of annexin V and 5 µL of propidium iodide (PI) were added to cell pellets. Using FACSCaliber (BD Biosciences), absorption of annexin-v was estimated and finally analyzed by CellQuest software.

Results: It was observed that the survival rates among two concentrations of imiquimod (0.1 and 0.01 µg/ml) were 93 %, and 92 %, respectively, after 6 h of treatment whereas the control group (tachyzoites) without treatment had 99.86 % viable parasites. The percentage of live cells in groups treated with sulfadiazine, pyrimethamine and sulfadiazine plus pyrimethamine were estimated 90%, 82.12% and 82.22%, respectively.

Conclusions: Our findings showed that regarding to control group (tachyzoites without treatment), induction of apoptosis was increased in tachyzoites of T. gondii after exposure to drugs. Therefore, it is inferred that imiquimod could be effective against T. gondii by inducing apoptosis.

Keywords: Toxoplasma gondii, Imiquimod, apoptosis

P327 / #456

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THERAPEUTIC AND PREVENTIVE EFFECTS OF IMIQUIMOD ON THE INFECTED MACROPHAGES WITH TOXOPLASMA GONDII IN VITRO

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Introduction: Despite the remarkable advances made in the field of the therapy of diseases, few effective therapeutic strategies are available to combat of toxoplasmosis in both humans and animals. Imiquimod has the ability to moderate immune response and is effective against viral infections and tumors. This compound can stimulate the immune system by helping to activate immune cells including, macrophages to produce proinflammatory cytokines.

Methods: Macrophage cells treated with drugs before and after infection with tachyzoites. For evaluation of parasite load in both models, cDNA was generated using a cDNA Synthesis Kit from RNA samples. To create the standard curve, samples of the realtime PCR assay, 6-fold-dilutions ranging from 2×10^1 to 2×10^6 parasites (total DNA extract from a sample containing 10^7 tachyzoites of strain RH per ml) were prepared and then threshold cycle (Ct) values were calculated for these standard curves. SYBR-green real-time PCR using repetitive element (RE) gene primers were taken to calculate the number of parasites in the treated and control samples.

Results: Parasite load was significantly reduced in macrophages treated with drugs before infection compared with those in the cells treated with drugs after infection ($p<0.01$). However, the parasite load decreased in both groups treated with drugs compared with control group without drugs.

Conclusions: Low concentrations of imiquimod were more effective in reducing parasite load than high concentrations. These results demonstrate a significant anti-Toxoplasma activity for imiquimod against T. gondii infections.

Keywords: Toxoplasma gondii, Imiquimod, In vitro

P328 / #313

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

PREDATORY TRICHOMONAS VAGINALIS: WHAT ARSENAL DOES IT USE TO FEED ON OTHER CELLS?

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Introduction: Trichomonas vaginalis (TV) is the most common non-viral sexually transmitted human pathogen. It feeds on vaginal epithelial cells and microbiota through phagocytosis. Subsequent destruction occurs in lysosomes, eukaryotic organelles with an acidic pH and a set of degradative hydrolases. The secretion of virulence factors by TV plays a fundamental role in host-parasite interaction prior to phagocytosis. Active secretion of a variety of proteins has been observed in axenic TV cultures and includes phosphatases, amylases, and proteases. However, a comprehensive profile of the secretome under more natural conditions, namely in company with vaginal bacteria, remains elusive. A large subset of the vaginal microbiota is comprised of Lactobacilli. Here we investigated the influence of L. jensenii (LJ) on the secretion of virulence factors by TV.

Methods: TV and LJ were co-cultivated for multiple time frames. Phagocytosis was confirmed through fluorescence microscopy using Lysotracker as a lysosomal marker and DAPI to visualize both LJ and TV nucleus. To analyze the trichomonad secretome upon interaction with bacteria, the cells were removed and the proteins in the supernatant were analyzed by label-free quantitative mass spectrometry (MS).

Results: Fluorescence microscopy suggests active interaction of TV with LJ as phagocytosed LJ could be observed in TV lysosomes. Preliminary MS results indicate that Cathepsin L-like cysteine peptidases are involved in the early steps of the interaction.

Conclusions: As decolonization of Lactobacillus has been observed in vaginal TV infections, our analysis aims to provide a comprehensive set of secreted proteins that may be involved in parasite-bacteria interactions and responsible for the impaired microbial community.

Keywords: Proteases, Interaction, Trichomonas vaginalis, Secretion, Lactobacillus

P329 / #879

Topic: *AS02.4 Other neglected parasitic diseases (e.g., Leishmaniasis, toxoplasmosis, echinococcosis)*

THE EFFECTS OF FEXINIDAZOLE AGAINST TRYPANOSOMA CRUZI: AN ULTRASTRUCTURAL STUDY

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Introduction: Fexinidazole (FEX) is a drug candidate for the treatment of African trypanosomiasis, which encouraged the investigation of its potential for Chagas disease, caused by Trypanosoma cruzi. Although the in vivo effects of FEX against T. cruzi are known, description of its ultrastructural changes is lacking. In this work, we aimed to describe the effects of FEX on T. cruzi (Y strain) ultrastructure, also considering parasite proliferation.

Methods: For this, parasites were treated for up to 72 hours with different drug concentrations. Then, cells were submitted to viability assay, High Throughput Screening, and transmission and scanning electron microscopy.

Results: Our results show that proliferation was inhibited both in epimastigotes (IC_{50} = 23 µM) and amastigotes (IC_{50} = 1 µM). LLC-MK_2 viability assay indicated a selectivity index of 80 against the parasite. Transmission electron microscopy of epimastigotes revealed loss of rounded shape and content of the reservosomes, detachment of the plasma membrane, unpacking of heterochromatin, intense cytoplasmic disorganization, and Golgi disruption. By scanning electron microscopy, treated parasites were rounded and wrinkled. Currently, the anti-trypomastigote effect and the ultrastructural analysis of amastigotes and trypomastigotes are under investigation.

Conclusions: Our results reinforce the anti-T. cruzi activity of FEX and point to reservosomes as the main affected organelle, suggesting it as a possible cellular drug target.
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Keywords: Trypanosoma cruzi, Ultrastructure, Fexinidazole, Electron microscopy

P330 / #2059

Topic: *AS02.5 Other studies related to parasites of humans*

AN INSIGHT ON IXODES RICINUS BACTERIOME FROM PORTUGAL MAINLAND

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Introduction: Ixodes ricinus is the most medically important tick species, not only in Portugal, but also in Europe. These obligate ectoparasites harbor a complex variety of symbiotic, commensal, non-pathogenic and pathogenic microorganisms named microbiota.

Methods: Tick microbiota directly impacts on physiological processes such as, reproduction, nutrition, development, fitness and immunity, and interferes both in the maintenance and transmission of pathogens to vertebrate hosts. In this sense, a better understanding about the interactions among the triad encompassed by the microbiota, the tick and the pathogens it transmits, may contribute to give an input on strategies regarding to tick control and tick-borne diseases mitigation. The present work aims to describe the microbiota of questing female ticks in Ixodes ricinus ticks from Portugal mainland.

Results: Female ticks collected in Gerês and in Mafra were selected and washed to avoid external contaminants. DNA was extracted and the conserved bacterial 16S hypervariable regions (V3-V4) was sequenced by MiSeq Illumina, in a pairwise alignment sequence dissimilarity approach. The Minimum Entropy Decomposition (MED) algorithm was used for operational taxonomic unit (OTUs) identification and further assignments was performed using the QIIME software package. Preliminary results from questing I. ricinus microbiota profile suggest that most abundant OTU found, was the novel genera of the order Rickettsiales: Candidatus Midichloriacea. In addition, two more genera were identified: Rickettsia and Borrelia, thus constituting the main internal microbiota of I. ricinus from the two collection sites.

Conclusions: The Minimum Entropy Decomposition (MED) algorithm was used for operational taxonomic unit (OTUs) identification and further assignments was performed using the QIIME software package. Preliminary results from questing I. ricinus microbiota profile suggest that most abundant OTU found, was the novel genera of the order Rickettsiales: Candidatus Midichloriacea. In addition, two more genera were identified: Rickettsia and Borrelia, thus constituting the main internal microbiota of I. ricinus from the two collection sites.

Keywords: bacteria, microbiota, Portugal

P331 / #293

Topic: *AS02.5 Other studies related to parasites of humans*

STUDY ON INVASIVE ASPERGILLOSIS USING GALACTOMANNAN ENZYME IMMUNOASSAY AND DETERMINING ANTIFUNGAL DRUG SUSCEPTIBILITY AMONG HOSPITALIZED PATIENTS

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Introduction: The incidence of invasive aspergillosis (IA) has dramatically increased during the last decade. This infection is associated with high morbidity and mortality specieally in patients with delaid diagnosis and treatment.This study was aimed to assess the diagnostic value of Galactomannan EIA (GM) for early diagnosis of aspergillosis in hospitalized patients.

Methods: 22 broncho alveolar lavage (BAL) and 13 biopsies from infected sinuses were obtained from a total of 150 patients suffering from different types of hematologic malignancies. All the samples were subjected to microscopic examination and fungal culture. specimens were tested for the GM level. Fungal identified were confirmed through the PCR-sequencing.The susceptibility to anti-fungal agents were evaluated according to the Clinical and Laboratory Standards Institute document(M38-A2) broth microdilution protocol.

Results: The results showed that the incidentrate of IA was 23.33%. 35 patients with IA (12 proven cases and 23 probable cases) were diagnosed. AML (31.5%) was the most prevalent risk factor and Aspergillus flavus (65.7%) was the most prevalent causal agent. Ague (71%) and cough (60%) were the most common symptoms respectively. A sensitivity of 94% and a specificity of 98% was reported for GM ELISA in BAL specimens. A sensitivity of 58% and a specificity of 98% was reported for GM ELISA in serum samples.The lowest minimum inhibitory concentration (MIC) were observed for posaconazole and ravuconazole which showed the range of 0.008–0.0062 and 0.031–0.125, respectively.

Conclusions: The current study has demonstrated that determining the value of GM investigation in BAL and serum specimens can be promising in early diagnosis of IA.

Keywords: Invasiveaspergillosis, Galactomannan, Bronchoalveolarlavage, Hematologicalmalignancy, Organtransplantation

P332 / #846

Topic: *AS02.5 Other studies related to parasites of humans*

THE OLDEST EVIDENCE OF HEAD LOUSE INFESTATION IN ANCIENT IRAN FOUND ON THE SALT MUMMY NUMBER 2 DATED BACK TO 224-651 AD (SASANIAN EMPIRE)

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Introduction: Several natural mummies have been found worldwide that provide impressive information from ancient times to the present day. Mitochondrial DNA studies have shown six clades of head lice (A, B, C, D, E, and F). We investigated the possibility of migration based on the information obtained from the extraction of aDNA from lice obtained from the Sassanid mummy and compared it with modern samples.

Methods: Forty hairs were cut from the mummy's head and transferred to The Parasitology Laboratory of Tehran University of Medical Sciences for further examination. Nine embryonic head lice were then sent to the Biodiversity Institute of Ontario, University of Guelph, Guelph, ON, Canada for molecular studies. To further study modern lice samples from northern Iran and northwestern Iran, were taken and sent to this institute. The molecular method was used to determine the genotype of lice, cytochrome oxidase, in which the mitochondrial gene COI-5P was used. The ethics committee approved this study from Tehran University of Medical Sciences

Results: Sequence matching analysis of life barcode data systems showed that the samples belonged to class A. samples of modern lice were sent from the northern and western provinces. Sequence results in the BOLD data system showed that the lice samples in Kurdistan Province in the west of Zanjan Province L-W and L-N lice taken from northern Iran belonged to two different clades of P. humanus. Sample identifiers L-N correspond to clade A, while L-W corresponds to clade B.

Conclusions: Genetic studies of human lice populations can give us a good trace of human migration in the previous millennia. They can be used as appropriate data to study the past. Furthermore, Due to the Zagros Mountains, there may be an obstacle to migration between the two provinces

Keywords: ancient, mummy, Lice, aDNA, Genetics

P333 / #1053

Topic: *AS02.5 Other studies related to parasites of humans*

SIRNAS NANOCARRIER AS A THERAPEUTIC STRATEGY IN AMOEBIAN KERATITIS: PREPARATION, CHARACTERIZATION AND ASSESSMENT OF ACTIVITY AGAINST FORMS OF ACANTHAMOEBA CASTELLANII

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Introduction: Amoebic keratitis (AK) is a severe disease of the cornea caused by Acanthamoeba spp. Considered difficult to treat due to the presence of cysts, the resistant life form of the amoeba. The Acanthamoeba trophozoite membranes and cyst walls have some essential components such as ergosterol and cellulose. Therefore, the present work aimed to prepare and evaluate siRNA nanocarrier for the silencing of the enzymes 14 α-demethylase (14alpha) and glycogen phosphorylase (Glyco) involved in the encystment of Acanthamoeba castellanii.

Methods: Nanoemulsion (NE) was prepared and characterized for 28 days, stored at 4 °C. To evaluate the phenotypic effect, trophozoites were incubated NE with and without siRNAs. The cytotoxicity of NE was evaluated in rabbit corneal cells (SIRC). Trophozoites were incubated in encystment medium for 15 h, with and without siRNA-carrying NE, and gene silencing was evaluated. Confocal microscopy confirmed the system's ability to promote endosomal escape and deliver siRNAs.

Results: The trophozoite viability assay with NE demonstrated a 32% decrease in viability NE_Glico. In the presence NE_14alpha there were 33% decrease in viability, respectively. When treated with NE_G14 was observed decrease of 51% in trophozoites viability, respectively. After cystic induction, treatments with NE_Glico decreased cyst formation by and 13%. The NE_G14 formulation was able to reduce in 18% the number of mature cysts. Viability of SIRC cells remained above 90%. Furthermore, a 20% (NE_Glico) and 21% (NE_14alpha) reduction in the expression of glycogen phosphorylase, and 14 α-demethylase genes was observed compared to controls.

Conclusions: Therefore, can be concluded that nanocarrier NE was efficient in delivering siRNA in A. castellanii.

Keywords: Acanthamoeba spp., siRNA, gene silencing, amoebic keratitis, encystment

P334 / #1472

Topic: *AS02.5 Other studies related to parasites of humans*

ASSOCIATION OF COPPER(II) COORDINATION COMPOUNDS AND CHLORHEXIDINE AS STRATEGY FOR SYNERGIC THERAPY OF AMOEBIC KERATITIS

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Introduction: In recent decades, Acanthamoeba spp. emerged as clinically important pathogens for causing severe opportunistic and non-opportunistic infections, such as granulomatous amoebic encephalitis (EAG) and amoebic keratitis (AC). Although there are treatment options, the drugs are nonspecific and not very active against the cystic forms of Acanthamoeba spp. In this context, the present work aimed to evaluate the amebicidal activity copper(II) coordination compounds against Acanthamoeba castellanii and in combination with chlorhexidine.

Methods: For the selected compounds, the evaluation of the amebicidal activity against trophozoites and cysts, evaluation of the interaction of drugs against trophozoites, and cytotoxicity of the compounds using rabbit corneal cell lines (ATCC - CCL 60) were carried out.

Results: Copper(II) coordination compounds showed high amebicidal potential, with inhibition of trophozoite viability above 80%. The selected compounds (Cp12 and Cp13) had a minimum inhibitory concentration (CAIM) of 200 µM and mean inhibitory concentration (IC50) values below 10 µM, showing death by apoptosis. Against the cysts, the compound Cp12 showed a reduction in viability 47.6 ± 2.53% in the longest incubation period evaluated. The drug interaction assay demonstrated a synergistic effect for the compound Cp12 with chlorhexidine, reducing the CAIM concentrations of both drugs. In the cytotoxicity assay, it was possible to observe that the selected compounds have a dose-dependent effect against rabbit corneal cells.

Conclusions: The results obtained demonstrate that the copper(II) coordination compounds used are promising for anti-Acanthamoeba action and synergistic action with chlorhexidine, reducing the concentration of both compounds.

Keywords: coordination compounds, amoebic keratitis, Acanthamoeba spp.

P335 / #1240

Topic: *AS02.5 Other studies related to parasites of humans*

SECRETED MOLECULES OF THE LIVER FLUKE, OPISTHORCHIS VIVERRINI, INDUCE THE ABNORMALITIES OF HUMAN RENAL TUBULAR CELLS

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Introduction: The morbidity of infection with liver fluke, Opisthorchis viverrini, is mainly hepatobiliary disease. However, several previous studies demonstrate the contribution of O. viverrini in renal disease both in animals and in the human specimen. Therefore, this study aims to investigate the effect of secreted molecules from O. viverrini on the abnormality of renal tubular cells.

Methods: The human renal proximal tubule HK-2 cell line was co-cultured with the adult worm of O. viverrini using a transwell plate. The cell morphology, proliferation, and apoptosis were determined after co-culture for 24, 48, and 72 hours. In addition, the gene expression and protein levels of kidney injury marker-1 were also examined.

Results: After the co-culture, the morphological change of HK-2 cells was observed from the first 24 hours of the experiment. The secreted protein of O. viverrini also decelerated the growth of proximal tubular HK-2 cells, and the apoptosis was increased time-dependently. The gene expression and protein levels of kidney injury marker-1 also increase corresponded with the apoptosis of HK-2 cell

Conclusions: The co-culture with O. viverrini demonstrate the abnormalities of the renal proximal tubular cells, which might relate to the pathology findings in human. However, further study on molecular mechanisms and an animal module is required.

Keywords: Liver fluke, Opisthorchiasis, Renal disease, Proximal tubular injury

P336 / #1299

Topic: *AS02.5 Other studies related to parasites of humans*

TRANSCRIPTOME CHANGES OF THE LIVER FLUKE, OPISTHORCHIS VIVERRINI, IN DIABETIC HOST: A STUDY OF HOST-PARASITE RELATIONSHIP

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Introduction: A recent study in the hamster model demonstrated that the infection with Opisthorchis viverrini in diabetics hamsters exacerbates the severity of the hepatobiliary disease. However, the effect of host metabolism changes such as diabetic conditions on worm phenotypes is still lacking. Therefore, this study aimed to investigate the impact of diabetes on the global gene expression and the development of liver fluke O. viverrini.

Methods: The study was carried out on the diabetic hamster model. Diabetes was induced using intraperitoneal injection of streptozotocin. The effect of diabetes on O. viverrini was determined using conventional parasitological methods and mRNA sequencing by comparing the fluke from normal control and diabetes.

Results: The infectivity rates between normal and diabetes groups were not different. However, the worm size and the maturity of the worm's reproductive system in diabetes were smaller in the diabetes group. The transcriptomic analysis revealed the changes of multiple biological pathways, including cellular compartment pathways and macromolecule biosynthesis.

Conclusions: Diabetes can interfere with the growth and reproductive development of O. viverrini, as demonstrated by the global gene expression and the phenotypic changes.

Keywords: Liver fluke, Opisthorchis viverrini, Opisthorchiasis, Transcriptomics, diabetes

P337 / #1376

Topic: *AS02.5 Other studies related to parasites of humans*

EFFECT OF INSECTICIDES ON GABA-GATED CHANNELS FROM THE HUMAN BODY LOUSE PEDICULUS HUMANUS HUMANUS

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Introduction: Human louse Pediculus humanus is a cosmopolitan obligatory blood-feeding ectoparasite causing pediculosis. Control of infestation is difficult due to the developed resistance to insecticides that mainly targets the GABA receptors. In a previous work, we showed that Phh-RDL is the target of lotilaner.

Methods: To enhance our understanding of how insecticides act on GABA receptors, we cloned and characterized two other GABA receptors subunits: Phh-grd and Phh-lcch3.

Results: The relative mRNA expression levels of Phh-rdl, Phh-grd and Phh-lcch3 revealed that they were expressed throughout the developmental stages and in the different parts of adult lice. When expressed individually in the Xenopus laevis oocytes, Phh-GRD, and Phh-LCCH3 were unable to reconstitute functional channels, whereas the subunit combinations Phh-GRD/Phh-LCCH3, Phh-GRD/Phh-RDL and Phh-LCCH3/Phh-RDL responded to GABA in a concentration dependent manner. The three heteromeric receptors were similarly sensitive to the antagonistic effect of picrotoxin and fipronil, while Phh-GRD/Phh-RDL and Phh-LCCH3/Phh-RDL were more sensitive to ivermectin than Phh-GRD1Phh-LCCH3. Moreover, the heteropentameric receptor constituted by Phh-GRD/Phh-LCCH3 was found to be permeable and highly sensitive to the extracellular sodium concentration.

Conclusions: These findings provided valuable additions to our knowledge of the complex nature of GABA receptors in human louse that could help in understanding the resistance pattern to commonly used pediculicides.

Keywords: LCCH3, Insecticides, Pediculus humanus, GABA receptors, GRD

P338 / #1325

Topic: *AS02.5 Other studies related to parasites of humans*

AN UPDATE ON THE BED BUGS CONTROL MANAGMENENT

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Introduction: Bed bugs are hematophagous insects that feed on the humans. Resistance to insecticides and ineffectiveness of current control practices makes their control a difficult mission. In this study, we conducted a literature review on various chemical and non-chemical based methods to detect and manage bed bugs in urban settings. We evaluated various control methods and discussed in detail on their advantage and inconvenient and their effectiveness in successful removing the bed bugs

Methods: As a part of our research activities, we evaluated the effectiveness of different non-chemical methods to eliminate bed bugs. These methods included heating, freezing, heat drying and laundering. Furthermore, the medical databases, including PubMed, Science Direct, Web of Science, Springer, MEDLINE, and Google Scholar, were searched for articles published from 1900 to 2022.The search strategy was performed using keywords such as bed bug control strategy, non-chemical and chemical control of bed bugs, their spellings (e.g., synonyms, etc.), also including various scientific research topics.

Results: The evaluation of various control methods together with a compilation of findings from a database including 435 scientific publications from seven major medical databases, allowed us to compare and do an update on the effective methods, mostly based on non-chemical methods, to eliminate the bed bugs

Conclusions: We highlight the intensive use of insecticides to fight bed bugs infestation and the appearance of resistance phenomena for almost all insecticide families worldwide. We underline the effectiveness of non-chemical methods against bed bugs that can be considered as an alternative to chemical treatments without harmful effects on human health and environment.

Keywords: Bed bugs, chemical, non-chemical, control

P339 / #1576

Topic: *AS02.5 Other studies related to parasites of humans*

INVESTIGATION OF ETIOLOGIC AGENTS AND CLINICAL PRESENTATIONS OF OTOMYCOSIS AT A TERTIARY REFERRAL CENTER IN TEHRAN, IRAN

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Introduction: Otomycosis is a superficial infection of the ear caused by a spectrum of various fungal agents and its epidemiology depends on geographical region and climatic condition. The aim of this study was to investigate the causal agents and clinical manifestations of otomycosis at a tertiary referral center in Tehran, Iran.

Methods: From Apr 2016 to Jan 2017 a set of 412 subjects with suspicion of external otitis were included. Clinical examination and specimen collection were performed by an otorhinolaryngologist. Subsequently, direct examination and culture were performed on specimens and isolated molds were identified morphologically. Yeast isolates were identified using CHROMagar *Candida* medium and PCR-RFLP of ribosomal DNA whenever needed. Data were analyzed using SPSS.

Results: Otomycosis was confirmed in 117 cases (28.39%) including 64 (54.7%) males and 53 (45.3%) females. Patients were within the age range of 10-75 yr and the highest prevalence was found in the age group of 46-55 yr (30.77%). Pruritus (89.74%) and auditory manipulation and trauma (83.76%) were the predominant symptom and predisposing factor, respectively. Among 133 isolates from 117 patients, *Aspergillus niger* (n=50, 37.59%) was the most common etiologic agent and *Candida glabrata* (n=25, 18.8%) was the predominantly isolated yeast. Furthermore, 16 cases of mixed infection were identified and coinfection due to *A. niger* and *C. glabrata* (seven cases) was the predominant pattern.

Conclusions: Our results revealed the high prevalence of *C. glabrata* and mixed infections in otomycosis patients. Therefore, mycological examinations should be considered for proper treatment.

Keywords: Aspergillus niger, Otitis externa, Tehran, Iran, Candida glabrata

P340 / #986

Topic: *AS02.5 Other studies related to parasites of humans*

CLINICAL AND LABORATORY INVESTIGATIONS OF CASES INFECTED WITH CAPILLARIA PHILIPPINENSIS IN EGYPT.

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Introduction: Ω •Capillaria philippenesis is a nematode parasite of fish eating birds. In man it is a significant cause of fatal protein-losing enteropathy. Diagnosis is based on finding parasitic stages in stool of patients. Intermittent excretion of eggs in stool, cause delayed diagnosis. Different methods have been used to diagnose infection. This study compares different methods in the diagnosis of intestinal capillariasis in cases from Egypt. These methods include microscopic examination of stool, antigen detection in stool, PCR to detect ssRNA of C. philippinensis, the use of antigen from Trichinella spiralis and the use of antigen from T. trichuris.

Methods: Ω The number of 20 patients were diagnosed with positive intestinal capillariasis, 20 cases with other parasitic infections and 10 persons with no parasitic infection were used in this study. Microscopic examination of stool samples, preparation of crude worm antigen, and polyclonal antibodies and use to detect coproantigen of C. philippinensis. Antigens of Trichinella spiralis and Trichuris muris were also used in diagnosis of confirmed cases. PCR was another method of diagnosis

Results: Ω Highly specific but with low sensitivity. (Sensitivity was increased by making stool analysis after the administration of a dose albendazole). Detection of coproantigen was excellent in diagnosis of the cases. Antigen of T. trichura gave better results than the antigen of T. spiralis which failed to diagnose cases withintestinal Capillariasis. PCR was positive with all cases and negative with other parasitic infections.

Conclusions: Ω PCR is excellent for diagnosis of C. philippenesis, but the limitation is its cost, equippments & experience. Stool analysis is very specific. Sensitivity is increased by analysis after albendazole.

Keywords: Capillaria, malabsorption, Trichinella spiralis, Trichuris trichura, chronic diarrhea, Capillaria, malabsorption,, T. spiralis, Capilariasis, malabsorption, Trichinella spiralis, Trichuris trichura, diarrhea.

P341 / #951

Topic: *AS02.5 Other studies related to parasites of humans*

COMPARISON OF TWO IMMUNOBLOT TOXOPLASMA IGG ASSAYS (LDBIO TOXO II IGG® AND MIKROGEN DIAGNOSTIK RECOMLINE TOXOPLASMA IGG®) FOR THE CONFIRMATION OF LOW IGG TITLES.

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Introduction: In the Toxoplasma serology field, immunoblot (IB) tests are used as confirmation techniques to determine status of pregnant women for the pathogen.

Methods: We used 48 selected samples to compared 2 different IB techniques: LDBIO Toxo II IgG® (Toxo II) and Mikrogen Recomline Toxoplasma IgG® (Recomline).

- 24 samples with IgG titers close or above the threshold with Architect Toxo IgG® (1.2-3.6 UI/ml) and ≥1 positive second-line test: Vidas Toxo IgG®, Toxoscreen® or Toxoplasma ICT IgG-IgM® (= Architect +/-, second test +).
- 24 samples with equivocal or positive Architect IgG titers (1.6-21.3 UI/ml) and negative for all the second-line tests (= Architect +/-, second test -).

Results: Architect +/-, second test +

- 21/24 were positive with Toxo II (≥3 bands) and 3/24 had two bands, equivocal results.
- 10/24 were positive with Recomline (score ≥6), 5/24 were equivocal (score=4) and 9/24 negatives (score=0).

Architect +/-, second test –

- 24/24 were negative with Toxo II (0 or 1 band).
- 2/24 were positive with Recomline, 20/24 equivocal (GRA8 band, score=4), and 2/24 negatives.

Conclusions: LDBIO TOXO II IgG® was able to classify 45/48 samples (94%) compared to 23/48 samples (48%) for Recomline. Mikrogen Diagnostik Recomline Toxoplasma IgG® was suspected to have misclassified 11 samples (22.9%) including 2 false positives and 9 false negatives. If confirmed, it could lead to either delay in treatment initiation (false negative) or no follow-up of at-risk women (false positive).

Disclosure: The company LDBIO has agreed to finance the purchase of reagents for this study.

Keywords: Immunoblot, Toxoplasmosis, Serology

P342 / #475

Topic: *AS02.5 Other studies related to parasites of humans*

THE IMPACT OF COVID-19 PANDEMIC ON THE PREVALENCE OF HEAD LICE INFESTATION AMONG CHILDREN ATTENDING SCHOOLS AND KINDERGARTENS: DIRECT RESEARCH IN POLAND.

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Introduction: Background and aims: Human pediculosis is a parasitic disease caused by Pediculus humanus. The infection, spread by direct contact, occurs mainly among children aged 5 to 13 years old. Due to the high prevalence of Pediculus humanus in the environment and the parasite's ability to transmit pathogenic bacteria, the disease needs monitoring. Each year, nearly 12 million patients suffer from head lice infestation. The infestation causes potential social side effects such as embarrassment, discomfort, and necessary absenteeism from school or kindergartens. Various types of restrictions were used in Poland during the COVID-19 pandemic since 2020, including restrictions on interpersonal contacts, social isolation and functional restrictions on educational facilities. The aim of this study was to evaluate the relationship between COVID-19 pandemic and the prevalence of head lice among children attending schools and kindergartens in Poland.

Methods: The study group consisted of children aged 3 to 14 years old attending elementary school and kindergartens. A total of 5108 children were examined in educational institutions by registered nurses in period of 35 months.

Results: The results indicated that the restrictions applied during the COVID-19 pandemic reduced significantly the number of infected school children comparing to the pre-pandemic state. At the same time, restrictions and functional limitations of educational facilities did not significantly affect the incidence of head lice among preschool children.

Conclusions: It can be concluded that restrictions on direct human contacts also significantly reduced the incidence of human head lice infestation among the study population.

Keywords: head lice infestation, pandemic, Covid-19

P343 / #731

Topic: *AS02.5 Other studies related to parasites of humans*

EFFECT OF NADPH OXIDASE INHIBITION IN AMEBIC LIVER ABSCESS SUSCEPTIBLE MODEL.

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Introduction: Amebiasis is an intestinal infection caused by Entamoeba histolytica (E.h) that affects millions of people in developing countries. Amebic Liver Abscess (ALA) is the most common complication of intestinal amebiasis. In animal models of ALA have demonstrated that neutrophils (NPs) are the first cells to contact E.h. In a previous report, our group demonstrated that in a hamster model myeloperoxidase (MPO), an enzyme secreted by NPs, showed an inadequate activation due to a significant decrease in enzymatic activity and mpo expression. Moreover, NPs produce and release reactive oxygen species (ROS), being NADPH oxidase (NOX2) a ROS-producing oxidase enzyme. There is no information regarding the role of NOX2 in ALA evolution.

Methods: Male hamsters were distributed into two groups: I) inoculated with amebas (CT), 2) inoculated with amebas and treated with apocynin (AP), a NOX2 inhibitor. Animals were sacrificed at 3, 6, and 12 h post-infection. In samples of ALA, we determined: 1) the percentage of lesion, the morphologic changes during ALA evolution, and quantification of E.h number in ALA.

Results: The animals AP showed a lower percentage of lesion, and a lower number of inflammatory foci with fewer leukocytes. In addition, the parenchyma showed more hepatocytes with normal appearance, as well as fewer viable trophozoites than CT group.

Conclusions: Our results showed that during the pathogenesis of ALA, the absence of NOX2 favors the resolution of ALA, probably due to the lack of ROS.

Keywords: Entamoeba histolytica, Amebic Liver Abscess, Apocynin, Neutrophils, Reactive Oxygen Species

P344 / #723

Topic: *AS02.5 Other studies related to parasites of humans*

PREVALENCE OF TRICHOMONAS VAGINALIS AND CO-INFECTION WITH GENITAL MYCOPLASMAS IN SYMPTOMATIC AND ASYMPTOMATIC FEMALE PATIENTS IN VIENNA.

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Introduction: Trichomonas vaginalis causes trichomoniasis, the most recurrent sexually transmitted infection (STI) worldwide. Genital mycoplasmas are known as sexually transmitted agents, but also as commensals, frequently isolated from the female genital tract. Symbiosis between Mycoplasma hominis and T. vaginalis has been described and linked to numerous reproductive morbidities.

Methods: In the Outpatients Centre for Infectious Venero-dermatological Diseases (OCD) in Vienna, patients are routinely screened for STIs. The main aim of this two-phase retrospective study was to assess the prevalence of T. vaginalis and genital Mycoplasma species in swab specimens obtained from female patients attending the OCD in 2021. In total, 582 samples from female patients and additional 20 T. vaginalis isolates were analysed by culture, molecular and microscopic methods.

Results: T. vaginalis was detected in 4 (0.7%) of the collected samples. Additionally, 178 (30%) distinct Mycoplasma species including Mycoplasma hominis, Ureaplasma species and M. genitalium were found in Phase I and II of the study. The 16S rDNA sequence of the recently newly described species, Candidatus Mycoplasma girerdii was obtained for the first time in Austria, in a sample also positive for the protozoan T. vaginalis. Additionally, molecular analyses of the cultivated T. vaginalis strains confirmed the symbiotic relationship with M. hominis in two out of the 20 samples. The presence of Ca. M. girerdii was confirmed only directly in vaginal discharge and not in pure cultures of T. vaginalis.

Conclusions: Altogether, this study demonstrates the presence of T. vaginalis and genital Mycoplasma infections in women of reproductive age, detected by new diagnostic assays, thus enabling better screening of patients.

Keywords: co-infection, Trichomonas vaginalis, urogenital tract, Prevalence

P345 / #1540

Topic: *AS02.5 Other studies related to parasites of humans*

RECOGNITION OF MOLECULAR TARGETS AGAINST ACUTE LYMPHOBLASTIC LEUKEMIA WITH ANTI T. CRUZI ANTIBODIES

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Introduction: La LLA ocurre en el 80 % de las leucemias pediátricas y en el 20 % de los adultos; México reporta una tasa de supervivencia a cinco años del 60%. Las inmunoterapias basadas en anticuerpos para la LLA aumentaron la supervivencia de los pacientes.

Methods: Antibodies to T. cruzi of the CLB strain were induced in rabbits and the IgGs were subsequently purified by affinity chromatography. The recognition of neoplastic ALL B cells was carried out by flow cytometry, confocal microscopy and Western Blot; the protein band of the neoplastic cells were recognized by T cruzi antibodies demonstrated by Wester blot and analyzed by mass spectrometry.

Results: The anti T. cruzi antibodies showed recognition with the antigens of the neoplastic cells by three procedures Flow cytometry, confocal microscopy and Western Blot; by flow cytometry, the recognition was 60.7% and by confocal microscopy the results showed clear recognition of the T cruzi antibodies with the membrane proteins of the neoplastic cells, demonstrated by the Alexa 488 fluorochrome. As well as by Wester blot, a band of 100 kD that by mass spectrometry identified 4 proteins related to cancer.

Conclusions: Anti-T. cruzi antibodies recognize ALL B lymphoblasts at the cell surface level. Nucleolin, Hsp7kDa and XPO2 are proposed as possible therapeutic targets against ALL-B.

Keywords: Acute Lynphoblastic Leukemia, Anti T. cruzi antibodies, Flow citometry

P346 / #998

Topic: *AS02.5 Other studies related to parasites of humans*

IN-VITRO INVESTIGATION OF THE EFFECTIVITY OF POLYHEXAMETHYLENE BIGUANIDE BOUND TO GOLD NANOPARTICLES ON GROWTH INHIBITION OF ACANTHAMOEBA

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Introduction: The opportunistic free-living amoebas are widespread in natural resources. Acanthamoeba species can cause severe diseases, including amoebic keratitis (AK), granulomatous encephalitis, and cutaneous ulcers. The treatment includes Biguanides drugs such as Polyhexamethylene Biguanide (PHMB). The binding of gold nanoparticles to Biguanide drug compounds can increase the drug's efficacy.

Methods: Amoeba was collected from patients with Acanthamoeba keratitis in Tehran medical centers, and genotypes were identified. Then they were cultured. To synthesize Au-conjugated PHMB nanoparticles, the gold chloride solution (III) was added to the PHMB solution then reduced by Sodium Borohydride. Characterization of gold nanoparticles attached to PHMB was performed using measurement by transmission electron microscopy. The toxicity of PHMB, Au-PHMB, was evaluated using the MTT method.

Results: The effect of Au-PHMB on the trophozoites of Acanthamoeba T4 and T11 genotypes was more than PHMB ($P < 0.05$). Also, the effect of Au-PHMB on Acanthamoeba T4 genotypes cysts was more effective than PHMB. But there was no significant difference ($P > 0.05$). However, the effect of Au-PHMB on cysts of the T11genotype was more effective than the free form of the drug ($P < 0.05$).

Conclusions: The increased effectiveness of the Au-PHMB is due to the drug passing through the cysts' wall and better absorption. However, the exact mechanism of enhancing the efficacy of drugs with a gold nanoparticle conjugated has not been well understood.

Keywords: Acanthamoeba, Polyhexamethylene Biguanide, GOLD NANOPARTICLES

P347 / #1153

Topic: *AS02.5 Other studies related to parasites of humans*

DIAGNOSIS AND IDENTIFICATION OF ACANTHAMOEBA KERATITIS PATIENTS BASED ON NESTED-PCR METHOD

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Introduction: Acanthamoeba is one of the opportunistic free-living amoebae that has a wide distribution in natural resources such as soil, water, and dust. This amoeba can cause amoebic keratitis, granulomatous encephalitis, and skin ulcers. Nowadays, PCR assays and culture are two available routine laboratory tests for the diagnosis of amoebic keratitis. The goal of the study was to the comparison of Nested-PCR and culture methods for laboratory diagnosis of amoebic keratitis.

Methods: In this descriptive cross-sectional study, 42 patients with keratitis between April 2017 and April 2021 were studied. The patients' corneas were scanned using a confocal microscope to examine the different layers and the presence of amoebic cysts. Corneal scraping was performed by an ophthalmologist and the patients' samples were examined for Acanthamoeba using culture, JDP-PCR, and Nested-PCR methods.

Results: Out of 42 patients with keratitis, 32 patients were suspected to Acanthamoeba keratitis, and 10 patients with non-amoebic keratitis. The mean age of patients was 29.8 years. In this study, 33 patients (78.57%) were female and 9 patients (21.42%) were male. After reviewing and analyzing the results, 21 cases with a confocal microscope (sensitivity 77.78%), 10 cases with culture (sensitivity 37.04%), 17 cases with JDP-PCR (sensitivity 62.96%), and 26 cases with Nested-PCR (sensitivity 96.3%) Were positive.

Conclusions: Taken together, the use of molecular methods such as Nested-PCR with high sensitivity and specificity for diagnosing this disease is strongly recommended. During this study, the Nested-PCR method was set up for the first time and was used to diagnose amoebic keratitis in Iran.

Keywords: Acanthamoeba, keratitis, Nested-PCR

P348 / #754

Topic: *AS02.5 Other studies related to parasites of humans*

DIFFERENTIALLY EXPRESSED GENES IN ACANTHAMOEBA DURING PHAGOCYTOSIS AND ENDOSYMBIOSIS

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Introduction: Acanthamoeba spp. feeds on various microbial organisms, but several pathogenic microorganisms can survive and multiply within Acanthamoeba. Yet, factors contributing to survival and proliferation of these microorganisms in Acanthamoeba remain unclear. The objective of this study was to identify effective factors for the survival of microorganisms in Acanthamoeba.

Methods: Differentially expressed genes (DEGs) in A. castellanii infected by Escherichia coli or Legionella pneumophila were identified based on mRNA sequencing. DEGs were classified based on the Gene Ontology (GO) terms. To confirm whether regulated genes are involved in microbial digestion or survival, Acanthamoeba were transfected with small interfering RNAs (siRNAs).

Results: A total of 1,878 and 2,342 DEGs were identified in Acanthamoeba with E. coli and L. pneumophila, respectively. GO analysis revealed that genes belonging to the molecular function domain were upregulated, while cellular component genes were down-regulated in Legionella-infected Acanthamoeba. Endosymbiotic Legionella induced noticeable changes in genes involved in phagosomal maturation. Transfecting siRNAs targeting vacuolar proton ATPase, Rab1/RabD family small GTPase, and cysteine proteinase into Acanthamoeba inhibited the phagocytosis of E. coli. Similarly, transfecting siRNAs interfering with R-SNARE VAMP72 family, V type ATPase A subunit, and SNARE domain-containing protein expressions resulted in the inhibition of the L. pneumophila.

Conclusions: Taken together, our results provide directions for further research to understand the survival strategy of L. pneumophila in A. castellanii.

Keywords: Acanthamoeba, phagocytosis, endosymbiosis, Differentially expressed genes

P349 / #774

Topic: *AS02.5 Other studies related to parasites of humans*

ONE-STEP QPCR FOR THE DIAGNOSIS OF ACANTHAMOEBA GENOTYPE T4

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Introduction: Acanthamoeba spp. are ubiquitous and opportunistic free-living amoebae (FLA) that can cause Acanthamoeba keratitis. Quick and efficient diagnosis of Acanthamoeba is often challenging. The aim of our study was to establish a qPCR assay to detect and quantify the predominant Acanthamoeba genotype T4, and also to assess and compare published assays and one commercial kit.

Methods: The methods were evaluated using surplus anonymized DNA from clinical corneal scrapings and Acanthamoeba reference strains (T4, T11, T6, T5, T12, and T10) from the strain collection at our institution. All samples were subjected to qPCR using primers and probes of a newly designed Acanthamoeba T4 method, and also those from Qvarnstrom, Karsenti, and ParoReal Kit Acanthamoeba from Ingenetix Gmbh.

Results: The T4 forward and reverse primers were selected to specifically amplify all Acanthamoeba T4 sub-species, no amplification was observed with the Acanthamoeba genotypes T3, T5, T6, T10, T11, and T12. The range of efficiency, slope, and R2 obtained with all three reference strains were 92.01 to 97.59%, -3.3810 to -3.5703, and 0.9768 to 0.9951. The calculated LOD range was 3.63 (2.12 – 62.04) with T4 primers to 33.27 (19.34 – 78.65) with Karsenti primers. The Qvarnstrom assay revealed the lowest Cq values proving its higher sensitivity compared to the other assays.

Conclusions: We successfully developed and validated a qPCR assay specifically targeting the Acanthamoeba genotype T4, which could be plexed with the Qvarnstrom assay to efficiently and simultaneously diagnose Acanthamoeba genotype T4 and other genotypes from clinical corneal scrap samples.

Keywords: Acanthamoeba, diagnostics, qPCR, genotype T4

P350 / #1171

Topic: *AS02.5 Other studies related to parasites of humans*

IMPACT OF THE PHARMACEUTICAL FORMULATION ON IVERMECTIN SYSTEMIC EXPOSURE IN HUMANS

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Introduction: Ivermectin (IVM) has been extensively used as an antiparasitic agent both in human and veterinary medicine. IVM repurposing is currently considered a relevant issue due to its pleiotropic pharmacological actions, such as the antiviral activity against the SAR-CoV2. There are different IVM formulations available for use in humans. Objective: To compare the systemic availability of IVM orally administered as different commercial formulations (tablet, capsule or solution) to healthy volunteers.

Methods: 12 healthy volunteers participated in a 3 phases crossover designed study. Volunteers were randomly assigned to each formulation and treated with a single dose of IVM at 0.4 mg/kg (14-day washout period among phases). Capillary blood was collected between 2 to 48 h post-treatment and analyzed by HPLC.

Results: IVM mean plasma concentrations and Cmax values were higher ($P<0.05$) after the solution administration compared to tablet and capsule treatments. Similar IVM systemic availability were measured after the treatment with the two solid formulations ($P>0.05$). The solution showed a significantly higher IVM systemic exposure (AUC: 1653 ng.h/mL) compared to the tablet (1046 ng.h/mL) and capsule (1026 ng.h/mL). The simulation of the repeated administration of each formulation for 5 days did not show a significant systemic accumulation.

Conclusions: Conclusion: IVM formulated as solution may be beneficial in humans when the therapeutical success depends on the systemic drug exposure.

Keywords: Pharmacokinetics, formulations, Ivermectin

P351 / #1040

Topic: *AS02.5 Other studies related to parasites of humans*

GASTROENTERITIS INDUCED BY KUDOA SEPTEMPUNCTATA INFECTION IN ANIMAL MODELS

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Introduction: Kudoa septempunctata in olive flounders is known to cause trasient diarrhea in humans. However, whether K. septempuctata can affect human gastrointestinal systems is still controversial.

Methods: In this study, K. septempunctata were isolated from the flounders raised in Korea and orally administrated to the suckling mice (ddy, ICR strain) and adult Asian house shrews.

Results: It was found that 80% of ddY and 70% of ICR suckling mice produced diarrhea with a minimum provocative dose 2x105 Kudoa. Asian house shrews produced emesis two times and emesis-like motion 3 times when administrated with 2x107 Kudoa.

Conclusions: These results indicated that K. septempunctata may cause gastroenteric symptom.

Keywords: Kudoa septempunctata, suckling mice, diarrhea, emesis

P352 / #1062

Topic: *AS02.5 Other studies related to parasites of humans*

BIOLOGICAL PLAUSIBILITY OF KUDOA SEPTEMPUNCTATA AS A CAUSATIVE AGENT OF ACUTE GASTROENTERITIS

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Introduction: Kudoa septempunctata are considered a pathogen that can cause acute gastroenteritis when eaten raw fleshes of infected flat fishes. To understand the mechanism of food poisoning caused by these protozoa, we have implemented a series of experiments.

Methods: The spores of K. septempunctata were purified from fleshes of Paralichthys olivaceus. The purified spores were orally administered to suckling mice or musk shrews (Suncus murinus). The activity of these protozoa was measured through TEER (Trans-Epithelial Electrical Resistance) in the intestinal cell lines. The structural changes of the spores was observed through holotomographic microscopy. The expression of membrane-bound proteins and serotonin was analysed via immunofluorescence or ELISA.

Results: When K. septempunctata were administered to two kinds of suckling mice, diarrheal reactions increased in a dose-dependent manner. The activity of these protozoa, which is measured through TEER, is important for inducing the diarrhea reaction. The sporoplasm of K. septempunctata was released within 30 minutes after being exposed to 37℃, which is a temperature to be exposed during human infection. After 30 minutes to exposure these protozoa, the permeability of intestinal epithelial cells was increased and recovered. A deletion of Zo-1, the membrane-bound proteins, was occurred. The emetic response was increased in the musk shrews depending on the dose-dependent manner. The secrettion of serotonin was more than doubled in the intestinal cell line exposed to spores.

Conclusions: To sum up the results, when raw fleshes of sea fishes infected by K. septempunctata be eaten, sporoplasm, which be released from these protozoa, stimulates the intestinal epithelial cells and induces acute gastroenteritis.

Keywords: causative agent, acute gastroenteritis, Kudoa septempunctata

P353 / #1105

Topic: *AS02.5 Other studies related to parasites of humans*

AN OPPORTUNISTIC URINARY INFECTION IN HUMAN WITH UNKNOWN SPECIES OF NEMATODE

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Introduction: In July 2021, an unknown nematode was found in the urine of a patient who was recuperating at Chamsarang care hospital, Cheongju, Korea.

Methods: Eggs and two types of larvae were confirmed in the specimen, suggesting urinary tract infection.

Results: Here, we report a case of infection with an unknown nematode species, the family Rhabditidae in a Korean patient, confirmed by morphological observation and ITS region sequencing that has not yet been registered in GenBank.

Conclusions: As drinking water is considered the most suspected source of infection in hospital settings where opportunistic infections with the nematode are low, the implications of this case are discussed in particular about the possibility of nematode infection by drinking water.

Keywords: NEMATODE, opportunistic infection, urinary infection, Human, rhabditidae

P354 / #828

Topic: *AS02.5 Other studies related to parasites of humans*

OXYGEN SCAVENGING AND METRONIDAZOLE RESISTANCE IN TRICHOMONADS

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Introduction: The trichomonads Trichomonas vaginalis and Tritrichomonas foetus are anaerobic/microaerophilic parasites in humans and cattle or cats, respectively. Metronidazole is the most commonly used drug in the treatment of trichomonadal infections. In fact, metronidazole is only a prodrug and requires reduction at its nitro group in order to become toxic. Due to the very low redox potential of the nitro group this only takes place quantitatively in anaerobic organisms. Metronidazole resistance has been linked to impaired oxygen scavenging mechanisms which lead to elevated levels of intracellular oxygen and, consequently, impaired reduction of metronidazole. Two enzymes were identified to have oxygen scavenging activity in T. vaginalis: NADH oxidase which reduces molecular oxygen to water, and flavin reductase (FR) which reduces molecular oxygen to hydrogen peroxide via FMN as cofactor. Indeed, FR activity was found to be downregulated or absent in metronidazole-resistant T. vaginalis.

Methods: We wanted to test if similar mechanisms are in place in T. foetus which also can become resistant to metronidazole.

Results: We found that a homologue to Tv FR exists in T. foetus but that it is only weakly active and not decreased in metronidazole-resistant T. foetus. When we assessed various strains of T. vaginalis and T. foetus for oxygen scavenging capacity no decrease in metronidazole-resistant cell lines was found, even if NADH oxidase and FR activities were nearly or completely absent. This indicates that metronidazole resistance might be independent of oxygen scavenging capacity and that NADH oxidase and FR are not critical factors for oxygen scavenging in trichomonads.

Conclusions: The current model of metronidazole resistance in trichomonads must be revised accordingly.

Keywords: trichomonads, metronidazole, resistance, oxygen scavenging

P355 / #1286

Topic: *AS02.5 Other studies related to parasites of humans*

CHARACTERISATION OF TRICHOMONAS VAGINALIS ISOLATES COLLECTED FROM PATIENTS IN VIENNA BETWEEN 2019 AND 2021

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Introduction: Trichomonas vaginalis (TV) is the causative agent of trichomoniasis. TV can carry symbionts: TV virus (TVV), Mycoplasma hominis and probably also Candidatus Mycoplasma girerdii. The presence of symbionts is discussed to impact TV virulence, pathogenesis and drug resistance. We characterised TV isolates collected in Vienna for the presence of symbionts, and investigated possible impacts of the symbionts on metronidazole susceptibility and cytotoxicity against HeLa cells in vitro.

Methods: 82 TV isolates were collected. Detection of TVVs, M. hominis and Candidatus M. girerdii was performed by PCR. All amplified products were sequenced and compared to reference sequences in GenBank. For metronidazole susceptibility screening TV cells were incubated with increasing metronidazole concentrations and viability of the trophozoites in the culture plate wells was assessed by visual examination of motile cells. Assay based on the release of lactate dehydrogenase (LDH) upon cell lysis was used for the determination of cytotoxicity of TV against HeLa.

Results: In 35% of the TV isolates TVVs were detected: TVV1, TVV2, and TVV3; no TVV4 was detected. M. hominis was detected in 37% of the respective vaginal/urethral swabs of TV positive patients; in 28% intracellulary in TV by PCR. No Ca. M. girerdii DNA was detected in the cultured TV isolates. No significant difference in metronidazole sensitivity between TV with and without symbionts was seen. No statistically significant differences in cytotoxicity between the investigated TV isolates were found.

Conclusions: We provided a first insight into the distribution of TV symbionts from Austrian patients. We did not observe significant effects of the symbionts on metronidazole susceptibility or cytotoxicity.

Keywords: TVVs, Mycoplasma hominis, Trichomonas vaginalis

P356 / #728

Topic: *AS02.5 Other studies related to parasites of humans*

KAEMPFEROL ANTIAMEBIC ACTIVITY AGAINST ENTAMOEBA HISTOLYTICA TROPHOZOITES

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Introduction: Entamoeba histolytica (E. histolytica) is a protozoan parasite in humans that provokes amebiasis. The most widely drug employed against E. histolytica is metronidazole, however, studies have reported that it induces genotoxic effects, DNA damage, and furthermore some ameba strains are resistant to metronidazole. Due to this, it is necessary to explore new biological compounds without toxicity that can eliminate E. histolytica. Flavonoids are polyphenolic natural compounds which have demonstrated inhibition of amebic growth and dysregulation of different amebic proteins. Despite the knowledge acquired, its target and action mechanisms are not completely dilucidated.

Methods: Trophozoites were incubated with kaempferol or metronidazole (reference drug) for 90 min at different concentrations for: viability analysis using WST-1 and genic expression of amebic enzymes as Thioredoxin (Trx), Peroxiredoxin (Prx), Rubrerythrin (Rr), Thioredoxin reductase (TrxR).

Results: Our study demonstrated a significant reduction of amebic viability of trophozoites incubated with kaempferol at 130, 140, and 150 µM concentrations, compared with metronidazole at the same concentrations ($p<0.0001$). The gene expression analysis showed a significant downregulation of Prx and Rr enzymes. No differences were observed in Trx and TrxR enzymes gene expression

Conclusions: Our results revealed that kaempferol has an anti-amebic activity associated to the downregulation of amebic gene expression like Prx and Rr enzymes, which participate in detoxification of ROS and defense of parasite.

Keywords: thioredoxin, Peroxiredoxin, Rubrerythrin, Thioredoxin reductase, kaempferol

P357 / #1722

Topic: *AS02.5 Other studies related to parasites of humans*

SENNA PLANT ALTERS MITOCHONDRIAL ACTICITY OF HYMENOLEPIS DIMINUTA

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Introduction: The ability to undergo transition from aerobic to anaerobic by the helminth parasite inside the host is due to Fumarate reductase and succinate dehydrogenase even under hypoxic environment. The present study explored at the mechanism of three *Senna* plant leaf extracts *S. alata*, *S. alexandrina* and *S. occidentalis* on the mitochondrial activity of the cestode parasite *Hymenolepis diminuta* under stress condition as these plants were earlier reported to have anthelmintic efficacy.

Methods: The structure of mitochondria, were studied through electron microsopy, and its density was detected through confocal microscopy, spectroflourimetry and spectrophotometry, while its enzyme activities as well as mitochondrial antioxidant enzyme activities were assayed through native gel and spectrophotometric assays. Praziquantel was tested on the parasites as a reference drug to compare its effects with that of the plant extracts.

Results: The mitochondria architecture was altered and the intensity decreased. Further, enzyme activity showed more than 60% decrease in all three plant species of *Senna* treated parasites. Activities of superoxide dismutase, catalase and nitric oxide synthase increased in mitochondria. Expression of AIF in the mitochondrial fraction and cytochrome c in cytosol indicated the initiation of apoptosis within the worms

Conclusions: Thus this suggested that these three *Senna* plant species disrupted the transition of aerobic to anaerobic metabolism of the parasite

Keywords: enzymes, anthelmintic, Parasite, Mitochondria

P358 / #1644

Topic: *AS02.5 Other studies related to parasites of humans*

MOST PREVALENT NEGLECTED ZOONOTIC INFECTIOUS DISEASES IN UKRAINE: ADVICES FOR PUBLIC HEALTH PROVIDERS

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Introduction: Due to the invasion of Russia over Ukraine, the United Nations High Commissioner for Refugees (UNHCR) estimated that over five million refugees have left Ukraine in the last two months of the conflict (20 April 2022). Due to the war, health system infrastructures in Ukraine were disrupted and among these the surveillance and control of infectious diseases. This study aims at reviewing the situation in Ukraine in the recent years with regard to parasitic zoonoses, from an epidemiological, clinical and diagnostic point of view.

Methods: A literature search was conducted in English and Ukrainian for the identification of the most relevant parasitic infectious diseases from 2000 to 2021.

Results: According to official statistics, around 200,000 new cases of parasitic diseases have been registered every year during the period 2006-2015. . During this period, 1,302 cases of echinococcosis, as well as 5,386 of opisthorchiasis, 2,676 of toxocariasis and 1,599 of dirofilariasis were reported by the official health statistics. Among protozoan infections, 932 cases of cryptosporidiosis, 230 cases of pneumocystosis, and 17,829 cases of blastocystosis were recorded.

Conclusions: This overview on endemic parasitic zoonotic infections should serve as a guide for the physicians and in general for the public health providers for the treatment of war-displaced persons from Ukraine, so that they can adapt their diagnostic algorithms accordingly.

Keywords: parasitic infectious diseases, Ukraine, zoonotic infectious diseases

P359 / #400

Topic: *AS02.5 Other studies related to parasites of humans*

EVALUATION OF MOLECULAR ASSAYS FOR DIAGNOSIS OF AMOEBIC LIVER ABSCESS USING BAYESIAN LATENT CLASS ANALYSIS IN AN ENDEMIC SETTING IN INDIA

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Introduction: The most common extra-intestinal complication caused by Entamoeba histolytica is amoebic liver abscess (ALA), and unlike pyogenic liver abscess (PLA) which has a global distribution, the burden of ALA is predominantly in low to middle income countries. For a rapid and confirmatory laboratory diagnosis of ALA, the performance of multiple molecular methods that detect Entamoeba histolytica DNA in liver abscess pus were evaluated with Bayesian latent class analysis (BLCA).

Methods: Patients were recruited for the study based on clinical diagnosis, ALA (n=27) pyogenic liver abscess (PLA, n=14) and 'probable ALA' (n=13). DNA extracts were tested by nested PCR, qPCR, digital droplet PCR (ddPCR) and loop-mediated isothermal amplification (LAMP) assays all targeting the SSU rRNA gene.

Results: In the latent class analysis, qPCR and ddPCR showed the highest sensitivity (98 – 98.1%) and specificity (96.6%) compared to other molecular assays and although clinical diagnosis had a comparable sensitivity to qPCR and ddPCR (95.2%), poorer specificity (64.3%) was seen. Further, kappa (Cohen's agreement analysis) showed that qPCR and ddPCR has a perfect agreement of 1 followed by an agreement of 0.76 (95% CI 0.64-0.88) with PCR.

Conclusions: We compared a range of molecular assays for laboratory diagnosis of ALA and showed that both qPCR and ddPCR performed with high sensitivity and specificity by Bayesian analysis. Due to the identical results obtained from qPCR and ddPCR assays, taking into consideration the lower cost and relative ease of performance as well as the wider availability of equipment needed, qPCR would be the most optimal assay for molecular diagnosis of ALA at the tertiary care level laboratory setting in India.

Keywords: Bayesian latent class analysis, Entamoeba histolytica, Amoebic liver abscess

P360 / #252

Topic: *AS02.5 Other studies related to parasites of humans*

THE THIOREDOXIN REDUCTASE SYSTEM IN NAEGLERIA

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Introduction: Primary Amoebic Meningoencephalitis is an acute fulminant, necrotizing and haemorrhagic meningoencephalitis caused by Naegleria fowleri. It is a rare disease with a high fatality rate due to delayed initiation of treatment and lack of a specific treatment. The main aim of this study is the characterization of the key factors of the thioredoxin-mediated redox system in Naegleria spp. and its evaluation as a drug target.

Methods: The NCBI and AmoebaDB databases were searched to identify factors involved in the redox system of N. gruberi strain NEG-M. Then, they were amplified from cDNA by PCR for insertion into the multiple cloning site of the pET-17b expression vector. After that, expression was performed in an appropriate E. coli expression strain. Finally, recombinant proteins were isolated and DTNB functional assays were performed to confirm the functions. Also, the thioredoxin reductase and glutathione reductase activity of cell extracts were measured.

Results: The genome data predicted two different TrxRs, one is a small type (TrxR-S) and of bacterial origin, and the other one is a large type (TrxR-L) containing selenocysteine in its C-terminal active site, such as the mammalian TrxR. The expression of these enzymes was confirmed by PCR amplification from cDNA. The TrxR-S and Trx-1 were expressed in BL21-AI™ cells, Trx-2 could be expressed in E. coli Origami™ and TrxR-L could not be expressed. TrxR-S showed no activity, but TrxR and GR activity was readily measured in cell extracts and was higher when the extract was exposed to H_2O_2 or diamide during 6 h.

Conclusions: Naegleria has an unusual redox system, it has two kinds of thioredoxin reductases with different origins and lacks glutathione reductase, but it is able to reduce glutathione disulphide.

Keywords: Naegleria, Thioredoxin reductase, redox system

P361 / #2060

Topic: *AS02.5 Other studies related to parasites of humans*

TICK-BORNE RICKETTSIAE IN A NATIONAL PARK IN PORTUGAL

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Introduction: Tick-borne rickettsioses are vector-borne zoonoses, caused by an obligate intracellular, bacteria belonging to the spotted-fever group (SFG) of the genus Rickettsia. Not only ixodid ticks are the main vectors of SFG rickettsiae, but also are considered reservoirs as many species of the genus Rickettsia can be vertically transmitted.

Methods: The present study aimed to use molecular methods to determine the prevalence and identities of SFG rickettsiae in ticks collected at Tapada Nacional de Mafra (TNM), as well as, to identify tick fauna of potential vectors of SFG rickettsiae.

Results: From May 2019 until May 2021, questing ticks (females, males, and nymphs) were collected from the vegetation by dragging method at TNM (N, 38o 57´ 884”, W 009o 18´ 162”). After collection, ticks were identified using tick taxonomic keys and submitted to DNA and RNA extraction. All samples were tested for the presence of Rickettsia spp. by TaqMan qPCR targeting a fragment of the citrate synthase gene (gltA). Samples that displayed to have the rickettsial DNA fragment by qPCR, were after used for separated amplifications of a larger fragment of the gltA gene and the amplification of the major outer membrane protein (ompA).

Conclusions: From a total of 668 questing ticks collected at TNM, Ixodes ricinus was the most prevalent species found (597/89.91%). Rickettsiae screening, by qPCR, have shown a prevalence of approximately 20% (47/231) in I. ricinus. Preliminary sequencing results of both rickettsial genes (gltA and ompA) have identified the presence of Rickettsia slovaca and R. monacensis in I. ricinus collected at TNM.

Keywords: Portugal, rickettsiae, SFG

P362 / #1713

Topic: *AS02.5 Other studies related to parasites of humans*

GROUP 2 INNATE LYMPHOID CELLS EXACERBATE AMEBIC LIVER ABSCESS IN MICE

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Introduction: *Entamoeba histolytica*, a protozoan parasite in the human large intestine, occasionally spreads to the liver via a portal vein and induces amebic liver abscesses (ALA). Upon liver infection with *E. histolytica*, we found that high levels of type 2 cytokines are induced from early after infection. However, neither sources nor functions of initial type 2 cytokines in the formation of ALA remain unclear.

Methods: In this study, we examined the roles of group 2 innate lymphoid cells (ILC2s) in ALA formation in Rag2 knockout (KO) mice after inoculation of *E. histolytica* into the portal vein of mice.

Results: The number of ILC2s was significantly increased in the liver in Rag2 KO mice on day 4 after inoculation. ILC2s spontaneously produced robust levels of IL-5 in the liver at the early phase of ALA formation. The *in vivo* transfer of ILC2s into Rag2 and common γ chain double KO mice aggravated the ALA formation accompanied by eosinophilia and neutrophilia. Furthermore, IL-33-deficient mice and IL-5-neutralized mice had less ALA formations. IL-33-deficient and IL-5-neutralized mice revealed the mechanism of ILC2-mediated ALA formation in which IL-5-producing ILC2s activated by IL-33 exacerbated ALA.

Conclusions: These results suggest that ILC2s contribute to exacerbating the pathogenesis of ALA by producing early type 2 cytokines and promoting the accumulation of eosinophils and neutrophils in the liver.

Keywords: Amebiasis, Innate lymphoid cells, Pathogenesis, Amebic Liver Abscess

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Topic: *AS02.5 Other studies related to parasites of humans*

CRISPR-CAS9 VERSION 2.0 IN TRICHOMONAS VAGINALIS

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Introduction: Trichomoniasis is a sexually transmitted infection which is caused by a motile protist Trichomonas vaginalis. It consists one of the biggest genomes among the protist in which the size is roughly around 160Mbp. It has nearly 30,000 protein coding genes and 65% of the genome is considered to be highly repetitive. Despite all this genome complexity, Janssen et.al., established genome editing using CRISPR-Cas9 in this protist. Human Cas9 was implemented in the vector and the expression was stabilized using Shield-1 to avoid the cell toxicity. Homology Directed Repair (HDR) was used to repair the dsDNA break. Our aim is to improvise this method in order to establish the rapid mutant cell line.

Methods: We optimized the human Cas9 coding sequence according to the codon frequency of this protist. Next, ligation of three PCR fragments (5'+Selection marker +3') was performed to amplify full length HDR without cloning. With this approach, workload was trimmed to just two days prior to nucleofection. The selection marker gene was flanked with succinyl thiokinase (STK) gene promoter for its independent expression.

Results: As a proof of principle, we selected 6 genes small Tim A (TVAG_287510), small Tim B (TVAG_026080), Frataxin 1 (TVAG_114560), Frataxin 2 (TVAG_182150), IscS2 (TVAG_365590) and TAX (TVAG_247390) for KO. We have achieved stable expression of Cas9 without any toxicity to cells. We succeeded to have KO clones for all the genes and their phenotypes were investigated.

Conclusions: With this improved version 2.0, genetic manipulation in this protist will be easily adapted among the trichomonads lab community. Our future direction will be towards establishing endogenous tagging, CRISPRi, CRISPRa in Trichomonas vaginalis.

Keywords: Version 2.0, CRISPR-Cas9, Trichomonas vaginalis

P364 / #353

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A RETROSPECTIVE STUDY ON HUMAN DERMATOLOGICAL DISORDERS SUSPECTED OF DEMODICOSIS AND SCABIES IN TEHRAN UNIVERSITY- IRAN 2009-2020

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Introduction: Ectoparasites that live on the host skin are important problems worldwide.We have made following aims in this research: Determine the frequency of demodicosis and scabies in different age and sex groups. Examine their seasonal trend during 2009-2020

Methods: Demographic informations of Individuals suffering from itching,redness recorded and imported into an excel file analysed by SDATA version 14, using the Chi-square test. Sampling was performed by skin surface scraped using scalpel.Then placed on a microscopic slide in lactophenol solution to be enough transparent for microscopical examination. Direct parasitological identification was conducted, followed by photography for each.

Results: In the present study, 494 patients were assessed. 20 (4.04%) and 99 (20.04%) samples were reported with positive scabies and demodicosis respectively.Scabies was more common among males with (5.6%) and demodicosis was more common in females with (20.3%) infestation rate. Moreover, scabies infestation rate was found highest in people under 5 age group (21.7%) and demodicosis was highest in 46-60 age group (29.9%). Our study illustrated a significant decline in the incidence of Demodex spp. and S.scabiei mites after the Quid 19 pandemic. The highest positive samples of demodicosis have been recorded in fall. In addition winter along with spring were 2 seasons which showed higher positive samples for scabies.

Conclusions: Demodicosis and scabies can be considered as multifactorial disorders affected by sex, age, environmental conditions.A significant reduction in the incidence of demodicosis and sarcoptic disease during the Quid 19 pandemic in our study shows that adherence to health protocols play an important role in reducing the incidence of these two ectoparasites.

Keywords: ectoparasite, Scabies, Demodicosis, Iran

P365 / #104

Topic: *AS02.5 Other studies related to parasites of humans*

STUDIES ON ANAEMIA IN PREGNANT WOMEN ATTENDING A SECONDARY HEALTH FACILITY IN A PERI-URBAN SETTING OF SOUTHEAST NIGERIA

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Introduction: Anaemia in pregnancy is a major contributor to maternal deaths in developing countries. It has a great impact on the foetus which results to impairment and foetal death.

Methods: A comparative cross- sectional study was done to determine the causes and consequences of anaemia in pregnant women attending ANC in Mater Misericoidae Hospital, Afikpo, southeast Nigeria. Four hundred and six pregnant women with and without anaemia were enrolled in the study. Data on obstetric, demographic and socio-economic characteristics was collected and analysed using a structured questionnaire. Capillary technique was used for the estimation of the packed cell volume (PCV), malaria parasite test and stool examination for hookworm infection was done

Results: Anaemia was found in 245 (60.3%) women, 45 (11.1%) of the women tested positive for malaria and 68(54.4%) out of the 125 respondents who provided their stool samples had hookworm infection. Their nutritional status showed the ignorance that existed amongst the respondents as many had no knowledge of anaemia. The p-value is 0.024; this means that there exists significant relationship between pregnant women with anaemia and the risk factors associated with anaemia.

Conclusions: Educating women on early antenatal booking and the effect of anaemia on both the mother and foetus should be encouraged. Emphasis should be made about de-worming and compliance with the use of prescribed medications during antenatal to reduce the problem of anaemia in pregnancy in Nigeria

Keywords: Anemia, hookworm, Parasites

P366 / #843

Topic: *AS02.5 Other studies related to parasites of humans*

FACTORS OF METRONIDAZOLE RESISTANCE IN TRICHOMONAS VAGINALIS

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Introduction: Trichomoniasis is one of the most common sexually transmitted infections treated by metronidazole (MTZ) despite the high resistance rates. The aim of this study is to evaluate the roles of nitroreductases (NR) and efflux pumps (EP) in MTZ resistance and multidrug resistance (MDR) modulators will be tested for their use to combat resistance by blocking EP.

Methods: NRs will be expressed in E. coli and characterized using cytochrome c-based assay. MTZ-sensitive/resistant strains will be used in drug susceptibility assay to determine the effects of EP inhibitors and MDR modulators on MTZ resistance.

Results: An NCBI/Genbank search identified 11 NRs and 3 oxidoreductases with only one NR having proven activity whereas the others may be involved in MTZ activation. The T. vaginalis genome encodes 48 MATE EP homologs, two of which are increased in resistant strains. The MDR modulators Cremophor EL and Verapamil lowered the MTZ MIC in strain B7268. These findings provide a starting point for larger-scale research into the function of MDR in MTZ resistance. Cimetidine and pyrimethamine will be investigated for their potential to block MATE efflux pumps. IC_{50} will be determined separately with modulators and MTZ, then together.

Conclusions: NRs encoded in the T. vaginalis genome are likely to participate in MTZ activation but could be less strongly expressed or active. In at least some of the resistant isolates, EPs are implicated in MTZ resistance and therefore, EP inhibitors should be studied for their use to combat MTZ resistance in these isolates. Importantly, all modulators/inhibitors planned to be examined have approval for medical use in humans and might therefore be allowed for off-label use in the treatment of MTZ-resistant trichomoniasis.

Keywords: metronidazole, Nitroreductase, efflux pumps inhibitors, Trichomonas vaginalis

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Topic: *AS02.5 Other studies related to parasites of humans*

BLOOD NUTRIENTS REGULATING THE EXPRESSION OF OUTER SURFACE PROTEINS IN BORRELIA AFZELII

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Introduction: Uncovering the factors modulating gene expression of Borrelia spirochetes is crucial for fighting Lyme disease. As the interface between Borrelia and its host is its outer surface, proteins localized to the outer membrane must play an important role in transmission. It has previously been shown that the expression of outer surface proteins can be stimulated by temperature, changes in pH, and tick feeding. However, just a little is known about what exactly during the tick feeding causes these changes in expression.

Methods: Here, we show the effect of different blood nutrients on Borrelia afzelii spirochetes using in vivo experiments. Infected nymphs were fed using membrane or capillary feeding, and the gene expression was measured using RT- PCR.

Results: Before feeding, Borrelia spirochetes in tick gut display low expression of OspC and Bbk32 molecules, but the expression of OspA is high. Our results demonstrate how the expression of OspC is enhanced while feeding the ticks on sugar-rich solutions. We show that even a small amount of sugars passing through the tick gut can lead to a huge increase in OspC expression. In contrast, we haven't seen any effect on other surface proteins, such as Bbk32 or OspA. This work was supported by the Ministry of Health of the Czech Republic, grant nr. NU20-05-00396, and by the Czech Science Foundation grant no. 22-30920S.

Conclusions: Based on our data, we assume that in addition to temperature, the main cause of the increase in OspC expression is the presence of sugars in the tick's blood meal. However, the regulation of Bbk32 and OspA appears to be controlled differently.

Keywords: Borrelia, gene expression, Blood nutrients, Ticks, sugars

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Topic: *AS02.5 Other studies related to parasites of humans*

RISK OF TRANSMISSION OF BORRELIA BURGDORFERI SENSU LATO FROM IXODES RICINUS TICKS TO HUMANS.

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Introduction: Lyme borreliosis (LB) is the most common vector-borne disease in Europe. The analysis of the prevalence of Borrelia-positive ticks in humans is an important indicator for assessing the risk of spirochete transmission from ticks to humans, due to the often non-specific symptoms of Lyme borreliosis (LB) and the inconclusive results of diagnostic tests in relation to the patient's clinical condition. The aim of our study was to assess the prevalence of B. burgdorferi s.l. in I. ricinus removed from humans and to analyse the influence of environmental and population factors that may affect the risk of Borrelia infection.

Methods: Ticks parasitic on humans were collected in Poland from June to November 2021. The presence of Borrelia was detected by PCR (amplification of flagellin gene fragment). A questionnaire method (at the beginning of the study and after 4-6 weeks), conducted among the study participants was used to obtain data on the location and time of tick parasitism, health status of the patient, diagnosis for LB and implemented treatment.

Results: There was a total of 855 I. ricinus collected, Borrelia was confirmed in 20% of them. In 12% of persons bitten by Borrelia-positive ticks, LB was diagnosed on the basis of the presence of ER (erythema migrans) and/or serological tests, in 9% only on the basis of ER. Interestingly, 13% of people, had prophylactic antibiotic therapy ordered, only on the basis of a positive tick test.

Conclusions: The obtained results indicate the necessity of studies aimed at estimating the real risk of B. burgdorferi infection in humans after contact with ticks and, when justified, to extend the diagnostics for LB in patients with a positive tick test result. This research was funded by the National Science Centre (Poland), grant no. 2020/37/B/NZ6/01587

Keywords: Ixodes ricinus, Ticks, transmission, Borrelia burgdorferi

P369 / #133

Topic: *AS02.5 Other studies related to parasites of humans*

ABSTRACT TITLE: EVALUATION OF PATIENTS WITH NASOPHARYNGEAL MYIASIS AND CUTANEOUS MYIASIS IN ZAHEDAN, IRAN

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Introduction: Myiasis is caused by the presence of fly larvae (maggot) in body tissues. The larvae usually enter through wounds and skin lesions or through natural cavities in the body such as the mouth, ears, eyes, and genitourinary tract. Dermal myiasis is the most common clinical form of the disease. Nasopharyngeal myiasis involves infections of the nose, mouth, sinuses, and ears. Sometimes, ophthalmomyiasis and intestinal myiasis caused by swallowing the organism are seen. The present study set to identify and determine the species of myiasis-causing flies in patients referred to Imam Ali Hospital in Zahedan, Iran.

Methods: To identify abundant species of myiasis-causing flies in people referred to Imam Ali Hospital in Zahedan, in terms of developing different kinds of myiasis from 2020 to 2022, 19 larvae samples were collected from five patients and were then examined and identified. Morphological traits such as anterior and posterior respiratory holes and oral sections were used to identify the larvae. The larvae then became adult flies by keeping them in proper conditions after the larval and pupa stage.

Results: All patients were infected in the suburb areas or outside the city. Analysis of the population composition in terms of abundance of myiasis-causing species revealed that Calliphora vicinia species from the Calliphoridae family with 47% infection was the cause of nasopharyngeal (Ear) and cutaneous myiasis, and Lucilia sericata with 32% infection was the cause of nasopharyngeal (Nose) and cutaneous myiasis.

Conclusions: due to the risk of damage to vital organs of the body and the possibility of infection, physicians should ask patients to refer to entomology laboratories for further examination and diagnosis of fly species when they show clinical signs.

Keywords: Nasopharyngeal Myiasis, Cutaneous Myiasis, zahedan, Iran

P370 / #917

Topic: *AS02.5 Other studies related to parasites of humans*

DIAGNOSTIC IMAGING IN PARASITIC DISEASES

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Introduction: In parasitic diseases, the etiological diagnosis is mainly laboratory, but in some of them imaging can determine, improve and expand the clinical and diagnostic information. The aim of the study was to show the potential of the routine imaging methods in clinical parasitology.

Methods: The presented imaging data were from patients with some intestinal and tissue parasitic diseases, for whom outpatient or inpatient care was provided. Abdominal ultrasound, radiography or computed tomography were used in the diagnosis of these diseases.

Results: The combination of parasitological methods, laboratory tests and diagnostic imaging according to organ pathology contributed to the correct diagnosis in patients with liver or lung cystic echinococcosis, cysticercosis localized in the CNS and muscles, ascariasis - in intestines, intestinal and urogenital schistosomiasis, paragonimiasis, toxocariasis, amoebic liver abscess, splenomegaly and hepatomegaly in visceral leishmaniasis and malaria.

Conclusions: The potential of imaging methods for exact diagnosis and monitoring of patients confirms the need for their targeted use in clinical parasitology.

Keywords: Clinical parasitology, parasitic diseases, imaging methods

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Topic: *AS02.5 Other studies related to parasites of humans*

SIMULTANEOUS TARGETED AMPLICON DEEP SEQUENCING AND LIBRARY PREPARATION FOR A COST-EFFECTIVE UNIVERSAL PARASITE DIAGNOSTIC SEQUENCING APPROACH

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Introduction: We recently described a metagenomics-based targeted amplicon deep sequencing method called nUPDx for detecting parasites in human blood. However, this assay performs poorly when applied to non-blood specimens. It also requires expensive reagents and time-consuming manual steps that prohibit its routine diagnostic use.

Methods: nUPDx uses the NEB Illumina library method to facilitate sequencing on the MiSeq platform. We developed an alternative approach, called AdUPDx, that incorporates Illumina barcodes and adapters during the PCR steps, making the amplicons immediately ready for sequencing. We compared AdUPDx to nUPDx on blood specimens and applied it to human tissue and body fluid specimens confirmed positive for parasites by established methods.

Results: AdUPDx correctly identified nine unique parasites in 21 out of 24 non-blood specimens, including Angiostrongylus sp. in cerebrospinal fluid, Hymenolepis sp. in duodenal aspirate, Leishmaniinae sp. in tissue and bone marrow, Schistosoma and Taenia spp. in unpreserved tissue, and Acanthamoeba sp., Balamuthia mandrillaris, Trypanosoma cruzi and Toxoplasma gondii in formalin-fixed paraffin-embedded tissue. Both nUPDx and AdUPDx correctly identified the human bloodborne parasites Plasmodium spp., Babesia spp., kinetoplastids, and filarial nematodes, with a detection limit of ~0.6 parasites/µl. AdUPDx reduced per sample assay cost and turnaround time from $40 and 4 days to $11 and 3 days, respectively, compared to nUPDx.

Conclusions: AdUPDx detected a broad range of parasites in different specimen types. It also markedly reduced costs and turnaround times, making the assay more amenable to routine diagnostic applications.

Keywords: Next Generation Sequencing, targeted amplicon deep sequencing, Metagenomics, diagnosis

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GENOMIC PROFILING OF ACANTHAMOEBA ISOLATES BY NEXT-GENERATION SEQUENCING

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Introduction: Acanthamoeba are amphizoic amoebae responsible for causing Acanthamoeba keratitis (AK) and Granulomatous amoebic encephalitis (GAE). Despite its ubiquitous nature, the frequency of infections is not high, probably due to existence of non-pathogenic isolates. The whole-genome sequencing and an annotated genome assembly can unravel the biological functions and help in identifying probable genes related to pathogenicity.

Methods: The Illumina and Nanopore sequencing was performed for a keratitis, encephalitis, and non-pathogenic environmental isolate. Hybrid assembly was prepared for the AK and GAE isolate while only the Illumina reads were utilized for non-pathogenic environmental isolate. Protein coding genes identified using GeneMark-ES program and BLASTx module of Diamond used for gene prediction. Additionally, Kyoto Encyclopedia of Genes and Genomes annotation and Cluster of orthologous group's annotation using RPS-blast against CDD database was performed. The subsequent data analysis and validation helped in identifying probable pathogenic genes.

Results: The genome assemblies of 9.67, 8.34, and 8.89GBs were reported for GAE, AK and non-pathogenic isolate respectively. KEGG reported 22,946 in GAE, 24,231 in keratitis, and 9367 genes in the environmental isolate. The COG annotation revealed 3232 in GAE, 3403 in keratitis, and 1314 genes in the non-pathogenic isolate.

Conclusions: The present study has attempted to generate de novo hybrid genome assemblies of Acanthamoeba for the first time that would help decode the genome of free-living amoeba and will provide genomic data for a better understanding of virulence-related factors.

Keywords: Acanthamoeba, Next Generation Sequencing, genomic profiling

P373 / #1630

Topic: *AS02.5 Other studies related to parasites of humans*

AN IN VITRO LUCIFERASE-BASED SYSTEM FOR DETERMINING TOXOPLASMA GONDII BRADYZOITE SURVIVAL

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Introduction: It is estimated that more than 1 billion people are infected worldwide with chronic toxoplasmosis. Despite this, there is currently no vaccine to prevent infection in humans and there is no recognized curative treatment to clear tissue cysts. A major hurdle for identifying effective drug candidates against chronic-stage cysts has been the low throughput of existing *in vitro* assays for testing the survival of bradyzoites. We have developed a luciferase-based platform for specifically determining bradyzoite survival within *in vitro* cysts in a 96-well plate format.

Methods: The cystogenic Type II *T. gondii* strain PruΔ*ku80*Δ*hxg* was genetically modified for the development of a dual-luciferase expressing strain. Parasites were modified to express firefly and nano luciferase (ffLuc and nLuc) under the bradyzoite-specific promoters *bag1* and *ldh2,* respectively. FfLuc expression was cytosolic, whereas nLuc was coupled to either BPK1 or MAG1 for trafficking to the cyst wall. We reasoned that cytosolic levels of the ffLuc would decrease in dead/dying parasites, while the secreted nLuc-coupled proteins would remain fixed in the cyst wall.

Results: Ratiometric luminescence (ffLuc/nLuc) decreased following treatment with anti-bradyzoite compounds. A panel of 12 compounds were tested in the assay, 5 of which were effective at decreasing ratiometric luminescence (atovaquone, LHVS, pyrimethamine, salubrinal and an experimental triazine nitrile compound). Dose-response assays allowed us to determine relative sensitivity to compounds based on calculated EC_{50} values.

Conclusions: We have developed an *in vitro* platform for testing the efficacy of compounds specifically against *T. gondii* bradyzoites, towards the first curative treatment for chronic toxoplasmosis.

Keywords: In vitro model, toxoplasma, chronic infection, bradyzoites, drug screen

P374 / #1232

Topic: *AS02.5 Other studies related to parasites of humans*

RNA-SEQUENCING-BASED TRANSCRIPTOME ANALYSIS OF ACTIN-BINDING PROTEINS FROM PATHOGENIC N. FOWLERI.

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Introduction: Naegleria fowleri, a pathogenic free-living amoeba, causes fatal primary amoebic meningoencephalitis (PAM) in humans. Actin and its regulatory proteins play a key role in several essential cellular processes such as cell movement, intracellular trafficking and cytokinesis in most eukaryotes. However, not much was known about the existence of these proteins in N. fowleri.

Methods: In this study, we examined the expression of actin binding protein in the transcriptome database of N. fowleri. RNA-seq database, the assembly procedure resulted in mean full length of 74,499,760 nucleotides in total 74,594 transcript contigs and 36.54 % of GC contents.

Results: RNA-seq. indicated that actin binding proteins (149 genes) in 2-fold expression and 42 genes in 10-fold expression were identified. In the actin binding proteins of N. fowleri, profilin, ADF/cofilin, coronin and formins were related. Especially N. fowleri profilin, known as an actin-binding protein involved in the dynamic turnover and restructuring of the actin cytoskeleton. The nf-proflin gene is composed of 450 bp (encodes 150 amino acids) and produces 22.5 kDa recombinant protein (rNf-profilin). The Nf- profilin was localized on the pseudopodia in N. fowleri trophozoites using immunofluorescence assay. In contrast, the nf-actin was localized on cytoplasm pseudopodia and food-cup structure.

Conclusions: Finally, these results suggest that actin binding protein is very important for understanding the N. fowleri infection and pathogenicity mechanism of this parasite.

Keywords: Naegleria fowleri, RNA-sequencing, actin, Actin-binding protein

P375 / #617

Topic: *AS02.5 Other studies related to parasites of humans*

IN VITRO EVALUATION OF CHANGES IN CYTOKINE SECRETION INDUCED BY RECOMBINANT CLP (MULTI CYSTATIN-LIKE PROTEIN) FROM TRICHINELLA BRITOVI

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Introduction: Trichinellosis is a well-known parasitic zoonosis. Trichinella species have developed some specific mechanisms, which enable them in successful completion of the life cycle. Those mechanisms are in great part involved in modulation of immunological response of the host and studying those mechanisms will help with better understanding of functioning of the immune system. Different proteins of Trichinella genus are worth scientific investigation according to their immunoreactive and immunomodulatory potential and important role in infection process. In this study the recombinant CLP derived from T. britovi was investigated and the aim of the study was to examine whether the CLP has anti-inflammatory properties in vitro.

Methods: Mouse CD11b+ monocytes, splenocytes and bone marrow cells were stimulated with lipopolysaccharide (LPS) and treated with the recombinant CLP for 24, 48, 72 or 96 h. The culture supernatant was harvested for the determination of the cytokine level using ELISA. The panel of cytokines tested included IFNγ, TNFα, IL-2, IL-6 and IL-10.

Results: The secretion of inflammatory cytokines TNFα and IL-6 was significantly decreased after co-stimulation with CLP in some cases, e.g. for CD11b+ monocytes after 72 h. In contrast, the secretion of the regulatory cytokine IL-10 was significantly increased after administration of CLP protein in some cases, e.g. for splenocytes after 48 h. The level of IFNγ and IL-2 was rather low and inconclusive.

Conclusions: Obtained results indicate that CLP has some immunomodulatory and anti-inflammatory properties and future research on this protein and its specific function is reasonable. *Financial support for this study was provided by the National Science Centre Poland (Grant no 2020/04/X/NZ6/00084).

Keywords: cytokines, cystatin-like protein, immunomodulation, Trichinella

P376 / #1158

Topic: *AS02.5 Other studies related to parasites of humans*

DIVERGENT PROTEIN IMPORT MACHINERY IN HYDROGENOSOMES OF TRICHOMONAS VAGINALIS

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Introduction: In model yeast mitochondria, matrix preproteins are translocated across inner membrane (IM) via TIM23 complex, which is membrane potential- and ATP- dependent process, whereas IM preproteins are recognized by small Tims, and translocated via TIM22 (membrane potential dependent only). *Trichomonas vaginalis* with anaerobic type of mitochondria named hydrogenosomes lack IM associated respiratory complexes and consequently IM membrane potential is minimal or absent. Here we investigated how the absence of membrane potential shaped the hydrogenosomal TIM complex.

Methods: In vitro protein import assay, immunoprecipitation (IP) and proteomic analysis (LFQ-MS), single particle analysis by electron microscopy.

Results: There are five divergent paralogs of Tim17/22/23 protein family in *T. vaginalis*. Three of them are closer to Tim23, and two displayed similarity to Tim17. In vitro import confirmed that TvTim23 is involved in the substrate translocation. Proteomic analysis of IP complex revealed 58 interacting proteins including small Tims, the presequence translocase-associated motor (PAM), and ATP/ADP carrier (AAC). Unlike TIM23 complex in other eukaryotes, the receptor protein Tim50 is absent. Reciprocal IP using Tim44 and AAC as baits confirmed their interaction with TvTim23. TvTim23 migrates in large protein complexes of about 250 and 800 kDa. The TvTIM23 complex measures 14 X 12 nm in size and is decorated with a ring-like structure resembling small Tim hexamer.

Conclusions: The hydrogenosomes most likely possess only a single type of TIM complex and it can stably associate with small Tim chaperones. This modification may facilitate it to import both matrix proteins and inner membrane proteins.

Keywords: TIM, Trichomonas vaginalis, hydrogenosomes

P377 / #562

Topic: *AS02.5 Other studies related to parasites of humans*

THE TRICHOMONS VAGINALIS MICROPROTEOME

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Introduction: Microprotein (MP) is defined as a protein composed of less than 100 amino acids. Accumulated experimental data showed that microproteins play important regulatory roles in various biological processes However, microprotein has been understudied because classical genome annotation methods do not consider open reading frames (ORF) less than 100 amino acids as putative genes. The present work used Trichomonas vaginals: the causing agent of the most common sexually transmitted infection (STI) of nonviral origin, as a model system to explore the microproteome in protozoan and to characterize the putative functional roles of MP.

Methods: To identify putative microprotein-encoding genes that are expressed, we developed a virtual MP database bioinformatics pipeline for the identification of expressed microproteins from RNAseq and proteomics data. Putative predicted ORF and annotated ORF coding less than 100 amino acids are extracted from the genome database to construct a virtual MP database. The landscape of expressed MP genes was identified by a multi-omics approach using second/third-generation sequencing technology and LC/MS/MS.

Results: ORFs identified by the genome-wide search were mapped to the reference transcripts. Novel ORFs shorter than 300 nucleotides were extracted to construct a T. vaginalis virtual microprotein database (vMPdb) composed of 179,917 unique putative microprotein-encoding genes. A total of 2266 MP transcripts and 497 MP were identified from the Illumina RNAseq, Oxford nanopore direct RNAseq, and proteomic datasets, respectively.

Conclusions: Experimental results from the present proposal will help illustrate the existence of microprotein-coding genes in T. vaginalis and advance studies on the biology of this parasite.

Keywords: Trichomonas vaginalis, microprotein, multi-omics

P378 / #1574

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

SEROPREVALENCE OF TOXOPLASMOSIS AMONG CHILDREN WITH AUTISM

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Introduction: Toxoplasmosis is one of the common public health problems. Toxoplasmosis is reported to be associated with many neuropsychiatric disorders like autism spectrum disorder (ASD). This study was performed to detect seroprevalence of toxoplasmosis among autistic children.

Methods: The study was conducted on 100 children grouped in two groups, 50 autistic children and a control group of 50 healthy children. All children included in this study were subjected to history taking (personal data, past and present history of any general, neurological and/or mental diseases and family history), clinical examination (neurological and mental assessment) and laboratory investigations to detect serum IgG and IgM specific to T. gondii.

Results: Among symptomatic autistic children the detected anti-T. gondii. antibodies demonstrated that there were 18 children out of 50 (36%) were infected with T. gondii. 16 children with an old infection of toxoplasmosis (positive IgG), and 2 of them (4%) demonstrated recent toxoplasmosis (positive IgM). Meanwhile among healthy control group there were 5 out of 50 healthy children (10%) were T. gondii.positive. 4 children were having an old toxoplasmosis and only one proved to have recent toxoplasmosis. with old infection of toxoplasmosis. The study revealed a significant increase in the prevalence of IgG seropositive among autistic children with positive family history than children with no family history. A significant increase in the prevalence of both the old and recent toxoplasmosis among autistic children with low socioeconomic class was demonstrated than children with moderate or high classes.

Conclusions: Our study clearly demonstrated a significant correlation between old toxoplasmosis and autism among children.

Keywords: Seroprevalence, toxoplasma, Autism

P379 / #1236

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

PHYLOGENETIC ANALYSIS OF SPIROCERCA LUPI FROM THE AMERICAS: WHAT´S THE STORY BEHIND?

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Introduction: Spirocerca lupi is a carcinogenic nematode of canids that causes esophageal spirocercosis and is distributed mainly in the tropics and subtropics of the world. The phylogenetic history of S. lupi from the Americas is currently unknown.

Methods: To study this, S. lupi specimens from Mexico, Costa Rica, and the United States obtained from domestic dogs were molecularly characterized using 18S and cox1 gene fragments. Maximum likelihood (ML) and Bayesian inference (BI) phylogenetic trees, as well as Templeton-Crandall-Sing (TCS) haplotype networks were constructed for each locus separately.

Results: ML and BI cox1 trees grouped the S. lupi worms from the Americas in genotype 1, together with Israeli specimens, and in the TCS network, formed a separate cluster which located them between Israeli S. lupi and Spirocerca vulpis. 18S sequences of Mexican and US S. lupi could not be obtained. But, ML and BI 18S trees separated Costa Rican worms from African, Asian and European specimens and the TCS network demonstrated a shared haplotype of Israeli, South African, Costa Rican and Indian specimens.

Conclusions: This study demonstrates that S. lupi from the Americas belong to genotype 1, together with the specimens from Israel, India, South Africa and Australia and apart from European S. lupi and S. vulpis. Therefore, we suggest that the worms from the American continent might have originated from wild or domestic canids of Asia or Africa, or vice versa, by migration or dispersal of intermediate or definitive hosts.

Keywords: spirocerca lupi, Phylogenetic analysis, parasite of dogs

P380 / #925

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

OESTRUS OVIS INFESTATION: CLINICAL SIGNS AND PCR AS A DIAGNOSTIC METHOD IN NATURALLY INFESTED LAMBS

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Introduction: The diagnosis of most of oestrids causing internal myiasis relies on the post mortem examination. The PCR may be useful to determine the efficiency of therapy against Oestrus ovis by monitoring the parasite-specific DNA in nasal mucus, thus avoiding the need to sacrifice them. The goals of this study were to analyse: clinical signs of oestrosis by the score of nasal discharge; IgG production; larvae number and morphology; and PCR as a diagnostic method of this parasitosis.

Methods: The experiment occurred from December to April with 39 male and female lambs. They were divided in infested group and treated group, that received closantel (Diantel® - Merial) 10 mg/kg orally every 28 days.

Results: The clinical signs varied individually and independently of the amount of recovered larvae post mortem, however, the thick mucus and mucopurulent nasal discharge scores were lower in the treated group. Out of the 26 animals not treated, 24 were parasitized (1-54 larvae/animal). No L3 was recovered. There was a gradual increase of IgG (anti-antigen of O. ovis larvae) in the animals of the infested group after the third week of study, whereas the treated lambs with closantel kept low levels of IgG until the end of the experiment. The PCR presented low sensibility (22%) and high specificity (100%) and slight agreement (κ=0,149) when compared to the recovered larvae counts (after the lamb slaughter). Clinical signs were not related to the amount of larvae, although the treatment of oestrosis have avoided the clinical symptoms.

Conclusions: PCR was not effective as diagnostic method of oestrosis, even though results from recent studies have shown that PCR is a valid technique to detect the presence of O. ovis larvae.
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Keywords: oestrosis, nasal discharge, PCR

P381 / #345

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

USE OF COPRO-LAMP REACTIONS TO IMPROVE THE DIAGNOSIS AND SURVEILLANCE OF ZOONOTIC HELMINTH PARASITES IN ENDEMIC AREAS

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Introduction: Approximately one third of the world's human population is infected with helminths, particularly in socioeconomically disadvantaged regions. Helminths have been extensively investigated, although, their actual distributions are still unknown and a specific, accurate, sensitive and affordable diagnosis is urgently needed. Our group has developed and implemented three LAMP reactions for DNA detection of Echinococcus granulosus sensu lato[1], Toxocara canis-Toxocara cati[2] and Ancylostoma caninum from feces[3].

Methods: The primers were design using software Primer-ExplorerV5. The design was developed ensuring the inclusivity and exclusivity to DNA detection from the helminths studied. The in silico validation was carried out by alignment of sequences of the target genes with the sequence of the primers and the sequences of orthologous genes of other parasites. For in-vitro validation, analytical sensitivity was evaluated using serial dilution of DNA and specificity was evaluated using DNA from host and other parasites. The LAMP reactions were compared to other standard coprological methods

Results: With the in silico validation strategy, it was possible to develop three specific and sensitivity LAMP reactions for the detection of E. granulosus s. l., Toxocara sp. and A. caninum. The LAMP reactions demonstrated an improved detection of the studied helminths compared to classical coprological studies.

Conclusions: The development and implementation of LAMP reactions is possible in laboratories with scarce equipment. This enables diagnosis and surveillance of zoonotic helminths in endemic areas with limited resources. References: [1] doi:10.1016/j.vetpar.2019.109017 [2] doi: https://doi.org/10.1017/S0031182021000342 [3] doi:10.3389/fvets.2021.770508

Keywords: Zoonotic helminths parasites, LAMP reactions, Diagnosis and Surveillance

P382 / #1380

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

DETECTION OF NEOSPORA CANINUM IN DIAGNOSTIC MATERIAL FROM BOVINE ABORTIONS: COMPARISON OF METHODS

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Introduction: The protozoan parasite Neospora caninum causes abortions in cattle globally. In Denmark, as part of the national surveillance program of bovine abortions, aborted fetuses and placentas can be submitted for examination to the Danish Veterinary Consortium – comprising Statens Serum Institut (SSI) and the University of Copenhagen. The aim of this study was to evaluate the level of agreement between histopathological findings, metabarcoding, and conventional PCR for the detection of N. caninum in the material submitted.

Methods: The submitted aborted fetuses and placentas underwent histopathological examination at the University of Copenhagen. DNAs from a subsample of the placentas and pooled tissue samples from the fetuses were analyzed at SSI by metabarcoding followed by Illumina sequencing for the detection of bacteria, parasites and fungi, as well as by conventional PCR for detection of N. caninum-specific DNA.

Results: Material from 67 abortions were included in the study, 15 of which had histopathological findings consistent with protozoal abortion (likely neosporosis). Neospora caninum DNA was detected in five of the samples by metabarcoding and in 13 of the samples by conventional PCR.

Conclusions: The preliminary results indicate that a supplementary PCR test may be a good addition to the histopathological approach.

Keywords: Protozoa, Bovine abortion, histopathology, Molecular diagnostics

P383 / #1024

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

GENETIC CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF T. VITULORUM ISOLATES FROM BUFFALOES FOR THE FIRST TIME IN BANGLADESH

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Introduction: Toxocara vitulorum infection in water buffaloes lead death among buffalo calves in Bangladesh and responsible for zoonotic visceral larva migrans. This study aimed to characterize mitochondrial cox1 gene in T. vitulorum isolates from buffalo of Bangladesh for phylogenetic analysis.

Methods: Worms were collected from calves from different regions of Bangladesh. Genomic DNA of parasite was extracted. Primer pairs JB3 F (5′-T TTTTTGGGCATCCTGAGGTTTAT-3′) JB4.5 & R (5′-TAAAGAAAGAACATAATGAAAATG-3′) of cox1 were employed and after DNA amplification, a 446 bp fragment of cox1 (mt gene) of T. vitulorum was obtained. The sequenced data were then analyzed using Mega X and the phylogenetic relationship was studied comparing with the gene sequences available in the GenBank (NCBI).

Results: Sequence alignment and phylogenetic tree revealed that the present ascarids were T. vitulorum. The pairwise distance of the current representative parasites of different region of Bangladesh were homologous and 100%, 99. 00%, 96.99% similarities were found with T. vitulorum from China (AJ920062.1), SriLanka (FJ664617.1), Turkey (MG911730.1) and Germany (KY313642.1), respectively. Phylogenetic analysis of cox1 sequences also illustrated that proportion of identity of representative T. vitulorum of different regions of Bangladesh were 96.99%, 92.21%, 91.46%, 91.46% and 91.48% with T. cati (AJ920057.1), T. malaysiensis (AJ920060.1), T. leonina (MK516267.1), Ascaridia gali (OM004027.1) and A. suum (HQ704901.1), respectively. It indicates the various species of Toxocara are host-specific and each member of the genus Toxocara were dissimilar with the molecular sequences.

Conclusions: It is imperative to explore the diversity and anthelmintic resistant genes in T. vitulorum.

Keywords: phylogenetic, Buffalo, Bangladesh, Toxocara vitulorum, mitochondrial cox1 gene

P384 / #1209

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

FASCIOLA SPECIES INTROGRESSION: JUST A FLUKE OR SOMETHING MORE?

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Introduction: The continued neglect of fasciolosis in low- and middle-income countries, combined with increased live animal export from Fasciola hepatica endemic regions to meet a growing demand for animal derived protein, has expanded areas of parasite sympatry in Southeast Asia and beyond. As a result, there are increasing reports of hybridization and possible introgression between F. hepatica and F. gigantica. Quantification of the extent of these events is limited by the requirement for access to adult parasites for species identification in a system that inherently relies on the movement of live animals. Furthermore, the One Health impacts of hybridization and introgression in Fasciola spp. remain unknown due to the absence of genetic and biological characterization of these forms.

Methods: Newer molecular methods enabling Fasciola spp. differentiation from faecal samples have extended our ability to conduct parasite surveillance beyond the abattoir. Next Generation and whole genome sequencing will help to elucidate implications of interspecific mating events.

Results: This opinion demonstrates that the identification of hybridization or introgression in these hermaphroditic parasites is far from trivial and over-interpretation of interspecific mating events identified with limited genetic markers should be avoided. Further, updated estimates reveal the economic impacts of fasciolosis on livestock in Southeast Asia alone ranges from $0.31-1.88 billion USD, excluding the cost of anthelminthics.

Conclusions: Given the economic, human and animal health impacts of this parasite and increased areas of species overlap, it is time we considered the longevity, functional and epidemiological implications of Fasciola spp. hybridisation and possible introgression.

Keywords: One Health, fasciolosis, food security, hybridisation, introgression

P385 / #1066

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

INFECTION STATUS OF AUSTRALIAN WILD DEER: EVIDENCE OF NOVEL PATHOGENS

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Introduction: Australia is currently free of many of the world's worst animal pathogens. However, animal pests and diseases remain a major threat to Australia's livestock industry as well as to human and wildlife health. Deer can act as reservoirs for various livestock diseases. This project assessed the infectious status of wild deer populations in Australia and evaluated the biosecurity risk posed by deer to livestock.

Methods: A total of 543 tissue samples were collected among 5 deer species across 4 Australian states. The presence of viruses and parasites was investigated combining traditional laboratory methods (ELISA, PCR) with next generation sequencing.

Results: Traditional laboratory methods demonstrated that wild deer may currently be an incidental spill-over host for Pestivirus and Neospora caninum but not a reservoir host for other pathogens commonly detected in livestock. Entamoeba bovis was found to be highly prevalent in wild deer and cattle; however, our analysis suggests a lack of current E. bovis transmission between these two animal species in Australia. Next-generation sequencing revealed a novel Picobirnavirus and a novel species of Bopivirus, with potential risk for domestic animals.

Conclusions: Our research highlights that deer could be a future source of infections for wild and domestic animals, extends our knowledge on known and novel viruses and parasites associated with Australian deer, and provides vital baseline data for future research.

Keywords: wildlife, Parasites, virus, Wild deer, Zoonosis

P386 / #1221

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

OVERCOMING CHALLENGES IN HELMINTH DIAGNOSTICS AND EPIDEMIOLOGY IN MOUNTAIN GORILLAS

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Introduction: Parasitological studies of endangered wildlife rely heavily on noninvasively collected samples, usually feces. The type of samples (identified/unidentified) and the number of sampling events per individual have a significant impact on the information that can be gained from the sample set. Depending on the taxon, parasite stages shed in feces can be determined to the level varying from the order to the species using optical examination methods. However, interspecies transmission, including zoonotic or wildlife-domestic animal cycles, cannot be evaluated, so DNA barcoding followed by sequence analysis is required. Unfortunately, the utility of molecular taxonomy tools is limited by the pre-existence of reference sequences usually tied to obtaining adult helminth specimens that can be properly morphologically determined and subsequently subjected to DNA sequencing.

Methods: We used adult specimens collected in necropsies, more than 60 proglottids extracted from feces, and a comprehensive set (n=1500) of both identified and unidentified fecal samples to investigate the epidemiology of anoplocephalid cestode infections in mountain gorillas in Volcanoes National Park, Rwanda.

Results: All adults and proglottids were identified as Anoplocephala gorillae, which was the dominant species. One individual showed to be infected by a Bertiella sp. Less than 1% of fecal samples contained neither cestode eggs nor DNA. The number of eggs shed by individual gorillas varied widely over time, and some animals even had negative and positive samples recorded within a short period of time.

Conclusions: Study aims should be reflected in the sampling strategy and examination methods. Repeated sampling and DNA-based diagnostics decrease the risk of false-negative results.

Keywords: Mountain gorilla, cestode, Anoplocephalidae

P387 / #1605

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

FIRST EVIDENCE AND MOLECULAR IDENTIFICATION OF TRYPANOSOMA THEILERI IN CATTLE FROM ECUADOR

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Introduction: *Trypanosoma theileri*, a cosmopolitan blood parasite in America, infects several species such as cattle, buffalo, and bats. Molecular studies have shown that TthI and TthII lineages are associated with geographic origin and host origin. The objective of the present study was to determine the presence of *T. theileri* in cattle and its lineage by amplification of the CatL gene and ITS from its 18S.

Methods: Between February and April 2021, 221 bovine blood samples were collected in two slaughterhouses located in Quito (n= 83) and Santo Domingo (n=138). Direct diagnosis was made by Woo technique and molecular diagnosis by PCR. DNA samples were extracted using the GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific™) and analyzed by PCR-CatL and Nested PCR-ITS. The PCR products were sequenced by Sanger methodology.

Results: Overall, 7 (3.16%) samples were positive for *Trypanosoma* spp. by the Woo technique; and 34 (15.59%) were positive for *T. theileri* by PCR-CatL. Out of the samples positives by PCR-CatL, 20/83 (24.10%) were from Quito and 14/138 (10.37%) from Santo Domingo. The sequences of the positive samples (n=13) for PCR-CatL and Nested PCR-ITS showed that 7 were related to TthI and 6 to TthII lineage.

Conclusions: The trypanosomes from the two areas were more closely related to the isolates from Sri Lanka, Brazil, and USA. Besides, the Ecuadorian isolates were associated with bovine genotypes IC, IB, and IIB, previously reported in Brazil, Venezuela, and Colombia. CatL gen and ITS sequences analysis identified *T. theileri* belonging to the TthI and ThII lineages in cattle in Ecuador for the first time.

Keywords: Trypanosoma theileri, Bovine trypanosomosis, TthI and TthII

P388 / #1551

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

BLOOD-MEAL HOSTS AND PARASITES OF THE TSETSE FLY IN TANZANIA

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Introduction: Tsetse flies cause trypanosomiasis in humans, wild animals, and livestock. Amplicon deep sequencing of the 12S ribosomal RNA gene can detect mammalian hosts, and the 18S rRNA gene can be used to detect eukaryotic pathogens, including *Trypanosoma* spp.

Methods: Tsetse flies were collected from the Serengeti National Park (n=48), Maswa Game Reserve (n=42), and Tarangire National Park (n=49) in Tanzania in 2013. Amplicon deep sequencing targeting 12S rRNA and 18S rRNA genes was performed to screen the blood-feeding sources of tsetse flies and eukaryotic parasites in tsetse flies, respectively.

Results: 12S rRNA gene deep sequencing revealed that various mammals were blood-feeding sources of the tsetse flies, including humans, common warthogs, African buffalos, mice, giraffes, African elephants, waterbucks, and lions. Genes of humans were less frequently detected in Serengeti (P=0.0024), whereas African buffaloes were found more as a blood-feeding source (P=0.0010). 18S rRNA gene deep sequencing showed that six tsetse samples harbored the *Trypanosoma* gene, which was identified as *Trypanosoma godfreyi* and *Trypanosoma simiae* in subsequent ITS1 gene sequencing.

Conclusions: Various mammalian animals were identified as blood-meal sources of tsetse flies and two *Trypanosoma spp.* were detected. It may provide essential data for formulating better strategies to control African trypanosomes.

Keywords: tsetse fly, Tanzania, Amplicon deep sequencing, Trypanosoma, Trypanosomiasis

P389 / #874

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

WHOLE GENOME SEQUENCING OF A FELINE STRAIN OF TRITRICHOMONAS FOETUS REVEALS MASSIVE GENETIC DIFFERENCES TO BOVINE AND PORCINE ISOLATES

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Introduction: Tritrichomonas foetus is a protozoan parasite of the reproductive tract of cattle as well as the gastrointestinal tract of cats. Furthermore, T. foetus is known as commensal of pigs. Transmission of T. foetus between the different hosts has to be considered a realistic scenario that may have important implications for the epidemiology of infections and disease. In our study, we generated whole genome sequencing data from bovine, feline and porcine T. foetus strains to investigate the genetic (dis)similarities among three strains.

Methods: As a reference, we used draft assembly from a bovine T. foetus strain K. We used WGS and in particular, we identified single nucleotide polymorphisms (SNPs) and the insertion-deletion (indel) variations within the genomes of the three strains.

Results: A low degree of polymorphism (68 SNPs and indels) was found between the bovine and the porcine strains in terms of variants with a predicted impact of moderate or high. Conversely, however, a 964 times higher number of such differences was detected by comparing the feline with either the bovine (65,569) or the porcine (65,615) strain. The latter observation was confirmed by PCR-based sequencing of 20 in silico selected indel markers and five in silico-selected SNP markers that uniformly demonstrated a relatively distant phylogenetic relationship of three independent feline T. foetus isolates in comparison to the bovine and porcine strains investigated.

Conclusions: Our study identified a large number of SNP- and indel-containing sequences that may be useful molecular markers for future epidemiological studies aimed at the elucidation of the transmission patterns of T. foetus with in different host species.

Keywords: hosts, NGS, Tritrichomonas foetus, GENOME

P390 / #1033

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

INTESTINAL PARABASALIDS IN PIGS DETECTED AND DIFFERENTIATED BY METABARCODING OF NUCLEAR SMALL SUBNIT RIBOSOMAL RNA GENES

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Introduction: Gut microbiota play a key part of an animal's health. The bacteria in the digestive system are well described, whereas data on intestinal eukaryotic communities are still relatively limited. In this study, we used metabarcoding of small subunit ribosomal DNA (SSU rDNA) to detect and differentiate intestinal parabasalids of pigs and study associations between parasite and bacterial communities.

Methods: Faecal DNAs were available from 273 pigs from four herds in Denmark. Eukaryotic SSU rDNA was amplified and sequenced using ILLUMINA sequencing. Sequences reflecting parabasalid DNA were aligned and clustered, and consensus sequences were subject to genetic analysis. Associations between these parasites and gut bacteria will be carried out mainly by standard alpha and beta diversity analyses.

Results: We identified Trichomitus batrachorum, Tetratrichomonas buttreyi, Hypotrichomonas imitans, and Tritrichomonas suis verified based on reference sequences available in the NCBI database. Colonisation rates differed substantially across the herds (range, 40%-88%). The presence of Giardia, which was not detected by metabarcoding assay but which had been detected by real-time PCR prior to the study, did not influence colonization rates byother parabasalids (P = 0.1096). Data analyses on intrageneric genetic variation and microbiota profiling are pending.

Conclusions: This is the first report on non-Giardia parabasalids in pigs sampled in Denmark. The parabasalid species identified here correspond well to those reported in pigs in Europe and Asia. Data on potential differences in bacterial microbiome between parabasalid-positive and –negative animals will be presented.

Keywords: genetic diversity, DNA-based taxonomy, 18S, NGS, host specificity

P391 / #1104

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

THE TRICHINELLA BRITOVI RECOMBINANT PROTEINS PRODUCED IN YEAST PICHIA PASTORIS EXPRESSION SYSTEM AS POTENTIAL DIAGNOSTIC MARKERS OF TRICHINELLOSIS IN ANIMALS

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Introduction: The Trichinella genus nematode cause trichinellosis among humans and animals. An ingestion of raw or undercooked meat infected with encysted muscle larvae (ML) results in the parasite invasion. The currently recommended serological diagnostic tests base on excretory-secretory (ES) products from T. spiralis ML. However, it enables late stage invasion diagnosis and can result in cross-reactivity with antigens from other parasites and pathogens. In our research we aimed to produce the T. britovi recombinant proteins and apply them as a specific diagnostic markers of trichinellosis.

Methods: Three proteins with a hypothetical immunodiagnostic potential have been chosen: TbCTRL, TbES21, TbHSP20, cloned and produced in yeast Pichia pastoris expression system. Subsequently, their immune reactivity with sera from mice and pigs experimentally infected with T. spiralis or T. britovi species were verified with ELISA by testing the specific IgG antibodies abundance.

Results: The rTbCTRL protein was detected at 41 dpi for T. britovi, and 45 dpi for T. spiralis infected pigs, and turned out to be most useful for serodiagnostic purposes. The highly expressed rTbES21 presented limited reactivity with animal sera, whereas the initially effectively produced rTbHSP20 protein degraded during further testing. Interestingly, the IgG level for sera samples from T. britovi infected mice were higher for combined rTbCTRL+rTbES21 proteins than for single antigens, and the results were close to the reference protein rTbCLP.

Conclusions: Due to limited cross-reactivity with other parasitic diseases, the approach of multiple-antigen usage may be suitable for Trichinella infection diagnostic test development. The research was funded by NSC Poland grant (UMO-2015/18/E/NZ6/00502).

Keywords: Pichia pastoris, Recombinant protein, Trichinella britovi, ELISA, Immunoglobulin G

P392 / #228

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

MOLECULAR DETECTION OF CIRCULATING CELL-FREE DNA IN HYDATID CYST NATURALLY INFECTED SHEEP SERA

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Introduction: Cystic echinococcosis (CE) is a worldwide parasitic zoonosis disease caused by the larval stage of Echinococcus granulosus sensu lato affecting human and livestock, particularly sheep and goats. The disease mostly remains asymptomatic in intermediate animal hosts and humans for a long period of infection. The diagnosis of the disease in human is complicated and according to WHO recommendations is mainly base on imaging finding along with serological tests. However, these methods have some disadvantages as an example they are not applicable for post-surgery follow up. In recent years there have been several publications on the detection of Echinococcus cell free DNA in the samples of echinococcosis patients but their sensitivity was about 20% to 25%. So, we investigated the presence of E. granulosus-specific cfDNA in the serum of CE sheep, for the first time, by detecting the NADH dehydrogenase (nad) mitochondrial gene.

Methods: Five mL of peripheral whole blood were collected from a total of 35 sheep naturally infected with hydatid cyst. The blood samples were centrifuged at 1600 g for 10 min and the sera were transferred in to new tubes and stored at -20 °C. The Cell free DNA was extracted by the modified phenol-chloroform-isoamyl alcohol method. The DNA of E. granulosus was detected by nested PCR amplification of a mitochondrial gene, nad, in sheep sera.

Results: Echinococcus DNA was detected in 33 out of 35 sheep serum by nad gene. So sensitivity of 94% was obtained for this test.

Conclusions: With high sensitivity of cell free DNA detection method for diagnosis of sheep hydatid cyst, this test can be investigated for diagnosis of human echinococcosis in future.

Keywords: detection, echinococcosis, Echinococcus granulosus, cell free DNA, NADH dehydrogenase,

P393 / #1191

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

SARCOCYSTIS SPP. FROM BELUGA WHALES (DELPHINAPTERUS LEUCAS) IN THE EASTERN BEAUFORT SEA, CANADA.

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Introduction: Beluga whales (Delphinapterus leucas) are important indicators of health in Arctic marine ecosystems. As well, these whales play an important role in food security and culture in Inuit communities in the Canadian North. Sarcocystis spp. are apicomplexan parasites composed of more than 100 species with a variety of hosts, including marine mammals. Most infections are asymptomatic in marine mammals; however, protozoal encephalitis has been reported.

Methods: The aim of the study was to determine prevalence and identity of Sarcocystis spp. in the eastern Beaufort Sea beluga population, in collaboration with the annual harvest in Hendrickson Island and East Whitefish, Northwest Territories, Canada between 2017 and 2019. Heart and muscle samples were collected from 42 whales.

Results: DNA of Sarcocystis was detected in 62% of animals by melt curve analysis (MCA), and positive samples were confirmed using nested PCR assays targeting the ITS-1 region and ITS-1 500. Further analysis is under way to sequence and identify species of Sarcocystis in belugas. Histopathology suggests that these Sarcocystis spp. infections were not associated with clinical disease in beluga, which are serving as intermediate hosts in the life cycle. Definitive hosts are unknown but could include polar bears.

Conclusions: This study contributes to the knowledge of the available tools to detect tissue coccidians as well as our understanding of beluga whales as sentinels for Sarcocystis in marine ecosystems in the Canadian North.

Keywords: Sarcocystis, Beluga whale, Arctic

P394 / #712

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

THE INCIDENCE OF NOSEMA SPP. IN THE HONEY BEE COLONIES IN SLOVAKIA DURING 2021

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Introduction: Microsporidia are unicellular obligate intracellular parasitic fungi which infect a wide range of vertebrates and invertebrates. There are two species infecting the honey bee in our geographical conditions- Nosema apis and Nosema ceranae. While Nosema apis was the first to appear and is accompanied by clinical signs of the disease such as diarrhea and altered bee movement, Nosema ceranae, which gradually supersedes Nosema apis, is not accompanied by clinical signs of disease, but the presence of disease weakens the colony and increases mortality. Our aim was to examine samples of honey bees collected from those who breed queen bees in the Slovak Republic during 2021.

Methods: 81 samples were examined using molecular methods. The samples of crushed honey bee abdomens were processed using an isolation kit in order to extract genomic DNA. Then the samples were processed by multiplex PCR using primer pairs for both species- Nosema apis (321 bp amplicon) and Nosema ceranae (218 bp amplicon) simultaneously.

Results: 73 positive samples showing the presence of Nosema ceranae (218 bp) were assessed using ELFO visualization. After sequencing the positive PCR products and comparing the sequences (BLAST) with the sequences stored in the gene bank, Nosema ceranae species was detected in all positive samples.

Conclusions: Nosematosis is an important determinant leading to the weakening of the colonies and alongside other factors, to their collapse.

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Disclosure: This paper was created with the support of the grant projects VEGA no. 1/0113/20, and APVV-21-0185.

Keywords: Nosema apis, Multiplex PCR, nosema ceranae, bees

P395 / #1590

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

REAL-TIME PCR INVESTIGATION ON STRONGYLUS VULGARIS IN HORSES, ROMANIA

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Introduction: Currently, the emerging development of anthelmintic resistance in equine parasites (i.e. small strongyles, ascarids) reported in numerous countries has changed deworming management by reducing the treatment frequency. In this context, more accurate diagnostic tools are required for detection of parasite infection, especially of the highly pathogenic *Strongylus vulgaris* (Nematoda: Strongylidae). In the present study a Real-Time PCR investigation was carried-out to asses the occurrence of *S. vulgaris* in Romanian horses.

Methods: For this, 168 animals, including horses from sport and recreational units (n=44) and working horses (n=124), originated from 11 premises in 8 counties of Romania were enrolled in a coprological study. Individual fresh fecal samples were collected and analyzed using a modified McMaster method for strongyle egg per gram (EPG) counting. Overall, of the tested horses from sport and recreational units and working horses, 59.1% and 74.8% respectively, were positive for strongyle infection. Ninety-four of the strongyle positive samples (24 from sport and recreational horses and 70 from working horses) were further subjected for molecular analyzes by using a species-specific real-time PCR technique for the presence of *S. vulgaris* DNA in strongyle eggs isolated from fecal samples.

Results: Subsequently, *S. vulgaris* DNA was detected in 8.33% (2/24) and 58.6% (41/70) of the tested horses from sport and recreational units and working horses, respectively.

Conclusions: These findings emphasize on the high prevalence of strongyle infection among Romanian horses and on higher frequency of *S. vulgaris* in working horses. Additionally, it is suggested that the high sensitive RT-PCR method is a good tool for *S. vulgaris* monitoring in horse populations.

Keywords: Strongylus vulgaris, Real-time PCR, horses, Romania, equine strongyles

P396 / #1662

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

FATAL GRANULOMATOUS AMOEBIC ENCEPHALITIS CAUSED BY SIMULTANEOUS INFECTION OF BALAMUTHIA MANDRILLARIS AND ACANTHAMOEBA SPECIES, REPORTED IN KOREA

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Introduction: *Balamuthia mandrillaris* and *Acanthamoeba* species are well known among the amoeba species to cause amoebic encephalitis. *Acanthamoeba* species mainly causes keratitis, but rarely (mainly in immunocompromised patients) causes intracranial infection called GAE, while with *B. mandrillaris*, regardless of immunocompetence status, infection can occur sporadically with very high mortality rate. A 45-year-old healthy female patient who visited Seoul National University Hospital showed symptoms of hemianopia on the left side, and an MRI scan revealed an occipital lobe brain abscess. Despite continuous antibiotic treatment, uncontrolled infection spread to the left frontotemporal lobe and occipital lobe, and multiple hemorrhages accompanied by necrotic changes were found.

Methods: The patient's brain tissue samples were histologically examined by PAS staining. Culture test and PCR diagnosis were also performed. Necrotic brain tissue was cultured in PYG medium to serve as a culture test. Afterwards, DNA from necrotic brain tissue were extracted and used to confirm the identified amoeba by RT-PCR using Balamuthia (Balspec16S) and *Acanthamoeba*-specific primer (JDP)

Results: HE of patient’s brain sample confirmed the presence of amoeba trophozoites. Also, Amoeba trophozoites and cysts were observed in the cultured samples. Amplicons of specific primers were used to check the similarity with reference genomes through blast search and phylogenetic tree of the nucleotide sequence obtained through RT-PCR confirmed the co-infection of *Acanthamoeba* and *B*. *mandrillaris*.

Conclusions: *B*. *mandrillaris* and *Acanthamoeba* were identified through RT-PCR using the patient's brain tissue, making this study as the first case of co-infection with GAE reported in Korea.

Keywords: Balamuthia mandrillaris, amoebic encephalitis, Acanthamoeba, GAE

P397 / #563

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

USE OF RECOMBINANT VIRUS-LIKE PARTICLES FOR SENSITIVE SERODIAGNOSIS OF TOXOPLASMOSIS

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Introduction: The diagnosis of toxoplasmosis mainly relies on serological testing by enzyme-linked immune assay (ELISA), in which antigen use is critical in improving the sensitivity and specificity of ELISA reaction.

Methods: In this study, we generated Toxoplasma gondii virus-like particles (VLPs) displaying AMA1 (AMA1 VLPs) of T. gondii and evaluated the use of VLPs for the serodiagnosis of toxoplasmosis. BALB/c mice were orally infected with T. gondii ME49 (10, 50, 100, 150, 300 cyst) or and RH ($5x10^3$, $1x10^4$, $5x10^4$). Sera were collected and the ELISA were performed using T. gondii AMA1 VLPs antigen.

Results: We found that AMA1 VLPs antigens are highly sensitive to react with mouse sera infected with T. gondii (ME49) or T. gondii (RH) with low infectious dosage (10 cysts for ME49, $5x10^3$ for RH), resulting in significantly higher levels of IgG and IgA antibody responses compared to T. gondii lysate antigen (TLA). Interestingly, AMA1 VLPs can detect both T. gondii RH and T. gondii ME49 infections as early as week 1 post-infections. AMA1 VLPs showed no IgG or IgA cross-reactivity with Plasmodium berghei infected sera. Importantly, AMA1 VLPs highly reacted with T. gondii - infected human sera compared to TLA, in which AMA1 VLPs showed 90% of sensitivity whereas TLA showed 70%.

Conclusions: These results indicated that AMA1 VLPs can detect the infections of T. gondii ME49 or RH at early stage of infection in mice, and it could be used for diagnosis of toxoplasmosis in humans.

Keywords: Toxoplasma gondii, AMA1, virus-like particle, serodiagnosis

P398 / #1275

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

SPECIES OF FAMILY ANISAKIDAE IN MARINE MAMMAL SPECIES FROM THE NORTH WESTERN PACIFIC OFF KOREA: PHYLOGENETIC AND PHYLOGEOGRAPHICAL ANALYSES

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Introduction: Members of family Anisakidae are well known human infecting parasites that cause anisakiasis. Thus, the majority of the researches have been focused on its infection source rather than other hosts. In particular, the information of the definitive host status of Korean seas were almost neglected. Our study aims to elucidate the infection status of anisakids in marine mammals inhabiting Korean waters, and to conduct phylogenetic and phylogeographic studies of them.

Methods: Anisakid specimens were recovered from a total of 215 carcasses of 11 marine mammal species collected from Korean seas from 2016 to 2021. They were identified by morphological and molecular analyses. Three gene markers; nuclear ITS, mitochondrial rrnS and cox2 were used for molecular identification, phylogenetic and phylogeographical analyses.

Results: The results indicated they could be classified into three species, Anisakis pegreffii, A. simplex (sensu stricto), and Pseudoterranova decipiens. They have been known as the major pathogens of anisakiasis in Korea. Mitochondrial cox2 gene marker revealed same tendency in the haplotype and nucleotide diversity with global average value. Haplotype survey showed both of geographical panmixia and genetic distance related to geographical distance.

Conclusions: This is the first implementation of comprehensive analyses on anisakids from definitive hosts in Western North Pacific. Our study provided the important information which possible to fill the gaps on the anisakids life-cycle off Korea.

Keywords: Parasite, Anisakidae, anisakis, pseudoterranova, Marine mammal

P399 / #515

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

INVESTIGATION OF SHEEP PIROPLASMOSIS IN ERZURUM PROVINCE BY MICROSCOPIC AND MOLECULAR TECHNIQUES

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Introduction: This study, it was aimed to determine the presence and prevalence of the agents causing piroplasmosis in sheep in Erzurum, also to reveal the tick species infesting the sheep, and to investigate the role of these ticks for piroplasmosis.

Methods: Sampling was conducted on randomly selected sheep from 20 districts of Erzurum province. A total of 1621 blood samples and 1696 ixodid ticks were collected. Thin blood smears were stained with %5 Giemsa and examined. Each sample was subjected to PCR for amplification of partial fragments of 18S ribosomal RNA gene of Babesia and Theileria spp with BJ1/BN2, Thei F1/R1 and Thei F2/R2 primers. Female ticks were oviposited. 115 tick pools were created. Ticks were screened for the presence of Babesia and Theileria spp with PCR. The selected PCR positive amplicons were sent to a commercial company for bidirectional sequencing.

Results: The microscopic analysis revealed 0.06% B. ovis and 2.77%Theileria spp. positivity in blood smears. A total of 307 blood samples were positive for Babesia spp. and Theileria spp. by molecular analysis. With sequence analysis B. ovis (0.41%), B. crassa (0.41%), B. canis (0.41%), T. ovis (69.26%), Theileria sp. (26.64%) and Theileria sp. OT3 (2.87%) were detected in 244 samples. Ticks were identified as D. marginatus, Hae. parva, Hae. punctata, Rh. turanicus and H. marginatum. In tick samples, T. ovis and T. annulata positivity in D. marginatus, B. crassa and T. ovis positivity in Hae. parva and T. ovis positivity in Hae. punctata were detected.

Conclusions: The presence and the prevalence of Babesia/Theileria species and genotypes in sheep and ticks of Erzurum were determined. Tick species infesting sheep were identified. The results of this study were provided up-to-date data on related diseases for the region.

Disclosure: This study is supported financially by a grant (TSA-2018-6883) from the Ataturk University Scientific Research Projects Unit.

Keywords: piroplasmosis, PCR, Erzurum, sheep, Microscopy

P400 / #1088

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

ESTIMATION OF THE EFFICACY OF THE FLOTAC BASIC TECHNIQUE

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Introduction: The FLOTAC technique is a quantitative coproscopic method for the diagnosis of parasitic infection. The aim of this study was to estimate the efficacy of this method, for the detection of common intestinal parasite eggs with respect to its accuracy, precision, recovery, sensitivity, and limit of detection and quantification.

Methods: An investigation was conducted using feces samples enriched with a known number of parasite eggs: 3, 15, 50, or 100 parasite eggs of 3 nematode genera (Toxocara, Trichuris, and Ascaris) per 1 g (EPG) of feces. In addition, 80 samples of dog feces were prepared consisting of 20 repetitions for each level of contamination. The samples were analyzed using the FLOTAC basic technique.

Results: The percentages of recovered eggs for 1 chamber and for the whole apparatus ranged from 11.67 to 21.90% and from 21.33 to 40.10%, respectively, depending on dose enrichment and genus of parasite. The limit of detection calculated for the whole FLOTAC device was 3 EPG and was 15 EPG for 1 chamber for each of the 3 parasite genera. The limit of quantification calculated for whole FLOTAC was 15 EPG for each of 3 kinds of eggs. For 1 chamber, the limit of quantification was 15 EPG for Ascaris and Toxocara eggs and 50 EPG for Trichuris eggs. Multiplication factors for calculation of the number of eggs in 1 g of feces calculated for whole FLOTAC were 3 (for Toxocara and Ascaris eggs) and 4 (for Trichuris eggs).

Conclusions: Experimentally calculated parameters of the method differ from the theoreticaly estimated parameters of the FLOTAC technique. This does not alter the fact that the FLOTAC technique is the most effective parasitological quantitative method, which can be used to detect parasitic forms in feces.

Keywords: FLOTAC, coproscopy, quantitative parasitological method

P401 / #839

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

EPIDEMIOLOGICAL AND MOLECULAR CHARACTERIZATION OF CRYPTOSPORIDIUM SPECIES OF PIGS FROM KARNATAKA, INDIA

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Introduction: Cryptosporidiosis is an emerging protozoan disease, responsible for neonatal diarrhea in vertebrate animals. It is an apicomplexan parasite distributed worldwide and composed of many species and genotypes. In pigs, infections are often asymptomatic, but may result in diarrhoea and poor growth. So far, no report on cryptosporidiosis in pigs from Karnataka state, work was taken to study the epidemiology and molecular characterization of Cryptosporidium spp. in pigs and zoonotic significance.

Methods: A total of 336 faecal samples collected from pigs of different age groups and rearing system were screened by modified cold Ziehl-Neelsen staining. The positive samples were subjected nested PCR by targeting 18s SSU rRNA gene. The sequence analysis of nPCR product and phylogenetic profile was carried out by NCBI BLAST.

Results: Among, 336 samples screened by modified Z-N method, 16 (4.76%) were positive for Cryptosporidium spp. and observed that free range pigs were most susceptible. High prevalence of cryptosporidium infection was seen in Indigenous and Yorkshire breeds of pigs between 1 to 2 months old age. Totally, 60 samples were subjected to nPCR targeting 18s SSU rRNA gene, 07 (11.6%) were positive by yielding a single amplicon of 834bp. Sequence analysis and phylogenetic profile indicated Cryptosporidium scrofarum (pig genotype-II). Pig genotype-II isolate with accession numbers OL69117, OL691173 and OL691174 were confirmed based on 100% homology with Indian isolate and 94.6 to 98.9% homology with other existing isolates.

Conclusions: The species Cryptosporidium scrofarum (pig genotype-II) identified for the first time from Karnataka state is of zoonotic significance, and hence the people who are closely associated with pigs are under higher risk of infection.

Keywords: Epidemiology, Nested PCR, Cryptosporidium Pig genotype-II, Karnataka, mZN staining

P402 / #265

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

COPROLOGICAL DIAGNOSIS OF COCCIDIA AND HELMINTH INFECTIONS IN GALLIFORMES AND RATITES, USING MINI-FLOTAC

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Introduction: Mini-FLOTAC (MF) has recently been proposed for coprological identification and quantification of avian gastrointestinal (GI) parasites, as an alternative to the traditional McMaster (McM) method. This research aimed to test the use of MF in routine diagnosis of coccidia and helminth infections in several domestic and exotic bird collections in Portugal.

Methods: Between July 2020 and April 2021, a total of 142 fecal samples from organic layers, peacocks, ostriches and emus were collected in four Portuguese bird collections. Samples were processed using MF protocol for exotic species (MF 2/38) to calculate GI parasite shedding and prevalence, and compare with the McM method.

Results: MF implementation allowed to identify an average coccidia shedding higher in peacocks from collection 2 (502 OPG), followed by peacocks from collection 1, organic layers, and peacocks from collection 3. Peacocks were also positive for Capillaria spp., Trichostrongylus tenuis and Strongyloides pavonis, whereas ostriches and emus were infected by Libyostrongylus douglassii. The MF and McM techniques did not differ significantly for each parasitic agent and bird species. Higher L. douglassii EPG was identified using the MF protocol for exotic species, followed by McM, and MF protocol for large and small animals.

Conclusions: This was the first European study to implement MF in routine diagnosis of GI parasitic infections in several bird species, and MF 2/38 is suggested as most suitable protocol for avian fecal samples. Funding: CIISA/FMV Project UIDB/00276/2020 and LA/P/0059/2020 - AL4AnimalS (both funded by FCT); Project ED431B 2021/07 (Consellería de Cultura, Educación e Universidades, Xunta de Galicia); João Lozano owns a PhD Research Fellowship 2020.09037.BD (funded by FCT).

Keywords: Exotic birds, Gastrointestinal parasites, Mini-FLOTAC, Portugal, poultry

P403 / #855

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

MOLECULAR ANALYSES OF TAENIID LARVAL CESTODES IN WILD RODENTS FROM SERBIA

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Introduction: Rodents play an important role as intermediate hosts in the transmission of many taeniid species, some of which have great zoonotic potential. Their larval stages develop in the host's body cavities or internal organs. This study aimed to investigate wild rodent populations for cystic parasitic lesions and give insights into taeniid diversity.

Methods: A total of 770 wild rodents belonging to the species Apodemus flavicollis (469), Apodemus agrarius (152), Apodemus sylvaticus (33), Myodes glareolus (51), Microtus arvalis (48), and Microtus subterraneus (17) were captured from 42 sites in Serbia, from 2013-2021, dissected, and examined for cysts and lesions. For confirmation of parasite species, DNA was extracted and mitochondrial marker 12S rDNA was amplified and sequenced.

Results: The total number of rodents that contained cysts or visible lesions were 47 (6.1%). 12S rDNA fragments amplified successfully in 13 larval samples, and three Taenia species were identified. Taenia taeniaeformis (1.03%;8/770) was dominantly present, followed by Taenia martis (0.51%;4/770), and Taenia crassiceps (0.12%;1/770). No cysts were found in A. sylvaticus and M. subterraneus.

Conclusions: We present here the first molecular identification of Taenia species in Serbia from larvae found in rodents. Since the applied mitochondrial marker did not amplify successfully in all samples, additional analyses using other genetic markers are needed, as well as further phylogenetic analyses. Acknowledgements This study was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract Nos. 451-03-68/2022-14/ 200007

Keywords: Taenia, Rodents, larval, PCR, Serbia

P404 / #1015

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

INDIVIDUAL PARASITIC FOLLOW-UP OF MEXICAN AXOLOTLS (AMBYSTOMA MEXICANUM).

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Introduction: The Mexican axolotl (Ambystoma mexicanum) is a native critical endangered amphibian, that it is bred and kept across world research labs as a model for regeneration, aging and cancer. However, little is known about its parasitic agents and their dynamic across time.

Methods: Twenty-two adult axolotls were evaluated for 14 months, from August of 2020 to September of 2021, feces were collected from water and analyzed by centrifugation/flotation technique for the diagnosis of helminth eggs and protozoan cysts/oocyst.

Results: Three parasitic agents were detected: Cosmocercoidea egg, Eimeriidae oocyst and ciliates, its overall prevalence were 68.7%, 62.5% and 81.2%, respectively. Parasite prevalence fluctuated across-time with different pattern by parasite. Eimeriidae oocyst and ciliates disappeared for some months, but Cosmocercoidea eggs were constant across the study.

Conclusions: This is the first follow-up individual parasitic study realized in Mexican axolotls. It is necessary to continue the study towards determining the effect of these parasites in axolotl health.

Keywords: axolotl, ambystoma, Eimeriidae, Cosmocercoidea, Amphibian

P405 / #522

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

DEVELOPMENT AND VALIDATION OF AN EHRLICHIA CANIS REAL-TIME PCR ASSAY

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Introduction: Ehrlichiosis is a potentially fatal zoonotic tick-borne disease, caused by a pleomorphic gram-negative bacterium. It occurs worldwide and affects humans, domestic and wild animals. Dogs infected with Ehrlichia canis develop canine monocytic ehrlichiosis (CME), a significant infectious disease of canines. Direct detection of the bacterial antigen by ELISA has been used successfully for diagnosis of CME, however, cross-reactivity of the serology assays makes it difficult to make species-specific diagnoses.

Methods: Real-time PCR assays to detect Ehrlichia spp. affecting dogs were developed and a real-time PCR assay specific for E. canis validated. The assays were designed with a set of group-specific primers that targeted a conserved region of the Ehrlichia 16S rRNA gene and species-specific TaqMan® minor groove binder probes.

Results: The efficiency of the E. canis assay was 93% and the 95% limit of detection was 33 E. canis plasmid copies/µl of blood (95% confidence interval: 23 - 58). The assay was specific for E. canis when tested against other haemoparasites. Consistent repeatability was observed, with an inter-run standard deviation (SD) range between 0.33 and 1.29 and an intra-run SD range between 0.04 and 1.14. Field samples were tested in parallel by both the E. canis real-time PCR assay and a reverse line blot hybridization assay. The results were in agreement for the two assays, with an exception of two out of 121 samples. Bayesian latent class analysis was used to calculate a diagnostic sensitivity of the E. canis real-time PCR assay of 90% and a specificity of 92%.

Conclusions: This assay is a sensitive and reliable molecular detection method for E. canis and will be a useful tool for early diagnosis that will aid with timely treatment for this haemoparasite.

Keywords: TaqMan®, Tick borne diseases, Dogs, Haemoparasite, Ehrlichiosis

P406 / #1686

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

MORPHOMETRY AND MOLECULAR IDENTIFICATION OF HAEMONCHUS COBB, 1898 (TRICHOSTRONGYLIDAE: NEMATODA) ISOLATES FROM SMALL RUMINANTS IN TANZANIA

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Introduction: The genus *Haemonchus* is the major abomasal parasite of ruminants responsible for substantial economic losses in tropical and temperate regions. This study was conducted to clarify the morphometry and molecular characterization of *Haemonchus* species isolated from sheep in Babati district, Tanzania.

Methods: A total of 486 trichostrongylid nematodes were recovered from five sheep. Of the total worms, 106 nematodes were distinguished by 37 males and 69 females. The asymmetrical length of the dorsal ray and the distance of the bulb at the apex of spicules were used for male identification.

Results: In females, the linguiform vulvar flap was most predominant with 33 out of 69 (47.8%) than knobbed morph type, which was 25/69 (36.2%), and smooth morph type with 11/69(15.9%). Partial *cox1* sequences fragments of *H. contortus* isolates showed 98.8%, 99.3%, 99.7%, 99.5%, 99.3%, and 98.4% in male, smooth, knobbed, linguiform A, linguiform B, and linguiform C respectively; with the average nucleotide divergence ranged from 1.0% to 2.4%. The amplified fragments of ITS2 genes in knobbed, linguiform A, and smooth morphotypes revealed 99.4%, 98.5%, and 98.3% respectively. Phylogenetic analysis was evaluated by employing Bayesian inference (BI) and maximum-likelihood (ML), and the tree was distinctly separated into 3 clusters focusing on *H. contortus* in cluster I within the family Haemonchidae.

Conclusions: Genetic drifting, mutation, and modification of the morphological features of the *Haemonchus* species are described to have an impact on the development of drug resistance. Species identification is necessary to understand which species infect animal hosts. We recommend more studies on the parasites intensity and the strategies for controlling *Haemonchus* species in Tanzania.

Keywords: linguiform, Haemonchus contortus, Tanzania, sheep

P407 / #830

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

MALDI-TOF MS AS A USEFUL DIAGNOSIS TOOL FOR THE IDENTIFICATION OF TRICHURIS SPECIES

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Introduction: Trichuriasis is considered a Neglected Tropical Diseases, caused by Trichuris trichiura, being the second most common nematode of humans. Detection of Trichuris in routine diagnosis is usually done by microscopic detection of eggs in fecal samples. Other molecular analyses are more reliable and could be used. Nevertheless, requires basic laboratory infrastructure with technical equipment. The matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) is a widely routinely employed technique for rapid and accurate identification of microorganisms, and more recently, arthropods and helminths. Hence, the aim of this work was to identify four new whipworm species using MALDI-TOF MS analysis and updated the internal database for Trichuris specific identification.

Methods: MALDI-TOF MS was applied to identify four Trichuris species (Trichuris sp. from Hystrix cristata, T. trichiura from Macaca sylvanus, Trichuris vulpis from Canis lupus familiaris and Trichuris ovis from Ovis aries), and to update, with the main spectra profiles obtained of each species, the internal database generated, with Trichuris suis previously created. To validate de internal database, a blind test of 63 specimens was carried out.

Results: The 100% of specimens were accurately identified, obtaining log score values (LSVs) greater than 1.70, and the percentage of samples with LSVs ≥ 2.00 was higher than 72%. To confirm the results, a dendrogram with all Trichuris species was elaborated, providing separated clades among the species.

Conclusions: The results confirmed the usefulness of MALDI-TOF MS technique as a rapid and accurate identification tool for Trichuris species and thus, for the diagnosis of trichuriasis and other Trichuris species.

Keywords: MALDI-TOF MS, Trichuris, diagnosis, identification tool

P408 / #1548

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

MOLECULAR CHARACTERIZATION OF TRICHURIS SP. FROM CAMELS (CAMELUS BACTRIANUS) FROM A ZOOLOGICAL GARDEN OF SPAIN.

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Introduction: Whipworms are widespread soil-borne helminths that can be found in a wide range of hosts and cause relevant disease and important economic losses. Nowadays, the systematics of the genus *Trichuris* Roeder, 1761 at species level is especially controversial. More than 23 *Trichuris* species have been described from ruminants. Whipworms of domestic ruminants may be found parasitizing different hosts, and *Trichuris* species from sheep or cattle have been cited in camelids. In addition, synonymies, cryptic species, different lineages, and new species of *Trichuris* have been reported.

Methods: The aim of this study was to characterize molecularly a population of *Trichuris* from *Camelus bactrianus* from a zoological garden of Spain and carry out the molecular analyses and phylogenetic relationships among the different *Trichuris* species. Two ribosomal DNA markers (ITS1 and ITS2) and three mitochondrial DNA gene (*cox*1, *co*b and *rrn*L) were analyzed. Phylogenetic trees were inferred by ML and BI methods.

Results: The phylogenetic trees showed that the population of *Trichuris* studied in this work revealed a higher similarity with *Trichuris* sp. from *C. bactrianus* from Czech Republic and related to *Trichuris skrjabini* but in two different subclades. All other *Trichuris* species sequences from camelids obtained in GenBank appeared in a different clade with *Trichuris globulosa*, *Trichuris* sp. from *Addax nosomaculatus* and *Trichuris* sp. from *C. bactrianus*, and separated from *Trichuris discolor*.

Conclusions: In conclusion, in camelids, morphological and molecular studies have reported several *Trichuris* species, suggesting a complex of *Trichuris* species parasitizing *C. bactrianus*.

Keywords: Camelus bactrianus, molecular, Trichuris, camelids, phylogeny

P409 / #1061

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

MOLECULAR CONFIRMATION OF TAENIA CRASSICEPS CYSTICERCOSIS IN A ZOO LEMUR IN POLAND

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Introduction: Taenia crassiceps is a cosmopolitan tapeworm endemic to the northern hemisphere. It has an indirect life cycle. The definitive hosts are carnivores, mainly red foxes, wolves and other canids, harboring the adult tapeworms in their small intestines. The intermediate hosts are rodents and rabbits, harboring in their body cavity, muscles or nervous system cyst-like larvae. Non-human primates in zoos appear to be highly susceptible for T. crassiceps cysticercosis, therefore they can be perceived as sentinels for the zoonotic potential of cestode parasite species. Factually, several cases of infection in zoo primates have been reported. The case of cysticercosis in the lemur described in this article was discovered during surgical operation that the animal had to undergo. In Poland, such case of cysticercosis in lemur has not been reported so far. This prompted us to conduct the research described in this paper.

Methods: The material containing cysticerci was collected, fixed in 70% ethanol, and sent to the Department of Parasitology of the NVRI in Puławy. In the laboratory cysts were microscopically examined to determine morphological features. Subsequently, the obtained material was frozen at –20 °C in individual tubes for further molecular identification and sequencing analysis.

Results: On the basis of morphological features, the cysticerci were identified as T. crassiceps metacestodes. Analysis of the sequencing results also confirmed this initial diagnosis. The phylogenetic analysis of the received seqences identified a new haplotype.

Conclusions: The received data can be used to the supplement the species description. To our knowledge, this is a first molecular confirmation of T. crassiceps metacestodes infection in zoo lemur in Poland.

Keywords: Taenia crassiceps, lemur, molecular detection, Poland

P410 / #852

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

ROUTINE PROCEDURES AT THE INTERNATIONAL TRICHINELLA REFERENCE CENTRE (ITRC) PRESERVES THE GENETIC VARIABILITY OF THE WILD STRAIN

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Introduction: The International Trichinella Reference Centre (ITRC) was created as a repository for Trichinella strains, source of material and information for international research in 1988. The maintenance of strains is ensured by in vivo-passages in mice, however, knowledge about the long-term influence over generations of infections on the genetic richness and diversity of the wild strains was missing.

Methods: We examined the effect of 25 years of in vivo passages in CD1 mice on the allele content of a Trichinella britovi (ISS107) reference strain maintained at the ITRC. Basing on single-larva microsatellite genotyping, analyses were performed by comparing: 1) larvae recovered from the oldest and the last generation 2) larvae recovered from individual mice after a single generation in one experimental infection. Genetic diversities among groups were evaluated estimating allele frequencies.

Results: The results showed a substantial similar allelic content and genetic structure from the oldest to the most recent generation, with no evidence of lost genetic variability. Otherwise, the analysis of individual mice evidenced that a genetic depletion in one generation-time, in absence of repooling of larvae after digestion, is possible and expected.

Conclusions: Although human manipulation could drive unintentional selection and regular inbreeding would lead to genetic drift, routine procedures carried out at the ITRC efficiently maintain the diversity and richness of the original genetic pool.

Keywords: Genetic variability, Trichinella, microsatellite, ITRC

P411 / #301

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

CHARACTERIZING THE EQUINE NEMABIOME AND DETECTING PARASITE-SPECIFIC ANTHELMINTIC RESISTANCE IN DOMESTIC HORSES

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Introduction: Drug resistance is increasing in many equine parasites, including small strongyles. Current diagnostic methods detect and quantify parasite eggs in fecal samples but can't differentiate between the 53 strongyles species infecting horses with nearly identical eggs. We will use DNA metabarcoding to characterize the nemabiome of domestic horses in Saskatchewan, Canada.

Methods: Fecal samples were collected from horses in fall with fecal egg counts (FECs) done before and after owner-administered treatment. Online surveys were sent to owners to create a database of horses' management, demographics, and deworming history. Third-stage larvae (L3s) were cultured from samples with>300 strongyle eggs per gram (EPG). The larval DNA will be extracted, sequenced, and compared to an equine GI nematode database and taxonomically classified.

Results: 107 horses have had FECs done and L3s cultured from 42 pre- and 4 post-treatment samples with observed lack of efficacy. Mean pre-treatment FEC for adult horses (age>3) was 707 EPG. Counts ranged from 0 to 4775 EPG. FEC counts differed in age groups; mean FEC was higher in young (0-3 year olds, $p = .003$; 4 – 7 year olds, $p = .01$) and old animals (> 20 years, $p = .04$). Nemabiome results will be presented with small strongyles expected to dominate. Early survey data shows 51% of owners use an ivermectin based dewormer, while 39% are unsure of the drug used (N=49).

Conclusions: This work characterizes the GI nemabiome of domestic horses to species level, demonstrates high strongyle FEC in adult horses (mean>500 EPG), and shows lack of efficacy of owner administered treatment in at least 4 horses. It also provides information about current deworming practices of horse owners and veterinarians in western Canada.

Keywords: Parasite, diagnostics, molecular, sequencing

P412 / #1032

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

SEROLOGICAL AND MOLECULAR DETECTION OF TOXOPLASMA GONDII IN ABORTED FETUSES OF SHEEP AND CATTLE IN BOJNURD CITY, NORTHEAST OF IRAN

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Introduction: Toxoplasma gondii is a prevalent protozoan parasite among all mammals, especially in livestock. It can lead to abortion in pregnant animals and has a considerable economic impact on traditional husbandry. The present study was conducted to determine the prevalence of T. gondii in aborted sheep and cattle fetuses in Bojnurd, Northeast of Iran.

Methods: This cross-sectional study was performed from 2020 to 2021 on 68 spontaneously aborted fetuses. The thoracic fluid and brain tissues from sheep (N= 52) and cattle (N=16) were considered as examined samples. Thoracic fluid samples were tested for anti-T. gondii IgG antibody by enzyme immunoassay. At the next step, IgG-positive samples were tested for the presence of Toxoplasma DNA by nested PCR method targeting the B1 gene and a positive sample yield of 197 bp amplified DNA products consistent with T. gondii.

Results: Using the serological method, anti-Toxoplasma IgG antibodies were detected in 11 (16.2%) out of 68 sera from the thoracic fluid of aborted fetus. The nested PCR method detected T. gondii DNA in the brain tissue samples of 2 seropositive cases of sheep.

Conclusions: Our finding revealed the presence of T. gondii DNA in tissues of animal husbandry in Bojnurd. Since meat animal and their product represent the direct source of infection for humans, this epidemiological data could help healthcare services to reduce the monetary burden of infection in humans and also in the livestock industry.

Keywords: Aborted fetus, molecular, Toxoplasma gondii, Bojnurd, Serology

P413 / #1101

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

GENETIC VARIABILITY OF ECHINOCOCCUS GRANULOSUS SENSU LATO AND ECHINOCOCCUS MULTILOCULARIS IN EASTERN EUROPE INFERRED FROM MITOCHONDRIAL SEQUENCE DATA.

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Introduction: The study was undertaken to broaden knowledge about the species/genotype spectrum of Echinococcus granulosus sensu lato and E. multilocularis tapeworms in eastern Europe to better understand their epidemiology and ways of spread.

Methods: Mitochondrial genes cox1, nad1, 12S rRNA and atp6, depending on the causative agents surveyed, were amplified and sequenced.

Results: In patients from southwestern Romania (Caras-Severin county), genotypes G1 and G3 of E. granulosus sensu stricto were recorded. The finding of E. granulosus s.s. G1 in cattle from eastern Ukraine (Dnipro region) followed up on its recent records in the two pigs in the Kyiv region. In four pigs from the Sumy region, E. canadensis G7 was identified. In reindeer from the herding farm in the Nenets Autonomous Okrug (European part of Russia), E. canadensis G6/7 was detected. The reindeer came from area near the Pechora river, where reindeer herds graze. In 11 E. multilocularis isolates from 4 administrative areas of the European part of Russia (Nizhny Novgorod, Ryazan, Vladimir, Tver) from red fox, raccoon dog, domestic dog and wolf hosts, 5 isolates gave patterns of Asian E. multilocularis haplotypes (sensu Nakao et al., 2010), 4 isolates clustered with European haplotypes and in 2 isolates a mixture of Asian and European haplotypes was recorded.

Conclusions: The findings of virulent E. granulosus s.s. is important for the epidemiology of cystic echinococcosis in Ukraine, where almost exclusively less virulent E. canadensis was found so far. The distribution of E. multilocularis haplotypes suggests that the frequency of the Asian variants in the European part of Russia is more pronounced than previously thought, also due to the paucity of genetic data from this area. The study was supported by VEGA grant 2/0157/22.

Keywords: Echinococcus, genotype, Eastern Europe

P414 / #1706

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

ASCARIS POPULATIONS FROM PIGS AND HUMANS IN SLOVAKIA: GENETIC STRUCTURING AND TRANSMISSION PATTERNS.

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Introduction: Although Slovakia is globally considered an area with low endemicity to human ascariasis, there are several foci of hyperendemicity, mostly linked to Roma settlements. The study was designed to examine the gene diversity of *Ascaris* from Slovakia in relation to the hosts and to consider whether infected pigs represent a significant reservoir for human infection.

Methods: The segments of cox1 of mitochondrial DNA (384 bp) and ITS1 of ribosomal DNA (312 bp) were examined in 59 pig isolates and 29 human isolates.

Results: The ITS1 analysis showed that 81.3% (48/59) pigs from Slovakia were infected with Ascaris suum (AS), 10.2% (6/59) pigs with human Ascaris lumbricoides (AL) and in 8.5% (5/59) of pig, hybrid patterns bearing the characteristics of AL and AS were detected. The increased occurrence of cross-infections and introgressive cases in pigs from several localities in eastern Slovakia was related to the proximity of Roma settlements and is indicative of significant environmental contamination by Ascaris eggs. All 29 patients were found to be infected with ´human´ AL parasites. Using cox1 sequencing, 8 isolates were grouped into cluster A, 11 isolates into cluster B and most isolates (40) were linked to cluster C. AL worms predominated in cluster A, while AS worms predominated in clusters B and C.

Conclusions: Comparison of species identification by ITS1-RFLP and distribution to clusters by cox1 sequences in the same isolates showed that cluster A was diagnostic at 88.9% for AL, cluster B at 87.8% for AS and cluster C at 82.5% for AS, for Slovak territory. The obtained data indicate the Ascaris circulation in Slovakia through human-human and pig-pig transmissions and in some hyperendemic foci also through human-pig transmission. The study was supported by VEGA grant 2/0157/22.

Keywords: cox1, ITS, Genotyping, transmission, Ascaris

P415 / #1575

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

PROFILING MICRORNAS FROM THE EQUINE BLOODWORM (STRONGYLUS VULGARIS)

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Introduction: The migrating larval stages of the equine bloodworm (*Strongylus vulgaris)* cause injury to the intestinal arteries, in some cases leading to intestinal necrosis and death of the horse. The available diagnostic options are not effective during the pre-patent phase, and biomarkers of pre-patent infection are much needed. Studies have shown that parasite-derived microRNAs (miRNAs) can be detected in blood samples from animals and humans with parasitic infections. The aims of this project were 2-fold: 1) Explore miRNAs excreted by *S. vulgaris* and 2) Investigate the presence of *S. vulgaris* miRNAs in blood samples from infected horses.

Methods: During post-mortem examination of seven naturally infected foals, live 4th and 5th stage larvae were collected from the Cranial Mesenteric Artery. Larvae were snap-frozen in liquid nitrogen or incubated for 72 hours for collection of excreted miRNAs. Citrate stabilized blood was collected from the foals before euthanasia. RNA was extracted from larvae, media, and plasma using miRNeasy kits, and small RNA sequencing was performed using Illumina technology. Bioinformatics analysis was conducted to identify nematode-specific miRNAs.

Results: Nematode miRNAs were identified in larvae (48 miRNAs), incubation media (26 miRNAs), and plasma (5 miRNAs).

Conclusions: Compared to other nematodes, the 48 miRNAs found in the larval samples were significantly fewer than expected, which could be explained by degradation of the samples. This study demonstrated that nematode miRNAs are detectable in plasma samples from infected horses, indicating a diagnostic potential, which should be investigated further in future studies.

Keywords: Horse, colic, Non-strangulating intestinal infarctions, diagnostics, Peritonitis

P416 / #429

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

DIGENEANS OF THE GENUS MESOCOELIUM ODHNER, 1910 IN JAPANESE AMPHIBIANS

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Introduction: The genus Mesocoelium Odhner, 1910 (Digenea: Mesocoeliidae) is one of the most complex taxa in trematodes. In Japan, a single species (M. brevicaecum Ochi in Goto and Ozaki, 1929) of this genus is hitherto recognized from amphibians, and the other species (M. elongatum, M. japonicum, M. lanceatum, M. minutum, M. ovatum, M. pearsei) are regarded as synonyms of M. brevicaecum (Freitas, 1963). The present study aims to elucidate the validity of this species parasitizing various amphibians in Japan.

Methods: Flukes were collected from the digestive tract of hynobiid salamanders, salamandrid newts, and toads. Fixed flukes were examined using morphological and molecular approaches.

Results: All specimens studied were morphologically identified as M. brevicaecum. There was no significant morphological variation in all of the examined specimens. The specimens recovered from Caudata were molecularly identical in 28S rDNA regardless of the locality and host species. The specimens recovered from Anura had a sequence slightly different from that of Caudata in 28S rDNA (p-distance 0.002).

Conclusions: Mesocoelium parasitizing Japanese amphibians could be morphologically regarded as M. brevicaecum. However, the genotypes slightly differ depending on host taxa, Caudata or Anura, suggesting host segregation between different lineages of this species. More data would be needed to clarify if this genetic difference is within the range of intraspecific variation or not.

Keywords: Amphibian, Taxonomy, Platyhelminth

P417 / #620

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

DIVERSITY OF HAEMOGREGARINE SPECIES (APICOMPLEXA: ADELEORINA) INFECTING BRAZILIAN AQUATIC TURLES.

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Introduction: Hemoparasites of the genus Haemogregarina (Adeleorina: Heaemogregarinidae) are commonly reported infecting aquatic chelonians. In Brazil, only three species are recognized using both molecular and morphological tools. Thus, this study aimed to bring new insights on Haemogregarina species diversity infecting Brazilian chelonids from Mato Grosso and Goiás states.

Methods: In 2017 and 2019, eleven aquatic turtles Podocnemis unifilis (n = 10) and Podocnemis expansa (n =1) were screened, and Haemogregarina was identified in the blood smears and histological slides of tissue samples (liver) through morphological analysis. The PCR was executed using two pairs of primers targeting the 18S gene, of the eleven tutles.

Results: Of the total, ten have shown similar developmental stages, with the presence of trophozoites, meronts, microgamonts, and macrogamonts in blood smears, and liver merogony with meronts and one cyst with cystozoites. Only in one P. unifilis turtle, the haemogregarine did not present the same morphology, with trophozoites, microgamonts, and two shapes of macrogamonts observed in blood smear. Moreover, the molecular tool identified two different species of Haemogregarina. Of the eleven sequences analysed, ten isolates were considered identical to Haemogregarina embaubali (100% of similarity); and one isolate was different from all known species and sequences deposited in Genbank (98% of similarity), indicating a putative new species of haemogregarine.

Conclusions: Therefore, this study increases the knowledge of Brazilian chelonids blood parasites and demonstrates the importance of using integrative approaches for the hemoparasites diagnosis, with the identification of an undescribed species [FAPESP 2018/00754-9; 2018/09623-4].

Keywords: Haemogregarina, turlte, Brazil, 18S, Diversity

P418 / #791

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

IDENTIFICATION OF IMMUNE-RELATED GENES IN THE FAT BODY OF TICK IXODES RICINUS

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Introduction: The tick Ixodes ricinus is the most serious vector of tick-borne pathogens in Europe. The transmission of pathogens depends on their ability to evade or inhibit tick immune response. A crucial role in invertebrate immunity is played by antimicrobial peptides that are mainly produced by the fat body or hemocytes. The tick fat body is a diffuse organ associated with the tracheal trunks or surrounding other internal organs. Despite its importance, the physiological function of the tick fat body is fairly unexplored.

Methods: To overcome the knowledge gap, we performed RNAseq analysis of the fat body/trachea complex dissected from partially fed adult I. ricinus females (NCBI Bioproject PRJNA748353). The obtained transcriptomic data were complemented by the proteomic analysis of proteins secreted to the tick hemolymph.

Results: In total, we identified about 45.000 transcripts out of which about 1% of contigs were qualified as immune-related genes. About 20 abundantly expressed transcripts representing different classes of immune molecules were selected and examined in the response to microbial immune challenge at different time points. The highest immune response was recorded after Escherichia coli Gram (-) bacterial infection in 13 from 20 selected genes. Only 4 genes were significantly upregulated after Micrococcus luteus Gram (+) bacterial challenge and 4 genes after the yeast Candida albicans challenge. The expression of 7 immune genes was not affected by any microbe.

Conclusions: These data may aid in better understanding the immune processes and their regulation in ticks and their role in the interaction between the ticks and pathogens they transmit.
Acknowledgment: This work was supported by the Grant Agency of the Czech Republic (grants No. 20-05736S to P.K.).

Keywords: tick, Transcriptome, fat body, immunity

P419 / #1615

Topic: *AS03.1 Diagnostics, phylogeny and genomics*

LUCIFERASE-LINKED ANTIBODY CAPTURE ASSAY (LACA) FOR THE SERODIAGNOSIS OF TOXOPLASMA GONDII INFECTION IN PIGS

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Introduction: Toxoplasmosis is a widespread protozoan zoonosis. Since ingesting undercooked meat harboring *Toxoplasma gondii* cysts is considered one of the major transmission routes to humans, the screening of *T. gondii* infection in meat-producing animals can reduce the risk of food-borne toxoplasmosis in humans. Serological tests are recognized as the most effective approach for the diagnosis of toxoplasmosis in those animals. In this study, we aimed to develop a novel Luciferase-linked Antibody Capture Assay (LACA) for the serodiagnosis of *Toxoplasma* infection in pigs.

Methods: Recombinant nanoluciferase fused-*T. gondii* antigens (rNluc-GRA6, rNluc-GRA7and rNluc-GRA8) were applied to the assay and examined with the sera from uninfected pigs and experimentally infected pigs with *T. gondii* and other parasites. Ninety-nine pig sera, collected from growing-finishing pigs aged over 6 months at two slaughterhouses in Japan from June to July 2014, were also applied in this study. The Sabin-Feldman dye-test was performed as a reference test, and the cut-off > 4 IU was defined as a positive for the test.

Results: The sensitivity of GRA6-, GRA7- and GRA8-LACA were 70.0%, 80.0% and 80.0% with specificity 87.0%, 81.5% and 74.1%, respectively. Then, a mixture of rNluc-GRA6, rNluc-GRA7 and rNluc-GRA8 were tested as a cocktail antigen. The cocktail LACA indicated higher sensitivity (90.0%) and a similar specificity (96.3%) in comparison with the commercial ELISA kit. Compared to the Sabin-Feldman dye-test, the cocktail LACA showed strong agreement (kappa value = 0.811) when pig sera collected at the slaughterhouses were assessed.

Conclusions: The developed cocktail LACA seems to be a promising platform for diagnosis of *T. gondii* infection in pigs with high sensitivity and specificity.

Keywords: Luciferase-linked Antibody Capture Assay (LACA), Toxoplasma gondii, pigs, serodiagnosis

P420 / #1348

Topic: *AS03.2 Epidemiology, pathology and impact*

PROGESTERONE INDUCES AN INCREASE IN WORM BURDEN AND EGG SHEDDING OF HAEMONCHUS CONTORTUS IN MALE ORCHIECTOMIZED LAMBS.

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Introduction: The increase in serum prolactin has been related to the increase in Haemonchus contortus egg shedding that occurs in sheep around parturition (peripartum rise). Progesterone also increases during pregnancy, however, its role in the peripartum rise has not been studied. The aim of this study was to evaluate the effect of hyperprogesteronemia on the worm burden in H. contortus infection.

Methods: Ten orchiectomized male lambs were divided into two groups (n=5). At week -2, experimental progesterone group lambs (EPGL) received two subcutaneous progesterone implants, control group lambs (CGL) received only SSF. At week 0, all lambs were infected with 5000 L3 of H. contortus. Serum progesterone and fecal egg count (FEC) were measured weekly and at week 7, adult worms in the abomasum were counted.

Results: EPGL showed an elevation ($p<0.05$) of serum progesterone between weeks -1 and 2 observing the maximum peak at the time of infection (week 0). The EPGL showed higher FEC's ($p<0.05$) than the CGL at week 7 In GPG, the total adult worms (1524 ± 204) and the number of females (770±80) in the abomasum was higher ($p<0.05$) than in the CGL (990±200 and 450±120 respectively). The correlation between progesterone levels and FEC was 0.92 ($p<0.001$).

Conclusions: The results sugest that progesterone increases the worm burden in abomasum and the shedding of eggs in the feces and that it is involved in the peripartum rise in sheep. Funded by PAPIIT-UNAM IN210322 and IN211222.

Keywords: progesterone, peripartum rise, hyperprogesteronemia, Haemonchus contortus

P421 / #284

Topic: *AS03.2 Epidemiology, pathology and impact*

HYDATID CYST OF RARE LOCALIZATIONS: ABOUT 19 CASES

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Introduction: The diagnosis of erratic localizations of the hydatid cyst (HC) is difficult because they are rare and often clinically asymptomatic. The objective of the study is to determine the circumstances of the diagnosis and the therapeutic and evolutionary modalities of the cases.

Methods: We report 19 cases of patients treated and followed for HC with erratic locations between 2018 and 2021. All patients underwent imaging (ultrasound and MRI), serology (haemaglutination and ELISA and biopsy with excision of the piece and its histological analysis

Results: Mean age 39 years (E: 2-81 years), male predominance (sex-ratio: 2.88). Rural origin of patients with established contact with animals. Insidious symptomatology with onset of variable symptomatology depending on location: bone (n=7) dominated by spinal involvement dorso-lumbar (4), muscular (n=6), cerebral (n=5) and ophthalmic in a 2-year-old child (n=1). MRI confirmed organ involvement and serology is strongly positive in all cases. The diagnosis is certain intraoperatively and by histology of the excised specimen. The patients received albendazole, in 3 courses, of 400mg/day for 14 days, spaced out by a 28-day break. Good drug tolerance. The radio-clinical and serological evolution is favorable over 12 months of follow-up. Rare neurological and musculoskeletal sequelae.

Conclusions: Early diagnosis of erratic locations of echinococcosis allows surgical treatment associated with albendazol to avoid the risk of recurrence.

Keywords: hydatid cyst, rare localizations, treatment,

P422 / #759

Topic: *AS03.2 Epidemiology, pathology and impact*

MORPHO-MOLECULAR IDENTIFICATION OF PSOROPTES CUNICULI INFESTATION IN NATIVE (BALADI) AND GABALI RABBIT BREED IN EGYPT

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Introduction: Ear mange caused by Psoroptes cuniculi (Acari: Psoroptidae, Delafond, 1859) is a common parasitic disease in all rabbit breeders worldwide. In Egypt, mange infestation (Psoroptes and Sarcoptes) have been found in large numbers at rabbit farms. However, few studies on the molecular identification of mange mites, particularly Psoroptes spp., have been conducted in Egypt. This research aimed to determine how common Psoroptes mites are in Native (Baladi) and Gabali rabbit breed in Egypt.

Methods: In Egypt's Cairo and Monufia governorate, 1665 Native (Baladi) and Gabali rabbit breeds were investigated for mange infestation raised at rabbit farms. Most lesions were identified on the ear, nose, and face's edges. The collected mites were examined by light and scanning electron microscope and confirmed by DNA sequence analysis of the cytochrome oxidase subunit I (COX1) gene.

Results: Overall 19% of had mange, whereby (2.11%) in summer and (34.92%) in winter. The majority of lesions were identified on the ear, nose, and face's edges. Hair loss, thickening of the skin, irregular dried dirty encrusted scabs with erythema, and facial and ear deformity were all signs. The mite was identified as Psoroptes cuniculi after being examined under a microscope and SEM. The average adult female size is ~550 µm (400–808 µm) × ~420 µm (270–505µm), and the male adult size is ~550 µm (545–606 µm) × ~380 µm (384–404 µm).

Conclusions: This is the first molecular report of the presence of P. cuniculi in rabbits in Egypt. An extensive survey should be conducted to investigate the prevalence of P. cuniculi in Native (Baladi) and Gabali rabbit breeds and other rabbits breeds in Egypt.

Keywords: Psoroptes cuniculi, Egypt, Rabbit, Mange

P423 / #1626

Topic: *AS03.2 Epidemiology, pathology and impact*

CRYPTOSPORIDIOSIS IN YOUNG CALVES IN FRANCE: SCIENTIFIC AND PRACTICAL ASPECTS

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Introduction: Nowadays, animal health is more than ever a burning issue and overlap with human health is well recognized. In France, 10 to 20% of calves do not reach the age of 6 months. Infectious diarrheal diseases are the main causes of death in young animals. Many questions remain unanswered. For example, the proportion of cryptosporidiosis linked to symptomatology or the mode of contamination of young animals.

Methods: In this context, the HealthyCalf project financed by APIS-GENE was developped to try to answer to these questions. Overall, data were collected regarding 836 calves (Holstein + Charolais) over a period of 4 years. Numerous health and epidemiological data were collected such as the age of the animals at the time of the microbiological analyses, their sex, the infectious status of their mothers, their symptoms, the performance of curative or preventive treatments. The performances of two diagnostic tests were evaluated for the detection of *Cryptosporidium*.

Results: The prevalence of *Cryptosporidium* reached 77% in symptomatic animals, far ahead the ones of Rotavirus, *E. coli* and *Coronavirus*. Among animals for whom DNA of *Cryptosporidium* was detected, only 42% were symptomatic. Speciation and genotyping analyzes demonstrated that calves were not contaminated by same genotypes than their mother. Contamination from the environment seems the most probable origin. Practically, the sensitivity of quick diagnostic test (Speed V-Diar 4®) was better when animals were symptomatic. Vaccination of cows against enteric viruses and bacteria were few effective.

Conclusions: To our knowledge, this work represents the most complete field study carried out to date on cryptosporidiosis French farms. Concrete applications can be drawn from the obtained results.

Keywords: Cryptosporidium, calves, Prevalence

P424 / #669

Topic: *AS03.2 Epidemiology, pathology and impact*

MOLECULAR IDENTIFICATION OF EIMERIA IN SEVERAL BAT SPECIES IN SPAIN

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Introduction: Species of the genus Eimeria are ubiquitous in vertebrate hosts and represent some of the most prevalent parasites known. However, Eimeria is less common in Chiroptera than in other mammalian orders. This is the first study reporting the detection and molecular characterization of Eimeria in bats in Spain, specifically in 12 of the 32 chiropteran species described in the Iberian Peninsula.

Methods: A total of 76 faecal samples were collected from different bat roosting sites across Spanish territory. The DNA was extracted from the samples and sequenced by targeting the Eimeria SSU-rRNA gene.

Results: Eimeria spp. were detected in 38.2% (29/76) of the samples, and the bat-specific Eimeria rioarribaensis and rodent-specific Eimeria jerfinica were detected in 4 (5.3%) and 25 (32.9%) of the faecal samples, respectively.

Conclusions: This is the first report of E. rioarribaensis in the bats Rhinolophus euryale, Myotis myotis and Nyctalus lasiopterus, extending the host and geographical ranges for this bat coccidian parasite. The identification of rodent-species E. jerfinica in bats indicates the presence of this species in wild rodents in Spain, although their presence has not previously been reported in this country.

Disclosure: The study was funded by the Autonomous Government of Galicia (grant ED431C 2021/26). SC-P is granted by the Programme for the requalification, international mobility and attraction of talent in the Spanish university system, modality Margarita Salas.

Keywords: *Eimeria*, Chiroptera, Molecular characterization, Spain

P425 / #715

Topic: *AS03.2 Epidemiology, pathology and impact*

FIRST REPORT OF EIMERIA MYOXI IN THE GARDEN DORMOUSE (ELIOMYS QUERCINUS LINNAEUS, 1766) IN SPAIN

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Introduction: More than 400 Eimeria species have been described in rodents. However, data on this protozoan parasite in members of the dormouse family, Gliridae, are very scarce. This work reports for the first time the presence and molecular characterization of Eimeria myoxi in the garden dormouse (Eliomys quercinus) from the Doñana Natural Area (SW Spain).

Methods: Fresh faecal samples were collected from a total of 28 garden dormice, which were caught following current guidelines for the ethical use of animals in research. A standard flotation technique with saturated saline solution for microscopical examination and molecular characterization of the SSU-rRNA gene of Eimeria were performed.

Results: Microscopic examination revealed the presence of Eimeria oocysts in 16 of the 28 (57.1%) faecal samples, which were morphologically compatible with E. myoxi. By molecular analysis of the SSU-rRNA gene, E. myoxi was identified in 22 of the 28 (78.6%) dormice, including 15 samples in which the oocysts were observed.

Conclusions: The present study contributes to current knowledge about Eimeria in rodent species, identifying for the first time E. myoxi in the garden dormouse (E. quercinus) in Spain and reporting high prevalence values.

Disclosure: The study was funded by the Autonomous Government of Galicia (grant ED431C 2021/26). SC-P is granted by the Programme for the requalification, international mobility, and attraction of talent in the Spanish university system, modality Margarita Salas.

Keywords: *Eimeria*, Molecular characterization, *Eliomys quercinus*, Spain

P426 / #807

Topic: *AS03.2 Epidemiology, pathology and impact*

PREVALENCE OF TRITRICHOMONAS FOETUS INFECTION IN ANIMAL HOSTS IN POLAND.

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Introduction: Tritrichomonas foetus is an intriguing protozoan parasite that is pathogenic in cattle and cats and is a commensal organism of pigs. T. foetus is the causative agent of the bovine veneral disease called tritrichomonosis. In Poland, no cases of tritrichomonosis have been found since 1997. However, tritrichomonosis in cattle is a notifiable disease on the OIE list. Feline tritrichomonosis has a worldwide occurrence and T. foetus mainly causes large bowel diarrhea. T. foetus was also identified in the nasal cavity, stomach, and intestines of pigs as a commensal. There is no obligation to examine and report porcine and feline tritrichomonosis. In the present study we report the prevalence of T. foetus in animal hosts in Poland.

Methods: A total of 117 freshly voided feline fecal samples from cats, 172 swabs from the nasal cavities of pigs, and 180 bovine specimens from the area of Poland were collected for the study. All samples were examined by our own method LAMP (Dabrowska, 2019). The statistical analysis was performed using Statistica v10 (StatSoft Inc., Tulsa, OK, USA).

Results: The prevalence of feline tritrichomonosis was 20.51%, and statistically significant differences were obtained between groups of animals regarding age, breed, the number of cats, diarrhea, and place of living. Positive LAMP results were estimated for 16.28% of pigs, and the data were significantly correlated with age. Furthermore, no cases of bovine tritrichomonosis were found.

Conclusions: In conclusion, our survey demonstrates the presence of T. foetus in cat and pig populations from Poland. Therefore, despite Poland being considered free of tritrichomonosis, the occurrence of parasites in pigs may increase the risk of T. foetus transmission to cattle.

Keywords: Cattle, pigs, Tritrichomonas foetus, Prevalence, cats

P427 / #1702

Topic: *AS03.2 Epidemiology, pathology and impact*

ISOENZYMATIC TYPING OF LEISHMANIA STRAINS ISOLATED FROM VECTORS AND RESERVOIRS IMPLIED IN THE LEISHMANIASIS OUTBREAK OF THE MADRID REGION (SPAIN)

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Introduction: The epidemiological cycle of leishmaniasis in Spain includes *Leishmania infantum*, the dog (principal reservoir) and *Phlebotomus perniciosus* and *P. ariasi* (proven vectors). However, in 2009, a large outbreak was declared in Madrid region, as consequence of environmental changes in the area. Among other particularities, hares and rabbits acted as reservoirs and *P. perniciosus* as vector. Studies on human isolates of the foci showed the presence of *L. infantum* genotype Lombardi.

Methods: Nineteen strains isolated from *P. perniciosus* (11), hares (5) and rabbits (3) from the outbreak area in Madrid were characterized using Multilocus Enzyme Electrophoresis and *hsp70* sequencing. Strains had been previously typed by ITS as Lombardi.

Results: revealed the presence of four different zymodemes due to different mobilities of the NP1 enzyme: MON-34 (NP^{100}, n=11), MON-80 (NP^{130}, n=6), MON-24 (NP^{140}, n=1) and another strain (n=1) with NP^{150} representing a new zymodeme. All zymodemes are quite common in Spain and the Mediterranean basin. For the first time both MON-34 and MON-80 were found in *P. pernicious*, hares and rabbits. Molecular typing showed a unique genotype.

Conclusions: Our results showed the existence of four zymodemes of *L. infantum*, one representing a new zymodeme. Therefore, a more comprehensive study, that includes a higher number of strains from vectors, reservoirs and humans, is needed in order to obtain a complete understanding of the outbreak circulating strains.

Keywords: Leishmania, MLEE, outbreak, Spain

P428 / #493

Topic: *AS03.2 Epidemiology, pathology and impact*

GLOBAL PREVALENCE OF TOXOPLASMA GONDII IN BIRDS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Introduction: Toxoplasma gondii is a zoonotic parasite that can cause severe health problems especially in high-risk groups, including immunosuppressed people. Among the potential animal reservoirs of T. gondii, birds are interesting due to their dispersion in different environments. So, this systematic review and meta-analysis aimed to assess the global status of T. gondii prevalence in different birds.

Methods: The standard protocol of preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines were followed. Scopus, PubMed, Web of Science, and Google Scholar were searched from 1 January 2000 to 30 December 2021. All peer-reviewed original research articles describing the prevalence of T. gondii infection in birds were considered. Inclusion and exclusion criteria were applied. The point estimates and 95% confidence intervals were calculated using a random-effects model. The variance between studies (heterogeneity) was quantified by I^2 index.

Results: Finally, 197 articles (including 248 datasets) were eligible for inclusion in the systematic review and meta-analysis.The highest prevalence of T. gondii was found in goshawks, 98.3% (77.7–99.9). Considering the diagnostic methods, the highest prevalence of T. gondii was found by real-time PCR methods 89.6% (95% CI: 77.3–95.6%).

Conclusions: The results highlight that birds should be remembered as a relevant host for the zoonotic parasite T. gondii.

Keywords: Toxoplasma gondii, Birds, Prevalence, Meta-analysis

P429 / #1591

Topic: *AS03.2 Epidemiology, pathology and impact*

CLINICAL BOVINE BABESIOSIS IN THE DANUBE DELTA AREA (ROMANIA): A CASE SERIES REPORT

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Introduction: Bovine babesiosis is a severe tick-borne disease caused by intra-erythrocytic apicomplexan protozoan (Piroplasmida: Babesiidae) that affect animals' health and their productivity. The aim of this paper is to provide new insights on clinical bovine babesiosis in South-eastern Romania.

Methods: During of July-August 2019, a case series of clinical bovine babesiosis were registered in two premises in an endemic area in the Danube Delta (Romania). The cattle, aged between 6 month and 6.5 years, four females (of which one pregnant) and one male, displaying clinical signs compatible for babesiosis were subjected for clinical examination and laboratory investigations. Blood samples were collected for the cattle and tested for the presence of piroplasms using the blood smear Giemsa stained method. Main haematological biochemical and parameters were also determined.

Results: The clinical symptoms of the affected cattle included depression, anorexia, fever (40.5°C-41.5°C), weakness, pale mucous membranes, haemoglobinuria, and icterus. Two animals showed moderate disease, while three had severe disease, of which the pregnant female aborted. In all affected animals the hematological examination revealed marked anemia, trombocytopaenia, lymphopaenia, and elevated values of liver enzymes. The microscopic examination of the blood smears revealed small (1.0–2.5 µm) intraerythrocyte parasites consistent with *Babesia bovis* in all six clinically affected cattle. All animals were successfully treated with diminazen aceturate (3.5 mg/kg b.w., IM).

Conclusions: Our results provide new insights on the clinico-pathological aspects of bovine babesiosis which must be taken in consideration for parasitological control programs to be implemented in tick-endemic areas.

Keywords: Pathology, clinical outbreak, babesiosis, Cattle, Danube Delta (Romania)

P430 / #822

Topic: *AS03.2 Epidemiology, pathology and impact*

MOLECULAR DETECTION AND CHARACTERIZATION OF CRYPTOSPORIDIUM SPP. IN WILD LAGOMORPHS FROM SOUTHERN SPAIN: A PRELIMINARY STUDY

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Introduction: Lagomorphs, including European rabbit (Oryctolagus cuniculus) and hares (Lepus granatensis, L. europaeus and L. castroviejoi) are key wild species present in the Iberian Peninsula with ecological and public health significance since they have been confirmed as hosts of zoonotic pathogens. Limited information is currently available on the epidemiology and zoonotic potential of Cryptosporidium spp. infection in wild lagomorphs in Spain.

Methods: To bridge this gap of knowledge, a total of 277 faecal samples were collected from European wild rabbits (n = 236) and hares (Lepus granatensis) (n = 41) from Andalusia, southern Spain during the period 2018 to 2020.

Results: The presence of Cryptosporidium spp. was assessed using a nested-PCR protocol to amplify a 587 bp fragment of the ssu rRNA gene. Cryptosporidium spp. DNA was detected in 2.5% (7/277) of the faecal samples from lagomorphs analysed. Sequence analyses of the Cryptosporidium-positive samples by ssu-PCR revealed the presence of C. cuniculus (2.2%, 6/277) and C. andersoni (0.4%, 1/277). Cryptosporidium andersoni infects mainly cattle but has also been described in rodents and humans. All C. cuniculus showed 100% identity with GenBank reference sequences. Three samples were successfully subtyped using nested PCR analysis of the 60-kDa glycoprotein (gp60) gene, revealing the presence of subtype VaA18.

Conclusions: This study confirms the occurrence of C. cuniculus and C. andersoni in wild lagomorphs from southern Spain, providing new information on the geographical distribution and genetic diversity of these zoonotic parasites. Further studies are required to better understand the epidemiology of Cryptosporidium spp. in wild lagomorphs and their possible public health repercussions.

Keywords: Wild lagomorphs, Cryptosporidium cuniculus, Cryptosporidium andersoni, Molecular characterization

P431 / #834

Topic: *AS03.2 Epidemiology, pathology and impact*

PREVALENCE AND GENOTYPING OF ZOONOTIC MICRO-EUKARYOTIC PARASITES IN WILD UNGULATES IN SPAIN.

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Introduction: Wild ungulates may act as suitable hosts for a wide range of microeukaryotic parasitic species. Molecular epidemiological studies assessing the frequency and molecular diversity of microeukaryotic parasitic in wildlife are scarce. The aims of this cross-sectional epidemiological study were to determine the diversity, frequency and molecular characterizartion of microeukaryotic parasitic in wild ungulates in Spain.

Methods: Faecal samples were collected from eight wild ungulate species as well as from semi-extensively farmed red deer. Microeukaryotic parasitic occurrence was investigated by molecular methods

Results: Giardia duodenalis was the most prevalent found in two chamois species [Rupicapra rupicapra, 14.3%, 3/31; R. pyrenaica, 12.2%, 5/41), followed by mouflon (10.0%, 1/10), Iberian wild goat (9.0%, 8/89), roe deer (7.7%, 7/91), wild boar (5.6%, 20/359), fallow deer (5.2%, 5/96), and red deer (3.8%, 24/651). Cryptosporidium spp. was more prevalently detected in roe deer (7.8%, 7/91), wild boar (6.7%, 24/359), and red deer (1.7%, 11/651). Enterocytozoon bieneusi was found in red deer (10.7%, 70/651) and wild boar (0.8%, 3/352). Balantioides coli was detected in wild boar (2.9%, 9/312). Six species of Cryptosporidium were identified, namely C. ryanae in red deer, roe deer and wild boar, C. parvum in red deer and wild boar, C. suis in red deer, C. scrofarum in wild boar, and both C. canis and C. ubiquitum in roe deer. Within E. bieneusi, eight known genotypes (EbpA, S5, HLJD-V, BEB6, BEB17, Wildboar3, Type IV, and MWC_d1) and three novel genotypes were detected in red deer and wild boar

Conclusions: Our results indicate that infections by enteric microeukaryotic parasites including zoonotic variants are relatively frequent in Spanish wild ungulates

Keywords: Wild Ungulate, Microeukaryotic Parasites, PCR, sequencing

P432 / #1080

Topic: *AS03.2 Epidemiology, pathology and impact*

WILD MICROMAMMAL HOST SPECTRUM OF ZOONOTIC MICRO-EUKARYOTIC PARASITES IN SPAIN. OCURRENCE AND GENETIC CHARACTERIZATION

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Introduction: Micromammals have historically been recognized as highly contentious species in terms of maintenance and transmission of zoonotic pathogens to humans. However, limited information is currently available on the epidemiology and potential public health significance of intestinal microeukaryotes parasites in wild micromammals.

Methods: We examined 491 faecal samples (grouped in 155 pools) from 11 micromammal species captured in Spain to detect and molecularly characterized Cryptosporidium spp., Giardia duodenalis, Enterocytozoon bieneusi, and Blastocystis sp. Additionally, the presence of Leishmania spp. was investigated in individual spleen samples.

Results: Cryptosporidium spp. was the most prevalent species found (3.7%), followed by G. duodenalis (2.8%) and E. bieneusi (2.6%). Sequence analyses for Cryptosporidium-positive samples (n = 17) identified C. andersoni (5.9%), C. ditrichi (11.8%), C. muris (5.9%), C. parvum (5.9%), C. tyzzeri (5.9%), rat genotypes III (5.9%) and IV (5.9%), vole genotypes V (11.8%) and VII (35.3%) and Cryptosproridium spp. (17.6%). For E. bieneusi, two known genotypes C (50.0%), Peru11 (25.0%), and novel genotype (25.0%) were identified. Blastocystis sp. was not detected in any of the samples. The presence of Leishmania spp. was detected in one Microtus arvalis and one Apodemus sylvaticus in northwest Spain.

Conclusions: Molecular data indicate that wild micromammals were primarily infected by rodent-adapted species/genotypes of microeukaryotic pathogens and have a limited role as source of human infections. The presence of ruminant-adapted species (e.g. C. andersoni, C. parvum) is indicative of an overlap between domestic/peridomestic and sylvatic transmission cycles of these agents.

Keywords: Wild Micromamals, Microeukaryotic Parasites, sequencing, PCR, Zoonotic parasites

P433 / #1600

Topic: *AS03.2 Epidemiology, pathology and impact*

FELINE DIARRHEA ASSOCIATED WITH TRITRICHOMONAS FOETUS IN UKRAINE

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Introduction: Diarrhea in cats is one of the most common pathologies that occur in a veterinarian's practice. Along with infectious and parasitic diseases and digestive disorders, the causative agent of chronic diarrhea is often *Tritrichomonas foetus*. To our best knowledge, the present study is the first investigation of the occurrence of *T. foetus* in cats from Ukraine.

Methods: Data were collected from two hundred and seventeen cats to estimate the prevalence and determine the risk factors for parasite infection. Cats from the West part of Ukraine were tested with copromicroscopic methods for common intestinal parasites and a specific PCR for *T. foetus*.

Results: *T. foetus* was molecularly recorded in thirty-one cats (14% of all cats within the study) and the obtained data were significantly correlated with age and place of living. Infected cats were one year old or younger and lived in large catteries. In nine cats, *T. foetus* was found in association with *Toxocara cati* and five with *Cystoisospora* spp. Animals with chronic gastrointestinal signs were more frequently infected with *T. foetus* at 68% in comparison to cats with no gastrointestinal distresses – 19%.

Conclusions: Feline trichomonosis constitutes a serious health threat to pet owners, causing long-term diarrhea and significant emotional and financial losses. In Ukraine, *T. foetus* is still a newly emerging enteric pathogen in cats. Acknowledgements: The MEMOVA project, EU Operational Programme Research, Development and Education No. CZ.02.2.69/0.0/0.0/18_053/0016982.

Keywords: trichomonosis, Tritrichomonas foetus, Feline diarrhea

P434 / #1359

Topic: *AS03.2 Epidemiology, pathology and impact*

AVIAN PLASMODIUM CO-INFECTIONS: DEVELOPMENT AND INDUCED DISEASE

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Introduction: Understanding the development of co-infections is essential because the same pathogen, during single infection and interacting with another parasite, can develop differently and can cause different effects on host health. Avian malaria parasites are diverse and infect different bird species forming various co-infections with other haemosporidians. Most experimental studies have been performed using single infections, for this reason there is little information about parasite interactions during co-infections.

Methods: In this presentation, we review several of our experimental studies on Plasmodium co-infections and reveal that the development of erythrocytic stages and caused disease in a vertebrate host depend on the species of malarial parasites.

Results: Depending on the combination of Plasmodium species used in the experiment, we can determine parasite interactions and their effects on host health. For example, during co-infection with P. relictum, the intensity of P. elongatum parasitemia increases, whereas the parasitemia of P. relictum does not change compared to a single infection. The increase in virulence during co-infection is often similar to that of a more virulent parasite, but there are other examples which will be discussed.

Conclusions: Experimental studies of co-infections reveal interactions between avian malaria agents and contribute to a better understanding of pathogen-induced diseases in the wild. This study was funded by the European Social Fund (grant 09.3.3-LMT-K-712-01-0016).

Keywords: plasmodium, co-infection, bird, virulence

P435 / #73

Topic: *AS03.2 Epidemiology, pathology and impact*

SPATIO-TEMPORAL ANALYSIS OF BOVINE THEILERIOSIS (THEILERIA PARVA) IN ZIMBABWE FROM 1995 TO 2018

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Introduction: One of the most important tick-borne transboundary cattle diseases in Zimbabwe & yet its distribution information remains outdated. The study aims to: • analyse historical data on bovine theileriosis cases in Zimbabwe from 1995 - 2018 • describe spatio-temporal patterns & identify high-risk areas of the disease • evaluate the significance of potential risk factors associated with disease occurrence

Methods: • Permission to use theileriosis database was obtained from the Department of Livestock & Veterinary Services. • Analysis of theileriosis cases & deaths was done to describe the temporal & spatial distribution of the disease using the R software. • Space-time permutation models were used to detect high rates of theileriosis using SaTScan® version 9.4.6. • Association between the number of theileriosis outbreaks & the risk factors was assessed using a generalized linear model with Poisson distribution for count data using the R software.

Results:

- Communal farmers, adult cattle, year 2018 & hot wet season had the highest proportion of cases recorded.
- 7/10 provinces & 36/59 districts were affected.
- Disease observed to lose seasonality when cases rose exponentially in 2018.
- 5 & 4 high-risk clusters of disease were detected using 1-year & 1-month time aggregate, respectively, all within the last 8 years of the study.
- 2 potential risk factors (province & farming system) were significantly associated with the disease.

Conclusions:

- Bovine theileriosis was found to be rampant & if left unchecked will spread & adversely affect the whole country.
- Improved disease surveillance & control is warranted.

- Control & prevention strategies revolve around better disease awareness, correct & consistent use of acaricides, cattle movement control & disease surveillance among others.

Keywords: communal cattle, Zimbabwe, bovine theileriosis, transboundary disease, high-risk cluster

P436 / #288

Topic: *AS03.2 Epidemiology, pathology and impact*

BAYLISASCARIS PROCYONIS INVESTIGATIONS IN RACCOONS FROM NORTHEASTERN FRANCE REVEAL THE PRESENCE OF THE NEMATODE … BUT MORE AT EAST THAN WHERE WE WERE SEARCHING

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Introduction: The nematode Baylisascaris procyonis was introduced in Europe with its main definitive host, raccoon (Procyon lotor). In Europe, the zoonotic parasite was identified in Germany, Poland, Denmark, Netherlands and Italy. In France, three raccoon populations are established and in expansion: in Nouvelle Aquitaine (southeast), in Auvergne (center) and in northeastern France. This last population has recently merged with those from Belgium, Luxembourg and western Germany. In absence of data, investigations on B. procyonis were implemented in a study area bordering Belgium.

Methods: Raccoons have been trapped from October 2019 to June 2021 in the African Swine Fever boar-free zone along the Belgian border. After necropsy, presence of the parasite was investigated by macroscopic analysis of intestinal content followed by molecular confirmation. Population genetic analyses of the worms and of the raccoons were performed by microsatellites analyses and compared to data from neighboring populations.

Results: None of the 156 raccoons analyzed exhibited B. procyonis. However, during the trapping period, a road-killed raccoon found at 5 km at the east of the study area, at 10km from the Luxembourg border, was also analysed and exhibited three worms, two males and one female. The genetics analyses are still in progress to identify the origin of the raccoon and of the worms.

Conclusions: This first detection of an infected raccoon in France questions its origin as no other animal of this population or of those in Belgium and Luxembourg has been found infected. Further studies around this case and bordering Germany will be implemented soon. In parallel, investigations are in progress on raccoons from other areas in the Northeast and in Nouvelle Aquitaine, and has started in Auvergne.

Keywords: Baylisascaris procyonis, raccoon, population genetics, invasive species

P437 / #1468

Topic: *AS03.2 Epidemiology, pathology and impact*

CONTAMINATION OF PUBLIC SPACES WITH INTESTINAL PARASITES FROM CANINES IN THE CITIES OF LA SERENA AND COQUIMBO

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Introduction: The contamination of urban soils with canine feces implies a problem for public health, due to the wide variety of species of protozoa and intestinal helminths that are pathogenic for animals and humans. In the cities of La Serena and Coquimbo there is a large number of dogs, with or without owners, that defecate in the streets and squares of the cities, leaving the waste in these places.

Methods: 1034 samples of dog feces were taken in both cities to determine the prevalence of intestinal parasites that contaminate the environment. Both cities were divided into north and south zones, taking random samples. The parasitological diagnosis was made using the modified Télemann method.

Results: Endoparasites were found in 26.5% of the total samples. Vacuolar forms of Blastocystis sp were observed in 25.04% of the samples, 1.55% presented Giardia duodenalis, Entamoeba coli or Toxocara canis cysts. 0.97% presented two parasites. The ANOVA test showed that there are significant differences between the four sampled sectors, with the South Coquimbo sector being the one that presented the largest number of parasitized samples (N= 101; 9.77%), of which 92.1% presented Blastocystis sp. La Serena Norte, Sur and Coquimbo Norte presented 8.12%; 5.71% and 2.3% of positive samples, respectively. Tukey's a posteriori test showed a subgroup composed of La Serena Norte, Sur and Coquimbo Norte.

Conclusions: These results show a high prevalence of zoonotic enteroparasites that are contaminating public spaces, especially Blastocystis sp., whose pathogenic potential and clinical importance are not clear, despite numerous studies reporting that it can cause digestive symptoms. It is necessary to carry out deworming, education and prevention campaigns for the population of both cities.

Keywords: Blastocystis, enteroparasites, environmental contamination

P438 / #615

Topic: *AS03.2 Epidemiology, pathology and impact*

LEISHMANIOSIS IN PARMA UNIVERSITY HOSPITAL: 5 YEARS RETROSPECTIVE STUDY

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Introduction: Leishmaniosis is a zoonotic disease caused by the protozoa Leishmania spp. worldwide diffused and of particular interest in pets, dogs overall. A retrospective study on the leishmaniosis clinical cases from 2015 to 2020 was performed using the medical records management software of the Veterinary Medicine Hospital of Parma University (Italy).

Methods: A total of 46 cases were identified and subjects were divided into two groups. In the first group (21) there were different breeds of dogs and in the second group only boxer (25). The study analyzed data on age, sex, geographical origin, symptomatology, chemical and blood parameters alterations and follow up after 3, 6 and more than 9 months from the beginning of therapy.

Results: Dogs from endemic areas represent almost half of the subjects, but there is a small percentage of individuals who have never moved from the area of Parma, that means that the sand fly is starting to colonize new geographical areas, due to climate change and other factors.Discordant symptomatology, alteration in laboratory tests parameters and a different follow-up has emerged between the two groups considered. The symptomatology turns out to be more disabling in the group called 'other dogs' than boxers, while laboratory alterations are more significant in the boxer group. Regarding therapy and follow-up, the most responsive group is that of dogs of different breeds, while boxer group showed a recurrence of the disease after 9 months from the start of therapy.

Conclusions: A clear difference in the mode of action of L. Infantum towards different dogs' breeds was evident, in particular for boxer dogs, and this issue must be investigated more in depth.

Keywords: clinical cases, follow-up, Leishmaniosis, boxer breed

P439 / #862

Topic: *AS03.3 Immunology, immunity and vaccine development*

A COMBINED TRANSCRIPTOMIC AND PROTEOMIC APPROACH TO IDENTIFY CANDIDATES FOR ANTI-TICK VACCINE.

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Introduction: Ornithodoros moubata is the main vector of African swine fever and human relapsing fever in Africa. The development of an effective vaccine against this argasid can ease the control of these diseases. We describe the implementation of a reverse vaccinology strategy to select tick salivary antigens as potential vaccine targets, based on the integration and analysis of recently obtained salivary transcriptomic and proteomic datasets from O. moubata.

Methods: According to our hypothesis, the salivary transcripts whose expression increases after feeding encode bioactive proteins necessary for the tick to feed again. Therefore, our selection of targets was based on these genes, which were further filtered by applying the following criteria: (i) transcripts identified in the saliva proteome, and (ii) transcripts not identified in the proteome, but presenting signal peptide and lacking transmembrane domains and GPI anchors. The list of candidates was refined, prioritizing those predicted to be extracellularly expressed and to play functions related to blood feeding and evasion of host defense mechanisms.

Results: From the list of candidates, four antigens have been selected, molecular and structurally characterized and obtained as recombinant proteins to be tested in animal vaccine trials.

Conclusions: Vaccinomics applied to ticks is a useful holistic approach in which omics datasets are integrated and analyzed to identify key molecules involved in tick-host relationships and to characterize them as possible vaccine targets. Funding: Grant “RTI2018-098297-B-I00” by MCIN/AEI (Spain) and ERDF. Grant “CLU-2019–05–IRNASA/CSIC Unit of Excellence” by Junta de Castilla y León (Spain) and ERDF

Keywords: Transcriptome, Vaccinomics, proteome, Ornithodoros moubata

P440 / #867

Topic: *AS03.3 Immunology, immunity and vaccine development*

FOUR SALIVARY PROTEINS FROM THE ORNITHODOROS ERRATICUS ARGASID TICK SELECTED AS VACCINE TARGETS BY REVERSE VACCINOLOGY INDUCE PROTECTIVE RESPONSES IN RABBITS

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Introduction: Ornithodoros erraticus is the main vector of African swine fever (ASF) and human relapsing fever (HRF) in the Mediterranean Basin and Middle East. Chemical acaricides are ineffective against this species, prompting the development of vaccines as an alternative control method. To identify potential vaccine targets, the recently obtained O. erraticus sialome was scrutinized following a vaccinomics approach, and a set of 37 salivary secretory proteins predicted to be antigenic and involved in the regulation of the tick-host relationship were selected. The aim of this work was to analyze the protective potential of 4 of these candidates, namely, acid tail salivary protein (ATSP), multicoagulation factor deficiency (MCFD), superoxide dismutase (SOD), and sulfotransferase (SULF1).

Methods: The candidates were produced as recombinants, formulated with Montanide, and administered individually to different groups of rabbits. Adult and nymphal-3 specimens of O. erraticus and Ornithodoros moubata (the African vector of ASF and HRF) were fed on the vaccinated rabbits and the tick feeding performance, survival and reproduction rates were evaluated.

Results: Protective efficacies of 46.8% (ATSP), 45.7% (MCFD), 54.3% (SOD) and 31.9% (SULF1) against O. erraticus and of 0% (ATSP) 3.9% (MCFD), 3.1% (SOD) and 8.7% (SULF1) against O. moubata were obtained.

Conclusions: These results proved the usefulness of this vaccinomic pipeline and validated the four selected candidates as protective antigens for the development of vaccines against O. erraticus.
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Keywords: Ornithodoros, vaccines, Vaccinomics, antigens, Ticks

P441 / #1349

Topic: *AS03.3 Immunology, immunity and vaccine development*

THE CIRCADIAN REGULATION OF THE IMMUNE RESPONSE TO PLASMODIUM IN A MOUSE MODEL OF CEREBRAL MALARIA

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Introduction: Malaria is a parasitic disease caused by Plasmodium which leads to intense immune responses. Studies have shown that circadian clocks regulate immune functions. Therefore, our aim was to study the circadian regulation of the immune responses to Plasmodium by using a mouse model of experimental cerebral malaria.

Methods: 1. To evaluate the circadian control of the inflammatory response in response to Plasmodium, we stimulated macrophages with Plasmodium berghei ANKA-infected red blood cells (iRBCs) at different times across 24h. 2. To investigate the circadian regulation of Plasmodium infection in vivo, mice were infected at different times across 24h. 3. To investigate the effects of circadian disruption, we subjected mice to chronic jet lag prior to infection.

Results: for aim 1. We found rhythms in proinflammatory cytokines in both RNA and protein level, suggesting the involvement of the macrophage clock in regulating the response to iRBCs. Results for aim 2. Mice infected at night had lower parasite load, parasitemia and enhanced resistance for the development of cerebral malaria, indicating that time of infection influences disease progression. Results for aim 3. Surprisingly, circadian disruption led to reduced parasite load, parasitemia and frequency of multi-infected cells, in comparison to mice subjected to a normal lighting schedule.

Conclusions: In conclusion, we showed that host clocks and the time of infection influence the progression of malaria and that circadian disruption influences parasite growth and development.

Keywords: immune cells, Mouse model, Malaria, Circadian rhythms

P442 / #1031

Topic: *AS03.3 Immunology, immunity and vaccine development*

INTESTINAL MICROBIOTA IN MICE INFECTED WITH HELMINTH SUPPRESS TYPE 1 DIABETES BY INDUCING CD8+ TREGS VIA PRODUCTION OF ACETIC ACID

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Introduction: Type 1 diabetes (T1D) is an autoimmune disease where insulin-producing pancreatic b cells are destroyed. Considering the recent increased incidence of T1D in developed countries, environmental factors appear to affect autoimmunity. One possible explanation for the involvement of environmental factors is the “hygiene hypothesis”, suggesting that reduced exposure to pathogens because of improved hygiene increases the risk of inflammatory disorders such as autoimmunity. Among numerous pathogens, intestinal helminths cause asymptomatic chronic infections, evoke immune suppression, and suppress T1D in rodent models. However, the underlying regulatory mechanisms of helminthic infections in T1D remain largely elusive.

Methods: Here, we found that a rodent intestinal nematode prevented the onset of drug-induced T1D in a CD8+ regulatory T cell (CD8Treg)-dependent manner.

Results: In this study, we show that a rodent intestinal nematode prevents the onset of STZ-induced T1D in a $CD8^+$ regulatory T cell (CD8Treg)-dependent manner. Infection with the nematode and its derivative, trehalose, affects the intestinal microbiota, resulting in the induction of CD8Tregs. Among microbiota Ruminococcus is markedly increased in infected mice and is responsible for induction of CD8Tregs. In addition to preventive ability, trehalose exhibits therapeutic effect not only in STZ-treated, but also in NOD mice. We also demonstrate that patients with T1D have a significantly lower number of CD8Tregs and intestinal Ruminococcus compared with healthy volunteers.

Conclusions: We will also talk about the mechanistic insights how CD8Tregs are induced.

Keywords: Helminth, immunity, microbiota, hygiene hypothesis

P443 / #698

Topic: *AS03.3 Immunology, immunity and vaccine development*

DIAGNOSTIC USEFULNESS OF TRIVALENT RECOMBINANT CHIMERIC T. GONDII PROTEINS TO DETECT IGG AND IGM ANTIBODIES

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Introduction: Toxoplasmosis is one of the most common and neglected parasitoses, caused by intracellular parasite Toxoplasma gondii. This parasite poses threat for immunodeficient people, and at the same time it can lead to economic losses in case of animal breeding. Certain problems are associated with infection with T. gondii, such as the lack of effective immunoprophylaxis or inadequate diagnostic methods requiring continuous improvement. Both problems can be overcome by recent development of T. gondii proteomics, which enabled designing of various recombinant antigens that can be used for both serodiagnosis and immunoprophylaxis of this invasion. In this study, we evaluated the potential usefulness of trivalent recombinant chimeric T. gondii proteins for serodiagnostics.

Methods: Several different trivalent chimeric T. gondii proteins were designed, produced in a prokaryotic Escherichia coli expression system and purified by one-step metal affinity chromatography. As part of the immuno-screening, the proteins were used for the detection of IgG and IgM T. gondii-specific antibodies in the sera obtained from laboratory mice at 2, 3, 6 or 12 weeks post-infection, using ELISA test.

Results: These proteins have been shown to react with specific IgG and IgM anti-T. gondii antibodies and comparison of obtained results enabled the selection of the most promising antigenic compositions for further testing.

Conclusions: Chosen chimeric proteins will be further evaluated for their antigenic properties, using immune human and animal sera, as well as their immunogenic potential in vivo. This work was supported by the National Science Centre, Poland (grant number UMO-2018/31/D/NZ6/02839).

Keywords: Recombinant protein, Serology, Toxoplasma gondii

P444 / #1389

Topic: *AS03.3 Immunology, immunity and vaccine development*

GLYCOLYSIS, MONOCARBOXYLATE TRANSPORT AND PURINERGIC SIGNALLING ARE KEY EVENTS IN EIMERIA BOVIS-INDUCED NETOSIS

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Introduction: The protozoan parasite Eimeria bovis is the causative agent of bovine coccidiosis, an enteric disease of global importance that significantly affects cattle productivity. Previous studies shown that bovine NETosis, - an important early host innate effector mechanism of PMN- is elicited by E. bovis stages. The metabolic requirements of E. bovis- triggered NET formation are unknown.

Methods: Metabolic activity of activated PMN was studied by Seahorse-based experiments. The NET formation was analyzed by confocal, 3D microscopy (Nanolive). Chemical inhibition of E. bovis induced NETosis was estimated by extracellular DNA detection in the presence of drugs blocking glycolysis, glutaminolysis, pyruvate dehydrogenase kinase, pyruvate dehydrogenase, lactate dehydrogenase and mitochondrial ATP-synthase. In addition, drugs targeting MCT-1, MCT-2 and purinergic receptor inhibitors were also evaluated.

Results: Seahorse-based experiments revealed a rapid induction of both neutrophil oxygen consumption rates (OCR) and early glycolytic responses thereby reflecting immediate PMN activation and metabolic changes upon confrontation with sporozoites. Overall, the sporozoite-induced NET formation was significantly diminished via PMN pre-treatments with OmA and OXA, thereby indicating a key role of ATP- and lactate-mediated metabolic pathways. In addition, AR-C141900, AR-C155858, theobromine and NF449 targeting MCT and purinergic receptors led to blockage of sporozoite-triggered DNA release from exposed bovine PMN.

Conclusions: Carbohydrate-related metabolic pathways and purinergic receptors play a pivotal role in E. bovis sporozoite-induced NETosis.

Keywords: Cattle, Eimeria Bovis, NETs, Innate Immunity

P445 / #1147

Topic: *AS03.3 Immunology, immunity and vaccine development*

IMMUNOPREVALENCE OF TRICHINELLA NEMATODES IN RACCOONS (PROCYON LOTOR) AND RACCOON DOGS (NYCTEREUTES PROCYONOIDES) IN CENTRAL EUROPE

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Introduction: The raccoon (Procyon lotor) and the raccoon dog (Nyctereutes procyonoides) are animals native to North America and East Asia, respectively. Due to their fast spread and sylvatic lifestyle, both species can be a reservoir of many parasites, which could be dangerous to humans and domestic animals. The aim of the study was to examine occurrence of anti-Trichinella antibodies in meat juice samples of raccoons and raccoon dogs.

Methods: The study was carried out on 139 raccoons and 26 raccoon dogs. To detect the presence of antibodies against Trichinella meat juice samples were tested using commercial ELISA kit (IDvet), according to the manufacturer's instructions. The positive and doubtful results from ELISA were confirmed by immunoblot. The most frequently detected bands were analyzed by LC-MS/MS to identify the specific proteins in meat juice raccoon dogs infected with T. britovi.

Results: The results of the ELISA combined with immunoblot found anti-Trichinella antibodies in 9.35% of raccoons and in 46% of raccoon dogs. Different proteins related to Trichinella infection have been discovered in the study (eg. heat shock proteins, proteases).

Conclusions: Our results show that raccoons and raccoon dogs were exposed to Trichinella nematodes and meat juice samples are useful to detect the immune response to Trichinella infection in these animals. Additionally, analysis by LC-MS/MS showed different proteins in meat juice samples, which are characteristic for Trichinella infection and can be used as potential biomarkers.

Keywords: immunoprevalence, Trichinella, raccoon, raccoon dog, proteins

P446 / #928

Topic: *AS03.3 Immunology, immunity and vaccine development*

GENETIC ANALYSIS OF ANTIGENS FROM LOCAL STRAINS OF LEPTOSPIRA SPP. OBTAINED FROM CATTLE IN THE STATE OF RIO DE JANEIRO: ARE WE CLOSE TO A LOCAL VACCINE?

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Introduction: Leptospirosis is a bacterial zoonotic disease caused by Leptospira spp. In bovines, it is commonly associated to chronic reproductive disease, as abortion and subfertility, resulting in economic losses in animal production. Vaccination has limitations in terms of effectiveness, since vaccine is serogroup specific and the genus present high serological diversity. Therefore, greater knowledge about the antigenic diversity of local strains is essential. The aim of the present study is to evaluate the genetic variability of antigens from Leptospira sp. obtained from cattle in the state of Rio de Janeiro for evaluation of vaccine candidates for veterinary use.

Methods: Strains of Leptospira spp. (n=24) were from circulating pathogenic species - Leptospira interrogans, L. santarosai and L. borgpetersenii – and from the most common serogroups associated with chronic diseases in cattle in Brazil- Sejroe, Icterohaemorrhagiae and Pomona. Nine antigenicity genes (lipL21, lipL32, loa22, ompL37, lemA, ligA, lipL45, ompL1 and ligB) were amplified and sequenced. Genetic variability was analyzed together with sequences available in GenBank, using bioinformatics tools.

Results: The following aminoacid similarity degrees were obtained: LigB – 76%; ompL1-91%; LipL45 - 92%; LigA-92%; LemA - 95%; OmpL37 - 96%; Loa22 - 96%; lipL32 - 98%; lipL21 - 99%.

Conclusions: The gene with the minimum genetic distance at amino acid level was lipL21, being considered the best vaccine candidate for bovine leptospirosis in the Brazilian Southeast region, since it is presumed to be more conserved in these strains. Importantly, it will be the target of further studies based on reverse vaccinology.

Keywords: animal leptospirosis, antigenicity, Genetics

P447 / #1065

Topic: *AS03.3 Immunology, immunity and vaccine development*

MODULATORY EFFECT OF BENEFICIAL ENTEROCOCCI AND THEIR ENTEROCINS ON THE BLOOD PHAGOCYTES IN MURINE EXPERIMENTAL TRICHINELLOSIS

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Introduction: Bacteriocins (enterocins) represent a new strategy to combat or prevent various intestinal and non-intestinal infections. In antiparasitic defense, an oxidative inflammation of phagocytes is effective in destroying newborn Trichinella spiralis larvae. Therefore we evaluated the phagocytic and oxidative activity of blood leukocytes after enterococci/enterocins therapy.

Methods: The strains Enterococcus faecium CCM8558 and E. durans ED26E/7 (10^9 CFU/ml) and their enterocins Enterocin M and Durancin-like (50 µl) were administered daily and mice were infected with Trichinella spiralis (400 larvae) on 7^{th} day of treatment. Phagotest and Bursttest kits were used to detect the phagocytosis and respiratory burst of blood leukocytes by flow cytometry.

Results: Phagocytosis was inhibited from day 11 post T. spiralis infection (dpi), i.e. in the time of newborn larvae's migration into the muscles. E. faecium CCM8558, E. durans ED26E/7 and Durancin-like increased phagocytic activity of leukocytes from day 11 dpi. Both strains and their enterocins (Enterocin M, Durancin-like) stimulated the ingestion capability of phagocytes from 18 to 32 dpi. Enterococci/enterocins therapy prevented the reduction of cells with respiratory burst caused by T. spiralis infection from 11 dpi. The enzymatic activity of phagocytes was stimulated on 18 and 25 dpi with the maximum effect of E. faecium CCM8558 and Enterocin M.

Conclusions: Enterocin M and Durancin-like were as effective in stimulating phagocytosis as the bacterial strains that produce them. The stimulation of phagocytosis could contribute to decrease larval migration and then reduce parasite burden in the host. The work was supported by the projects VEGA 2/0056/19 and APVV-17-0028.

Keywords: enterocins, Enterococcus, Trichinella spiralis, phagocytosis

P448 / #1079

Topic: *AS03.3 Immunology, immunity and vaccine development*

CHANGES IN CYTOKINE PRODUCTION IN MICE TREATED WITH ENTEROCINS/ENTEROCOCCI IN EXPERIMENTAL TRICHINELLOSIS

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Introduction: The immunomodulatory activity of bacteriocins (enterocins) as a new therapeutic strategy in trichinellosis was studied. Recognition of bacterial components (enterocins, exopolysaccharides) through Toll-like receptors leads to the activation of transcription factors that trigger cytokine production.

Methods: The strains Enterococcus faecium CCM8558 and E. durans ED26E/7 (10^9 CFU/ml) and their enterocins Enterocin M and Durancin-like (50 µl) were administered daily and mice were infected with Trichinella spiralis (400 larvae) on 7^{th} day of treatment. Cytokine production in vitro was detected in splenocytes by capture ELISA.

Results: Splenocytes from untreated mice produced high levels of Th1 (IFN-γ, TNF-α) and Th2 (TGF-β, IL-4, IL-5, IL-10) cytokines in the early intestinal phase of trichinellosis (5 – 11 dpi). IL-10 production slightly decreased in the migration phase (18 – 25 dpi), but IL-4 levels were increased, IL-5 production decreased slightly in the muscle phase (25 – 32 dpi). E. faecium CCM8558 and Enterocin M stimulated IFN-γ production in the acute phase of T. spiralis infection on day 5 post infection (dpi), thereby promoting the inflammatory response, which prevents the parasite from settling in the intestinal epithelium and producing newborn larvae. E. durans ED26E / 7 and Durancin-like reduced IL-10 and IL-4 production in the muscle phase from 18 to 25 dpi.

Conclusions: The beneficial effect of enterocins/enterococci therapy in T. spiralis infection was confirmed by regulating inflammatory responses in the intestinal phase and by reducing the Th2 response useful for muscle larvae in the muscle phase. The work was supported by the projects VEGA 2/0056/19 and APVV-17-0028.

Keywords: enterocins, Enterococcus, cytokines, Trichinella spiralis

P449 / #1694

Topic: *AS03.3 Immunology, immunity and vaccine development*

IRRADIATION OF ZOONOTIC PARASITES WITH LOW ENERGY ELECTRONS FOR THE DEVELOPMENT OF VACCINE CANDIDATES

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Introduction: *Cryptosporidium parvum* and *Toxoplasma gondii* are two relevant zoonotic parasites for animals and humans alike. Vaccination would be an efficient method of disease prevention against these pathogens, so there is a high demand for innovative approaches in vaccine development. For efficient protection, vaccines against parasites have to induce immune responses against a variety of antigens because, in their complex life cycle, they undergo severe changes in their antigen composition. Attenuation would allow them to keep enough metabolic capacity and virulence to undergo several of these changes without causing disease symptoms. One approach to attenuate pathogens is treatment with ionizing radiation, since nucleic acids are mainly damaged and protein structures stay intact. By adjusting the radiation dose, the parasites can stay active enough for subclinical infection and induce protective immune responses.

Methods: We developed a process using low energy electron irradiation (LEEI) to treat pathogens in liquid solution. Tachyzoites of *T. gondii* and oocysts of *C. parvum* were treated with different radiation doses to identify a range for attenuation. After LEEI treatment the parasites were characterised *in vitro*, analysing intracellular replication, antigen conservation and the capacity to change into different life stages after LEEI-treatment.

Results: LEEI treatment of both *T. gondii* and *C. parvum* resulted in a dose dependent reduction of their reproductive capacity in cell cultures. A good conservation of antigenic structures after LEEI was shown via ELISA.

Conclusions: Promising attenuation parameters for both *T. gondii* and *C. parvum* using LEEI were identified. The following step is testing the vaccine candidates in animal immunization studies.

Keywords: Toxoplasma gondii, Cryptosporidium parvum, Attenuated Vaccines, Irradiation

P450 / #930

Topic: *AS03.3 Immunology, immunity and vaccine development*

COMPARATIVE PROTEOMICS ANALYSIS OF ANISAKIS SIMPLEX S. S. – EVALUATION OF THE RESPONSE OF INVASIVE LARVAE TO IVERMECTIN

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Introduction: Ivermectin (IVM) - an antiparasitic agent - has a beneficial effect on Anisakis simplex s. s. infection and is used for the treatment and prevention of anisakiasis in humans. However, the molecular mechanism of the action of IVM on A. simplex s. s. remains unknown.

Methods: The experiment was performed with live A. simplex s. s. L3 larvae obtained from herring (Clupea harengus membras). Ivermectin was added at a concentration of 12.5 µg/ml of the culture medium. The tandem mass tag labeling followed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was used to identify the effect of IVM on the proteome of A. simplex s. s. in vitro.

Results: During the study, 3,433 proteins, including 1,247 with at least 2 unique peptides, were identified. Comparative proteomic analysis showed that 59 proteins were differentially regulated (DRPs) in IVM-treated larvae, of which 14 and 38 proteins were up- and downeregulated after 12 hours of culture, respectively, but after 24 hours, 12 proteins were up- and 22 were downregulated. The mRNA level of five randomly selected DRPs was determined by real-time PCR. Functional enrichment analysis showed that most DRPs were involved in oxidoreductase activity, immunogenicity, and protein degradation.

Conclusions: This study provided, for the first time, comprehensive proteomics data on the response of A. simplex s. s. to IVM and may provide us with new insights into the molecular mechanism by which IVM acts on invasive larvae of A. simplex s. s. A detailed description of the presented study can be found at https://doi.org/10.3390/genes11060710. This work was funded by the GAIN-Xunta de Galicia, project number IN607D 2017/01. M. C. was supported by the Ramón y Cajal contract (Ministry of Science and Innovation of Spain).

Keywords: Anisakiasis, proteome, antiparasitic drugs

P451 / #1652

Topic: *AS03.3 Immunology, immunity and vaccine development*

FERMENTABLE DIETARY INULIN MODULATES MUCOSAL IMMUNE RESPONSES AND PREVENTS TRICHURIS MURIS EXPULSION

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Introduction: The role of fibre in gut health is becoming increasingly appreciated, especially in populations consuming processed 'Western-style' diets lacking whole grains and complex fibres. Soluble, fermentable fibres (e.g. inulin) may modulate host microbiome and immune function, potentially reducing inflammation and enhancing immunity to infection. However, the interactions between diet and immunity to parasites are not well understood. Here, we investigated the effect of dietary inulin on infection with the murine whipworm *Trichuris muris.*

Methods: Intestinal immune cell profiling using flow cytometry, transcriptomic analysis and 16S rRNA sequencing were utilised to investigate the immunomodulatory impact of inulin in the gut during *T. muris* infection.

Results: Dietary inulin prevented worm expulsion in C57BL/6 mice, resulting in chronic infection, gut dysbiosis and a dominant Th1-immune state. Th1 polarisation was characterised by increased *IFNG* and *NOS2* caecal gene expression at day 21 post-infection (p.i.), and increased gut-derived Th1 and Th17 cells as a result of inulin & T*. muris* treatment at day 7 p.i. Through neutralization of IFNγ, we reversed the effect of inulin and restored host resistance. Furthermore, infected mice fed a cellulose-supplemented diet (insoluble fibre) expelled worms normally, indicating the importance of microbial fermentation of inulin for inducing susceptibility to *T. muris*.

Conclusions: Our results indicate a profound effect of diet on *T. muris* infection and immune regulation. Elucidation of the inulin-mediated mechanisms causing *T. muris* persistence is still required, nevertheless these findings have clear implications for the use of soluble dietary fibres as a potential therapeutic for inflammatory-mediated conditions.

Keywords: inflammation, Trichuris muris, Dietary inulin

P452 / #340

Topic: *AS03.3 Immunology, immunity and vaccine development*

VACCINOMICS: MINING THE ORNITHODOROS ERRATICUS SIALOME FOR VACCINE CANDIDATES.

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Introduction: The argasid tick Ornithodoros erraticus transmits African swine fever and Human relapsing fever in the Mediterranean Basin. Development of vaccines for tick and tick-borne disease control starts by identifying highly protective antigens. Tick salivary proteins secreted into the host during feeding play critical functions for tick physiology and pathogen transmission and, in adult argasids, these proteins must be replaced for the tick to be able to feed again. Thus, these proteins are potential targets for new tick vaccines.

Methods: Reverse vaccinology explores omics datasets using bioinformatic tools for the hypothesis-driven selection of proteins potentially capable of inducing a protective response in vivo. Herein, reverse vaccinology was applied to the recently obtained salivary transcriptomic and proteomic data from O. erraticus for identification of vaccine targets. The following filters were sequentially applied: (i) feeding-induced transcript upregulation, (ii) be detected in the saliva proteome or show secretory signals, (iv) Vaxijen antigenicity prediction and (v) functional annotations related to blood-feeding and modulation of host-defensive response

Results: Up to 37 candidates met all the above-mentioned criteria. Four of them were selected and their structure and immunogenicity were characterized in order to be later produced as recombinants and tested in animal vaccination trials.

Conclusions: We contribute four new potential protective antigens to the currently scant repertoire of soft tick vaccine candidate antigens. Funding: Grant “RTI2018-098297-B-I00” by MCIN/AEI (Spain) and ERDF. Grant “CLU-2019–05–IRNASA/CSIC Unit of Excellence” by Junta de Castilla y León (Spain) and ERDF

Keywords: Ticks, Ornithodoros erraticus, Transcriptome, reverse vaccinology, proteome

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Topic: *AS03.3 Immunology, immunity and vaccine development*

ARGASID TICK AQUAPORINS AS VACCINE CANDIDATES: THE ORNITHODOROS MOUBATA MODEL

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Introduction: Aquaporins (AQP) are evolutionarily conserved proteins that transport water and small solutes through cell membranes. Numerous AQPs have been recently identified in ixodid ticks, which are expressed in tick salivary glands, midgut and Malpighian tubules and participate in multiple vital processes. Accordingly, ixodid AQPs are considered rational targets for tick vaccines and recent studies have confirmed their protective efficacy. By contrast, argasid AQPs have been little studied, so their structure, function and protective potential remains unexplored. This work aims to identify and characterize the AQPs expressed by the argasid tick Ornithodoros moubata (OmAQP).

Methods: The transcriptomes of O. moubata salivary glands and midgut (BioProjects PRJNA377416, PRJNA667315) were screened for full OmAQP ORFs. The ORFs were PCR amplified, cloned and sequenced, and their tissue expression analyzed. BLASTp searching for homologues, multiple sequence alignment, phylogenetic and structural analysis and epitope prediction were performed to identify conserved domains and immunogenic peptides.

Results: Seven OmAQPs were found, all of them were expressed in midgut and salivary glands, and 3 of them also in coxal glands. They classified into 4 well-defined clades, and for each clade, 3 clade-specific immunogenic peptide motifs were found.

Conclusions: This is the first comprehensive analysis of the AQPs expressed by an argasid species. It provided abundant novel information on argasid AQPs, including 12 immunogenic motifs that could be useful for developing AQP peptide-based tick vaccines. Funding: Grant “RTI2018-098297-B-I00” by MCIN/AEI (Spain) and ERDF. Grant “CLU-2019–05–IRNASA/CSIC Unit of Excellence” by Junta de Castilla y León (Spain) and ERDF

Keywords: argasid ticks, Ornithodoros, aquaporins, peptide-vaccines

P454 / #1634

Topic: *AS03.3 Immunology, immunity and vaccine development*

INTERPLAYS BETWEEN CRYPTOSPORIDIUM PARVUM AND EIMERIA ACERVULINA DURING INFECTION OF A POULTRY MACROPHAGE CELL LINE (HD11)

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Introduction: *Cryptosporidium parvum* and *Eimeria acervulina* are apicomplexan intestinal parasites that can infect chickens. In chickens, *C. parvum* is a zoonotic parasite, commonly found in cattle. In chickens, infection is chiefly a neglected malady. *E. acervulina* is a ubiquitous parasite in domestic fowl that infects the upper part of small intestine. Chicken coccidiosis caused solely by *E. acervulina* is mostly mild. However, it could evolve into a moderate to fatal illness in birds jointly infected with *E. acervulina* and other pathogens of importance in chicken health (e.g. *E. tenella*, *Clostridium* spp., *Salmonella* spp.). The aim of the present project is to study possible interactions during in vitro co-infection of *C. parvum* and *E. acervulina* in a chicken macrophage cell line (HD11)

Methods: HD-11 monolayers were infected with *C. parvum* and *E. acervulina* incubated for 2, 6, 24, and 48 h. Three groups of infection were set: single infection of *C. parvum* (Cp), single infection *E. acervulina* (Ea) and co-infection (CoInf). Quantification of *C. parvum* and *E. acervulina* genomic copies and cytokine expression were conducted using qPCR

Results: *C. parvum* multiplication started lower ($P > 0.0001$) in the CoI group than the Cp group. However, the number of *C. parvum* copies increased ($P > 0.0001$) at 6 hpi. During all time points *E. acervulina* DNA copies in the CoI group were lower than the single infection ($P > 0.0001$). Furthermore, there was no statistical difference in cytokine expresion between groups.

Conclusions: Our findings show that there is a possible interplay between *C. parvum* and *E. acervulina* replication during infection of chicken macrophages. Which highlights the need for attention in the poultry industry neglected co-infections like in the case of *E. acervulina* and *C. parvum*.

Keywords: Chicken coccidiosis, Eimeria, Innate Immunity, Cryptosporidium

P455 / #1109

Topic: *AS03.3 Immunology, immunity and vaccine development*

CHARACTERIZATION OF LIVER FLUKE FASCIOLA GIGANTICA SECRETED EXTRACELLULAR VESICLES

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Introduction: Fasciola are obligate blood feeders and regular regurgitation of parasite gut contents is thought to inject molecules into the bloodstream where they can exert an immunosuppressive activity on the host immune system. Extracellular vehicles (EVs) are now recognized as important mediators of intercellular communication by transferring molecular signals. This study aimed to characterize EVs from F. gigantica through analysis of proteomic and genomic content that involved in F. gigantica host relationships.

Methods: Fluke spit were collected from live flukes from RPMI1640 culture media and preserved in the freezer for subsequent analysis. Isolation and characterization of some peptides of extracellular vesicles by SDS-PAGE and fractionations of peptide profiles were done

Results: The analysis of incubation medium following SDS-PAGE revealed that the ES products of F. gigantica separated into 24 bands with a molecular weight range of 15±180 kDa. Proteomics analysis of the EVs revealed proteins in both replicates. A total of 68 proteins were found in both replicates, whereas 32 and 48 proteins were uniquely found in replicate 1 and 2 respectively, resulting in 148 proteins in total. The liver fluke EVs will be useful to gain new insight into the molecular information and proteomics.

Conclusions: These EVs will be used to explore some immunogenic proteins and that may help early diagnosis of liver fluke disease, anthelmintic resistance and subsequent vaccine development research in Bangladesh Acknowledgmens:We gratefully acknowledge the financial support for this research from the Grants for Advanced Research in Education (LS-20191118 /2019), Ministry of Education, Govt. of the People's Republic of Bangladesh.

Keywords: Proteomics, immunogens, Fasciola gigantica, extracellular vesicles, intercellular communication

P456 / #405

Topic: *AS03.3 Immunology, immunity and vaccine development*

RECEPTOR FOR ADVANCED GLYCATION PRODUCTS (RAGE) CONTRIBUTES TO NEGATIVE REGULATION OF TYPE 2 MUCOSAL IMMUNITY TO THE INTESTINAL NEMATODE NIPPOSTRONGYLUS BRASILIENSIS

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Introduction: Receptor for advanced glycation end products (RAGE), which is expressed in fibroblast and endothelial cells, mediates inflammatory responses by regulating the expression of pro-inflammatory cytokines and endothelial adhesion molecules. However, role of RAGE on type 2 mucosal immunity to intestinal nematodes is poorly understood. In this study, we performed Nippostrongylus brasiliensis infection experiments in RAGE-deficient ($RAGE^{-/-}$) and wild-type (Wt) mice to clarify the role of RAGE.

Methods: N. brasiliensis infective larvae were administrated subcutaneously to Wt and $RAGE^{-/-}$ mice (1.000 larvae/mouse). Worms were recovered from small intestines at 3 and 7 days post-infection (dpi) and counted under a stereomicroscope. RT-quantitative PCR and histochemical analysis were performed on small intestinal tissues at 7 dpi.

Results: Although the larval migration rate to small intestine was unchanged between Wt and $RAGE^{-/-}$ mice at 3 dpi, the adult worm burden was significantly lower in $RAGE^{-/-}$ mice compared with Wt mice at 7 dpi. Expression levels of interleukin (IL)-25 and IL-13 were upregulated in $RAGE^{-/-}$ mice compared with Wt mice. Goblet cells significantly increased in $RAGE^{-/-}$ mice compared with Wt mice.

Conclusions: Our results suggest that RAGE prevents goblet cell hyperplasia through downregulation of IL-25 and IL-13 in mouse small intestine after N. brasiliensis infection. Intestinal nematodes may make use of host RAGE for successful parasitism.

Keywords: Nippostrongylus brasiliensis, Receptor for advanced glycation products (RAGE), Helminth, type 2 mucosal immunity

P457 / #777

Topic: *AS03.3 Immunology, immunity and vaccine development*

AN ADVANCEMENT ON RECOMBINANT VACCINES AGAINST PARASITIC GASTROENTERITIS IN CATTLE

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Introduction: Treatment of gastrointestinal nematode infections in cattle relies heavily on anthelmintic drugs, but the spread of anthelmintic resistance hampers its future perspectives. As a more sustainable alternative, two experimental vaccines have been developed against Ostertagia ostertagi and Cooperia oncophora that consistently reduce faecal egg output by 55 to 91%. As these vaccines are based on native ASP proteins purified from parasite culture medium, commercialization relies on the possibility to produce recombinant versions with adequate antigenic properties.

Methods: Key antigenic properties of the antigens were evaluated through mass spectrometry, peptide microarrays and glycan microarrays. The results were used to steer antigen expression in Nicotiana benthamiana. The recombinants produced were evaluated via in vitro immunological assays followed by in vivo studies in mice and cattle, in which the immunogenicity of the recombinants and the potential to induce a protective immune response were evaluated.

Results: Highly immunogenic epitopes were found on the ASP antigens and subsequently adopted in the recombinant expression system. The immunogenic potential of the generated recombinants was positively evaluated through in vitro assays and in vivo assays in mice demonstrated the induction of a clear humoral and cellular immune response. Furthermore, a recent bovine vaccination study showed a clear systemic and local antibody response in conjunction with a 45% reduction in faecal egg output.

Conclusions: Recent insights on the antigenic properties of these ASP antigens, their recombinant expression and their ability to reduce the faecal egg output are promising for the further development of vaccines against parasitic infections in livestock.

Keywords: Cattle, Activation-associated secreted protein, N-glycosylation, Recombinant vaccine, Ostertagia ostertagi

P458 / #592

Topic: *AS03.4 Prevention and treatment, drug resistance*

ANTI-TRICHOMONAL ACTIVITY OF SEVERAL ESSENTIAL OILS, EXTRACTS AND PURE COMPOUNDS OF GERANIUM MACRHORRIZHUM

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Introduction: Trichomonas gallinae is a flagellated oropharyngeal parasite of birds than induce granulomas and starvation. Treatment is based on nitroimidazoles, but no preventive treatment is approved in the EU. In this context, our group is searching for new anti-trichomonal compounds from natural products, among them, Geranium machrorrhizum, was selected.

Methods: One hundred and fifty microlitres/well of T. gallinae trophozoites culture in TYM were used in 96-well plates, and Essential oils (EOs), ethanolic extracts, and pure compounds were assayed. The composition of active EOs was obtained by gas chromatography-mass spectrometry (GC-MS). EOs and extracts were assayed from 800 ug/ml to 200 ug/ml, while pure compounds were employed at 100 ug/ul to 1 ug/ml. Trophozoite viability was assessed by the MTT colorimetric assay, based on the reduction of MTT in formazan salts by the parasite metabolic activity.

Results: EOs of G. macrorrhizum showed good anti-trichomonal effect (>75%) from 800 µg/ml to 200 µg/ml, but the EO obtained from flowers was also active at 100 µg/ml. Only 4/5 extracts showed good results at higher concentrations (800 and 400 µg/ml). Of them, only AFB222 and AFB223A showed good activity at lower concentrations. Germacrone and β-elemenone were the main components of the EOs. Germacrone showed good activity from 100 µg/ml to 50 µg/ml, and low activity at 25 µg/ml, while β-elemenone was not active. Also, products AFB140 and AFB32 were tested, but only the first one showed good anti-trichomonal activity at 100 and 75 µg/ml, while at 50 µg/ml the activity was moderate (49%).

Conclusions: EOs from G. macrorrhizum, AFB222 & AFB223A extracts and Germacrone and compound AFB140 showed good anti-trichomonal activity and could be good candidates for treatment.

Keywords: Natural products, anti-trichomonal activity, Trichomonas gallinae, Geranium macrorrhizum

P459 / #363

Topic: *AS03.4 Prevention and treatment, drug resistance*

GENETIC CHARACTERISTICS OF THE THEILERIA ANNULATA AND TRYPANOSOMA EVANSI RESISTANCE CANDIDATE LOCI AND THEIR IMPACT ON THE BUPARVAQUONE AND DIMINAZENE RESISTANCE

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Introduction: Buparvaquone is the only commercially available drug for the treatment of theileriosis, but resistance has now been reported in T. annulata. Diminazene is widely used for the treatment of trypanosomiasis, resistance has now been considered to be a serious challenge threatening the viability of ruminant production in different parts of the world.

Methods: We have analysed the allele frequencies of cytochrome b in T. annulata and adenosine transporter-1 in T. evansi field isolates using high throughput “Resistome” deep amplicon sequencing.

Results: Buparvaquone resistance-associated mutations 129G (GGC), 253S (TCT) and 262S (TCA) were present in 21/75 buffalo-derived T. annulata and 19/119 cattle-derived T. annulata isolates. Eighteen buffalo-derived and 6 cattle-derived isolates contained 129G (GGC) resistance haplotype and one buffalo and cattle derived isolate contained 253S (TCT) resistance haplotype at a high-frequency demonstrating evidence of hard selective sweep. Two buffalo-derived isolates contained 253S (TCT) and 129G (GGC)/253S (TCT) resistance haplotypes and 11 cattle-derived isolates contained 253S (TCT), 129G (GGC) and 262S (TCA) resistance haplotypes had equally high frequencies demonstrating evidence of soft selective sweep. Similarly, diminazene resistance-associated mutations GAT(239Y) and AGC(286S) or AGC(286H) were present in 7/24 cattle-derived T. evansi isolates with the evidence of a single origin of resistance-conferring mutations.

Conclusions: Knowledge of the basic parasite genetics through which buparvaquone and diminazene drugs cause resistance is urgently needed, not only to generate new formulations but also to inform sustainable parasite control strategies.

Keywords: Buparvaquone, Diminazene, T. annulata, T. evansi

P460 / #511

Topic: *AS03.4 Prevention and treatment, drug resistance*

RELEVANCE OF FARMERS CRITERIA FOR TARGETED SELECTIVE TREATMENT AGAINST GASTROINTESTINAL NEMATODES IN SHEEP

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Introduction: The increase of gastrointestinal nematode (GIN) resistance to anthelmintics is a worldwide challenge. Targeted selective treatment reduces anthelmintic use and has been shown to slow down anthelmintic resistance development. The choice of animals to be treated is often made by farmers. However, few studies have studied the relevance of the criteria they use. Thus, the aim of this study was to assess the relevance of criteria used by farmers to apply targeted selective treatment.

Methods: The study was conducted in the Drôme Valley, France, in October 2018, on a mixed flock of 98 Mourenous and Merinos sheep (58 ewes, 21 ewe lambs and 19 cull ewes). Five experienced sheep farmers individually estimated the GIN infection level (low, medium, high) of each animal and stated their evaluation criteria guiding their estimate. Concurrently, faecal egg counts (FEC) were performed individually for all sheep using a McMaster method. Linear regression analyses were performed in order to evaluate the correlation of farmers infection estimate and the guiding criteria with FEC.

Results: Average FEC of sheep was quite high with a large variability (687 ± 702 egg per gram). The farmers estimated that 66 % ± 9.3 % of the animals had a medium to high level of GIN infection. FAMACHA and body condition score were used as evaluation criteria by all farmers. Only the estimated infection level ($p = 0.03$) and body condition score of ewes ($p = 0.006$) were significantly correlated with FEC.

Conclusions: Body condition score seems to be a relevant indicator used by farmers for targeted selective treatment.

Keywords: Gastrointestinal nematode, Body Condition Score, Targeted Selective treatment, sheep, Evaluation criteria

P461 / #38

Topic: *AS03.4 Prevention and treatment, drug resistance*

PHOSPHOENOLPYRUVATE CARBOXYKINASE AS A DRUG TARGET IN MANAGEMENT OF HELMINTH PARASITES

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Introduction: Phosphoenolpyruvate carboxykinase (PEPCK, EC: 4.1.1.32) catalyzes the first committed step in gluconeogenesis in higher vertebrates and catalyzes decarboxylation of oxaloacetic acid (OAA) in cestode parasites. This differential activity between the parasites and their hosts in glucose metabolism makes the enzyme a plausible anthelmintic target for management of helminth parasites.

Methods: In order to characterize this important enzyme, PEPCK has been biochemically purified and characterized from the parasite and its host. In this study, protein expression and purification, siRNA, molecular docking, and enzyme kinetics were used in this study.

Results: Biological significance of the enzyme and a functional RNAi pathway by silencing of PEPCK has been demonstrated in the cestode parasite, R. echinobothrida. Comparative specific activities (U/mg of protein) of RePEPCK (PEPCK from R. echinobothrida) and GdPEPCK (PEPCK from Gallus domesticus, the host for R. echinobothrida) for both carboxylation and decarboxylation reactions have also been demonstrated. Molecular docking of few probable inhibitors has also been done in order to elucidate their role in the enzyme kinetics and for survival of the parasite.

Conclusions: Though PEPCK is considered a potential anthelmintic target, recently, it has been explained the requirement of PEPCK for survival of the cestode parasite, which might be considered essential for management of the parasitic infection. Biological significance and in vivo studies need to be performed, so as to exploit the enzyme as a robust therapeutic agent against helminth parasites.

Keywords: Helminths, PEPCK, Anthelminthics

P462 / #1633

Topic: *AS03.4 Prevention and treatment, drug resistance*

IVERMECTIN-INDUCED GENE EXPRESSION IN ADULT PARASCARIS UNIVALENS AND CAENORHABDITIS ELEGANS: A COMPARATIVE APPROACH FOR ANTHELMINTHIC RESISTANCE STUDIES

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Introduction: The nematode *Parascaris univalens* infects horses, but anthelmintic resistance renders treatment challenging. Due to the lack of experimental in vitro models, the molecular mechanisms behind drug activity and resistance remain unknown. The anthelmintic ivermectin was evaluated in *Caenorhabditis elegans* as a model for *P. univalens* drug metabolism/resistance research.

Methods: *P. univalens* adults were treated with ivermectin (10^{-13}, 10^{-11}, and 10^{-9} M) for 24 h at 37 °C, and RNA was sequenced. Differentially expressed genes (DEGs) with *C. elegans* orthologues were identified as candidate genes for IVM metabolism/resistance. At 20°C, IVM (10^{-9}, 10^{-8} and 10^{-7} M) was exposed to 300 C. elegans worms for 4 h. The expression of candidate genes was compared between the worm species after drug exposure.

Results: Adult P. univalens worms had 1085 DEGs after IVM exposure, however the relative gene expression changes were minor and there was large variability amongst worms. Despite dose-dependent behavioral effects seen in C. elegans after IVM administration, none of the 15 DEGs chosen in P. univalens for further characterization responded to IVM in *C.elegans*, including the putative drug target lgc-37. The overlap in IVM induced gene expression was minimal in adult worms of both nematode species.

Conclusions: Using *C. elegans* as a model, we used comparative gene expression to understand IVM metabolism/resistance in *P. univalens*. However, finding conserved genes involved in IVM metabolism/resistance in *P. univalens* and *C. elegans* proved difficult. The modest number of genes studied (n=15) limits the approach's potential. Future research comparing more genes between the two species may find additional drug metabolism and/or resistance genes.

Keywords: equine roundworm, gene expression, anthelmintic resistance, RNA sequencing, Parascaris

P463 / #1332

Topic: *AS03.4 Prevention and treatment, drug resistance*

DETERMINING ALBENDAZOLE SENSITIVITY IN FASCIOLA HEPATICA ISOLATES BY IN VITRO EGG HATCH TEST

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Introduction: Fasciola hepatica causes severe morbidity and mortality in ruminant livestock, costing >£40.4 million annually. Reliance on triclabendazole (>99% effective) against F. hepatica has led to resistance. Thus, farmers rely on alternatives e.g. albendazole (ABZ). Here, we validated the published egg hatch test in house (Ceballos et al, 2019) and determined ABZ sensitivity of four F. hepatica isolates.

Methods: For each isolate ~200eggs/well for 5 replicates were exposed to three drug concentrations, 5, 0.5 and 0.05 µM ABZ, or methanol only. Sensitivity was determined based on: ovicidal activity (%OA) at the discriminating dose, 0.5µM = [(%eggs developed in control - %eggs developed after drug incubation)/ %eggs developed in control] x100 and a significant difference between egg development in ABZ-treated vs controls by pairwise t-test. Isolates were defined as a) resistant = %OA ≤40% and no statistical difference, b) susceptible = %OA ≥70% and a significant difference or c) equivocal = neither criterion met.

Results: One isolate, E, was ABZ-sensitive %OA 74.0% at 0.5 µM, ($p < 0.001$). One isolate, D21, showed reduced sensitivity to ABZ, %OA 32.4% at 0.5 µM ($p = 0.5063$) and three were equivocal at 0.5 µM, D20, %OA 43.8%, ($p < 0.001$), B, 59.0%, ($p < 0.001$) and S OA%, 35.6% ($p < 0.001$).

Conclusions: Our in vivo findings are consistent with our in vitro observations for isolate E, giving confidence that we can conduct the egg hatch test to determine ABZ sensitivity on isolates without the need for in vivo trials. Our results indicate that at least one isolate (D21) lacks sensitivity to ABZ and the other isolates (D20, B and S) are equivocal, indicating further evaluation of ABZ sensitivity is required.

Disclosure: The authors acknowledge funding from BBSRC DTP, University of Liverpool & Hybu Cig Cymru.

Keywords: Fasciola hepatica, Albendazole, egg hatch test, drug resistance

P464 / #1339

Topic: *AS03.4 Prevention and treatment, drug resistance*

A SURVEY TO EVALUATE FASCIOLA HEPATICA CONTROL PRACTICES OF SHEEP FARMERS ADOPTING PROACTIVE ANIMAL HEALTH PLANNING.

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Introduction: Fasciola hepatica, liver fluke, causes severe morbidity and mortality in livestock, particularly sheep. The Hybu Cig Cymru (HCC) Stoc+ project supports farmers to prioritise sheep health, welfare, and productivity. This study aimed to identify how farmers currently control liver fluke infection and assess the risk flukicide drug resistance poses to effective control.

Methods: From January 2022 - March 2022, 302 HCC Stoc+ sheep farmers were invited to complete an online survey of twenty closed and open-ended questions to evaluate their current liver fluke control practices. The survey was advertised through Stoc+ emails and monthly newsletter.

Results: Most respondents reported the presence of liver fluke infection on farm (47.9%), with 52.1% reporting either uncertainty or absence of liver fluke infection. Almost all respondents (95.9%) reported treating at least once in the last five years, with the majority treating twice a year for liver fluke (57.8%). Of the 129 treatment events reported by farmers, 35.7% reported using triclabendazole and 30.2% used closantel. 60.6% of respondents reported not using diagnostic testing prior to treatment. Few respondents reported concerns about drug resistance (15.1%), but of those, 80% reported having TCBZ resistance.

Conclusions: The results showed that anthelmintic drugs are used to target fasciolosis in the absence of diagnostic testing either before or following treatment, pointing to unnecessary use of flukicides on farm. To best protect the limited number of flukicides, we must advocate more strongly for targeted and selective treatment plans.

Disclosure: The authors acknowledge funding from BBSRC DTP, University of Liverpool & HCC.

Keywords: flock health, drug resistance, Fasciola hepatica, Survey, control practices

P465 / #1475

Topic: *AS03.4 Prevention and treatment, drug resistance*

GIARDIA INFECTIONS IN AN ANIMAL SHELTER IN AUSTRIA: OCCURRENCE, CLINICAL SYMPTOMS AND TREATMENT SUCCESS

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Introduction: Giardia is a common cause of diarrhea in dogs however, infections can also have an asymptomatic course. Treatment failure is reported frequently. As a result, many animal shelters struggle with Giardia-infections. The aim of this study was to detect the frequency of diarrhea in Giardia-positive dogs in an animal shelter and to document the efficacy of treatment.

Methods: For this purpose, samples of 104 dogs were examined with flotation and the Megacore Giardia-AG FASTest®. Fecal consistency was documented. Animals positive for Giardia in flotation or animals with diarrhea were treated with fenbendazole. Three days after treatment feces was examined. Giardia-positive animals with diarrhea and/or positive flotation after this treatment were treated with metronidazole, still positive animals with a combination of fenbendazole+metronidazole.

Results: 17.3% of samples were positive in at least one examination method and 11.5% in both. 59% of animals with positive flotation but no animal with just a positive AG-test showed diarrhea. 50% of animals positive for Giardia had an age of less than one year. 12 animals were treated. First treatment was successful in 8 animals, 4 animals received a second, one a third treatment.

Conclusions: Giardia infections do not always cause diarrhea and a positive AG-test without detectable cyst-excretion might not be an indication to treat. However, as Giardia is a potentially zoonotic agent this recommendation should not be given for dogs living in a household with immunocompromised people unless a species specific genotype is detected

Keywords: treatment, diagnostics, shelter management

P466 / #529

Topic: *AS03.4 Prevention and treatment, drug resistance*

ALBENDAZOLE AND LEUKOCYTE EXTRACT THERAPY STIMULATES TH1/TH2 IMMUNITY IN MESOCESTOIDES VOGAE INFECTION VIA MODULATION OF MACROPHAGE POLARIZATION AND CYTOKINE PROFILES

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Introduction: Model flatworm Mesocestoides vogae infection associated with immunosuppression was used to investigate effect of albendazole (ABZ) and leukocyte extract (LE) and as adjunct therapy.

Methods: Peritoneal exudate cells, their adherent counterparts and peritoneal exudates were studied after termination of therapy.

Results: The highest reduction of larvae was achieved by ABZ+LE therapy, what was associated with expansion of CD11b+F4/80^{mid}MHCII^{high} macrophages of M1 type and diminished populations of CD11b+F4/80^{mid}MHCII^{low} M2 macrophages in comparison with infected and ABZ treated mice. Polarization of adherent macrophages after LE and ABZ+LE therapy was confirmed by decreased gene expression for M2 markers arginase-1, FIZZ-1,Ym-1, IL-10 and transcription factors STAT-3, STAT-6. Expression of M1 marker iNOS was up-regulated as well as INF-γ receptor, STAT-1 and IL-12. NO production by LPS stimulated macrophages from treated groups and naïve cells co-cultured in vitro with LE were suppressed. Therapy with ABZ, LE and their combination differentially modulated transcription profiles and concentrations of IFN-γ, TNF-α, IL -12p40, IL-6, IL-10, and TGF-β cytokines. LE strongly ameliorated ABZ-induced suppression of INF-γ and IL -12 and preserved downregulation for IL-10 and TGF-β. In vitro epigenetic study on adherent M2 macrophages from infected mice confirmed direct effect of ABZ, ABZ sulphoxide and LE on inflammatory gene transcription.

Conclusions: ABZ and LE therapy resulted in activation of proinflammatory cell markers and cytokines and stimulated macrophage polarization towards M1 type resulting in a balanced Th1/Th2 immunity.

Keywords: cestode, infection, mice, Albendazole, immunity

P467 / #473

Topic: *AS03.4 Prevention and treatment, drug resistance*

EPRINOMECTIN-RESISTANT HAEMONCHUS CONTORTUS IN FRENCH DAIRY SHEEP FARMS: CAN WE BALANCE PASTORAL TRADITIONS AND CONTROL OF GASTRO-INTESTINAL NEMATODES?

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Introduction: In French ovine dairy farms, eprinomectin is increasingly used during lactation as it is the only anthelmintic with a zero-day milk withdrawal period, and consequently to the increase in milk withdrawal periods for benzimidazole products that occurred in 2014. In France, eprinomectin is commercially available in sheep as a pour-on dosed at 1 mg/kg or as an injectable solution dosed at 0.2 mg/kg. This overreliance on one molecule in the 2 main sheep cheese regions of France – namely the Roquefort area and the Pyrénées Atlantiques département – where sheep grazing is culturally deeply imbedded and mandatory to comply with European specifications, brings deep concern about the rise of resistant isolates.

Methods: Fecal Egg Count Reduction Tests were conducted in dairy sheep farms of these areas, and eprinomectin concentration was individually measured in treated ewes 2 and 5 days after treatment.

Results: Fecal Egg Count Reduction Tests revealed eprinomectin-resistant isolates of Haemonchus contortus in 21/27 farms explored in this study (Pyrénées Atlantiques) and 1/6 farms (Roquefort area). Eprinomectin concentration in treated ewes 2 and 5 days after treatment in 9 farms confirmed implication of resistant isolates in the FECRT results in 8 farms, and demonstrated on one farm that the observed efficacy default was due to underexposure of the worms to the drug when the pour-on formula was used.

Conclusions: These results plead once more for implementation of Targeted Selective Treatment (TST) using an adapted form of eprinomectin for the control of sheep nematodes, the feasibility of which is currently evaluated by the multi-partner ANTHERIN program led by the Veterinary School of Toulouse (ENVT) as well as the French Agronomical Institution (INRAe).

Disclosure: Although the main author (SJ) is employed by CEVA, her PhD takes place in academic settings. The authors declare no conflict of interest in the present results.

Keywords: eprinomectin, Haemonchus contortus, dairy sheep, resistance, FECRT

P468 / #806

Topic: *AS03.4 Prevention and treatment, drug resistance*

OVICIDAL ACTIVITY OF ETHANOLIC AND ACETONE EXTRACTS OF LEAFS AND FLOWERS OF TANACETUM VULGARE L. AGAINST GASTROINTESTINAL NEMATODES OF SHEEP

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Introduction: One of the most important parasites of sheep is the gastrointestinal nematodes - Trichostrongylidae. Due to the high prevalence of gastrointestinal nematodes and the growing anthelmintic resistance today, new alternatives for their control are being sought. One such alternative method is phytotherapy. The aim of the study is to find out the ovicidal activity of tansy growing in Latvia on sheep gastrointestinal nematodes.

Methods: Extracts were prepared by Rīga Stradiņš University Faculty of Pharmacy. The leafs and flowers of the tansy were extracted separately in 70%, 50% and 30% ethanol and acetone. Six dilutions were prepared from each extract – 500 mg/mL, 200 mg/mL, 100mg/mL, 50 mg/mL, 20 mg/mL and 10 mg/mL. In vitro - the egg hatch test was used to determine the ovicidal activity of the extract. Non-embryonated, embryonated eggs and first stage larvae were counted to calculate the inhibition of eggs (%).

Results: All extracts showed ovicidal activity. Stronger activity showed leaf extract of tansy than flower extracts. According to preliminary data, the highest percentage of inhibition is 200 mg/ml dilution of leaf 50% acetone extract – 96%. Egg inhibition 92% are 500 mg/ml dilution of leaf ethanolic and acetone 50% extracts.

Conclusions: Tansy has ovicidal activity against the Trichostrongylidae of sheep. Future studies are required to determine the larvicidal activity of these extracts and in vivo testing should also be performed. Acknowledgements : This study was funded by the Latvia Ministry of Agriculture and Rural Support Service program LAD16.2 project: The support for pilot projects and for the development of new products, practices, processes and technologies “Development of herbal plant containing medical extracts with anti-parasitic effect”.

Keywords: tansy, sheep, ovicidal

P469 / #1226

Topic: *AS03.4 Prevention and treatment, drug resistance*

ASSESSING THE PREDATORY ACTIVITY OF FILAMENTOUS FUNGI AGAINST EIMERIA SPP. AFFECTING CHICKENS AND PEACOCKS

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Introduction: The use of filamentous fungi with predatory capacities is globally considered an accurate and sustainable approach in gastrointestinal (GI) parasite control programs. However, their testing against avian GI parasites has been scarce thus far. This research aimed to isolate filamentous fungi from avian fecal samples and evaluate their predatory activity against coccidia.

Methods: Between July 2020 and April 2021, a total of 93 fecal samples from free-range chickens and peacocks were collected in a poultry farm and 2 exotic bird collections, in Portugal, and processed using Mini-FLOTAC and Willis-Flotation techniques, to assess their positiveness for coccidia and obtain concentrated suspensions of oocysts, respectively. Fecal samples were also used for isolation of filamentous fungi and their in vitro testing against coccidia oocysts, using Water-Agar (WA) medium and coprocultures.

Results: A total of 7 Mucor spp. isolates were obtained, and all developed lytic activity against coccidia, namely hyphae adhesion on oocysts' walls (activity type 1), deformation (type 2) and lysis (type 3), in both assays. Isolates FR3, QP2 and SJ1 had significant efficacies higher than 70% on limiting oocyst sporulation ($p<0.05$), while isolates FR1, QP2 and QP1 had significant efficacies of 22%, 14% and 8%, respectively, on damaging oocysts' walls, only after 14 days of incubation.

Conclusions: More in vitro assays are needed to confirm the potential use of these fungal isolates in the biocontrol of avian coccidiosis. Funding: CIISA/FMV Project UIDB/00276/2020 and LA/P/0059/2020 - AL4AnimalS (both funded by FCT); Project ED431B 2021/07 (Consellería de Cultura, Educación e Universidades, Xunta de Galicia); João Lozano owns a PhD Research Fellowship 2020.09037.BD (funded by FCT).

Keywords: Mucor spp., Portugal, Birds, Coccidia, Predatory Fungi

P470 / #968

Topic: *AS03.4 Prevention and treatment, drug resistance*

PHARMACODYNAMICS OF BIOSYNTHESIZED SILVER NANOPARTICLES FROM THE CALYX OF ABELMOSCHUS ESCULENTUS: A NOVEL ANTIHELMINTIC STUDY

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Introduction: Helmintic infections are one of the most prevalent diseases in developing and developed countries. The tapeworm parasite Raillietina echinobothrida, which is endemic to countryfowl Gallus gallus domesticus, has caused catastrophic losses to the livestock industry in tropical countries such as India. Typical management strategies involve frequent usage of broad-spectrum synthetic anthelmintics that has shown several cases of drug resistance. Recently, green synthesis of nanoparticles has become popular for its therapeutic effect and environmental friendliness.Integrating the principles of green chemistry into interdisciplinary nanoscience research can pave the way for sustainable methods in the synthesis of nanoparticles.

Methods: In this study, the calyx of lady's finger (Abelmoschus esculentus) belonging to the Malvaceae family was used for biosynthesis of silver nanoparticles (Ag-NP), and the possibility of anthelmintic was evaluated. UV-Vis spectroscopy, XRD, FTIR, SEM, and EDX analysis were performed to determine the formation of AgNP.

Results: The drug has a significant effect on the normal physiological function of tapeworms, causing flaccid paralysis and subsequent parasite death. Changes in ultrastructure and enzymes have shown remarkable effects, thus establishing the potential of its anthelmintic. Histochemical localization study of tegument associated enzymes, i.e. AcPase, AlkPase, ATPase, and 5'NU showed a marked decrease in activity post drug exposure.

Conclusions: The significant loss of activity of neuronal components such as NSE and ChE indicates the role of this green synthetic AuNP as an anthelmintic. Pharmacodynamic studies have also shown that when administered at the same molar dose, it does not adversely affect the host.

Keywords: green chemistry, tegumental enzymes, histochemical localization, antihelmintic, silver nanoparticle

P471 / #848

Topic: *AS03.4 Prevention and treatment, drug resistance*

AN EXPLORATION INTO FARMER'S TRANSPORT PATTERNS BETWEEN FARM AND LIVESTOCK SALES AND THE POTENTIAL RISK OF ANTHELMINTIC RESISTANCE TRANSMISSION

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Introduction: Gastrointestinal nematode (GIN) infections are costly to the livestock industry, affecting the health, welfare, and productivity of ruminants. Resistance to anthelmintic drugs among sheep GIN species is growing. The aim of this project is to assess the risk animal movements through livestock markets may play in the transmission of GIN and resistance to the commonly used anthelmintic benzimidazole (BZ).

Methods: Applied parasitological techniques in conjunction with deep amplicon sequencing (DAS) molecular technologies and transportation data are being used to assess GIN prevalence, species composition and BZ resistance allele frequency in traded sheep in UK livestock marts.

Results: Thirteen UK marts were visited over a five-month period in 2019/20. Faecal egg counts (FEC) were conducted on composite faecal samples opportunistically collected from 491 sheep pens. 91% of the samples were strongyle positive (average 217 eggs per gram (EPG) [range 0-11,268 EPG], with Nematodirus and Monezia eggs also observed in some samples. On average, sheep travelled 61 km (range 0-512 km) to attend market, with only 23% attending their nearest market. DAS is currently underway on 300 sheep samples.

Conclusions: Most sheep sampled carried positive GIN FEC, some very high. The results highlight the need for the development of knowledge exchange strategies to raise awareness of potential spread of roundworm infections and BZ resistance due to livestock transportation and the ongoing role that effective quarantine treatments will play in slowing their spread. Acknowledgements: Veterinary Medicines Directorate and Scottish Government for financial support.

Keywords: Disease Control, anthelmintic resistance, Livestock Health, Next Generation Sequencing

P472 / #1001

Topic: *AS03.4 Prevention and treatment, drug resistance*

ANTHELMINTIC ACTIVITY OF CASSIA OCCIDENTALIS ON INFECTIVE LARVAE OF DONKEY CYATHOSTOMINS

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Introduction: Equids, including horses, donkeys, and mules are reared for production, for social activities, and as working animals. Gastrointestinal nematodes (GINs) are one of the main health problems in equids worldwide. Studies on GINs have uncovered a diversity of species in donkeys, especially small strongyles also known as Cyathostomins. Several authors have reported that drug resistance in GINs of equids has greatly limited the efficacy of synthetic anthelmintics. Alternatives to control GINs using plants extracts are studied in small ruminants and are extended to other species. The use of Cassia occidentalis extracts on GIN species is reported worldwide. One of the assay used to evaluate the activity of plant extracts is the larval exsheathment inhibition assay (LEIA) on infective larvae (L3) of different species of GINs.

Methods: The main objective of this study was to evaluate the anthelmintic (AH) activity of a lyophilized extract from C. occidentalis on donkey GINs by using LEIA. To this aim, fecal samples of donkeys naturally infected by GINs were cultured and L3 were collected and identified as belonging to the cyathostomin genera Cylicocyclus and Cylicostephanus. Ensheathed L3 were incubated with different concentrations (1200/600/300/150 µg/ml in PBS) of the plant extract.

Results: At the highest concentration (1200 µg/ml), C. occidentalis extract was able to inhibit completely (100%) the larval exsheathment, and significant AH effects ($p<0.05$) were observed to the concentration of 300 µg/ml. The EC50 of the extract was 394.649 µg/ml.

Conclusions: In conclusion, C. occidentalis lyophilized extract tested in this study showed interesting in vitro anthelmintic properties against cyathostomin infective larvae, and further studies are encouraged.

Keywords: medicinal plants, resistance to anthelmintic, alternative control, invitro assay

P473 / #606

Topic: *AS03.4 Prevention and treatment, drug resistance*

DEVELOPMENT OF A POUR-ON PHEROMONE FORMULATION TO IMPROVE ACTIVE SURVEILLANCE AND TREATMENT OF LIVESTOCK FOR THE MANAGEMENT OF THE TROPICAL BONT TICK

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Introduction: Research has established that pheromones in combination with pesticide greatly improves treatment efficiencies for the tropical bont tick (TBT), Amblyomma variegatum. Additionally, pheromone combined with CO_2 traps greatly improves active surveillance. However, pheromone technology is not currently used for the control of TBT. The goal of this work is to integrate pheromone technology into current efforts to survey for and eradicate TBT in the Caribbean.

Methods: Current pheromone technology uses pesticide-impregnated plastic strips attached to the tail of the host. The strips are difficult to attach and need to be reapplied often. We developed an easily applied pour-on pheromone formulation for animals and CO_2 traps.

Results: The pheromone pour-on has been tested for adhesion to animal hides and resistance to rain with acceptable results. We are currently optimizing the pheromone concentration of the formulation and will lab and field test them against live TBT this spring and summer.

Conclusions: The Island of St. Croix has a history of TBT outbreaks. Surveillance efforts are underway to determine the extent of TBT on St Croix. Puerto Rico is the next island in the archipelago and is at a greater risk of reinfestation due to active outbreaks on St Croix. From Puerto Rico the risk of TBT movement to the American continent increases significantly. The establishment of TBTs in the USA alone would cause $1.2 billion USD in losses to the livestock industry per year. We believe this technology could be used throughout the Caribbean and endemic regions of Africa to survey for and manage this harmful and dangerous tick more efficiently while using less pesticide, thus reducing direct losses due to tick feeding and the potential spread of human and animal disease vectored by this tick.

Keywords: Amblyomma variegatum, Pheromones, Surveillance, Integrated Pest Management

P474 / #1604

Topic: *AS03.4 Prevention and treatment, drug resistance*

MOLECULAR DOCKING OF LUPEOL AGAINST THREE ANTITRYPANOSOMAL TARGETS

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Introduction: Trypanosomiasis is a parasitic disease caused by the parasite members of the genus Trypanosoma which are broadly categorised into African and American. Efforts are ongoing to search for newer agents against the disease in response to the limitations of the available therapies. Some newer drug targets have been identified which include adenosine kinase, triose phosphate isomerase and uridylyl transferase. In the present study, molecular docking was used to understand the effect of lupeol on the three antitrypanosomal targets.

Methods: Chimera was used to prepare the ligand and the receptors followed by the molecular docking using Autodock Vina.

Results: Lupeol was found to bind to adenosine kinase, triose phosphate isomerase and uridylyl transferase with minimum binding free energy values of -8.7, -6.9 and -8.1 kcal/mol respectively. Hydrogen bond was not involved in the binding event for any of the ligand-receptor interactions possibly because of the hydrophobic nature of the ligand.

Conclusions: With the lower binding energies observed, the three enzymes are targets for the antitrypanosomal activity of lupeol reported in various preclinical studies available in the literature.

Keywords: lupeol, adenosine kinase, triose phosphate isomerase, uridylyl transferase, antitrypanosomal

P475 / #889

Topic: *AS03.4 Prevention and treatment, drug resistance*

EXPOSURE OF MEAT SHEEP TO STRONGYLICIDES IN THE DEUX-SÈVRES COUNTY (FRANCE) IN 2020: A PRESCRIBER SURVEY

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Introduction: Strongyle infections of grazing sheep are usually managed by anthelmintics (AH). However, due to resistance of strongyles, there is an urgent need to reduce their use by the development of the integrated management of parasitism. In the present survey, a method developed to monitor the antibiotics' sales was adapted to AH prescription to describe the sales of AH in 2020 in one of the main ovine French counties.

Methods: Five veterinary clinics and three structures allowed to prescribe strongylicides to their customers in the Deux-Sèvres county were asked to indicate through an online survey the number of units of each medicine containing strongylicide molecules they had prescribed to sheep owners in 2020. Using these data, sales and exposure indicators were calculated.

Results: The total volume of prescriptions for sheep in 2020 amounted to 115.5 kg of strongylicides with mebendazole, fenbendazole and closantel accounting for 70% of the tonnage. Regarding exposure, the sheep were mainly exposed to moxidectin followed by ivermectin, these two molecules representing 54% of the total body weight treated. Finally, results showed that, in average, each sheep could have received 2.56 AH treatments in 2020.

Conclusions: These data represent the first description of strongylicide exposure of sheep in France. We described the initial situation and using the same method in the same area, we will be able to follow over time the effects of the development of the integrated parasite management on the level of use of anthelminthics. The authors acknowledge all the veterinarians and structures who answered the survey and the Région Nouvelle-Aquitaine for its financial support.

Keywords: anthelmintics, sheep, sales, France

P476 / #397

Topic: *AS03.4 Prevention and treatment, drug resistance*

EQUINE CYATHOSTOMIN ENVIRONMENTAL CONTAMINATION IN ANDEAN GRASSLANDS OF ECUADOR: PRELIMINARY STUDY IN WINTER

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Introduction: Anthelmintic resistance (AR) in cyathostomins is a health problem in horses due to incorrect deworming programs and husbandry practices. Integrated control of cyathostomin includes fecal monitoring, manure removal and co-grazing, but deworming remains crucial practice. Climatic conditions can influence cyathostomin oviposition, development and larval survival in the environment. Therefore, it is essential to recognize the season when environmental contamination occurs to apply strategic control, which can lead to reduced risk of infection in horses under grazing and lowed selection of AR. This work aims to determine equine cyathostomin oviposition in the Andean grasslands environment.

Methods: This study was performed at Bombolí mountain (-0.455277, -78.675999) in the Ecuadorian Andes during winter (August 2021 to February 2022). Nine adult horses (not dewormed in the previous six months) were selected and maintained under extensive rotative co-grazing with cows. Fecal samples were collected every 15 days analyzed using the modified McMaster technique to determine strongyle eggs per gram (epg). Horses were classified as high (>500epg), moderate (200-500epg) and low (<200epg) contaminators. Temperature (TEM) and relative humidity (RH) were recorded.

Results: A high RH (95.0±6.6%) and low TEM (10.0±1.6°C) were recorded. Strongyle-type eggs (99.2%) and Anoplocephala spp (0.8%) were identified in fecal samples. High cyathostomin oviposition was recorded (1561.7±709.3 epg), where 98.4% of the horses were high contaminators (HPG>500epg) and 1.6% moderate (200-500epg).

Conclusions: There is high environmental contamination with cyathostomin in Andean Ecuadorian grasslands in winter; hence this season is optimum to apply a strategic deworming.

Keywords: Oviposition, weather, horses, HPG, cyathostomin

P477 / #1143

Topic: *AS03.4 Prevention and treatment, drug resistance*

EFFICACY OF NICOTIANA TABACUM AND ITS FRACTIONS AGAINST CYPERMETHRIN RESISTANT CATTLE TICKS (RHIPICEPHALUS MICROPLUS)

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Introduction: Among different species of ticks, cattle tick, Rhipicephalus (R.) microplus is considered to be of great economic significance. Unfortunately, R. microplus has developed resistance against all acaricides. So there is a need for alternate control programs. Nicotiana (N.) tabacum is widely used in ethnoveterinary medicine for the control of ticks, but its efficacy against acaricide-resistant ticks is not known. Therefore, the present study was designed to check the efficacy of N. tabacum and its various fractions against cypermethrin-resistant cattle tick.

Methods: Crude aqueous-methanol extract (CAME) of N. tabacum was prepared by soaking the powdered plant material in an aqueous-methanol (30:70) solvent. Different fractions (petroleum ether, ethyl acetate, chloroform, methanol and water) of CAME were also prepared by using standard phytochemical procedures. For all in vitro trials with crude extract or fractions, syringe test was used (modified larval immersion test). Mortality (%) results obtained in vitro were subjected to Probit analysis to calculate $LC_{50,}$ LC_{90} and LC_{99}. Qualitative analysis of these fractions was done to check the active group of compounds.

Results: The most effective fraction was found to be CAME of N. tabacum (LC_{50} = 0.02; LC_{99} = 0.56), followed by petroleum ether (LC_{50} = 0.07; LC_{99} = 2.2) and water fraction (LC_{50} = 0.10; LC_{99} = 6.2). Phytochemical analysis revealed the presence of secondary metabolites like carbohydrates, alkaloids, flavonoids, tannins, phenols, glycosides, volatile oils, fixed oils and saponins.

Conclusions: Crude aqueous methanol extract and various fractions of N. tabacum were found to be effective against cypermethrin-resistant R. microplus.

Keywords: Pakistan, ethnoveterinary medicine, plant extract, acaricide resistance

P478 / #587

Topic: *AS03.4 Prevention and treatment, drug resistance*

ON THE SUCCESSFUL ERADICATION CAMPAIGN OF ECHINOCOCCUS AND TAENIA CESTODES FROM DOGS IN ICELAND

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Introduction: In the past five cestode species have been reported to parasitize dogs in Iceland. Four of them have already been eradicated, Taenia ovis, probably introduced from USA in the 1930s, still sporadically occurs in dogs and sheep in Iceland.

Methods: In the 19th century Echinococcus granulosus was very common.

Results: Based on autopsy reports the infection prevalence of hydatid disease in humans born during 1861–1870 was estimated to be 22%, 28% of dogs were infected. During the late 19th century public education programs, reduction of the dog population, and a ban on feeding raw offal to dogs, complimented later in the 20th century by prohibiting the import of dogs from other countries, meat inspection and targeted intervention on infected farms, successfully reduced this high prevalence that finally resulted in elimination of this dreadful parasite on the island. Autopsies of humans born in Iceland in the 20th century revealed only eight cystic echinococcosis cases. The last human case acquired the infection in the 1950's, the last hydatid case in sheep was reported in 1979.

Conclusions: The long-standing eradication campaign also eliminated three other dog tapeworms. First to disappear, probably already prior to the Second World War, was Taenia multiceps. Somewhat later Dipilidium caninum also disappeared, not least due to the fact that its intermediate host, the human flea Pulex irritans, became extinct. Last to disappear was Taenia hydatigena, its cysts have not been detected during routine inspections in Icelandic slaughter houses since 2008.

Keywords: Echinococcus, Taenia, eradication, Iceland

P479 / #1263

Topic: *AS03.4 Prevention and treatment, drug resistance*

IN VITRO IMMUNE EFFECTS OF CAPRINE PBMC ON THE MOTILTIY OF INFECTIVE STAGE LARVAE OF TRICHOSTRONGYLID NEMATODES COMPARED WITH ANTHELMINTICS AND HERBAL EXTRACTS

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Introduction: Trichostrongylid parasites present a global animal health challenge amplified by development of anthelmintic resistance. To control resistant worm populations , alternative methods for livestock producers is imperative. Study aimed to explore the immune effects of caprine PBMC compared with anthelmintics and herbal extracts against trichostrongylid parasite.

Methods: Infective larvae from coproculture and PBMCs were harvested from caprine blood using density gradient medium. Anthelmintics viz albendazole, levamisol and ivermectin were exposed in vitro to five doses of 0.01 µg/mL, 0.1 µg/mL 1.0 µg/mL, 10.0 µg/Ml and100 µg/mL of each drug :and examined at 0h, 1h, 2h, 4h, 8h, 12h, 24h and 36h for larval sinusoidal movement under photo microscope. Azadirachta indica and Moringa oleifera leaf extract concentration were 1%, 5% and 10% concentration and PBMC concentration 1 x 10^6 cells, 2 x10^6 cells and 4 x10^6 cells in culture medium were applied and examined as described earlier.

Results: Ivermectin appears to be the most potent drugs that rapidly paralyze larvae than albendazole or levamisole at all doses in a dose-dependent manner. In herbal extracts of Azadirachta indica treatment, paralysis was started around 24-36 hours. In PBMC treatment, paralysis was started around 12-24 hours and all larvae were found dead. In addition, PBMC induced rapid paralysis earlier during 12-24 hours compared to anthelmintics and plant extracts.

Conclusions: Due to immune effects, PBMC may induce rapid paralysis of the Trichostrongylid larvae earlier compared to anthelmintis and herbal extracts.

Keywords: Trichostrongylid larvae, immune effects, PBMC, anthelmintics, herbal extracts

P480 / #386

Topic: *AS03.4 Prevention and treatment, drug resistance*

EFFICACY OF TOPICAL ADMINISTRATION OF PRALLETHRIN-PERMETHRIN-PIPERONYL BUTOXIDE COMBINATION FOR THE TREATMENT AND CONTROL OF FLIES AND OTHER NUISANCE INSECTS IN HORSES

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Introduction: Pyrethrins and pyrethroids have been widely used for many years to control insect pests. Their effectiveness against arthropods affecting different animal species is well known, but there is a lack of data regarding their use in horses. The aim of the study was to evaluate the repellent activity of a spray formulation based on prallethrin (0.033%) and permethrin (0.10%), synergized with piperonyl butoxide (0.50%), against annoying and harmful insects for horses in field conditions.

Methods: A horse stable in the Tuscany region, Italy, was chosen for the trial. Twelve horses infected with a minimum of 15 flies were selected and divided into two groups (control and treated). Insect counts were performed on Day 0 (before product administration), and for three subsequent days, at 1, 10, 20 and 30 minutes and at 1, 2, 3, 4, 5 and 6 hours after the administration. The product (Bronco® Equine Fly Spray, Farnam) was applied on all parts of the horses, including mane, head and tail. Insect counts were carried out at different time intervals post-treatment, by three different operators.

Results: One minute after the administration of the product, all the horses were negative for the presence of insects. The repellent efficacy for Hippobosca equina, tabanid flies and Simulium spp. remained higher than 93% for all 4 days pt. Efficacy against Musca domestica and M. autumnalis was 100% after 1-minute and remained at this level for M. autumnalis till 6 hours. The efficacy against M. domestica decreased to 89.1% at 10 minutes pt and only reached 53.5% at 6 hours pt.

Conclusions: The treatment is safe and effective in killing and repelling insect pests in horses. Residual activity lasted four consecutive days after treatment.

Disclosure: The field trial was performed by Prof. Marco Genchi together with GV and GA after having signed a contract registered at the Veterinary Medicine Department of Parma between the company and the parasitology unit.

Keywords: horses, flies control, nuisance insects control, prallethrin, permethrin

P481 / #695

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

MOLECULAR EPIDEMIOLOGY OF THEILERIA IN TICKS AND ITS POTENTIAL TRANSMISSION TO DOMESTIC ANIMALS IN THE REPUBLIC OF KOREA

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Introduction: Theileria spp. are tick-borne protozoan parasites and cause theileriosis in domestic and wildlife animals. The purpose of this study was to investigate prevalence of Theileria spp. in ticks in Korea and to assess their potential transmission from wildlife to domestic animals.

Methods: A total of 12862 hard ticks were collected in Chungcheong- and Jeolla-provinces in Korea from march to October 2021, on grazing close to livestock farms (horse, deer, goat, and cattle). Of the collected ticks, 4309 ticks (438 pools) were selected for screening of Theileria by PCR. Phylogenetic analysis was performed for species identification and molecular characterization of Theileria spp. identified.

Results: By PCR, Theileria spp. were detected in ticks (200/438 pools, 45.66%) and phylogenetic analysis showed the presence of T. luwenshuni (167/200; 83.50%) and Theileria sp. (47/200, 23.50%) which have close relationship with Theileria spp. identified in deer. Minimum infection rate (MIR) for Theileria spp. was the highest in Haemaphysalis longicornis (MIR: 8.74), followed by Ixodes nipponensis (MIR: 5.12), H. flava (MIR: 1.07), and Haemaphysalis sp. (MIR: 0.14). No positive case was identified in Amblyomma testudinarium. Sequence analyses showed high identity with the previously documented sequences in Korea.

Conclusions: This study showed that the hard ticks in Korea have responsibility on the transmission of Theileria, however, the possibility of Theileria transmission from wildlife to domestic animals is low especially in cattle, horse, and goat.

Keywords: tick, Tick-borne disease, Theileria, phylogeny, Haemaphysalis

P482 / #296

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

DETECTING OF LEISHMANIA PARASITE IN URBAN RATS EXHIBITING SPLENOMEGALY IN IRAN

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Introduction: Urban rats of the Muridae family (Rattus rattus, Rattus norvegicus) are known reservoirs for several pathological agents including parasitic ones. Amongst those parasitic infections exhibiting splenomegaly in rodents mainly rats, Leishmania sp. is of well-known example. In this survey, the carcasses of rats with abnormal size of spleen were investigated to find the possible causative parasitic agent.

Methods: Rats have previously been collected by the municipality capital city of Tehran in 2000 and 2012 under the ongoing pest control program. Tissues processing was performed using conventional laboratory techniques to obtain 4–5 µm-thick sections. Tissue sections were stained with hematoxylin and eosin (H&E) and finally examined under a light microscope. A molecular study was implemented aiming to identify hidden parasites using the PCR technique.

Results: Histopathological sections did not show any signs of parasitic infections while DNA extraction lightened the involvement of studied rats with Leishmania sp. to some extent. Although the presence of Leishmania sp. is confirmed until now nevertheless our investigation will be continued until a definite confirmation.

Conclusions: The present study exhibits the circulating of Leishmania parasite "most probably the major species" in rats in Tehran. Further studies should follow the procedure on more samples. This study has emphasized the transmission possibility of this vector-borne infection to humans and other capable mammalian hosts. In conclusion, aiming to prevent zoonotic infection transmission, pest control programs should be regarded intersectoral as recommended in One Health.

Keywords: Urban areas, Leishmania, Rattus

P483 / #1396

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

TOWARDS CONTROL OF TICKS AND TICK-BORNE PATHOGENS UNDER ONE HEALTH PERSPECTIVE IN MEXICO

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Introduction: Ticks and tick-borne pathogens (tbp) negatively impact humans, domestic and wild animals. In Mexico, associated problems with ticks include tick resistance to acaricides, pesticide contamination of products destined to human consumption, environmental contamination, and the increase of emerging tick-borne diseases. The recent outbreaks of Rocky Mountain Spotted Fever and increasing cases of Lyme borreliosis, anaplasmosis, ehrlichiosis and other emerging tick-borne diseases in humans require attention focused on control of tick vectors.

Methods: Herein we analyzed the information generated during the last ten years of research by public academic institutions including epidemiological surveillance in domestic and wild animals, with specific actions like appropriate use of acaricides, control of mobilization and translocation of wild animals; as well as education and collaboration of veterinarians, public health, and wildlife specialists to achieve tick control under one health perspective.

Results: Preliminary results indicate that Rhipicephalus microplus and R. sanguineus are the two most common ticks affecting domestic animals, while Amblyomma mixtum, R. sanguineus, and Ixodes spp. are found parasitizing domestic, wild animals, and humans. However, information on ticks in wildlife is still scarce.

Conclusions: Therefore, we conclude that a research agenda including tick surveillance and identification of tbp for a better understanding of pathogen transmission at the wildlife-domestic-human interface is required.

Keywords: Ticks, mexico, tick-borne-pathogens

P484 / #1361

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

FIRST RECORD OF DIROFILARIA IMMITIS IN CHILE IN AN IMPORTED DOG

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Introduction: Dirofilaria immitis is a zoonotic filarial nematode transmitted by mosquitoes to domestic and wild carnivores. It has a worldwide distribution and is reported in most countries in the Americas except Chile. Only Achantocheilonema spp. and a D. repens-like have been found. The country's geographic isolation and strict sanitary border control, have kept the country free of several pathogens, including D. immitis. However, the vector needed for its transmission is present in Chile.

Methods: In January 2022, a five-year-old female dog born in Venezuela and brought to Chile three years ago was presented to a veterinary clinic due to inappetence, emesis, and vulvar discharge after mating two months before the consultation. Suspecting a uterine infection, the veterinarian requested an ultrasound and bloodwork and proceeded with antibiotic treatment (enrofloxacin). The dog seemed to recover after the treatment. However, the animal deteriorated quickly and was taken to a second veterinary clinic. The presence of microfilariae was detected. Knott test, measuring of microfilariae and PCR-sequencing for D. immitis were performed.

Results: Ten microfilariae measured 285.6 µm as average. PCR amplified a gene section of the expected size for D. immitis, and the sequencing confirmed D. immitis showing 100% homology with the reference gene. Bloodwork showed severe anaemia and renal failure. An echocardiogram did not show the presence of adults worms. The dog deteriorated quickly and was euthanised after seven days.

Conclusions: This is the first confirmed report of D. immitis in Chile. However, the presence of the parasite was suspected before in imported dogs, but never published. It is possible that D. immitis is circulating in Chile, future surveillance work is needed.

Keywords: Dirofilariosis, Dirofilaria immitis, Zoonosis, Chile, vector-borne

P485 / #763

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

TICK-BORNE PATHOGENS IN CLIENT-OWNED DOGS VISITING VETERINARY CLINICS IN SRI LANKA

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Introduction: Tick-borne pathogens (TBP) cause significant diseases in canines from the tropics. In Sri Lanka, there has been limited or no data available to accurately evaluate the prevalence and associated risk factors of TBPs in pet dog populations. In this study we close this knowledge gap to better facilitate diagnosis, treatment, prevention, and control of TBPs and associated diseases in Sri Lankan dogs.

Methods: Whole blood samples obtained from 423 pet dogs across five provinces in Sri Lanka were screened using a previously validated multiplex qPCR assay able to detect the six most prevalent canine TBPs in the Asia Pacific. Animal data were obtained through an owner-centered survey.

Results: A total of 254 dogs (60.05%, 95% CI: 55.31-64.61) were infected with one or more TBP. Of these, Babesia gibsoni was the most prevalent (37.35% , 95% CI: 32.87-42.06) with older (> 2 years) dogs more likely to be infected than younger (≤ 2 years) dogs (OR 1.89, CI 95% 1.24- 2.90; p<0.05). Babesia vogeli, Hepatozoon canis, Ehrlichia canis, Anaplasma platys, and haemotropic mycoplasma were found in 4.96% (95% CI: 3.23-7.51), 21.04% (95% CI: 17.42-25.18), 4.26% (95% CI: 2.67-6.67),3.78% (95% CI: 2.30-6.10) and 9.93% (95% CI: 7.41-13.17), respectively. All TBPs were detected with statistically significant differences in prevalence between provinces (p<0.05), except for haemotropic mycoplasma (p=0.196). A statistically significant difference for infection and sex was only found for this latter pathogen, with males more likely to be infected than females (p<0.001).

Conclusions: Given the high endemicity of all investigated TBPs in client-owned dogs in Sri Lanka, utilization of effective products with repellent activity that would prevent tick feeding is strongly recommended.

Keywords: Babesia, Dog, tick, Sri-Lanka

P486 / #604

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

OCCURRENCE OF LEISHMANIA INFANTUM IN UNUSUAL WILD MAMMALS FROM SPAIN

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Introduction: Leishmania infantum is a zoonotic parasite that affect multiple species of domestic and wild animals, including humans. It is endemic in the Mediterranean basin, where interaction of wildlife, such as rabbits, hedgehogs, bats, minks, etc., and L. infantum has been detected in several studies, due to their proximity to human populations and their role in outbreaks of human leishmaniasis. The aim of this study is to analyse the presence of L. infantum in different tissues of mammalian species using various DNA targets.

Methods: Spleen and ear skin samples from 115 animals, including European hedgehog (Erinaceus europaeus), Algerian hedgehog (Atelerix algirus), red squirrel (Sciurus vulgaris), European badger (Meles meles), weasel (Mustela nivalis), stone marten (Martes foina), European polecat (Mustela putorius) and garden dormouse (Eliomys quercinus) from the Spanish provinces of Madrid, Valencia, Toledo, Guadalajara and Ávila were analysed by PCR and nested PCR, using the following targets: Repeat region, SSUrRNA and ITS1. Positive samples were sequenced.

Results: In total, 15 animals (13.04%) tested positive for any of the samples or targets used. The highest percentage of positives was detected in samples of spleen from badger, using nested PCR with SSUrRNA (33.3%), followed by spleen from red squirrels (12.5%) and ear skin from hedgehog (8.95%), both with the Repeat region target. The highest sensitivity was detected in spleen samples, except in hedgehogs, where skin reported the highest number of positives.

Conclusions: These results highlight the presence of L. infantum in different mammalian species and tissues and the need to analyse them employing various samples when leishmaniasis outbreaks occur.

Keywords: PCR, Nested PCR, Leishmania infantum, wildlife

P487 / #1747

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

DETECTION OF FASCIOLA HEPATICA EXOSOMES CARRYING SPECIFIC BIOMARKERS IN INFECTED MICE.

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Introduction: Although there are currently diagnostic methods for Fasciolosis new methods are necessary. The extracellular vesicles (EVs) are small cell secreted membrane-delimited structures detectable in many body fluids carrying specific markers. . Studies on extracellular vesicles, in particular the exosomes, looking for biomarkers had spred rapidly. Exosomes are small membrane-delimited vesicles (diammeter 30-100nm) secreted by most living cells. We postulated that exosomes released in vivo by the parasite may contain characteristic molecules that could differentiate them from exosomes released by host cells during infection.
The aim of this work was detection of *Fasciola hepatica* infection markers in serum exosomes of CD1 mice experimentaly infected.

Methods: Ten CD1 mice were infected with 20 F. hepatica metacercariae and bleed to obtained sera. Exosomes were purified following MISEV2018, from parasite excretion/secretion products, normal and infected sera at 20 weeks post infection. Protein profiles of exosomal fractions were compared by SDS-PAGE and inmunoblot with quimioluminicence detection using specific antibodies raised against two screted parasite enzymes.

Results: The results showed that althought both enzymes were detected whithin exosomes isolated from adult fluke culture, the exosomes circulating in the blood of the infected mice at 20 weeks p.i. carried one of the parasitic antigens studied. More encouragingly, we found that this biomarker was not present in the circulating exosomes of normal, uninfected mice.

Conclusions: These findings demonstrate that *F. hepatica* exosomes circulate in the blood of infected animals, that they are detectable and distinguishable from healthy animals and consequently carry biomarkers of diagnostic value.

Keywords: Biomarkers, diagnosis, Fasciola hepatica, exosomes

P488 / #1732

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

WOLBACHIA STRAIN W-ANGA IS NOT STABLE IN LABORATORY WITHIN MOSQUITOES BUT ITS PREVALENCE IS NEGATIVELY CORRELATED WITH PLASMODIUM FALCIPARUM WITHIN WILD MALARIA VECTORS

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Introduction: In a context of vector resistance to available insecticide molecules, biological control would be a complementary method to malaria control. In this perspective, the use of endosymbiotic bacterium *Wolbachia* has been explored. However, to optimize the performance of this bacterium in control strategies, it is imperative to evaluate the stability of its incorporation in the genetic background of *Anopheles*, the malaria vector. Also, having good knowledge on a potential impact of the bacterium on the presence of *Plasmodium* in would be necessary

Methods: *Anopheles gambiae* complex mosquitoes were collected in Western Burkina Faso. Then blood fed and gravid female mosquitoes were oviposited individually. After oviposition, the species of the parent females was determined, followed by their *w-Anga* infection status. Then, *Plasmodium falciparum* infection status was determined by PCR. Finally, the stability of *w-Anga* transmission was assessed by determining the generational infection rate of positive females and their offspring

Results: *Anopheles coluzzii* species was the most infected to *w-Anga* with a proportion of 92.45% of infected mosquitoes, and the transmission frequency of this bacterium from infected females of the F0 generation to F1 offspring was 13.67%. Furthermore, *w-Anga* positive *Anopheles coluzzii* females were less infected with *Plasmodium falciparum* compared to negative females.

Conclusions: Natural *w-Anga* infections are present in field mosquito populations and are negatively correlates with the presence of malaria parasites. However, these infections are not stable in the laboratory assays.

Keywords: Anopheles, Wolbachia, plasmodium, Burkina Faso, Malaria

P489 / #1414

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

SHELTER DOGS AS A SENTINEL RESERVOIR FOR TOXOCARIASIS RISK IN THE U.S. STATE OF MISSISSIPPI

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Introduction: Human toxocariasis is a primarily soil-transmitted helminth (STH) zoonosis which is highly prevalent among socioeconomically disadvantaged populations worldwide. Recent case reports of ocular toxocariasis and the high seroprevalence of Toxocara exposure in Mississippi, the poorest state in the U.S., have raised public health concerns. Infected dogs as a reservoir for Toxocara canis may be an important risk factor.

Methods: A total of 252 canine fecal samples from puppies and adult dogs were collected from animal shelters located in 14 Mississippi counties. The SAF-preserved fecal samples were transported to the Federation University and screened for intestinal parasites by examining a single coverslip of a formalin-ethyl acetate concentrate sediment by microscopy. Statistical analysis was performed using SPSS and Microsoft Excel.

Results: The prevalence of canine toxocariasis in Mississippi was 17%. A significantly higher prevalence of Toxocara infection was found in puppies (30%) when compared with adult dogs (7%). The geometric mean intensity of infection was 11 eggs per coverslip. Puppies had a significantly higher intensity of Toxocara infection when compared to adult dogs.

Conclusions: This pilot study demonstrated a prevalence of T. canis infection in MIssissippi shelter dogs was far higher than the diagnostic prevalence in dogs (1.8% and 2.0%) in a recent (2016) report. This may reflect a much higher burden of T. canis infection in dogs from lower income households, representing the group of people most vulnerable to human toxocariasis. To effectively control zoonotic transmission of T. canis from the domestic dog population, a ‘One-Health’ approach, involving a close collaboration between veterinary and public health professionals, is required.

Disclosure: This work was funded by the University of Mississippi Medical Center

Keywords: toxocara, Toxocariasis, South East United States

P490 / #856

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

UTILITY OF SERUM AMYLOID A (SAA) CONCENTRATIONS IN CATS WITH AELUROSTRONGYLUS ABSTRUSUS TO DETERMINE SEVERITY AND PARASITE LOAD.

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Introduction: Feline aelurostrongylosis (Aelurostrongylus abstrusus) is characterized by inflammatory cell infiltrates in bronchi and lung parenchyma. Serum amyloid A (SAA) is a major acute phase protein in the cat and has shown utility as a prognostic indicator. Its adequacy to evaluate the severity of aelurostrongylosis has not yet been demonstrated, so the aim was to determine concentrations of SAA in cats with A. abstrusus.

Methods: SAA was measured in serum from 8 symptomatic cats infected by A. abstrusus, detected on Baermann examination. Cats were grouped into low (n=3; <1000 larvae/gram of feces) and high parasite load (n=5; >1000 larvae/gram of feces). To determine the clinical status of the patients, thoracic radiographs, a complete blood count and biochemistry were performed.

Results: Cats with high parasite load showed pathological values of SAA (1826±1282 mcg/mL); furthermore, monocytosis was present in all hematological exams. Cats with low parasite load showed normal concentrations of SSA (<5 mcg/mL) and normal blood count. No anomalies were found in the biochemical analysis in any of the cats. A marked severe broncho-interstitial pattern and focal alveolar pattern were observed in thoracic x-rays of cats with high burden, and a mild-moderate broncho-interstitial pattern in cats with low burden.

Conclusions: SAA may be a reliable biomarker to determine lung inflammation and severity of infection in cats with high parasite load. The results indicate that lung inflammation in cats with a low parasite load may not be significant, although all cats studied showed clinical signs related to aelurostrongylosis. These are preliminary results and a higher number of cats are being studied to determine the real utility of SAA.

Disclosure: This research was funded by the Ministry of Science and Innovation. Government of Spain. CDTI IDI-20200056.

Keywords: Aelurostrongylosis, Serum amyloid A, cats

P491 / #372

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

PATHOGENS IN FLEAS COLLECTED FROM DOMESTIC DOGS IN UZBEKISTAN: PRELIMINARY RESULTS

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Introduction: Dogs live in close contact with humans putting them at risk by carrying fleas and zoonotic flea-borne pathogens into human settlements. The most common fleas infesting dogs are species of the genera Ctenocephalides and Pulex irritans, depending on the geographic location. However, some areas, such as Central Asia, were poorly investigated on this matter. The aim of the present study was to determine the species diversity of fleas infesting domestic dogs in Uzbekistan and to detect the pathogens transmitted by these.

Methods: Fleas were collected (December 2020 – February 2021) from 77 domestic dogs in different regions of Uzbekistan, and preserved in ethanol. DNA was extracted with the preservation of the exoskeletons. Morphological identification and molecular characterization cox1, was done for each flea individually. DNA was molecularly tested for the presence of Rickettsia spp., Bartonella spp. and Acanthocheilonema reconditum.

Results: A total of 198 fleas were collected and identified to species level. Most of them belonged to the Ctenocephalides genus (88.38%) and the rest were Pulex irritans (11.11%). Of Ctenocephalides genus, 64.6% were C. canis (n=113), 30.3% C. orientis (n=53) and 5.14 % Ctenocephalides spp. (n=9). The ones identified only to genus level failed to amplify. Bartonella spp. were detected in 2% (n=4) of the fleas of genus Ctenocephalides. In the fleas of the same genus, 2% (n=4) were found to be infected with A. reconditum. Rickettsia spp. were identified in 28.8% fleas (n=57) from both genera.

Conclusions: C. canis is the dominant flea – species in domestic dogs in Uzbekistan. Vector-borne pathogens were confirmed in fleas, highlighting the importance of further studies and pathogen surveillance.

Keywords: Uzbekistan, Ctenocephalides, fleas, pathogens

P492 / #528

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

CRYPTOSPORIDIUM SPP. IN YOUNG DOGS

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Introduction: Cryptosporidium is a protozoan parasite causing diarrhoeic disease in a broad range of hosts. The aim of the herein presented study was to monitor young dogs from Eastern Germany within their first year of life for Cryptosporidium infection. Furthermore, the association between infection status and age, or faecal score was examined.

Methods: In sum, 349 faecal samples of overall 171 dogs from Eastern Germany were screened. The samples were organized into 4 age groups depending on the dog's age at the sampling time point. Following a faecal scoring, samples were analysed by PCR targeting the small subunit ribosomal RNA gene (SSUrRNA), or the 60 kDa glycoproteine gene (gp60). The obtained PCR products were subsequently sequenced. Associations between infection and age, or faecal score were evaluated.

Results: In total, 10% of the examined faecal samples were positive for Cryptosporidium. Most infections were detected in the age group 10 weeks-5 months (p=0.009). The faecal scoring revealed that most samples were classified as firm (n=214) or soft (n=111), and no significant association between faecal score and the infection status was identified. In 94.3% (33/35) of Cryptosporidium positive samples, infection was caused by C. canis, whereas only two dogs were infected with the zoonotic C. parvum subtype IIaA15G2R1.

Conclusions: In this study, it was shown that C. canis as well as C. parvum were detectable in young German dogs. Recurrent detection of C. canis in two dogs hint at the possibility of re-infection or chronic infection in young dogs with this parasite. The C. parvum subtype IIaA15G2R1 found in this study is also the most common one in calves, leading to the suggestion that dogs living near to cattle farms may be at higher risk for infection with zoonotic C. parvum subtypes.

Keywords: Cryptosporidium, young dogs, zoonoses

P493 / #871

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

MOLECULAR EPIDEMIOLOGY OF LEPTOSPIRA NOGUCHII REVEALS IMPORTANT INSIGHTS INTO A ONE HEALTH CONTEXT

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Introduction: Leptospirosis presents a complex and dynamic epidemiology. Bovine leptospirosis has been described as a major infectious disease impairing reproductive efficiency. Although infections by Leptospira interrogans, L. santarosai and L. borgpetersenii are frequently reported in cattle, the presence of L. noguchii in these animals should not be neglected.

Methods: Serological (MAT) and molecular characterization (rrs and secY gene sequencing MLST and PFGE) of eight L. noguchii strains obtained from cows were performed. Intraspecific genetic diversity was evaluated, and haplotype networks were constructed based on hosts and geographical localizations.

Results: Strains were characterized as belonging to serogroups Australis, Autumnalis and Panama, and molecular characterization showed a high heterogeneity of these strains. Ten different STs were found as well as nine different pulsotypes. Two clonal complexes were found. Phylogenetic trees based on secY locus and concatenated MLST loci showed two main clusters. In general, there was no relationship between the geographical origin and the secY phylogenetic clusters, as well as between secY phylogenetic clusters and serogroups. Molecular diversity indexes confirmed a high variability (H > 0.8). Haplotype networks clearly demonstrated the circulation of genotypes between humans and animals, confirming the zoonotic potential.

Conclusions: The present study provides relevant data for the study of leptospirosis in the One Health context, where human, animal and environmental health is closely connected.

Keywords: bovine leptospirosis, Genotyping, Zoonosis

P494 / #1252

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

A PECULIAR CASE OF PROTOZOAN BOVINE EOSINOPHILIC MYOSITIS

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Introduction: Bovine eosinophilic myositis (BEM) is a specific inflammatory myopathy, often associated with Sarcocystis spp., with multifocal gray-green lesions which lead to carcass condemnation and considerable economic losses. Bovines are common intermediate host of this protozoan, which also includes zoonotic species such as S. hominis. Recent research found Sarcocystis spp. DNA in 90.7% of BEM condemned carcasses and speculated on the possible role of S. hominis and S. bovifelis as the major species involved. Here we describe a peculiar case of BEM occurred in a 16 months Limousine bull, born in France, fattened and slaughtered in Italy at the end of 2021.

Methods: At post-mortem inspection, multifocal gray-green lesions were observed in all the principal muscles. Samples were collected for cytological, histological, and molecular investigations. Meat juice was subjected to IFAT for Toxoplasma IgG. Genomic DNA was extracted and analyzed by PCR targeting 18S rDNA genes and sequenced.

Results: The cytology showed inflammatory cells mostly referable to eosinophils; at histology, protozoan cysts and severe granulomatous myositis were observed; the sequence obtained was compared by BLAST, giving 99.5% similarity with Toxoplasma/Hammondia. Finally, the IFAT resulted negative for Toxoplasma IgG.

Conclusions: Our finding suggested a non-Sarcocystis induced BEM, hypothesis also supported by the molecular results showing high similarity only with Toxoplasma/Hammondia genera. Further studies are in progress to confirm the exact etiology.

Keywords: BEM, Bovine, Apicomplexa, Meat Safety

P495 / #1409

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

PREVALENCE OF COXIELLA BURNETII AND TOXOPLASMA GONDII IN SLAUGHTERED ANIMALS FROM ABATTOIRS IN THE NORTH PROVINCE, SOUTH AFRICA

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Introduction: Background and Aims Coxiella burnetii is a small intracellular bacterium that causes zoonotic diseases called Q fever. Toxoplasma gondii is an obligate intracellular protozoan parasite that has felids is definitive host and causes toxoplasmosis in both animals and humans. The aim of this study is detect the prevalence C. burnetii and T. gondii infecting cattle, sheep and pigs in abattoirs in the North West province, South Africa.

Methods: This study was carried out of the red meat abattoirs located in the North West province, South Africa. A total number of 593 slaughtered animals was used to harvest 593 serum samples, 297 testicles, 331 penises and 261 vaginas for the study.

Results: A total 593 slaughtered animals from Abattoirs of the North West Province, South Africa was tested for seroprevalence of T. gondii and C. burnetii. An overall seroprevalence of was 7.42% {44/593 // 95%CI = ± 3.52} and 7.25% {43/593 // 95C% = ± 2.087} for T. gondii and C. burnetii respectively. For PCR results showed on three animals (0.51%) and ten animals (1.69%) were positive for C. burnetii and T. gondii.

Conclusions: Conclusion The presence of C. burnetii and T. gondii in slaughtered animals from abattoirs in the North West province indicates that more studies need to be conducted as both pathogens can be transmitted from animals to humans.

Keywords: Toxoplasmosis, Infections, Prevalence, Q fever, animals

P496 / #1187

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

IDENTIFIED VECTORS TRANSMITTING HAEMOPROTEID PARASITES OF DIURNAL RAPTORS

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Introduction: Biting midges (Ceratopogonidae, Culicoides) are vectors of Haemoproteus (Haemoproteidae) parasites, which infect birds. Among the hosts, diurnal raptors are scarce in numbers and difficult to handle, but their haemoproteid parasite prevalence is often high. However, transmission has never been investigated and their corresponding vectors remain unknown. This study investigates the natural vectors of Haemoproteus parasites and the lineage diversity in diurnal raptors.

Methods: Biting midges were collected by UV-light traps placed under the nests of the diurnal raptors. Parous females were dissected, and salivary glands preparations were done for sporozoites investigation, while remains were used for molecular analysis. Blood samples were collected from juvenile raptors during the breeding season and used for molecular analyses of haemosporidian parasites identification.

Results: Wild biting midges were identified morphologically and infection of biting midges with haemosporidian parasites was determined. Wild biting midges were infected with Haemoproteus minutus (TURDUS2) and the infective sporozoite stage was detected. Analysis from raptors juveniles blood samples (white-tailed eagle, lesser spotted eagle and common buzard) revealed their infection with Leucocytozoon, Haemoproteus and Plasmodium parasites.

Conclusions: This study determined vectors and prevalence of haemosporidian parasites of diurnal raptors. This research was funded by a grant (No. S-MIP-20-57) from the Research Council of Lithuania.

Keywords: Diurnal raptors, Culicoides, Haemoproteus

P497 / #335

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

THELAZIA CALLIPAEDA AND ITS VECTOR PHORTICA VARIEGATA IN AUSTRIA AND SOUTH TYROL (ITALY) – WHERE DO WE STAND?

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Introduction: Thelazia callipaeda (Spirurida, Thelaziidae) is a parasitic nematode causing infections of the conjunctival sac and associated ocular tissues in domestic and wild carnivores, rabbits, hares, and humans. Railliet and Henry first described this parasite in Asia in 1910, while the first European cases were documented in Italy in the 1980s. Since then, the parasite has been recorded in many European countries, where the drosophilid fly Phortica variegata acts as vector responsible for its transmission. Although potentially autochthonous infections of T. callipaeda in dogs were reported in the past decade in Austria, information about this parasite is lacking. Recently the first autochthonous case of ocular thelaziosis was reported in a cat. Moreover, there is virtually no information about the presence of P. variegata in entire Austria.

Methods: The status of this parasite and its vector in Austria and South Tyrol/Italy was evaluated. In addition, an online survey was conducted among veterinary practitioners and veterinary students in order to provide information about their knowledge of this zoonotic parasite and its vector.

Results: We summarize several autochthonous cases in dogs, which were also confirmed by molecular tools and document the first finding of P. variegata in several regions in Austria and South Tyrol/Italy (results of a two-year sampling period).

Conclusions: It can be concluded that Thelazia callipaeda and its vector Phortica variegata are present in Austria. Financial support was provided by the Austrian Federal Ministry of Education, Science and Research (Austrian Barcode of Life - Hochschulraum-Strukturmittel).

Keywords: Phortica variegata, Thelazia callipaeda, South Tyrol, vector-borne disease, Austria

P498 / #423

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

FIRST REPORT OF NATURAL INFECTIONS OF ANGIOSTRONGYLUS CANTONENSIS IN URBAN RATS, RATTUS NORVEGICUS AND R. RATTUS, IN CONTINENTAL EUROPE (VALENCIA, SPAIN)

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Introduction: The rat lungworm, Angiostrongylus cantonensis, a zoonotic parasite of the pulmonary arteries in rats, is the leading cause of human eosinophilic meningitis, a global emerging disease with serious implications for animal and public health. Rattus norvegicus and R. rattus are its definitive hosts. Globalization has facilitated the dispersion of the parasite mainly due to ship-borne rats.

Methods: As part of a rodent control campaign carried out by the Valencia City Council, Laboratorios Lokímica and in the cooperation of the Parasite and Health Research Group of University of Valencia, rat specimens caught through standardized snap traps placed in the sewage system of Valencia were analysed for endoparasites.

Results: After the dissection of the first 27 rats, A. cantonensis individuals (males and females in the pulmonary arteries and young adults in the brain) were found in two R. norvegicus and one R. rattus. Species identification was confirmed by PCR and ITS-2 sequences clustered with A. cantonensis.

Conclusions: This is the first report of autochthonous cases of A. cantonensis in continental Europe. This finding should encourage government entities, pest control agencies and experts in parasitic zoonoses to conduct epidemiological surveys on rat populations in European countries to prevent, not only neuroangiostrongyliasis, but other parasitic zoonoses transmitted by rats. Acknowledgements: We would like to thank the Health Service of Valencia City Council for facilitating and promoting this study.

Keywords: Rattus norvegicus, Rattus rattus, Valencia, Spain, Angiostrongylus cantonensis

P499 / #545

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

HIGH MOLECULAR LEVELS OF LEISHMANIA INFANTUM IN RATS AND SANDFLIES IN URBAN SEWERS: IS UNDERGROUND LEISHMANIASIS THE PERFECT STORM?

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Introduction: Leishmaniasis, a vector-borne disease transmitted by the bite of female sand flies (phlebotomine), is caused by 20 species pathogenic to humans, and the disease is among the top ten Neglected Tropical Diseases. Spain is one of the endemic countries of leishmaniasis in the Mediterranean basin where Leishmania infantum is the predominant species and, concerning the invertebrate hosts, Phlebotomus perniciosus and P. ariasi are the main vectors. Dogs have classically been considered the only reservoir of the protozoan in urban areas. However, we revealed a 33.3% prevalence of L. infantum in the spleens of the Norway rat, Rattus norvegicus, trapped in the sewers of Barcelona (Spain) in a previous study.

Methods: In this study, we have investigated the presence of L. infantum in the sand flies as well as in the skin of the rats, both trapped in the Barcelona sewers, by real time PCR (qPCR) and subsequent sequencing.

Results: L. infantum DNA was detected in 14 out of the 27 P. perniciosus individuals analysed (51.9%). Two of the sand flies harboured strikingly high parasite loads. Likewise, rats presented a 41.7% (10/24) prevalence. 40% of the infected rats presented the parasite in their ears. One of the rats exhibited a load of more than 430,000 estimated parasites per 150 ear tissue mg.

Conclusions: We conclude that Norway rats, in addition to dogs, are likely to act as reservoirs of leishmaniasis in cities where the sewer systems seem to offer the ideal scenario for a perfect leishmaniasis storm.

Keywords: Leishmania infantum, Rattus norvegicus, Phlebotomus perniciosus, urban sewers, Barcelona

P500 / #627

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

RATTUS NORVEGICUS, THE NORWAY RAT, AS RESERVOIR OF ZOONOTIC HELMINTHS AND INTESTINAL PROTOZOANS IN THE CITY OF BARCELONA, SPAIN

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Introduction: The Norway rat, Rattus norvegicus, which lives very close to humans, has been recognised as reservoir of several protozoan and helminth parasitic zoonotic agents. In urban areas, the Norway rat can transmit zoonotic parasitic species directly to humans or act as indirect reservoir.

Methods: As part of a study concerning the zoonotic potential of this synanthropic rodent in the city of Barcelona, Spain, a total of 271 R. norvegicus was helminthologically analysed by means of organ dissection. Moreover, the faeces of 100 of these rats were also molecularly analysed, by means of a multiplex qPCR, to investigate the presence of intestinal zoonotic protozoans.

Results: 10 parasitic zoonotic species were found: 6 helminths (Hydatigera taeniaeformis larvae, 1.5%; Rodentolepis nana, 7.4%; Hymenolepis diminuta, 21.4%; Calodium hepaticum, 46.9%; Gongylonema neoplasticum, 34.7% and Moniliformis moniliformis, 2.6%), and 4 protozoans (Blastocystis sp., 83.5%; Giardia duodenalis, 37.7%; Cryptosporidium spp., 34.1% and Dientamoeba fragilis, 14.1%). 67.8% of the rats analysed were found parasitized by zoonotic species.

Conclusions: Although the actual contribution of R. norvegicus to the zoonotic transmission of parasites is still unknown, public health authorities should be alerted with regard to rat control and surveillance, especially in deprived settlements close to cities. Moreover, correct hygienic and sanitary practices should be promoted, mainly, among sewage workers as well as the children population. Study supported by University of Valencia grant UV-INV-AE-19-1196278

Keywords: Rattus norvegicus, Helminths, Barcelona, protozoans, zoonoses

P501 / #477

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

PHYLOGENETIC RELATIONSHIPS AND EVOLUTIONARY PATTERNS OF THE GENUS PSAMMOLESTES (HEMIPTERA: REDUVIIDAE: TRIATOMINAE): TOOLS FOR VECTOR CONTROL OF CHAGAS DISEASE

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Introduction: The Triatomines are vectors of Trypanosoma cruzi, which causes Chagas disease. Vector control strategies could benefit from a characterization of the number of lineages inside of genera, phylogenetic relationships, and its evolutionary patterns. Here we studied the diversification of Psammolestes, a genus of triatomines endemic of the seasonal dry tropical forest and naturally infected with T. cruzi.

Methods: We collected 92 samples of Psammolestes from Venezuela, Colombia, and Brazil. DNA extraction, PCR, and sequencing of seven loci were performed. Phylogenetic relationships, divergence times, population genetics analyses, geographical diversification and niche modelling were evaluated using molecular and geographical data.

Results: We recovered P. coreodes and P. tertius in a monophyletic clade sister to P. arthuri. Demographic model predicted a scenario of divergence without gene flow in late Miocene, driven by geographical isolation and suggesting that mixed haplotypes may be the result of shared ancestral variation since the divergence of the subtropical-temperate species (P. coreodes/P. tertius). P. arthuri was highly differentiated from the other two identified a clear geographical barrier

Conclusions: Amazon basin is a climatic barrier that promoted allopatric speciation after long range dispersion. Each species of Psammolestes occupies different climatic niches, suggesting that niche conservatism is not crucial for species differentiation. These findings contribute knowledge on the speciation and dispersal patterns in triatomines and could be used to strength the vector surveillance programs of Chagas disease. Funding: DIeI from UR. Minciencias/Conv.727.

Keywords: Vectors, chagas disease, Psammolestes, evolution, phylogenetic

P502 / #832

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

STEPPING TOWARDS A WHOLE GENOME FOR PANSTRONGYLUS GENICULATUS (HEMIPTERA: REDUVIIDAE), THE TRIATOMINE WITH THE LARGEST GEOGRAPHIC DISTRIBUTION IN LATIN AMERICA.

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Introduction: Chagas disease is a vector-borne tropical neglected illness caused by Trypanosoma cruzi, transmitted by insects of the Subfamily Triatominae. Currently, there are only three triatomine species with whole genome sequences available. New genomes would provide new insights to triatomine evolution and possible targets for vector control. The aim of this study was to provide a draft whole genome of Panstrongylus geniculatus, a triatomine reported in 18 countries, inhabiting a variety of habitats, and currently reported as the major vector of T. cruzi in peri-urban environments in Colombia and Venezuela.

Methods: We extracted DNA from head/thorax of a field female adult captured at the Orinoco basin in Colombia, which was sequenced by both long-read and short-read platforms. We undertook hybrid de novo assembly of long and short reads with MaSuRcav4.0.4. Assembly statistics using assembly-statsv1.0.1 were determined and measured the quality by searching for conserved single-copy orthologs using BUSCOv5.2.2.

Results: Our draft assembly is composed of 93102 scaffolds, with 12684.1 bp average scaffold size, an N50 of 21741 bp and E-size of 33983.9. Completeness reached 87.9% compared to the Hemiptera database, composed of 2510 busco's, with 85.9% of single-copy genes sequenced as well as 2.0% duplicated and 6.1% fragmented.

Conclusions: Our work constitutes the first step towards the study of the whole genome of P. geniculatus. The use of long and short reads provides a feasible method to obtain genomes from a great diversity of Chagas disease vectors. The study of the P. geniculatus genome could provide insights into its environmental adaptability and new transmission scenarios. Funding: DIeI from UR. Minciencias/Conv.727. ANID/DoctoradoBecas/Chile 2019 72200391

Keywords: Vectors, Long-reads/Illuimina assembly, chagas disease, Panstrongylus geniculatus, GENOME

P503 / #1382

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

TRICHINELLA SPP. IN WILD BOARS (SUS SCROFA) IN ESTONIA

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Introduction: Trichinella spp. infections are endemic in game animals in Estonia. The wild boar is an important game animal and an indicator of the occurrence and spread and distribution of several Trichinella species. During 2007–2020, 236,000 wild boars were hunted in Estonia. Here we summarize the findings of Trichinella testing of wild boar samples at the Estonian Veterinary and Food Laboratory during 2007–2020.

Methods: The database includes two datasets: samples of 30,566 wild boars hunted in 2007–2014 (Kärssin et al., 2021), and samples of 21,272 wild boars hunted in 2015–2020. Muscle samples were tested using artificial digestion method. Larvae were counted, rinsed, and stored in ethanol. Trichinella species identification was done for isolated larval samples using multiplex polymerase chain reaction for Trichinella species detection.

Results: Trichinella prevalence was 0.9%. Trichinella species detected infecting wild boars in Estonia were T. nativa, T. britovi, T. spiralis and T. pseudospiralis.

Conclusions: Monitoring Trichinella prevalence and species distribution is necessary to have updated information. Reference: Kärssin, A., Häkkinen, L., Vilem, A., Jokelainen, P., & Lassen, B. (2021). Trichinella spp. in Wild Boars (Sus scrofa), Brown Bears (Ursus arctos), Eurasian Lynxes (Lynx lynx) and Badgers (Meles meles) in Estonia, 2007–2014. Animals, 11(1), 183. https://doi.org/10.3390/ani11010183

Keywords: Trichinella, Zoonosis, wild boar, foodborne

P504 / #1607

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

METAGENOMICS STUDY OF HAEMAPHYSALIS LONGICORNIS TICKS IN KOREA

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Introduction: Ticks can transmit pathogenic bacteria, protozoa, viruses that cause great suffering and potentially fatal diseases in humans worldwide. Ticks also cause considerable losses to the livestock industry by causing irritation and blood loss to their hosts at the same time acting as vectors of many pathogens. *Haemaphysalis longicornis* has been reported in Australia, New Zealand, New Caledonia, Fiji, Japan, Korea, Northeastern China, USSR, and USA.

Methods: In this study, we employed high-throughput sequencing of the V3–V4 regions of the 16S rRNA gene to investigate the bacterial abundance and diversity between the nymph stage and the adult developmental stages of *H. longicornis* to evaluate the changes in the bacterial abundance and the maintenance of the bacterial community throughout the life cycle of the tick.

Results: The Shannon index was significantly higher in nymphs than adult ticks. Principal coordinates analysis showed that the microbiome composition of female adult and male adult ticks were different. Notably, *Coxiella*-like bacterium, known as a tick symbiont, was found in all nymphs and female adult ticks, but only one out of four male adult ticks had *Coxiella*-like bacterium. In addition, *Rickettsia rickettsii*, *Coxiella burnetii,* and *Anaplasma bovis* were detected in this study.

Conclusions: In conclusion, in this metagenomics study on *H. longicornis*, different microbiome patterns were found among nymphs, female adult ticks, and male adult ticks.

Keywords: Korea, Metagenomics study, Microbiome, tick, Haemaphysalis longicornis

P505 / #1608

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

METAGENOMIC SCREENING OF BACTERIA AND PARASITES IN FECES OF APODEMUS AGRARIUS

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Introduction: *Apodemus agrarius* is a common type of wild rodent found in Korea. Wild rodent feces are known to carry various pathogenic bacteria and parasites. The aim of this study was to rapidly detect bacterial, fungal, and parasitic pathogens in the feces of wild mice using next-generation sequencing (NGS)-based metagenomic analysis.

Methods: We conducted 16S/18S rDNA-targeted high-throughput sequencing on fecal samples from *A. agrarius* (n = 48) collected in May and October 2017. The taxa of bacteria, protozoa, fungi, and helminths in the feces were identified.

Results: There were no statistical differences in microbial richness (number of operational taxonomic units) and Shannon diversity index between the spring and fall. However, beta diversity analysis revealed microbial composition differences between the two seasonal groups (p = 0.001 in PERMANOVA). According to the linear discriminant analysis effect size results, *Escherichia* and *Parabacteroides* were significantly more abundant in the feces of mice captured in the fall than in the spring samples. The following parasitic species were detected in the fecal samples: *Heligmosomoides polygyrus* (41/48), *Syphacia obvelata* (12/48), *Hymenolepis diminuta* (5/48), *Raillietina tetragona* (4/48), and *Giardia muris* (36/48). Additionally, *Lactobacillus gasseri* and *Lactobacillus intestinalis* were found to be more abundant in *H. polygyrus*-infected mice than in those not infected by this parasite.

Conclusions: These results highlight the advantages of the application of NGS technology in monitoring zoonotic disease reservoirs.

Keywords: Apodemus agrarius, pathogens, 18SrRNA, next-generation sequencing, 16SrRNA

P506 / #1134

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

MOLECULAR CHARACTERIZATION OF TICKS AND TICK BORNE PARASITES OF CATTLE FROM SOUTH KARNATAKA, INDIA

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Introduction: Ticks and tick-borne diseases (TBDs) of cattle are widely prevalent especially in the tropical and subtropical regions of the world, affect the productivity and significant adverse impact on livelihoods. In Karnataka, main TBDs include theileriosis, babesiosis and anaplasmosis mainly infect high yielders and exotic cattle. Therefore, the study was taken to identify the tick species of cattle and their vector potentiality by PCR.

Methods: Ticks were collected from the cattle in different parts of south Karnataka and identified by PCR using specific primers targeting 12S rRNA gene. The vector potentiality of ticks was studied by PCR targeting different genes like 18S rRNA for Theileria and Babesia sp., mpsp gene for Theileria orientalis, rpoB and groEL gene for Anaplasma and Ehrlichia sp. of cattle.

Results: A total of 300 cattle were examined, 166 (55.3%) harbored ticks. Species were identified as Rhipicephalus microplus, R. haemaphysaloides and Haemaphysalis longicornis by PCR product sequence analysis. Totally, 50 engorged above ticks DNA samples were subjected to PCR, 33 positive for Theileria orientalis, 19 for Babesia, 09 for Anaplasma sp. and 06 for Ehrlichia. PCR product sequence and phylogenetic analysis revealed the presence of Theileria annulata, T. orientalis, Babesia bigemina, Anaplasma marginale and Ehrlichia minasensis after aligning with respective reference sequences obtained from GenBank.

Conclusions: The present study indicate the presence of haemoprotozoan and rickettsial organisms in ticks and are of potential risk to animal health. Ehrlichia minasensis is recorded for the first time in cattle from Karnataka state. Effective control measures needed to reduce the risk of TBDs and can be achieved by regular surveillance and adequate treatment.

Keywords: Cattle, South Karnataka, Ticks, Vector potentiality, PCR

P507 / #495

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

RESULTS OF PROFICIENCY TESTING FOR TRICHINELLA IN POLAND, 2015-2019

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Introduction: Trichinellosis is a zoonotic parasitic disease caused by nematodes of the Trichinella genus. Each year, over 18 milion pigs, 120,000 wild boars and 40 000 horses are examined for Trichinella spp. in Poland. In 2009, the digestion method supported by a magnetic stirrer (MSD) was introduced to all abattoirs and wild game establishments (EU Regulation 2075/2005 replaced by EU Regulation 2015/1375). The purpose was to implement the diagnostic methods in the same manner in all EU countries in order to remove barriers in internal EU trade. The management system require laboratories to confirm their competence in proficiency testing (PT).

Methods: PT organized by Polish National Reference Laboratory for trichinellosis is based on the ISO/IEC 17043 Standard. Each laboratory received a set of samples (three levels and blank), individual codes and access the PT website. The laboratory was assessed negatively if reported at leat one incorrect result on the levels of zero, three or five larvae. Samples containing single larva were used to assess the quality of the management system.

Results: Over 2015-2019, 1895 laboratories participated in the PT's and in total, 7580 samples were sent to them. Over 95% of the samples were considered correct in qualitative assessments. The quantitative evaluations indicated 89% of correctly considered samples. In total, 88% of laboratories passed the PT. A slight decrease was observed in the examination of samples spiked with five larvae, and great progres in samples with three larvae.

Conclusions: The results of the PT tests carried out indicate that the quality of the tests performed in Polish veterinary laboratories does not differ from the results obtained in European l aboratories; however, the results indicate a possible need for further actions.

Keywords: Trichinella spiralis, proficiency testing, Poland

P508 / #99

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

HUMAN SPARGANOSIS AND A NEW MOLECULAR PHYLOGENETIC HYPOTHESIS OF SPIROMETRA: ZOOGEOGRAPHICAL DOES MATTER!

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Introduction: The assignment of species identity to the causative agents of sparganosis, a relatively neglected foodborne and waterborne disease, has been problematic or completely ignored by previous surveys. The current phylogenetic analysis indicates there are at least 6 well-defined molecularly well-defined lineages of Spirometra corresponding to separate species with a clear geographical pattern behind them

Methods: See Kuchta et al. (2021).

Results: 1) Spirometa mansoni is the most widespread lineage throughout Asia and Australia, but has also been discovered in Romania and Tanzania. 2) A new undescribed species of Spirometra is rarely recorded in Korea and Japan. 3) Spirometra enrianceieuropaei is restricted exclusively to Europe and all cases reported from other continents represent misidentification. 4) Spirometra folium has been detected in Africa (Sudan, Ethiopia, and Tanzania). 5) Two additional lineages of the Spirometra decipiens complex have been detected in North and South America.

Conclusions: From the phylogenetic analysis, the following patterns for the systematics of the genus Spirometra can be deduced:
(i) Species-level lineages follow a strong geographic pattern;
(ii) S. erinaceieuropaei does not show a cosmopolitan distribution, implying that all Asian and Australian specimens actually belong to S. mansoni and not to S. erinaceieuropaei, whose distribution seems to be restricted to Europe;
(iii) the existence of a second, relatively rarely sequenced species-level lineage, a new undescribed species;
(iv) the existence of at least two distinct lineages in the Americas;
(v) the erroneous identification of specimens reported from Asia and Africa as "S. decipiens" and "S. ranarum", which are in fact related to S. mansoni.

Keywords: emerging infectious disease, Epidemiology, Zoonosis, Cestoda

P509 / #1041

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

MOLECULAR EVIDENCE INTESTINAL EXPOSURE TO ANCYLOSTOMA CANINUM AMONG HUMANS IN JAMAICA

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Introduction: Canine hookworm infections are endemic in the tropics and are of major veterinary and public health significance as they are capable of causing intestinal infections in dogs and skin and enteric disease in humans. The most important zoonotic species include A. caninum, A. braziliense, and A. ceylanicum.

Methods: To determine the prevalence of Ancylostoma species in dogs and possible intestinal exposure of humans in Jamaica, stool samples were screened using PCR targeting the internal transcribed spacer (ITS-1), 5,8S and ITS-2 region of the ribosomal DNA genes. Genomic DNA was extracted using the QIAamp Fast DNA Stool Kit® (Qiagen, Hilden, Germany). A portion of the first and second ITS regions (450bp) were amplified using forward- RTHW1F (5-GAT GAG CAT TGC WTG AAT GCC G-3) and reverse- RTHW1R (5-GCA AGT RCC GTT CGA CAA ACA G-3) primers to hookworm genomic DNA as described previously. Positive amplicons were purified using the PCR and Gel Band purification kit.

Results: The prevalence of hookworm infections in dogs based on PCR was 60.9% (78/128). DNA sequencing revealed that A. caninum accounted for 88.2% (30/34) of infections in dogs. DNA of A. caninum targeting the same gene fragment was identified in 22.1% (17/77) of stool samples from patients presenting at hospital with the gastrointestinal symptoms. A subset of the sequence data obtained in this study was submitted to GenBank accession numbers: MH215563, MH215564, MH215565, MH215566, MH215567, MH215568, MH215569 and MH215570.

Conclusions: Analysis of sequences obtained showed that A. caninum identified from both, humans and dogs were 99% homologous. This first report of molecular identification of A. caninum in human stools suggests that intestinal infection with this parasite may be underreported in endemic areas.

Keywords: eosinophilic enteritis, molecular epidemiology, DNA sequencing, Ancylostoma caninum, Jamaica

P510 / #719

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

MOLECULAR AND SERO-EPIDEMIOLOGICAL SURVEY IN REINTRODUCED AND WILD-BORN LYNXES IN THE PORTUGUESE GUADIANA VALLEY

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Introduction: The Iberian lynx (Lynx pardinus) is an endangered species living in restricted areas of the Iberian Peninsula. Few animals survived hunting, exploitation of natural habitats and decline of prey population. Since 2014, LIFE the Lynxconnect programme has succeeded in reintroducing lynxes back into nature. To support conservation efforts, research and surveillance of infectious diseases are needed to prevent outbreaks and assess the impact of disease in these populations. This research aims at assessing the risk of exposure to Leishmania infatum of a group of wild Iberian lynxes living in Guadiana Valley Natural Park, Portugal.

Methods: Captures and blood collection were performed by ICNF between 2018 and 2020. DNA was extracted from 400 ul of 36 samples of whole blood (DNAeasy Blood and Tissue Kit) and plasma was separated for ELISA and IFAT. For ELISA, anti-Leishmania IgG detection was estimated by the seroreactivity produced against specific antigens: 1) SPLA (Leishmania promastigotes soluble antigens), 2) recombinant K39 protein (rK39), 3) Leishmania cytosolic peroxiredoxins (cPX); complemented by the seroreactivity against a none Leishmania related antigen, Escherichia coli soluble antigens (SECA). IFAT will be performed using whole L. infantum promastigotes as antigen. Molecular detection of Leishmania donovani sensu latu will be performed using specific primers targeting kDNA minicircles (RV1 and RV2).

Results: Preliminary ELISA results indicated 3% (1/36) exposure to L. infantum, supported by combined high seroreactivity towards SPLA, rK39 and cPX. IFAT serological evaluation and Leishmania PCR are ongoing.

Conclusions: A longitudinal study is necessary to evaluate the impact of L. infantum infection in this endangered species.

Keywords: Lynx pardinus, ELISA, IFAT, PCR, Leishmania infantum

P511 / #733

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

CURRENT EPIDEMIOLOGICAL SITUATION OF CANINE AND FELINE LEISHMANIA INFECTIONS IN PORTUGAL

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Introduction: Portugal is a hot spot for Leishmania infantum and its phlebotomine vectors. Canine leishmaniasis (CanL) is a serious veterinary and public health concern in the country, as this is a zoonotic parasite having dogs as primary reservoirs. This study aims to provide an updated epidemiologic survey on the prevalence of canine and feline exposure to L. infantum in mainland Portugal by means of screening apparently healthy and clinical suspected dogs and cats by ELISA. Molecular typing of clinical isolates obtained from canine and feline cases will be performed to monitor parasite diversity and spreading.

Methods: During 2020, 434 dogs and 157 cats living at municipal shelters distributed over mainland Portugal were sampled. Anti-Leishmania IgG detection in serum was performed by ELISA using Leishmania antigens: SPLA (Leishmania promastigotes soluble antigens), recombinant K39 protein (rK39), Leishmania cytosolic peroxiredoxins (CPX) and a non related antigen - Escherichia coli soluble antigens (SECA). Animals suspect of leishmaniasis had cells seeded in Schneider complete media for promastigotes isolation. Parasite strains will be studied by PCR-RFLP of kDNA minicircles.

Results: Preliminary results based on SPLA seroreactivity indicate 18% seroprevalence for CanL (78/434) and 3% (5/157) for FeL in 10 Portuguese districts (Vila Real, Bragança, Coimbra, Aveiro, Castelo Branco, Santarém, Portalegre, Évora, Setúbal, Beja). Thirty clinical isolates of L. infantum were obtained (28 canine and 2 feline).

Conclusions: This is the most updated seroepidemiological survey on the distribution of canine and feline Leishmania infections in Portugal and the first integrating molecular identification of the circulating strains of L. infantum and their distribution.

Keywords: Leishmania infantum, Zoonosis, Surveillance, ELISA, PCR-RFLP

P512 / #23

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

PREVALENCE, INTENSITY, AND DIVERSITY OF TRICHINELLA SPP. IN WILDLIFE IN NORTHERN NORTH AMERICA

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Introduction: A new, possibly freeze tolerant species of the zoonotic, muscle-dwelling nematode Trichinella, T. chanchalensis (T13), was recently described in wolverine in the Yukon and Northwest Territories (Sharma et al., 2020). In Northern Canada, Indigenous communities are involved in the harvest of bear and walrus, which are associated with human infection with freeze-tolerant Trichinella spp (T. nativa and T6). It is not known if the new species, T. chanchalensis (T13) can infect wildlife species other than wolverine, or where else it is present in Canada.

Methods: To better characterize the geographic and host range of T2, T6, and T13, we collected tongue, diaphragm, and/or forelimb from terrestrial carnivores (fox, wolf, lynx, and bear) from Alaska, British Columbia, Yukon, Northwest Territories, Nunavut, northern Quebec, and Newfoundland/Labrador. Larvae were recovered and quantified using the double separatory funnel tissue digestion method. DNA was extracted from pooled and individual larvae, and amplified using a multiplex PCR to identify species of Trichinella. The PCR RFLP was used to differentiate T2 from T13 (which share the same band on the multiplex PCR).

Results: Thus far, prevalence and intensity has been lower in fox and lynx than in wolf and wolverine. Molecular characterization to detect T2, T6, and T13 is in progress. We hypothesize that T. nativa (T2) dominates in the eastern North American Arctic, whereas T6 dominates in the west, although the two species can be sympatric.

Conclusions: Research into host-specific differences in prevalence, intensity, and diversity of Trichinella is essential to assess the risk for food safety in indigenous communities, especially in wildlife species consumed by people, such as lynx and bear.

Keywords: Animal Disease, Zoonotic disease, Epidemiology, Food Safety, Etiology

P513 / #1337

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

FIRST DATA ON CYTAUXZOON AND HEPATOZOON IN WILD CATS (FELIS SILVESTRIS SILVESTRIS) IN NORTH-EASTERN ITALY.

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Introduction: The vector-borne protozoa Cytauxzoon spp. and Hepatozoon spp. affect several wild felids worldwide. In Europe, both protozoa are recently reported in European wild cats (Felis silvestris silvestris) but data on epidemiology, life-cycle and pathogenicity are still fragmentary. In this study, both protozoa were investigated in wild cats in North-eastern Italy and potential target organs were evaluated.

Methods: Wild cats found dead in road accidents were collected. DNA was isolated from blood-clot, lung, liver, lymph-nodes, heart and spleen. A conventional PCR (18S-rRNA) was performed to detect both protozoa. Then, a nested PCR (Cytochrome B gene) was run to determine Cytauxzoon species and to consider potential co-infections. Amplicons were sequenced and compared to those deposited in GenBank®. Fisher exact test (R software, version 4.1.2) was performed to evaluate potential correlations between protozoan infections and positive tissues in order to identify target organ/s.

Results: Among 19 wild cats, 4 (21.05%) animals were infected by Cytauxzoon europaeus and 8 (42.11%) by Hepatozoon spp. (i.e. Hepatozoon felis, n=6; Hepatozoon silvestris, n=2). Only 1 co-infection by C. europaeus and H. silvestris was detected. Liver and spleen were target tissues for H. silvestris and C. europaeus, respectively.

Conclusions: In North-eastern Italy wild cats are infected by both protozoa with high prevalence rates, suggesting their potential role as reservoir. Since in the North-eastern Italy domestic and wild cats share the same habitat, the isolation of C. europaeus, H. felis and H. silvestris in wild cats highlights the potential health risk for domestic ones. The identification of target organs simplifies and accelerate Cytauxzoon and Hepatozoon diagnosis processes.

Keywords: Felis silvestris silvestris, Cytauxzoon europaeus, Hepatozoon felis, Hepatozoon silvestris, North-eastern Italy

P514 / #1060

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

GENETIC DIVERSITY OF CRYPTOSPORIDIUM SPP. IN CATTLE IN LATVIA

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Introduction: Cryptosporidium spp. is the fifth most important foodborne parasite in Europe. Main transmission routes are food and drinking water, transmission from person to person or, from domestic or wild animals to humans. To gain a better knowledge on zoonotic Cryptosporidium spp. molecular epidemiology, the aim of the study was to determine Cryptosporidium spp. species and subtypes in cattle in Latvia.

Methods: Overall, 135 DNA samples isolated from cattle feces were used for species identification targeting 18S RNA gene and C. parvum subtyping targeting gp60 gene. CryptoGenotyper tool was used to identify mixed infections.

Results: A total of six species were detected – C. parvum (type A and B), C. bovis, C. andersoni (type A and B), C. ryanae, C. scrofarum, C. ubiquitum – in single or mix infections. Mix infection were detected in 22.9% of cases. Eight different C. parvum subtypes of were identified where IIaA15G2R1 being most prevalent.

Conclusions: Potentially zoonotic C. parvum subtypes are found in Latvian cattle herds, therefore it poses a risk as a human health concern. This study was funded by the European Regional Development Fund postdoctoral research study (1.1.1.2/VIAA/1/16/204), COST action FA1408 and Latvian Council of Science project (lzp-2021/1-0055). Reference: Deksne G., et al. 2022. Prevalence, risk factor and diversity of Cryptosporidium in cattle in Latvia, Veterinary Parasitology: Regional Studies and Reports, Vol. 28., https://doi.org/10.1016/j.vprsr.2021.100677.

Keywords: Cryptosporidium, Epidemiology, molecular epidemiology, Cattle, Zoonosis

P515 / #308

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

INVESTIGATING THE ROLE OF INVARIANT SURFACE GLYCOPROTEINS IN TRYPANOSOMA CONGOLENSE

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Introduction: African trypanosomes are single-celled, eukaryotic pathogens of humans and animals in sub-Saharan Africa. As they are extracellular parasites, their cell surface forms the primary host-pathogen interface and must balance nutrient uptake with host innate and adaptive immune evasion. Invariant Surface Glycoproteins (ISGs) are a large family of surface proteins expressed in bloodstream forms. Characterisation of ISG65 in Trypanosoma brucei revealed an interaction with mammalian complement C3. In T. congolense, there has been an expansion of the ISG gene family but the functions are unknown. Do T. congolense ISGs also interact with the same or different components of the complement system? Is there functional diversity among the T. congolense ISGs?

Methods: A phylogenetic analysis of the full ISG gene family was generated. Pull-downs and mass spectrometry were used to identify interaction partners of recombinantly expressed T. congolense ISGs. Surface plasmon resonance was used to validate these interactions.

Results: The phylogeny of ISGs of African trypanosomes shows a recent expansion of T. congolense ISGs. Two T. congolense ISGs have been shown to interact with different components of the mammalian complement system.

Conclusions: The expansion of ISGs in T. congolense may correspond to functional diversity. Several T. congolense ISGs may have a role in host immune evasion, via their interactions with the complement system. Thanks to Eelco Tromer, University of Groningen, and Andrew Jackson, University of Liverpool, for advice on phylogenetics; and Katherine Stott, University of Cambridge, for assistance with SPR.

Keywords: Invariant Surface Glycoproteins, complement factors, innate immune response, African trypanosomes

P516 / #497

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

MOLECULAR DETECTION OF TICK-BORNE PATHOGENS AND ENDOSYMBIONTS FROM AMBLYOMMA (ACARI: IXODIDAE) TICKS COLLECTED FROM REPTILES IN SOUTH AFRICA

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Introduction: Tick-borne pathogens (TBPs) are among the most diffuse vector-borne diseases emerging worldwide; posing an increased health risk to both humans and animals. Particularly, the role of ectotherms in the epidemiology of these TBPs is unknown, despite reptilian ticks having been implicated with TBPs of livestock. Little is known about the presence/diversity of reptilian TBPs in South Africa (SA). As such, the aim of this research was to screen SA reptiles and associated ticks for these pathogens.

Methods: Two hundred and fifty-seven ticks were screened for Coxiella, Anaplasma, Rickettsia, and Borrelia spp., by amplification, sequencing, and phylogenetic analysis of the 16S and 23S rRNA, gltA, OmpA, and Fla genes.

Results: For the first time, the presence of reptile associated Borrelia sp., and Coxiella-like endosymbiont was recorded in SA. Furthermore, Rickettsia massiliae was observed in 7 Amblyomma marmoreum and 12 A. sylvaticum from tortoises. Franciella-like endosymbiont was observed from 2 A. latum collected from a snake. Coxiella burnetii and Anaplasma spp., were not detected.

Conclusions: Although direct evidence of reptiles acting as reservoir hosts remains to be determined, observations indicate reptilian ticks may play a role in transmission. The absence of Anaplasma spp. and C. burnetii should not be neglected. Accumulation of this epidemiological information from multiple reptile individuals and/or species and different developmental stages of ticks in future studies should be informative.

Keywords: Amblyomma, Tick-borne pathogens, Reptiles, Zoonosis, Endosymbionts

P517 / #1172

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

HUMAN BERTIELLOSIS – A RARE CESTODE INFECTION IN A SOUTH AFRICAN CHILD

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Introduction: Cestodes of Bertiella genus are parasites of non-human primates, which have been reported to cause zoonotic disease. Here, we report a rare case of bertiellosis in a 3-year-old South African child.

Methods: The child presented with a one-year history of rectal proglottid discharge and intermittent abdominal pain. After repeated failure with benzimidazole anti-helminthic treatment, praziquantel was administered. Stool samples and worm segments were sent for laboratory diagnosis.

Results: Macroscopically, proglottids displayed motility and had a ‘bowtie’ appearance. It was also noted that the width of the proglottids were much greater than the length. Microscopic examination showed oval to round ova measuring 45-50 µm. Using Nomarski differential interference contrast microscopy, a thick outer coat with an internal typical pyriform apparatus containing hooks were clearly visible. Based on these findings, the causative parasite was identified as Bertiella species. A one-year follow-up of the patient confirmed no further discharge of worm segments or abdominal pain.

Conclusions: Praziquantel treatment proved successful in this case, underscoring the importance of an accurate diagnosis. Reference: Naranbhai N, A Singh R, Moodley B, Han KSS, Archary M, Mvelase N. Case Report: Human Bertiellosis-A Rare Cestode Infection in a South African Child. Am J Trop Med Hyg. 2021 Oct 25;106(1):219-221. doi: 10.4269/ajtmh.21-0204.

Keywords: Bertiella, Zoonosis, cestode

P518 / #677

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

TRYPANOSOMA CRUZI DNA IN MULTIPLE ORGANS OF A BARN-OWL FROM MEXICO

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Introduction: Chagas disease, caused by the protozoan Trypanosoma cruzi, is a serious public health problem affecting 6 to 7 million people. This protozoan shows a primary wild enzootic cycle, maintained between Triatominae insects and Mammalia wild hosts. Birds infection resistance has been reported since the 70s, but there is only evidence for poultry and no studies were done in other bird species.

Methods: A fresh roadkill adult female American barn-owl [Tyto furcata] was collected in southeast Mexico. Heart, liver, spleen, intestine and breast tissues were subjected to PCR assay using a DNA satellite and Minicircles of T. cruzi. Amplicons obtained were sequenced and sequences were subjected to BLAST searches in GenBank. Phylogenetic reconstruction was conducted using a Bayesian approach with MrBayes version 3.2.

Results: All analyzed tissues, except the spleen, were positive for DNA-satellite amplification. Four DNA sequences were obtained from heart, liver, breast and intestine. Results showed a high percentage of identity, 98 to 99% (107/108 bp) with the Y strain of T. cruzi, DTU II (AY520070).

Conclusions: This is the first study that found evidence of T. cruzi natural infection in birds, and our results raises new questions about its role in the cycle dynamics. For barn-owls and family Tytonidae new studies must be conducted to discover if T. furcata has a role in disseminating, transmitting, maintaining and/or controlling the parasite. But there are still questions that need answers: Can other bird species become infected? In which ways do birds interact with Triatominae vectors?

Disclosure: Results of this study have been published in Martínez-Hernández, F., Martínez, B. O., Rendón-Franco, E., Villalobos, G., & Muñoz-García, C. I. (2022). Trypanosoma cruzi, beyond the dogma of non-infection in birds. Infection, Genetics and Evolution, 105239

Keywords: Tyto alba, wildlife, wild host, barn-owl, Trypanosoma cruzi

P519 / #678

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

LONG-TERM SEROPREVALENCE OF TRICHINELLA IN TWO WILD AMERICAN CARNIVORES, WHITE-NOSED COATIS (NASUA NARICA) AND COMMON RACCOONS (PROCYON LOTOR).

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Introduction: Trichinella is a zoonotic foodborne nematode that is transmitted by consumption of raw or undercooked meat. Animals from the order carnivora have been reported as the most important hosts for the parasite because their feeding behavior. Procyonids are carnivore members that have been reported consumed as bush meat in some Mexican and South America communities.

Methods: Two hundred forty-one coatis and a hundred and five raccoons from a park in southeast Mexico, were captured and recaptured during the summer 2010 to winter 2013. Serum samples were collected from each animal and tested by an indirect ELISA using a non-species-specific protein A conjugate for detection of IgG antibodies versus Trichinella sp. Samples were confirmed by Western Blot looking for the triplet strips of 45, 50 and 55 kDa.

Results: General prevalence was 18.2% for both species. A higher prevalence was observed in raccoons (24.8%) than in coatis (15.4%), with significant differences between species (P=0.041). The same pattern was observed for the summer, with a high prevalence in raccoons than in coatis (31.3% and 12.9% respectively, p=0.005). The seropositive samples form 69 coatis and 50 raccoons were confirmed by WB. During the evaluation period a Trichinella outbreak was detected in 2011 in both procyonid populations with a slight increase in prevalence across the time. The lowest prevalence was 0% in 2010 and the highest was 33% during the winter 2013, for coatis, and 46.7% during the summer of 2013, for raccoons.

Conclusions: This study provide indirect evidence about the Trichinella establishment in this procyonid community from southeast Mexico. However, further studies are needed to determine which Trichinella species is or are circulating in the area and its implications for public health.

Keywords: Trichinella, wildlife, raccoons, mexico, bush meat

P520 / #1614

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

ANTHROPOGENIC IMPASSE AND EMERGING PATHOGENS FROM CANIS FAMILIARIS AND PANTHERA LEO IN SERENGETI ECOSYSTEM IN TANZANIA

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Introduction: Wildlife has long history of co-evolving with parasites. This study was conducted to assess the species-area relationship and the exposure of people to novel infections and possible emerging pathogens that can be transmitted by carnivorous mammals to the community.

Methods: Fecal samples from the lions and dogs were collected from 2020 to 2021 in Serengeti ecosystem, Tanzania. Formalin-ether sedimentation method was used in the preparation of the fecal samples prior to examined under light microscopy. Data was analyzed using appropriate descriptive, static methods.

Results: Fecal samples from 117 domiciled dogs and 21 lions were collected from 5 villages adjacent to Serengeti National Park and from within the park. The overall prevalence was 77.5 (n = 107/138). *Spirometra* species was the most common with 27.2% followed by taeniid species (25.5%) and the nematodes (rhabditiform) revealed in the lowest rate of infection. Among the helminths, *Spirometra* sp. was the most prevalent 18.7% (n = 20), taeniid species 17.8% (n = 19), hookworms 10.3% (n = 11), *Spirocerca* sp. 11.2% (n = 12), and the trematode *Dicrocoelium* sp. 8.4% (n = 9). The protozoan species such as *Eimeria* sp. 7.5% (n = 8), *Balantidium* spp. 5.6% (n = 6) were observed in dog samples. Helminth infection rates in dogs were high in Ololosokwan village with *Spirometra* sp. (n = 9), taeniid species (n =7), and *Hymenolepis* sp. (n = 3) followed by hookworms (n = 4). Protozoan were highly observed in Loliondo village with *Balantidium* sp. (n = 4) and Arash village with *Eimeria* sp. (n = 4).

Conclusions: Human activities, loss of biodiversity and encroachment adjacent to the protected areas lead to the introduction of zoonotic diseases to human. Further investigation on the parasites is needed to clarify zoonotic parasites affecting human.

Keywords: Serengeti ecosystem, Zoonotic parasites, Loliondo area, Lion, dog, community

P521 / #687

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

THE ROLE OF GROUND BEETLES AS INTERMEDIATE HOSTS FOR MASTOPHORUS MURIS

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Introduction: Mastophorus muris, a cosmopolitan gastric spiruid nematode, requires an obligatory intermediate invertebrate host. Larvae can develop in Orthoptera, Dermaptera, Dictyoptera and Siphonaptera insects. Larvae of spirurid parasite may also develop in ground beetles (e.g. Geotrupidae). Bank voles (Myodes glareolus) are omnivorous rodents, mainly herbivorous. However, their diet includes also arthropods and annelids. Natural sources of proteins from invertebrates are usually the most important during breeding and lactation when rodents have significant protein requirements. Beetles are considered reservoirs of infection for their occasional predators, including rodents.

Methods: Our objectives were to monitor the prevalence of M. muris in the ground beetles species (Anoplotrupes stercorosus, Trypocopris vernalis) found in three separated locations in NE Poland and to assess the potential role of ground beetles as intermediate hosts for M. muris. Beetles were dissected, and then any parasites present were counted, recorded, transferred to a glass slide and examined under the light microscope. Recovered larvae were stored in 70% ethanol for polymerase chain reaction (PCR). Products of PCR reaction were visualised with the electrophoresis.

Results: We detected M. muris larvae in 18 of 240 dissected beetles, with an overall prevalence of 7.5% (23.8% for Pilchy, 40.0% for Tałty and 26.3% for Urwitałt). We sequenced M. muris isolates and obtained ten various sequences.

Conclusions: These results contribute to our understanding of the prevalence and abundance of M. muris in ground beetles in Poland and confirm that M. muris circulates in A. stercorosus and T. vernalis. Therefore, they may play a role as reservoirs of this parasite in the sylvatic environment.

Keywords: Parasite, ground beetles, Mastophorus muris, intermediate hosts

P522 / #980

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

INTERSTINAL PARASITES OF PET DOGS IN BELGRADE AREA IN PERIOD 2020 - 2021

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Introduction: In urban environment the largest population of pet animals are dogs. In urban environment green areas and parks are the main place where children play and present areas for recreation of city people, but also are the places where dog owners running their pets and those animals permanent contaminated thet places with its faeces. Besides its unpleasant appearance and odor, dog feces is a high epidemiological danger. Dogs are carriers and hosts of a large number of zoonotic parasites species which eggs were eliminated by faeces and contaminate urban areas. man health

Methods: In the aim of evaluating the intestinal parasites fauna of pet dogs from Belgrade area, in period 2020-2021 we examined fecal samples of 367 pet animals. All animals had clinical symptoms that indicated parasitic infections (weight loss, stunted growth, swelling of the stomach in puppies, foul-smelling diarrhea; feces with blood, with findings of swallowing, etc.). Fecal samples we examined with flotation methods by McMaster, Stoll and Richardson-Kendell. Determination of parasite eggs and oocysts was made on the basis of their morphological characteristics.

Results: During our examination presence of parasites we found at 39.32% animals. Ancylostomidae sp were found in 38.95%, Dypillidium caninum in 37.07%, Toxocara canis in 32.20%, Giardia intestinalis in 23.97%, Amoeba sp. in 16.85%, Taenia sp.in 5.61%%, Toxascaris leonina in 8.98%, Isosporas sp. in 6.14%, Strongyloides stercoralis in 4.11%, Trichuris vulpis in 3.74% and Cryptosporidium sp. in 2.99%.

Conclusions: In addition to the parasites themselves important to the health of dogs, they have a great epidemiological significance. The high prevalence of zoonotic parasites indicates a potential risk to human health

Keywords: Dogs, Parasite, zoonoses

P523 / #1388

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

A FIELD TRIAL TO REDUCE THE RISK OF INFECTION BY ZOONOTIC STHS AT A CANINE REFUGE BY USING PARASITICIDE FUNGI

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Introduction: Canine shelters provide a community service consisting of admitting and caring for lost or abandoned dogs, which are provided appropriate veterinary attention. Important levels of prevalence by digestive endoparasites have been reported in shelters from different countries, mostly caused by protozoa and helminths, mainly soil-transmitted helminths.

Methods: Along 17 months, dogs passing eggs of Toxocara canis, Toxascaris leonina, Trichuris canis and Ancylostoma caninum received five treatments based on febantel + pyrantel emboate + praziquantel. Group FGD was also provided chlamydospores of two parasiticidal fungi, Mucor circinelloides (ovicide) and Duddingtonia flagrans (larvicide) the fungi three days / week, and group CTD remained as control without fungi. The effect of deworming was assessed by estimating the Fecal Egg Counts Reduction (FECR) and the prevalence of dogs passing eggs by feces. A low-risky feces period (LRFP) was considered if FECR> 90% and <100%, and a non-risky feces period (NRFP) when FECR= 100%.

Results: The anthelmintic efficacy was 96-98% throughout the study. Similar values of helminths egg-output were reached in both groups of dogs until the 6th month, then reduced significantly to lower than 150 eggs per gram of feces in FGD. No NRFP differences were observed among the two groups, whereas the LRFP extended two-three times in FGD.

Conclusions: These results appear to point that the risk of infection by STHs among dogs maintained in a refuge can be decreased significantly by providing them a blend of chlamydospores of M. circinelloides and D. flagrans, thus it is strongly recommended. Partly supported by the Projects PID2020-120208RB-I00 (MCINN, Spain; FEDER) and ED431B 2021/07 (Xunta de Galicia, Spain).

Keywords: zoonoses, canine refuge, Mucor circinelloides, Duddingtonia flagrans

P524 / #140

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

MOLECULAR AND MICROSCOPIC INVESTIGATION OF SARCOCYSTIS SPECIES ISOLATED FROM SHEEP MUSCLES IN IRAN

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Introduction: Sarcocystis species is a genus of cyst-forming parasites infecting both humans and animals globally. Some of these species cause clinical and subclinical diseases in the host and may lead to economic losses. This study was carried out to identify the distribution patterns of Sarcocystis spp. in slaughtered sheep based on the digestion method and PCR-RFLP in Isfahan, the center of Iran.

Methods: In total, 150 fresh muscle samples (30 hearts, 60 esophagi, and 60 diaphragms) were investigated by naked eye observation and then scrutinized based on the digestion method. To this end, pepsin and HCl were used to observe the Sarcocystis parasite via a light microscope. The PCR was carried out to intensify a fragment of the 18S rRNA gene. Afterward, the PCR products were exposed to digestion by endonuclease TaqI, HindII, EcoRI, and AvaI. Consequently, the results of RFLP were confirmed by sequencing, and the phylogenetic placement of all species was analyzed.

Results: Through the examination by the naked eye, 5/150 (3.33%) macroscopic cysts were found in the samples. With the tissue digestion and microscopic examination, 116 (77.33%) samples were positive for Sarcocystis spp.; however, 125 (83.33%) samples were positive with PCR. Moreover, the results of sequence analysis on macrocysts and microcysts showed that 4% and 96% of the species belonged to S. gigantea and S. tenella, respectively. According to the results of the current study, sarcocystosis caused by S. tenella are highly prevalent among sheep in the Isfahan region.

Conclusions: Due to the high prevalence of Sarcocystis infection in the world and Iran, the development of disease control and prevention policies in sheep would be essential.

Keywords: Sarcocystis, PCR-RFLP, 18S rRNA

P525 / #1170

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

EXPANSION OF RACCOONS (PROCYON LOTOR) THREATENS ALREADY ENDANGERED NATIVE ANIMAL SPECIES AND POSES HEALTH RISKS FOR HUMANS

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Introduction: Raccoons (Procyon lotor) are native in North America and is an invasive alien species (IAS) in Europe. Due to its omnivorous diet, its adaptiveness and the lack of natural enemies, it has been spreading widely in the last decades and will continue in the future. The aim of this study was to investigate the impact of raccoons on protected or endangered native species, as well as on the economy and animal and human health, because of its role as a vector of parasites and pathogens and his increased spread in urban areas.

Methods: 350 raccoons were examined by dissection to investigate diet composition and parasite fauna. Blood and swab samples were taken to test for viral infections. Current distribution and land use was used to model possible future distribution.

Results: A predation on endangered and protected native species, like amphibians (e.g. yellow-bellied toad (Bombina variegata)) and birds could be proven through stomach content analyses. The parasite fauna shows a high number of parasite species from different classes. The key species is Baylisascaris procyonis, the human-pathogenic raccoon roundworm that appeared in over 95% of the samples. Some of the blood samples were tested positive for WNV (West Nile Virus) which reveals a vector-competence of raccoons for this pathogen.

Conclusions: The present study shows, that raccoons can have strong negative impacts on native species like amphibians and birds as well as on animal and human health caused by the spread of zoonoses. In the future, economic losses caused by the raccoon's expansion into densely populated urban areas could be increasing as well.

Keywords: raccoon, Parasites, invasive, zoonoses, endangered

P526 / #197

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

BABESIA NEGEVI INFECTION IN DOGS, PATHOPHYSIOLOGY AND RESPONSE TO TREATMENT

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Introduction: Babesiosis is an important protozoan tick-borne disease in animals and humans caused by various species of the genus, Babesia. We report babesiosis in three dogs from Israel with history of anorexia, lethargy and listlessness.

Methods: On clinical examination, dogs were noted to have pyrexia, pale or icteric gingival mucous membranes, tachycardia and tachypnea. Hematological analyses performed included complete blood count and microscopical examination of blood smears as well as serum biochemistry. Conventional and qPCR of blood samples targeted the piroplasmid 18S rRNA and mitochondrial cox1 genes as well as the Borrelia persica flagellin and Hepatozoon 18S rRNA genes.

Results: Microscopic examination of blood smears and PCR of blood confirmed Babesia infection caused by the recently described, Babesia negevi. Co-infections with B. persica was found in one dog and with H. canis in two other puppies. One puppy deteriorated and died despite initial imidocarb dipropionate treatment. Treatment of the other dogs with imidocarb dipropionate repeated after two weeks and with doxycycline resulted in dramatic clinical improvement. However, B. negevi DNA was still recovered from blood samples by qPCR one month after treatment in the surviving pup (27,486 parasites/µl) and 5.5 months post-treatment in the female dog (420 parasites/µl). Treatment with the combination of atovaquone and azithromycin resulted in a negative PCR for B. negevi twelve months post initial therapy in both dogs.

Conclusions: This study emphasizes that although B. negevi infected dogs may be clinically responsive to therapy effective against large Babesia spp., complete parasite elimination from the blood may require treatment similar to that used against other small canine Babesia.

Disclosure: There are no financial conflicts of interest to disclose.

Keywords: Babesia negevi, canine, 18S rRNA, mitochondrial cox1

P527 / #837

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

FIRST REPORTED CASE OF CANINE THELAZIOSIS IN THE CANARY ISLANDS: A POTENTIAL NEW INVASIVE SPECIES?

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Introduction: The oriental eyeworm (Thelazia callipeda) has rapidly extended throughout most of Europe since 1989, from its natural habitat in Asia. Ecological models suggest a further expansion of the parasite's range to infect dogs in new regions such as the United Kingdom, where thus far only imported cases have been diagnosed. In Spain this rapid range expansion is dramatically demonstrated by the report in 2010 of more than 180 infected dogs in just one city in western Spain (Cáceres). During 2021, on Gran Canaria (Canary Islands, Spain), an intact 3-year old female crossbreed dog presented to the referring veterinarian with a history of epiphora, mild conjunctivitis and blepharospasm. The owners recently moved from the north of the Iberian Peninsula. On ophthalmologic examination a motile white nematode was observed on the corneal surface.

Methods: The nematode was carefully extracted by forceps, fixed in 70% ethanol and delivered to the Laboratory of Parasitology, Faculty of Veterinary Sciences of the University of Las Palmas de Gran Canaria, on the island of Gran Canaria (Spain).

Results: The material received consisted in one gravid female, diagnosed as Thelazia sp. most likely T. callipeda.

Conclusions: T. callipeda depend solely on fruit flies (Drosophilidae: Steganinae) which feed on eye secretions as their intermediate hosts however, neither of these have so far been reported in the Canary Islands. It would suggest that currently the range of T. callipeda is unlikely to extend to the extreme south-western most point of Europe. Still, very year new species (endemic and exotic) are discovered in this territory, highlighting the need for further and ongoing vector surveillance.

Keywords: Thelazia, eyeworm, Canary Islands, zoonoses

P528 / #1176

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

RACCOON DOG (NYCTEREUTES PROCYONOIDES) AS A POTENTIAL THREAT TO NATIVE SPECIES AND A DISEASE AND PARASITE CARRIER IN EUROPE.

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Introduction: Raccoon dog Nyctereutes procyonoides is native to Asia but increasingly occurs in Europe. The introduction of this species in Europe was caused by anthropogenic influence which classifies him as an invasive alien species (IAS). These IAS are known for having an impact on native ecosystems based on their role as vectors of parasites and pathogens as well as predation of protected native species. The aim of this study was to reveal carried parasites as well as pathogens of raccoon dogs from a defined area in Germany through dissection and analysis of their diet composition. The results are used to assess the raccoon dog's impact on native ecosystems.

Methods: For investigation, 70 raccoon dogs were examined by dissection. The stomach content was separated and identified genetically. Found endo- and ectoparasites were identified morphologically as well as genetically. Based on the results, the prevalence, intensity and abundance of parasite infestation was calculated.

Results: Based on the dietary composition, a predation on native animal species such as the protected frog Rana temporaria could be shown. In total, 7 ectoparasite species as well as 10 endoparasite species could be identified to species level. Highest prevalences were found for Toxocara canis and Uncinaria stenocephala, the highest intensitiy was found for the zoonotic Echinococcus multilocularis.

Conclusions: The present study shows that Nyctereutes procyonoides could play an important role in the spread of zoonoses, because he serves as host for a high number of parasite species. In addition, he can cause a decline in native animal species and therefore has a negative impact on native ecosystems as well as on animal and human health.

Keywords: tanuki, zoonoses, predatory, diet, nyctereutes

P529 / #625

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

MOLECULAR DETECTION OF SELECTED ENDOPARASITES IN VESPERTILIONID BATS FROM CENTRAL EUROPE

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Introduction: Bats of the family Vespertilionidae can serve as potential source of infection for humans and other animals. The aim of this study was to test wild bats from the Czech Republic and Slovakia for the presence of selected parasites Toxoplasma gondii, Neospora caninum and microsporidia Encephalitozoon spp.

Methods: In total, tissues (brain or small intestine) of 100 bats (52 Myotis myotis, 43 Nyctalus noctula and five Vespertilio murinus) were used for the DNA isolation and PCR detection of above-mentioned agents. Brain samples were used for detection of T. gondii DNA targeting TGR1E region and for detection of N. caninum DNA, targeting Nc-5 region. Samples, positive to T. gondii were confirmed by real-time PCR targeting a 529 bp repeated fragment of T. gondii DNA. Small intestine samples were used for detection of Encephalitozoon spp. DNA, targeting ITS regions. Positive samples were sent for sequencing.

Results: T. gondii DNA was detected in the brain of one M. myotis (male); while all bats were negative for N. caninum. Encephalitozoon spp. DNA was detected in small intestine of 25% bats: 22 M. myotis, two N. noctula and one V. murinus. Sequencing showed homology with the genotypes E. cuniculi II and E. hellem 2C.

Conclusions: Our results show that members of the family Vespertilionidae can serve as an intermediate host of the protozoan parasites and come into a contact with the spores of microsporidia. To our knowledge, this is the first study on the wild vespertilionid bats from Central Europe with a relatively high positivity of Encephalitozoon spp. in bats. Acknowledgements: This research was supported by grant VEGA No. 1/0043/19

Keywords: PCR, Neosporosis, Toxoplasmosis, Insectivore, Microsporidiosis

P530 / #1594

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

ASSESSMENT OF THE RISK OF FASCIOLA AND SCHISTOSOMA SPP. CROSS-INFECTION BETWEEN LIVESTOCK AND WILD MAMMALS IN WESTERN UGANDA

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Introduction: A longitudinal first kind of study in the area was conducted for 10 months from November 2019 to establish; the prevalence of *Fasciola* and *Schistosoma* infection among definitive and intermediate host species, and the socio-ecological risk factors associated with the spread of *the parasites*.

Methods: Animals were followed, and freshly dropped feces collected and fixed in 10% formol saline solution. Parasite eggs were concentrated by formal ether sedimentation and identified morphologically. Animal drinking points were sampled monthly by scooping for 30 minutes, collecting *Radix* and *Bulinus* spp. Snails were exposed to light for 2 hours to shed cercariae. Questionnaires were administered to 110 stakeholders to document the risk factors associated with the parasite spread in animals.

Results: Cattle had the highest prevalence (56%) of *Fasciola* followed by sheep (50%) and lowest in goats (28.2%). For wild mammals, hippos (66%) had the highest prevalence of *Fasciola* followed by warthogs (8%) and baboons (6%) ($P<0.001$, $\chi^2=25.98$). *Fasciola was* not detected (0%) in elephants and monkeys*. S. bovis* was detected in cattle from Mpeefu (2.6%) and Ndaiga (4.3%); *S. mattheei* was detected in goats (1.4%) and cattle (0.39%) at Ndaiga*.* No human *Schistosoma spp.* was detected in the non-human primates. Only snails collected from Mpeefu shed cercariae: 2% (n =701) of the *R. natalensis* shed *Fasciola,* 2.56% (n =351) and 33.6% (n =122) of *B. tropicus and B. nasutus* respectively shed *Schistosoma* cercariae. Up to 100% of the respondents practiced free-range grazing on communal land, 62.7% and 6.5% knew about fasciolosis and schistosomiasis in animals.

Conclusions: In conclusion, livestock and wild mammals host *Fasciola* spp. and limited knowledge about the parasites is a risk for cross-infection.

Disclosure: This study was funded by the Belgium development cooperation through Action Towards Reducing Aquatic snail borne Parasitic diseases.

Keywords: Western Uganda, Fasciola, Schistosoma, species, mammals

P531 / #280

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

CHARACTERIZATION OF ANAPLASMA OVIS STRAINS USING THE MAJOR SURFACE PROTEIN 1A REPEAT SEQUENCES IN GOATS

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Introduction: Anaplasma ovis is a tick-borne obligatory intraerythrocytic bacterium that infects domestic sheep, goats and wild ruminants. Recently, various studies have been carried out using 16S rRNA and msp4 genes to determine the genetic diversity of A. ovis. However, since these genes are highly conserved among heterologous strains, msp1a gene, which is considered a stable molecular marker to classify A. marginale strains, has been used in A. ovis genetic diversity studies. The aim of this study was to assess the genetic diversity of A. ovis in goats based on the analysis of the msp1a gene.

Methods: Blood samples were taken from the vena jugularis to the EDTA tubes from 293 randomly selected goats (apparently healthy) in the Mediterranean region (Antalya, Mersin) of Turkey. The genomic DNA was extracted from 200 µl of EDTA anticoagulated blood samples from the goats using commercial kit. All DNA samples were analyzed by PCR with specific primer pairs AoMsp1aF/AoMsp1aR to amplify of the msp1a gene of A. ovis. Among the amplified products, well-defined bands with different band sizes were selected and sent for sequence analysis. The obtained sequence data were converted into amino acid sequences using the online bioinformatic program and the tandem regions were examined.

Results: The results showed that 135 (46.1%) goats were positive for msp1a gene of A. ovis. They were translated to amino acid sequences and analyzed based on the msp1a tandem repeats structure. As a result of the tandem analysis, 5 different tandems were determined and 3 of these tandems were defined as a new tandem.

Conclusions: Msp1a gene may be used as a marker for identifying A. ovis strains. This study provides important data for understanding the genetic diversity and evolution of A. ovis based on the tandem repeats.

Disclosure: This work was supported by founding from the Scientific and Technological Research Council of Turkey (TUBITAK) (project no. 118O871).

Keywords: Anaplasma ovis, goat, msp1a gene, Turkey

P532 / #2055

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

GIARDIA IN SYMPTATRIC GRAZERS – WILD REINDEER AND SHEEP: A WALK ON THE WILD SIDE.

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Introduction: Previous studies have found a low and widespread prevalence of *G. duodenalis* in wild reindeer in Norway. The aim of this study was to re-examine *Giardia* prevalence, burden, and genotype in two wild reindeer populations in Norway.

Methods: During 2018 - 2021, 168 faecal samples were collected from wild reindeer in Knutshø and Forollhogna, and 45 samples from sheep grazing in the same areas were collected in 2018 and 2019. Samples were analysed qualitatively and quantitatively using immunofluorescent antibody assay (IFAT) and molecular characterisation was performed by isolating DNA from all *Giardia*-positive samples, PCR and sequencing at two genes; glutamate dehydrogenase (*gdh*) and beta-giardin (*bg*).

Results: *Giardia* was detected in 25 of the168 reindeer samples (15%) . Of these, 18 (of 41 samples) were collected during a single day in July, 2021. Among the 45 sheep samples, 13 (29%) were positive for *Giardia* cysts. In the molecular characterisation, sequences were obtained from 27 samples using the *gdh* primer set and from 21 samples using the *bg* primers, revealing Assemblage A and B in reindeer and Assemblage E and A in sheep.

Conclusions: The overall results show a relatively low prevalence of *G. duodenalis* infection in wild reindeer in Knutshø, although one sampling occasion provided samples with high prevalence and high numbers of cysts, indicating that *Giardia* infections in wild cervids may be endemic and cause intermittent bouts of infections in a herd. The molecular results are interesting, as although the reindeer and sheep were sympatric, they appear not to share *Giardia i*n this situation. The findings of both Assemblage A and B in wild reindeer, may, however, point to a possible zoonotic potential with an infection risk to those enjoying a walk on the wild side.

Keywords: Giardia, reindeer, sheep, Zoonosis

P533 / #838

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

MUSKRATS ARE IMPORTANT HOSTS FOR ECHINOCOCCUS MULTILOCULARIS AND OTHER TAENIIDS IN CENTRAL EUROPE

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Introduction: Echinococcus multilocularis (Em) is the causative agent of alveolar echinococcosis, the most pathogenic zoonosis in northern temperate and arctic regions. Muskrats (Ondatra zibethicus), invasive in Europe, are known to be suitable intermediate hosts for various taeniid species including Em. Our aim was to assess the prevalence, infection intensity and genetic diversity of these parasites in muskrats in an area from central Europe.

Methods: Carcasses of 280 muskrats from Luxembourg collected from 2013 to 2017 were examined. Identification of metacestodes was done morphologically and by partial sequencing of the cox1 gene.

Results: Metacestodes of five taeniid species were found (prevalence): E. multilocularis (14.6%), Hydatigera kamiyai (45.7%), Taenia martis (8.9%), T. polyacantha (5.0%) and Versteria mustelae (0.7%). Cox1 sequences of Em showed very low diversity, in contrast to high diversity of H. kamiyai. Em infected muskrats harboured a mean number of 311,714 protoscoleces, with a maximum of >1.6 million protoscoleces in one animal. The prevalence of Em in muskrats in Luxembourg increased within the study period and compared with older data, this is in line with prevalence of Em in foxes.

Conclusions: These results confirm the suitability of the muskrat as a sentinel species for this zoonosis. The low genetic diversity of Em indicates a founder effect and, possibly, an unstable endemicity level. The putative importance of muskrats for the lifecycle of Em is discussed.

Keywords: muskrats, Prevalence, Echinococcus multilocularis, Taeniidae

P534 / #542

Topic: *AS03.5 Parasitic zoonoses and vector-borne diseases*

NEW RECORDS OF BACTERIA IN DIFFERENT SPECIES OF FLEAS FROM SOUTHWEST OF ANDALUSIA, SPAIN.

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Introduction: The public health importance of flea-borne infections has been reported in several studies in recent years; however, the role of fleas as competent vectors of several zoonotic pathogens species has been poorly studied. Moreover, epidemiological data is still missing for several flea species which may play an important role in the transmission of zoonotic vector borne pathogens. In this sense, the aim of this work was to assess the prevalence of several flea species in different domestic and peridomestic hosts from Southwest of Andalusia (Southwest of Spain) evaluating, at the same time, the presence and prevalence of several flea-borne pathogens and endosymbionts (Bartonella spp., Rickettsia spp., Yersinia pestis, Coxiella burnetii, Francisella tularensis, Borrelia spp., Leishmania spp., Mycobacterium spp. and Wolbachia spp.).

Methods: Fleas were collected from Julio 2021 to February 2022 from dogs (Canis lupus familiaris), cats (Felis silvestris) and hedgehogs (Erinaceus europaeus) from different localities of Southwest of Andalusia. Furthermore, all samples were screened using primers and probes targeting specific sequences of the previously mentioned microorganisms by quantitative PCR (qPCR).

Results: A total of 385 fleas were collected. From these samples, we identified 5 different flea species (Ctenocephalides felis, Ctenocephalides canis, Pulex irritans, Archaeopsylla erinacei and Spilopsyllus cuniculis). On the other hand, we detected the presence of Bartonella spp., Rickettsia spp. and Wolbachia spp. with different percentages of prevalence.

Conclusions: With this study we updated the knowledge of flea species prevalence and flea-borne pathogens present in different domestic and peridomestic hosts from the Southwest of Spain.

Keywords: Siphonaptera, Epidemiology, pathogens, Vector-Borne Diseases

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Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

DIETARY SUPPLEMENTATION WITH FERMENTED RAPESEED AND SEAWEED AND EFFECTS ON NATURALLY ACQUIRED PARASITE INFECTIONS AND GUT MICROBIOTA IN OUTDOOR PIGS

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Introduction: Outdoor pig production systems may lead to higher levels of infection with helminths such as Ascaris suum. Due to risk of anthelminthic drug resistance, alternative treatments need to be explored. This study investigated the potential anti-parasitic effects of a dietary supplement of 2% w/w fermented rapeseed-seaweed (FRS) (6% Saccharina lastissima, 6% Ascophyllum nodosum, and 88% rapeseed meal, based on dry matter) in naturally infected outdoor pigs, and its effects on gut microbiota (GM).

Methods: Groups were fed a control diet (n=25) or diet with 2% w/w FRS (n=25) and replicated 4 times over time (N=200). Each replicate/sub-study (SUB1-4) lasted 11-12 weeks. SUB1 was given a different FRS batch than SUB2-4. Weights and fecal samples for GM were collected on arrival and end of study. Fecal samples for fecal egg excretion (FEC) were collected at weeks 0, 5, 7, 9, and 11 or 12.

Results: A linear model found mean daily weight gain (DWG) affected by diet (FRS -40g/day, P=0.01), sub-study (P=0.02), sex (males +70g/day, P<0.01) and infection (-30g/day; P=0.11) in SUB2-4. Furthermore, FRS inclusion in SUB2-4 lowered incidence of infection for A. suum (P=0.19) and accumulative FEC (-45.3%, P=0.16). Analysis found GM not affected by infection but FRS increased relative abundance of a range of Bacteroidetes members, e.g. Prevotella and S24-7. Controls were enriched in various Firmicutes, such as Clostridium and Turicibacter spp.

Conclusions: FRS inclusion negatively affected DWG, tended to reduce parasite burdens and induced changes to GM, depending on the FRS batch.

Disclosure: The study was performed in collaboration with a commercial partner, Fermentationexperts A/S, using their commercially available product EP1199.

Keywords: bioactive forage, fermented seaweed product, fermented rapeseed product

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Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

PARASITIC INFESTATIONS IN BIRDS AND REPTILES OF THE NATIONAL ZOOLOGICAL GARDEN OF RABAT (MOROCCO) AND THERAPEUTIC CHOICES

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Introduction: The parasitic infestations of birds and reptiles were studied in the Rabat Zoological Garden where animals are often treated with antiparasitics but the frequency and the choice of these treatments could be better adapted if the data of parasitic infestations were better known.

Methods: Internal and external parasites were screened for 22 species of birds and 14 species of reptiles from November 2020 to July 2021, using three diagnostic techniques (coproscopic analysis by flotation enrichment method, blood smear and macroscopic and microscopic search for ectoparasites). To treat infected animals, Fenbendazole, Doxycycline, Chloroquine, Toltrazuril and Fipronil were administrated.

Results: The infestation rate in birds was 68% (n=15) of which 57% were related to gastro-intestinal parasites (Capillaria sp, coccidia), 24% to external parasites and 19% to Hemoparasites (Plasmodium sp). The infestation rate in reptiles is 79% (n=11) of which 50% are related to gastro-intestinal parasites (Oxyuris sp, coccidia), 33% to Hemoparasites (Hepatozoon sp, Plasmodium sp) and 17% to external parasites (Ophionyssus natricis). Chloroquine, Fenbendazole and Fipronil were totally effective; Doxycycline had no effect on Hepatozoon sp and Toltrazuril reveals a percentage of 64% efficacy

Conclusions: The present survey provides further insights of internal and external parasitism in birds and reptiles and orientates the practitioner towards the choice of certain therapeutic molecules

Keywords: Birds, Parasitism, Reptiles, zoological garden, Morocco

P537 / #411

Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

FIRST APPROACH ON CHOLESTEROL METABOLISM IN ARGASIDS: MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF THE N-TERMINAL DOMAIN OF THE NIEMANN-PICK C1 PROTEIN.

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Introduction: Cholesterol is an essential component of cell membranes, and in ticks it is a precursor of key molecules in various physiological processes. Ticks are unable to synthesize it, so they have to take it from the host blood meal. Proteins of the Niemann-Pick C1 (NPC1) family mediate the cholesterol transport from intestinal lumen to the inside of the enterocytes through its N-terminal domain. Cholesterol metabolism has been extensively studied in insects and other parasites, although there is hardly any information on ticks. The objectives of this work were the molecular and functional characterization of the intestinal NPC1 N-terminal domain (NPC1-NTD) of the argasid ticks Ornithodoros erraticus and O. moubata, and the assessment of the effect of NPC1-NTD gene knockdown and tick cholesterol metabolism blockage.

Methods: To this end, we: (i) cloned, sequenced and analyzed the NPC1-NTD from both species; (ii) analyzed gene function by RNAi gene knockdown; and (iii) assessed the phenotypic effect of blocking intestinal absorption of cholesterol with ezetimibe.

Results: As in other eukaryotes, the NPC1-NTD of both argasids showed highly conserved secondary and tertiary structures despite their low amino acid sequence identity. RNAi gene knockdown and blocking intestinal absorption of cholesterol caused slight reductions in the viability of O. moubata eggs but significant decreases in adult survival.

Conclusions: This study provides the first molecular and functional data on NPC1-NTD in argasids, which could be interesting targets for new vaccines aimed at tick control. Funding: Grant “RTI2018-098297-B-I00” by MCIN/AEI (Spain) and ERDF. Grant “CLU-2019–05–IRNASA/CSIC Unit of Excellence” by Junta de Castilla y León (Spain) and ERDF

Keywords: Ornithodoros, Argasids, NPC1, Cholesterol

P538 / #1661

Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

MOLECULAR DETECTION OF RICKETTSIA IN TICKS ASSOCIATED WITH BIRDS AND SEASONAL ABUNDANCE OF AMBLYOMMA DISSIMILE IN A RELICT OF TROPICAL DRY FOREST IN NORTHERN COLOMBIA

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Introduction: Birds are important hosts in the life cycle of several tick species and have been studied for their ability to disperse ticks and the pathogens they transmit. Due to the high diversity of bird species in the tropical dry forest, this work describes the finding and identification of ticks and *Rickettsia* in the ticks that parasitize birds in a relict of tropical dry forest in Northern Colombia. At the same time, sampling was carried out on the vegetation throughout the year to compare the abundance of ticks in vegetation and on birds.

Methods: Sampling was carried out between February and September 2021. Ticks were identified taxonomically using keys and morphological descriptions and confirmed molecularly by amplification of the 16S and CO1 genes. The detection of *Rickettsia* was carried out by sequencing of the gltA, OmpA and Sca1 genes.

Results: A total of 367 birds belonging to 41 species were captured, of which 60 were parasitized by 533 *Amblyomma dissimile* ticks (524 larvae and 33 nymphs). A slight seasonal relationship was observed between the abundance of ticks on birds and in vegetation. The presence of *Candidatus Rickettsia colombianensi* was detected with a prevalence of 66%.

Conclusions: This study reports for the first time the presence of *Rickettsia colombianensi* in *Ambyomma dissimile* ticks collected from wild birds in Northern Colombia. These birds showed a high mean abundance of tick parasitism compared to other studies. ***Acknowledgments.*** The authors thank Dr. Paula Sepúlveda and Tania Carelis for their support in taking photographs at the Unimag entomology laboratory and Dr. Diana Tamaris for her support in identifying the birds. We also thank Ángel Oviedo for his support in the field work.

Keywords: dry forest, Candidatus Rickettsia colombianensi, Amblyomma dissimile, Wild birds

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Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

ULTRASTRUCTURE OF THE TEGUMENT INCLUDING NOVEL EXTRACELLULAR VESICLES IN ADULT OOCHORISTICA ANOLIS (CESTODA: CYCLOPHYLLIDEA)

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Introduction: The tegument of parasitic platyhelminths is known to be a dynamic organ that participates in numerous critical functions, ranging from nutrient absorption to immunomodulation and molecular communication. Recent evidence suggests that some of these functions may involve extracellular vesicles released by the helminth from the tegument. However, few cestodes have been studied in this regard, and much remains unknown about the production, structure, and function of exosomes or microvesicles in these parasites.

Methods: The tegument of the scolex, neck, and strobila of adult *Oochorisitica anolis* cestodes from naturally infected *Anolis carolinensis* lizards from Louisiana, USA were examined by standard transmission electron microscopy.

Results: The overall tegumental structure was similar to that described for numerous cestodes, with the surface folded outward into distinct microtriches. Gladiate spinitriches and columnar filitriches predominated in ratios that varied with body region, and diverse vesicles filled the distal cytoplasm of all regions. The posterior gravid proglottids were similar to other regions, but the surface microtriches were disrupted by numerous membrane-bound surface vesicles extending into the exterior in three forms: 1) moniliform strands of ovoid vesicles; 2) multivesicular bodies containing many single pedunculated vesicles; 3) individual surface vesicles.

Conclusions: Based on recent studies of other trematode and cestode species, the ultrastructure of extracellular vesicles reported here suggests non-apoptotic secretion of materials that function outside the cestode's body, possibly interacting with the host. Moniliform strands and multivesicular bodies shown here have not been reported previously for any cestode.

Keywords: Ultrastructure, extracellular vesicles, tegument, cestode, Oochoristica

P540 / #575

Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

IMPLEMENTATION OF A MALLORCAN SNAIL FARM SURVEILLANCE PROGRAM TO DETECT THE ARRIVAL OF A. CANTONENSIS.

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Introduction: Since the first detection of Angiostrongylus cantonensis in hedgehogs from Mallorca (Spain), we implemented parasitological surveillance to assess the risk of zoonotic transmission on the island. This parasitic worm infects snails and rats as intermediate and definitive hosts, respectively. Our approach to surveillance included examining the risks associated with the arrival of A. cantonensis and other parasites in edible snail farm ecosystems.

Methods: We have used three methodological approaches to detect parasites in snails collected from three Mallorcan farms. First, to detect A. cantonensis, we submerged snails for 24h in water, followed by centrifugation of the water and microscopic examination of the sediment. Second, we conducted full snail necropsies to characterize if other parasites were present and if there was tissue specificity. Third, a modified Baermann technique was used for comparative purposes. Finally, a helminth COI fragment was amplified in some specimens.

Results: More than 800 gastropods have been analyzed to date. We have found that Mallorcan commercial snails can harbor several parasite species: Tetrahymena sp., Cryptobia sp., Tetratrichomonas sp., Brachylaima sp. and an unidentified trematode, Alloionema appendiculatum, Rhabditella sp., Caenorhabditis sp. and mites.

Conclusions: To date, A. cantonensis has not been detected in edible snails examined from three farms in Mallorca. However, two trematode species are present, which may indicate that vertebrates are entering the farms. Snail farmers confirmed that rodents are occasionally observed. We recommend several prevention strategies to avoid the risk of introduction of A. cantonensis and other parasites of public health concern in Mediterranean snail farms.

Keywords: One Health, emerging infectious diseases, Angiostrongylus cantonensis, zoonoses, snail farms

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Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

NEOSPORA CANINUM SEROPREVALENCE AMONG CATTLE IN UKRAINE

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Introduction: Neospora caninum could affect different animal species, among which sheep, goat and cattle. In cows it could be reason of abortions. The main idea of our research was to estimate the seroprevalence of N. caninum among cattle from the territory of Ukraine.

Methods: Blood samples of 174 cattle from Volyn, Khmelnytskyi, Cherkasy, Poltava and Ternopil regions of Ukraine were collected during December, 2021 to February, 2022. The farms had from 1300 to 4500 animals, and based on questionnaire data both dogs and cats can access to the territory of the farms. The samples were investigated using a commercial enzyme-linked immunosorbent assay, following the manufacturer's instructions.

Results: Of the samples, 50 were positive, yielding an apparent seroprevalence of 28.7%, and 2.3% of samples (4 samples) had doubtful result. The seroprevalence (only positive samples calculated, without doubtful) was 33.3% among animals from Volyn region, 48.9% from Khmelnytskyi region, 34.2% from Cherkasy region, 0% from Poltava region and 10.0% from Ternopil region. 164 of these samples were tested in parallel on presence of antibodies against Toxoplasma gondii. Among these samples, 2 (1.2%) tested positive for antibodies against both Toxoplasma gondii and Neospora caninum.

Conclusions: These are the first results from investigation of cattle for Neospora caninum from the territory of Ukraine The seroprevalence is high, and need for continuing studying it is clear.

Keywords: Neospora caninum, Cattle, Serology

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Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

PRESENCE OF THE ENZYME NADPH OXIDASE DURING THE EVOLUTION OF AMOEBIC LIVER ABSCESS IN BALB/C MICE.

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Introduction: Entamoeba histolytica is the causal agent of intestinal amebiasis, it remains one of the top three parasitic causes of mortality worldwide, which can cause a serious condition called: amoebic liver abscess (ALA). The BALB/c mouse was determined to be a model of resistance, in which amoebae are eliminated by the fourth day after inoculation. Several works have analyzed the role of neutrophils in amebiasis, they interact with amoebas. Serine proteases, myeloperoxidase (MPO), NADPH oxidase (NOX2) and superoxide dismutase (SOD) are the enzymes present in neutrophils. Currently, there is no information about the NOX2 enzyme in ALA.

Methods: Male Balb/c mice were divided into 2 groups: I) amoeba-inoculated animals, and 2) amoeba-inoculated animals treated with apocynin, an NOX2 enzyme inhibitor (0.41 mg/25 g). The animals were sacrificed at 3, 6, and 12 h post-infection. In ALA samples we determined: histological changes during the evolution of ALA, and quantification of amoebas.

Results: A significant difference was found in the histological sections between the two groups; in the one treated with the inhibitor, we observed an increase in the presence of amoebas without apparent damage, compared to the other group.

Conclusions: The results showed that NOX2 enzyme plays an important role interfering in the host acute immune response. Apocynin treatment in Balb/c mice developed a way to eliminate the amoeba.

Keywords: amoeba, Neutrophils, Apocynin

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PREDATION OF HAEMONCHUS CONTORTUS LARVAL STAGES BY THE MACROCHELES SP. MITE.

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Introduction: Haemonchus contortus is the most pathogenic gastrointestinal nematode of sheep. Its biological cycle includes a free-living phase in the faeces of the host. These faeces are exploited by dung beetles which commonly carry commensal predatory mites as Macrocheles species. The objective of this study was to investigate the predatory effect of Macrocheles mites on the free-living stages of H. contortus in the perspective of a biological control of this parasite.

Methods: Macrocheles sp. were collected on free-living dung beetles. First, the Macrocheles sp. feeding activity on third-stage H. contortus larvae (L3) was checked under a stereomicroscope: up to 10 L3 were exposed to two mites in Petri dishes containing water-agar. Secondly, an experimental unit was designed to quantify effects of mites' predation on the development of the H. contortus free living stages. Each unit consisted of a tube topped by a cell strainer in which fresh sheep faeces with a known amount of nematode's eggs was added. The tube was filled with water until the bottom of the cell strainer to allow vertical L3 dispersal. Of the 54 units, 27 were colonised with 10 mites each, the other half being controls. L3 were counted after 12 days at 24°C. A mixed model allowed to compare numbers of L3 between the control and mites units.

Results: In Petri dishes, mites showed an active predatory behaviour on the L3. The number of recovered L3 was significantly lower ($p < 0.0001$) in the presence of mites (84 ± 99) than in the control units (207 ± 158).

Conclusions: The predatory effect of a commensal dung beetles mite on a pathogenic nematode was demonstrated. Further studies are needed to better understand the accurate role of Macrocheles mites in controlling H. contortus on pastures.

Keywords: phoretic mites, Macrocheles sp., predation, Haemonchus contortus

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Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

LABEL-FREE QUANTITATIVE (LFQ) PROTEOMICS IDENTIFIES EXCRETORY-SECRETORY (E/S) PROTEINS OF ANOPLOCEPHALA PERFOLIATA TAPEWORMS

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Introduction: *Anoplocephala perfoliata* is a common tapeworm in horses capable of causing colic and increasing mortality. Current diagnostic tests to detect *A. perfoliata* infections have their limitations. Excretory/secretory proteins (E/S proteome) of this parasite are promising candidates for diagnostic tests.

Methods: We compared E/S proteins produced by small (length < 20 mm, width < 5 mm) and large (length 20 to 40 mm, width 5 to 10 mm) size *A. perfoliata* worms in vitro. E/S proteins collected after three and eight hours of incubation were compared by LFQ proteomics with a protein database composed of all predicted proteins encoded by *Hymenolepis diminuta*, *Echinococcus multilocularis/granulosus* and *Taenia aseatica* for protein identifications.

Results: Altogether, 509 E/S proteins produced by A. perfoliata were identified after three and eight hours of incubation. The greatest E/S proteome changes suggested both worm size- and time-dependent changes in cytoskeleton remodeling, apoptosis, and production of antigens/immunogens.

Conclusions: Our proteomic data show how the size (age) of the worms affect E/S protein production as well as how *A. perfoliata* responds to *in vitro* conditions (e.g., activation of apoptosis/stress-related pathways). The E/S proteins collected at the three-hour time point are proposed to contain the most relevant diagnostic targets.

Keywords: tapeworm, cestode, Horse, E/S proteins, LFQ-proteomics

P545 / #403

Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

IDENTIFICATION OF BITING MIDGES (CERATOPOGONIDAE: CULICOIDES) AND THEIR HOSTS TRAPPED NEAR SYLVATIC ANIMALS

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Introduction: Biting midges are not only indomitable bothers, but in Europe they transmit helminths of game, ruminants and horses; haemosporidia of birds and viruses (Schmallenberg, Blue tongue etc.). The results of some studies confirm the presence of these viruses respectively antibodies also in wild ruminants or exotic zoo animals. The present work is based on identification Culicoides fauna and host preference captured near sylvatic animals from Košice Zoo and game park Rozhanovce, between May 2019 and October 2021.

Methods: Biting midges were trapped using the CDC 1212 light traps. The species were identified according the morphological features and confirmed by the molecular identification based on the sequencing of partial mitochondrial cytochrome oxidase subunit I gene (COI). The host blood detection was carried out while focussing on a portion of the sequence of the MT-CYB gene using the cyt bb 1 and cyt bb 2 primers. In total, we trapped 11,320 midges. Females with fresh host's blood represented 2.8% of all females.

Results: We confirmed the occurrence of 15 species of biting midges. C. festivipennis (53.62%) in the Zoo Košice formed the majority of the fauna. At the same time, we confirmed the occurrence of rare species C. kibunensis and C. nubeculosus for the first time. On the game farm, the predominant species were C. obsoletus /C. scoticus (93.7%). Midges from zoo garden paratized mainly on zebras, cattle and humans; on a game farm on hares, pheasants and humans.

Conclusions: A knowledge of Culicoides fauna present their ecological preferences is required, so different control strategies can be applied effectively. This study demonstrates that midges are able to utilise blood of sylvatic host species as well as humans. This research was supported by grant VEGA No. 1/0043/19.

Keywords: Culicoides fauna, game, host preference

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TRACKING OF TOXOCARIASIS IN (PANTHERA PARDUS SAXICOLOR) THE ENDANGERED (VULNERABLE) FELINE SPECIES IN THE WILDLIFE OF NORTHWESTERN IRAN.

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Introduction: The Iranian Panther (Panthera pardus saxicolor) is a well-known rare feline species in Iran. They are known as the main host for several helminth parasites. They play an important role in maintaining of the life cycle of some zoonotic parasites in the nature.

Methods: In 2009, a single nematode resembling to ascarids were obtained from the stomach of an unwillingly killed panther. A single nematode resembling helminth in appearance was isolated and kept in 10% formaldehyde and Trans parented in lacto phenol. A precise drawing under the camera Lucida equipped microscope was performed and photographed to obtain further helminthological identification.

Results: Morphological characters and the measurements of the worm (90 mm length, 2.9 mm cervical alae, presence of two spicule, three prominent lips and the lack of ventricle between the oesophagus and intestine) have been finally revealed that the found helminth was male of Toxascaris leonina. The presence of Toxascaris leonina were finally confirmed according to morphological characters.

Conclusions: Finding of this helminth in a Persian panther in the northwestern Iran has brought the importance of wild carnivores in persisting of some zoonotic infections specifically visceral larval mingrans (VLM).

Keywords: Toxascaris leonine, Toxocariasis, Pantera pardus saxicolar, Iran

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Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

REDUCTION OF THE TWO MAJOR ALLERGENS BLA G 1, 2 IN GERMAN COCKROACH AFTER AMPICILLIN TREATMENT

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Introduction: The use of fecal and frass extracts of cockroaches for immunotherapy has been previously investigated but has not yet been fully standardized. Here, we treated cockroaches with ampicillin to produce extracts with reduced amounts of total bacteria.

Methods: We performed targeted high-throughput sequencing of 16S rDNA to compare the microbiomes of ampicillin-treated and untreated (control) cockroaches. RNA-seq was performed to identify differentially expressed genes (DEGs) in ampicillin-treated cockroaches. For the quantification of Bla g 1, 2, 5, in the whole body, an absolute quantitative real-time PCR approach, was developed.

Results: Analysis of the microbiome revealed that alpha diversity was lower in the ampicillin-treated group than in the control group. Beta diversity analysis indicated that ampicillin treatment altered bacterial composition in the microbiome of cockroaches. Quantitative polymerase chain reaction revealed that almost all bacteria were removed from ampicillin-treated cockroaches. RNA-seq analysis revealed 1,236 DEGs in ampicillin-treated cockroaches compared to untreated cockroaches. Among major allergens, the expression of Bla g 1, 2, 5 decreased in ampicillin-treated cockroaches.

Conclusions: In conclusion, ampicillin treatment changed the bacterial composition and reduced major allergens in *Blattella germanica.*

Keywords: cockroach, allergen, antibiotics, Microbiome

P548 / #1340

Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

A PARASITOLOGICAL SURVEY ON ZOOLOGICAL COLLECTION OF NORTHEASTERN ITALY USING MINI-FLOTAC METHOD AND MOLECULAR APPROACH

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Introduction: Wild species living in captivity are found in unusual close contact with humans, as well as with other animal species taken out of their natural environment. This might result in an increased susceptibility to parasitic diseases and transmission of zoonotic pathogens.

Methods: An overall number of 101 animals including carnivores, herbivores, non-human primates (NHP) and reptiles, from two zoological collections in Northern Italy were analysed seasonally in one year for helminthic and protozoan infections. Mini-Flotac method and Baermann technique were applied to fecal samples. Detection of Cryptosporidium spp. and Giardia intestinalis was performed targeting SSU rRNA through nested PCR and qPCR, respectively. Giardia assemblage was studied by sequencing βgiardine or TPI gene.

Results: Overall, 21% samples (76/363) were positive for at least one parasitic taxon. Forty-eight animals (47.5%) tested positive at least once during the whole study period. Gastrointestinal strongylids and Trichuris spp. were detected with the highest prevalence (11% and 7.7% respectively). Prevalence ranged along the year between 23-.7 and 30.8%. All samples tested negative for Cryptosporidium spp., while 23% were positive for G. intestinalis at qPCR, including species from all groups, with highest prevalence in NHP (61%), in which assemblage B was isolated. Assemblage E and F were also found.

Conclusions: The detection of gastrointestinal parasites without overt clinical signs of disease is suggestive of subclinical infection. Nematodes and monoxenous parasites were the most prevalent, as expected. The presence of Giardia assemblage B confirms that NHP are a potential reservoirs for zoonotic transmission. Work supported by the University of Padova, Italy (prot. BIRD200034)

Keywords: Giardia, Mini-FLOTAC, Italy, Zoo, Cryptosporidium

P549 / #641

Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

USEFULNESS OF COMPUTED TOMOGRAPHY PULMONARY VEIN TO PULMONARY ARTERY RATIO IN THE ASSESSMENT OF PULMONARY HYPERTENSION IN DOGS WITH HEARTWORM DISEASE

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Introduction: In dogs, Dirofilaria immitis causes severe cardiorespiratory symptoms related to the appearance and persistence of precapillary pulmonary hypertension (PH). Currently, there are not established computed tomography (CT) criteria to help detect PH specifically produced by D. immitis in the canine species. Therefore, the aim was to evaluate the potential use of the pulmonary vein to pulmonary artery ratio (PV:PA ratio) obtained by CT in the diagnosis of PH caused by D. immitis in dogs.

Methods: Thoracic CT scans with contrast were performed in 31 dogs with heartworm. The PV:PA ratio was determined using a cross-section following previously established protocols. The presence/absence of PH was determined using transthoracic echocardiography (TTE), based on the determination of the PV:PA ratio as previously described (Cut-off ≤ 0.72).

Results: PH was echocardiographically present in 64,5% of the dogs, showing a mean CT PV:PA ratio of 0.68 ± 0.18 (99% CI: 0.57–0.80). Normotensive dogs (35,5%) had a mean PV:PA ratio of 1.24 ± 0.16 (99% CI: 1.14–1.30). Additionally, A high correlation was observed between TTE and CT in the study of PV:PA ratio (R^2=0,806) (P<0.05).

Conclusions: The results showed significant differences in the PV:PA ratio between the presence or absence of PH in dogs with D. immitis, and demonstrate that the PV:PA ratio can be a useful tool to assess PH in patients with heartworm disease in images obtained by CT. New studies are indicated with a larger sample size, being able to standardize protocols and obtain reliable reference values to establish the presence and severity of PH in these dogs. This research was funded by the Ministry of Science, Innovation and Universities. Government of Spain-EQC2019-006512P.

Keywords: Dirofilaria immitis, Dogs, Computed Tomography, Pulmonary Hypertension

P550 / #1201

Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

MOLECULAR ANALYSIS OF DICROCOELIUM DENDRITICUM ISOLATED FROM RUMINANTS IN IRAN

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Introduction: Dicrocoelium dendriticum is a broadly distributed zoonotic helminth, which is mainly reported from domesticated and wild ruminants. there is little data about the molecular analysis of this trematode; therefore, current study aimed to molecularly analyze D. dendriticum in livestock ruminants: cattle, sheep, and goats

Methods: Totally, 23 isolates of D. dendriticum were collected and the discriminative fragment of the ITS2 fragment was amplified and sequenced. Phylogenetic tree and network analysis were employed to investigate genetic variations through the ITS2 fragment. All 23 isolates were successfully amplified and sequenced.

Results: Phylogenetic tree showed that our isolates were clearly grouped in a clade together with reference sequences. There was no grouping based on geographical origins and hosts. Network analysis confirmed the phylogenetic tree and showed the presence of 10 distinct haplotypes while most of sequences were grouped in the Hap-1.

Conclusions: Our findings indicated that although ITS2 fragment discriminate Dicrocoelium spp., at species level, this fragment is not suitable to study intra-species genetic variations. Therefore, exploring and describing new genetic markers could be more appropriate to provide new data about the genetic distribution of this trematode

Keywords: PCR, ITS1, Livestock, Dicrocoelium spp.

P551 / #274

Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

REFINEMENT OF A HIGH WELFARE, ON-HEN MITE FEEDING MODEL.

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Introduction: Poultry red mites (PRM) are small, highly mobile, blood feeding ectoparasites that live off-host, only seeking a bird to feed. PRM are therefore difficult to contain in a controlled experimental environment that allows natural feeding on the host. An 'on-hen' in vivo feeding device was developed for PRM (Nunn et al.,2019 Nunn et al 2020). The device attaches to the hen's thigh which is plucked to facilitate flush device attachment to the hen's skin enabling mite feeding. A study to determine analgesic effect of EMLA cream while plucking using an ethogram and respiration rates was carried out.

Methods: Three groups hens were used: an EMLA group, a placebo cream group and a feeding control group. Ethogram scorers were blinded to groups throughout. Baseline ethogram and respiration scores were taken during dummy handling day, with scores taken on separate plucking and treatment days. Mite feeding assays were carried out on three consecutive days after treatment day to establish any effect of cream on mites.

Results: Significant differences were demonstrated in ethogram scores in each group when comparing dummy handling day to plucking day (> P = 0.01). Only the EMLA group demonstrated no signficant difference between ethogram responses on treatment day and dummy handling day. Respiration rates were elevated on 'plucking' day in comparison to dummy handling day. All but one bird demonstrated a rise in percentage respiration rate on plucking day. On treatment day increases were demonstrated in the placebo and feeding control group. Mite feeding was decreased in the EMLA group although mite mortality and fecundity was not affected.

Conclusions: EMLA cream has a safe, analgesic effect in hens but does have an effect on mite feeding and more work is required to see if this can be mitigated.

Keywords: poultry, mite, In vivo

P552 / #531

Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

STEPS TOWARDS IN VITRO COLONY MAINTENANCE OF THE HEMATOPHAGOUS MITE DERMANYSSUS GALLINAE; OPTIMISING EGG LAYING.

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Introduction: Poultry red mites (PRM) are blood feeding ectoparasites that live off-host, only seeking a bird to rapidly engorge every few days. Recently we described utilising Baudruche membrane in an in vitro device to feed adult females (Nunn et al 2020) using goose blood as a food source, which led to improved and reproducible feeding rates. We then evaluated the device to feed the hematophagous nymph stages of PRM and demonstrated significant correlation between the feeding rates of deutonymph stages and adult females. Mite fecundity in vitro can be variable and here we describe steps towards optimising egg laying.

Methods: To see if fecundity of mites fed in vitro was improved by being kept in groups of fed females, we compared numbers of offspring per fed mite on two occasions by incubating fed mites individually (n = 60, n = 41 respectively) and then 5 replicate groups of 5, 15 and 30 mites and counting offspring after incubation for 7 days Repeated feeding of adult female mites was performed, with 6 flasks of 100 adult females fed on four consecutive days and 6 flasks of 100 adult females fed only once. Flasks were incubated at 20C and 75% relative humidity for 5 further days to allow eggs to hatch and for larvae to moult into protonymphs. Protonymphs were then counted and progeny per adult female was calculated.

Results: Using an unpaired t-test with Welch's correction, a significant increase (P = 0.02; t=2.844, df=6) in progeny/mite was demonstrated in those mites fed on four consecutive days (range 0.49-1.9, mean 1.115, SEM 0.21) compared to those fed once (range 0.23-0.49, mean 0.485, SEM 0.08).

Conclusions: Fecundity was improved by repeated feeding of females but not by housing groups of fed females together. Further work may determine other optimal conditions for mite egg laying.

Keywords: poultry, mite, In vitro

P553 / #1091

Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

SURVEY ON SARCOCYSTIS INFECTION IN RED DEER (CERVUS ELAPHUS) OF EASTERN SLOVAKIA BY MICROSCOPY AND PCR ANALYSIS

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Introduction: Consumption of game meat has increased in Slovakia in recent years. Meat of red deer is rich in nutrients and low in fat. It may, however, contain tissue parasites that can reduce its quality and make people ill if the meat has not been prepared in a hygienically flawless manner. This study offers an overview of the occurrence of Sarcocystis spp. in red deer of Slovakia.

Methods: A total of 69 muscle samples of red deer were collected during the years 2018-2021 years (Hunting Grounds of Markovec, Richvald, Rozhanovce, Sobrance) during duly authorized hunts. Samples were examined visually for macro tissue cysts and by the peptic digestion method to analyze fresh muscle for detecting micro tissue cysts followed by microscopic examination. DNA from selected samples was subjected to standard PCR amplification followed by sequencing of partial cytochrome c oxidase subunite I gene (cox1).

Results: In the visually examination, macroscopic cysts were not detected. By light microscopy, fusiform or oval sarcocysts were observed in the striated muscles. We found 97.3% prevalence of tissue cysts. The average tissue cysts load was 61.1 per gram of muscles. PCR analysis of isolated cysts indicated the presence of Sarcocystis linearis and Sarcocystis cervicanis.

Conclusions: Our results provide an estimation of sarcocystis infection prevalence (97.3%) in red deer and this is the first time that these species has been confirmed by molecular analysis in Slovakia. Acknowledgements This research was supported by grant VEGA No. 1/0043/19.

Keywords: PCR, cytochrome c oxidase subunite I gene, Sarcocystis linearis, Sarcocystis cervicanis

P554 / #1441

Topic: *AS03.6 Other studies related to parasites of domestic and wild animals*

TOXOPLASMA GONDII SEROPREVALENCE AMONG CATTLE IN UKRAINE

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Introduction: Toxoplasma gondii is a unicellular parasite that can infect humans and numerous warm-blooded animals, including cattle. The main idea of our research was to estimate the seroprevalence of T. gondii among cattle from the territory of Ukraine.

Methods: Blood samples were collected from 164 cattle, from farms with 1300 to 4500 animals, from Volyn, Khmelnytskyi, Cherkasy, Poltava and Ternopil regions of Ukraine, during time period from December, 2021 to February, 2022. Based on questionnaire data, cats had access to the territory of the farms.

Results: Of the samples, 17 tested positive, yielding an apparent seroprevalence of 10.4%. The seroprevalence was 10.3% among animals from Volyn region, 18.9% from Khmelnytskyi region, 2.6% from Cherkasy region, 10.0% from Poltava region and 10.0% from Ternopil region.

Conclusions: Since undercooked beef and unpasteurized milk could be potential sources of infection for humans and other animals, and the estimated seroprevalence is not low, these researches should be continued.

Keywords: Toxoplasma gondii, Serology, Cattle

P555 / #199

Topic: *AS04 Parasites of fish / AS04.1 Fish-borne zoonotic parasites*

ASCARIDOID NEMATODES INFECTING COMMERCIALLY IMPORTANT MARINE FISH AND SQUID SPECIES FROM OFF BANGLADESH

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Introduction: Parasitic ascaridoid nematodes occur in a wide range of marine organisms across the globe. Some species of the anisakid family can cause gastrointestinal disease in humans (i. e. anisakidosis). Despite their importance, the occurrence and infection characteristics of ascaridoids are poorly known from many host species and geographical areas. We investigated the diversity and infection levels of ascaridoids in commercial fish and squid species off Bangladesh.

Methods: Fish and squids were visually inspected for nematodes using the UV-press method. Nematodes were assigned to genus level based on morphology and identified by sequence analyses of the entire ITS and partial 28S rDNA and mtDNA cox2 genes.

Results: Third-stage larvae (L3) of Anisakis typica occurred at low prevalence (P=10% and 8%) in the viscera of Selar crumenophthalmus and Trichiurus lepturus, while Hysterothylacium amoyense occurred in the viscera of Sardinella fimbriata (P=1%) and the viscera and muscle of Harpadon nehereus (P=32%) and T. lepturus (P=76%). Lappetascaris sp. Type A L3 occurred in the mantle of the squid Uroteuthis duvaucelii (P= 11%).

Conclusions: Anisakis and Lappetascaris species, and H. amoyense were firstly identified in the Bay of Bengal. The potentially zoonotic A. typica was only found in fish viscera. H. amoyense and Lappetascaris sp., both generally regarded as non-zoonotic, occurred at low prevalence in the muscle or mantle of fish or squid, respectively. Since consumption of raw or lightly processed seafood seems to be rare in Bangladesh, the risk of acquiring anisakidosis from consuming fishery products from off Bangladesh appears to be low. Due to its reddish appearance, the visual presence of H. amoyense larvae in fish flesh may represent a food quality issue.

Keywords: fish parasite, anisakis, Hysterothylacium, Lappetascaris, anisakidosis

P556 / #1114

Topic: *AS04 Parasites of fish / AS04.1 Fish-borne zoonotic parasites*

CONTRACAECUM LARVAE (NEMATODA: ANISAKIDAE) INFECTING FARMED TILAPIA IN ISRAEL

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Introduction: Contracaecum larvae infecting fish may have zoonotic potential and are economically important causing market rejection in massive infection. In Israel, Contracaecum larvae have been found in several fish species both wild and farmed since the 1960s. Aims of this survey was to assess the presence and distribution of Contracaecum larvae in farmed tilapias.

Methods: From June 2020 to May 2021, 5,090 tons of tilapia (Oreochromis niloticus x O. aureus, O. niloticus and Oreochromis sp.) farmed in 17 tilapia farms were inspected by Official veterinarians in eight fish-sorting stations. The fish were subjected to routine visual inspection, including macroscopic examination for the presence of potentially zoonotic parasites. All the collected parasites were preserved in 70% ethanol for downstream analyses.

Results: Among 1894 batches of market-size tilapia examined, 170 were found to be infected by at least one Contracaecum larva. A total of 271 Contracaecum larvae were collected from 10,356 hybrids tilapias analyzed. Prevalence of infection varied among different farms and ranged from 1% to 32.8%.

Conclusions: Globally, Tilapines are the second most important group of farmed fish providing an inexpensive protein source and, in Israel, tilapia is the main farmed species. The presence of larval stages of Contracaecum spp. poses risks to human health and may deteriorate the quality and market value of these fish products, leading to high economic losses.

Keywords: farmed tilapia, Israel, contracaecum

P557 / #149

Topic: *AS04 Parasites of fish / AS04.1 Fish-borne zoonotic parasites*

RECORDS OF ZOONOTIC DIGENEAN METACERCARIAE FROM FISHES OF HUNGARIAN NATURAL FRESHWATERS

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Introduction: Previous surveys, like H2020 ParaFishControl project revealed that European aquacultures are mostly free of zoonotic trematodes. However, natural freshwaters ease the appearance of trematodes compared to the fish farms, because natural freshwaters are inhabited by a richer snail fauna, which are the first intermediate hosts of digenetic flukes. Water birds are their final hosts which also appear in abundance in wild habitats. The research aimed to investigate the occurence of the zoonotic flukes in fishes in the Hungarian natural freshwaters, like River Danube and in Lake Balaton and Lake Tisza.

Methods: Sampling of fishes was conducted since 2017 in River Danube and Lake Balaton and since 2020 in Lake Tisza. Dissected fishes were investigated for opisthorchid and heterophyid metacercariae by stereomicroscope and enzymatic digestion of fillets. Sequencing of ITS region, 28S rDNA and COI was carried out for identification.

Results: Metagonimus metacercariae were common on the scales of chub and nase from the river Danube. Apophallus sp. occured in all freshwaters, mostly on the surface of perch and pikeperch, and in a single case its presence was confirmed from the muscle of Wels catfish. Clinostomum complanatum was also found in the muscle of a perch.

Conclusions: Compared to aquacultures, fish from natural freshwaters are more prone to be infected by Digenean trematodes with zoonotic risk probably due to the presence of freshwater molluscs and water birds. Species identification encountered difficulties often as only a part of described species have available sequences and metacercariae do not provide sufficient morphological characteristics for identification. This project has received funding from the Hungarian Scientific Research Fund (OTKA FK 140350).

Keywords: metacercaria, Digenea, Metagonimus, Apophallus, natural freshwaters

P558 / #189

Topic: *AS04 Parasites of fish / AS04.1 Fish-borne zoonotic parasites*

MIGRATION OF ANISAKIS SIMPLEX (NEMATODA: ANISAKIDAE) LARVAE IN THE FLESH OF THREE FISH SPECIES FROM THE NE ATLANTIC: THE ROLE OF STORAGE TIME AND TEMPERATURE

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Introduction: Anisakis simplex (s. s.) is a zoonotic parasite infecting commercial fish species in the Northeast Atlantic. In fish, most of Anisakis larvae reside in viscera, while some larvae may migrate into the flesh both intra-vitam and post-mortem. Presence of this parasite in the fish flesh may represent a consumer health hazard. This study investigated the tissue localization of A. simplex (s.s.) larvae in relation to storage time and temperature in Atlantic herring, Atlantic mackerel and blue whiting from NE Atlantic fishing areas.

Methods: 300 fish pr species were divided in batches of 50 each, stored at different temperature conditions (2°C, 5°C, 15°C), and inspected by UV-press for anisakid larvae, genetically identified as A. simplex (s.s.), at different intervals, i.e. a control batch at 0h, then at 24h and 48h post catch.

Results: Presence of A. simplex (s.s.) larvae in the fish flesh examined at 0h post catch (control) confirms larval intra-vitam migration to occur. In herring and blue whiting, t larval intensity in the fillets significantly increased with increasing storage temperature (5°C, 15°C) and time 24h, 48h). In mackerel, no such variations were observed.

Conclusions: Storage temperature seems to be the single most important driver of post-mortem motility of A. simplex (s.s.) larvae in herring and blue whiting. Findings confirm that ≤ 2°C storage temperature of the fish efficiently prevents post-mortem larval migration during handling and transport.

Keywords: anisakis, Northeast Atlantic Ocean, herring, mackerel

P559 / #865

Topic: *AS04 Parasites of fish / AS04.1 Fish-borne zoonotic parasites*

NEMATODE PARASITES OF COMMERCIALLY IMPORTANT FISH FROM THE SOUTHEAST COAST OF BRAZIL: MORPHOLOGICAL AND GENETIC INSIGHT

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Introduction: Studies of nematofauna of teleost fish from the Brazilian coast are relatively scarce and limited to identification based on morphology. The objective of the present study was to determine the diversity and prevalence of nematode parasites in teleost fish from the southeast Atlantic coast of Rio de Janeiro, through morphological, molecular, and ecological approaches.

Methods: Parasites were collected from sixty specimens each of Genypterus brasiliensis, Micropogonias furnieri, and Mullus argentinae obtained in winters and summers of 2012–2014. Morphological and genetic characterization was conducted using light microscopy and the molecular targets 18S rDNA, ITS1, and mtDNA cox2.

Results: Nematodes identified in M. furnieri were Cucculanus genypteri (n =1575, P =98.3%) and Hysterothylacium deardorffoverstreetorum (s.l.) (n =2, P =3.3%); in G. brasiliensis were Dichelyne (Cucullanellus) sciaenidicola (n =99, P =33.3%), Cucculanus pulcherrimus (n = 45, P= 18.3%), Hysterothylacium deardorffoverstreetorum (s.l.) (n =3, P =5%), and Anisakis typica (n =1, P =1.7%); and, in M. argentinae, were H. deardorffoverstreetorum (s.l.) (n =146, P =48.3%), and Procamallanus (Spirocamallanus) halitrophus (n =4, P =6.7%). DNA sequence data of C. genypteri, C. pulcherrimus, D. (C.) sciaenidicola, and P. (S.) halitrophus were reported for the first time. No significant seasonal variation in parasitological indices was observed. Hysterothylacium specimens (n =6) were found in fish muscle, potentially a public health concern.

Conclusions: In Brazil fish are increasingly eaten uncooked, and its essential to make consumers aware of the risks of eating some fish species uncooked, and consumption of only appropriately frozen or cooked fish must be encouraged.

Keywords: fish parasite, marine biodiversity, Integrative taxonomy

P560 / #1206

Topic: *AS04 Parasites of fish / AS04.1 Fish-borne zoonotic parasites*

THE ZOONOTIC NEMATODE ANISAKIS PEGREFFII RELEASES EXTRACELLULAR VESICLES: MICROSCOPIC AND PROTEOMIC CHARACTERISATION

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Introduction: Anisakis pegreffii is a parasitic nematode belonging to the A. simplex (s.l.) species complex. Due to the risk of human health, the zoonotic species A. pegreffii has been subjected to many studies to investigate the biological signals involved in host-parasite interactions. Excretory-secretory proteins play a key role in these interactions. Nevertheless, many proteins are released through small extracellular vesicles (EVs). Their recent discovery reveals a new paradigm both in parasite-parasite communication and parasite-host relationships. In this frame, EVs from A. pegreffii larvae, their morphology and protein contents have been here characterized.

Methods: Live A. pegreffii L3 were cultured for 24h in PBS, 37°C, 5% CO_2. EVs were isolated by serial centrifugation and ultracentrifugation of culture media. Anisakis EVs were characterized for size and morphology by Transmission Electron Microscopy and Nanoparticle Tracking Analysis. Protein content was determined by shotgun proteomics. Multiple bioinformatics tools were used to characterize the detected Anisakis EVs.

Results: A. pegreffii released vesicles with rounded-shaped structures and size and concentration of 65-295 nm and 1,54x1011 particles/ml, respectively. The EVs proteome included 158 proteins. Among the others, Anis 14, Anis 13 and Anis 1 involved in the host immune modulation were found, which are also antigens of the IgE response in human anisakiasis. Heat shock proteins and C-type lectins as galectin, involved in host immune activation, were also detected.

Conclusions: These results suggest that the released EVs may deliver antigenic and immunomodulatory cargo to host tissue microenvironment. This study was supported by Italian Ministry of Health, Ricerca Finalizzata 2018-12367986

Keywords: TEM, Anisakis pegreffii, extracellular vesicles, NTA, Proteomics

P561 / #36

Topic: *AS04 Parasites of fish / AS04.1 Fish-borne zoonotic parasites*

TRANSMISSIBILITY OF ANISAKIDAE ALLERGENIC PEPTIDES FROM ANIMAL FEED TO CHICKEN MEAT

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Introduction: The family Anisakidae are zoonotic nematodes infecting many marine organisms. Besides being causative agents for gastrointestinal disease in human after ingestion of a live larva, and an allergic reaction after consuming/handling infected fish, there is proof-of-concept for hidden allergic concerns further down the food chain. Specifically, several anisakid allergens are highly resistant, and in this way may be transmitted to meat by use of fishmeal as feed for livestock. This may expose consumers to anisakid allergens not only in fish, but also in meat. To confirm this hypothesis, a chicken feeding trial using Anisakidae-contaminated feed was conducted.

Methods: Anisakid larvae were collected from infected codfish and freeze-dried according to fishmeal manufacturing conditions. This larvaemeal, in addition to a plant-based feed, was administered to a positive control (PC) group of five chickens. A negative control group only received the plant-based diet. After 3 weeks of exposure, blood and muscle samples were subjected to an in-house optimized liquid chromatography tandem mass spectrometry analysis targeting anisakid allergens.

Results: revealed the presence of peptides from 6 anisakid allergens in the meat and/or blood samples from the PC group. This demonstrates that anisakid peptides can withstand feed manufacturing procedures and can be transported across the gut mucosa towards the blood circulation and muscles.

Conclusions: While further investigations are required to confirm the allergenic potency of the chicken meat, these findings may change the importance of these zoonotic nematodes from originally a purely fishborne food risk to potentially a much wider risk from several food sources (e.g. chicken and pork meat, aquacultured fish).

Keywords: allergens, targeted proteomics, chicken feeding trial, Anisakidae

P562 / #584

Topic: *AS04 Parasites of fish / AS04.1 Fish-borne zoonotic parasites*

ARE LARGER FISH REALLY MORE HEAVILY INFECTED WITH ANISAKIS THAN SMALLER FISH?

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Introduction: Anisakis is one of the most important zoonotic parasites nowadays. Its life cycle includes 3 or more hosts. The fish is a paratenic or transport host for this worm, and it is known that it tends to accumulate worms with age. Our aims were to analyse parasite distribution in relation to fish age, and establish if larger fish are more prone to heavier parasite infections than smaller fish.

Methods: A survey of Anisakis infection was carried out in 45 hake (Merluccius merluccius). The UV-Press method was used to detect and count the worms. Host parameters (length and weight) were correlated with parasite variables (abundance and density) in muscle and viscera. Moreover, the fish were separated into two groups according to size, and their density and abundance values were compared.

Results: In total 473 worms were found including 194 worms in the muscle. The prevalence was 95.6% and the mean intensity was 11.0 worms/host. The mean density for the muscle was 0.05 worms/g, and the mean density for the viscera was 0.54 worms/ g. Larval abundance in viscera and muscle were correlated, but not the density. Fish size was significantly correlated with larval abundance in muscle and viscera, and larval density in viscera, but not with larval density in muscle. The muscle density was not significantly different between smaller and larger fish.

Conclusions: A greater abundance was observed in larger fish but not a greater density, thus it seems that the same portion of muscle from an larger fish is not more dangerous to eat than the one from a smaller fish.

Keywords: anisakis, site distribution, age distribution, Merluccius merluccius

P563 / #1127

Topic: *AS04 Parasites of fish / AS04.1 Fish-borne zoonotic parasites*

ANISAKIS SPP. IN DEEP-WATER CHONDRICHTYES FROM SOUTHERN PORTUGAL

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Introduction: The genus Anisakis is a zoonotic worm that is known to infect different Teleost fish (transport hosts). These fish establish the link in the parasite life cycle between the crustacean host, the first host, and the definitive host (marine mammals). However its record in Chondrichtyes (sharks, rays, and rabbit fish) in Portugal is unknown. Thus, we aim to study its occurrence in different Chondrichtyes species by measuring Anisakis spp. prevalence and mean intensity.

Methods: Chondrichtyes individuals were randomly sampled from the by-catch of crustacean bottom trawl fisheries, on the south coast of Portugal. They were dissected and the visceral organs and a piece of muscle were submitted to Anisakis survey, using the UV-Press method. Some worms were analysed molecularly.

Results: 266 Chondrichtyes individuals were surveyed, belonging to 14 different species. Anisakis spp were only detected in 6 of these species, and all were sharks. The host species with the highest prevalence /mean intensity was Scymnodon ringens with 57.9% /3.1, followed by Etmopterus spinax with 32.7% / 6.5, Etmopterus pusillus with 33.3% / 50.0, Deania profundorum with 18.2% / 9.5, Galeus atlanticus with 7.4% / 1.5, and in Deania calcea only one fish analysed, infected with 293 worms. The Anisakis found were: Anisakis simplex, A. pegreffi, A. simplex X A. pegreffi hybrid, A. physeteris and A. ziphidarum.

Conclusions: Four Anisakis species were found in 6 different species of Portuguese sharks, and some of those infected showed high mean intensity values. Ackowledgments: FCT - UIDB/04423/2020, UIDP/04423/2020, UIDB/04326/2020, SFRH/BD/147493/2019 and CEECIND/03501/2017, Ocean3R (NORTE-01-0145-FEDER-000064, ERASMUS +, Save our Seas Foundation (SOSF501), EEA Grants (PT-Innovation-0007).

Keywords: Zoonosis, anisakis, Chondrichtyes

P564 / #1185

Topic: *AS04 Parasites of fish / AS04.1 Fish-borne zoonotic parasites*

LEVEL OF KNOWLEDGE ABOUT ANISAKIS SSP., A PARALLEL BETWEEN THREE DIFFERENT GROUPS OF CONSUMERS

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Introduction: With the growth of international trade and the popularization of raw fish, there has been an increase in Anisakiosis in recent years. Thus, this study evaluated the level of knowledge that 704 people, divided into 3 groups (1 - eat any meat; 2 - eat raw fish; 3 - vegans/vegetarians), have about Anisakis spp. and its potential health risks.

Methods: Data were collected online, via a questionnaire.

Results: Texture and variation of menu are the main reasons for eating raw fish, while culture, allergy, and origin are the main reasons for not eating it. The transmission of parasites was the main problem associated with the consumption of raw fish, but 65.8% of Group 1, 64.96% of Group 2 and 64.28% of Group 3 said they did not know about Anisakis spp. Most of those who know, mentioned cooking and freezing as a prevention method. The majority of Group 3 would not buy fish resulting from a technology that would make it free of Anisakis spp. and its allergens; the majority of Groups 1 and 2 would buy and be willing to pay an increase of 11% to 12.5%. The presence of Anisakis spp. would be a reason to avoid buying fish for 100% of Group 3, 68.97% of Group 1 and 55.34% of Group 2. For most of Group 1 the risk of developing anisakiosis and/or allergy to Anisakis spp. is random, while for Groups 1 and 2 the risk is considered small. The presence of Anisakis spp. therefore reflects a loss in the commercial value of fish.

Conclusions: The knowledge about Anisakis dangerous is very low among the Portuguese population. Moreover, the need for scientific education programs on the subject is highlighted here.
Acknowledgments. Founding by Project, from FCT, with reference: UID/Multi/04423/2019. Thanks to the research grants awarded: CNPQ (PrP 006/2021 – PIBIC/CNPq) and UEG (UEG No. 001/2021).

Keywords: Zoonosis, Food Safety, Fishing industry, Risk management

P565 / #544

Topic: *AS04 Parasites of fish / AS04.1 Fish-borne zoonotic parasites*

PLEROCERCOIDS OF ADENOCEPHALUS PACIFICUS IN ARGENTINE HAKE, MERLUCCIUS HUBBSI FROM PATAGONIAN WATERS

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Introduction: Adenocephalus pacificus is the agent of a common fish-borne cestodosis, by consumption of raw fish. Its adults are known worldwide, but its larvae have been reported in fish only from Peru, where most human cases have been reported. In the Argentine sea, sea lions host this species, which allow infer that previous records of “pseudophyllidean” larvae in Argentine hakes, Merluccius hubbsi, belong to this species. Aim: To identify plerocercoids parasitizing hakes and, from a pubic halth perspective, to assess the risk of parasitism for consumers.

Methods: A total of 471 hakes caught in 11 samples in the southern Patagonian waters were measured, headed and gutted (H&G) and examined for plerocercoids. H&G fish were washed and parasites were retained in a sieve. Then, each fish was filleted and examined by transillumination. Larvae were characterized by sequencing the partial lsrDNA (D1–D3 domains) and almost complete cox1.

Results: Larvae were recovered from H&G fish in 5 samples at prevalence <10% and low mean abundance (0.02-0.08). No larvae were observed in the 942 fillets. Parasite prevalence increased with fish size. Specific identity was genetically confirmed as A. pacificus.

Conclusions: This is first record of A. pacificus in a fish out of Peruvian coasts. The higher prevalence in larger fishes could increase the risk for consumers, however it is minimum because fillets are devoid of larvae. The hypothesis of Argentine fish as source of diphyllobothriosis for Europeans, published after finding larvae in dolphins from Patagonia, is unfounded since hakes are exported frozen to Europe, as fillets and H&G. PICT 2018-1981

Keywords: Argentina, Argentine hake, Adenocephalus pacificus, fish-borne disease

P566 / #425

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

BACKTRACKING THE HISTORICAL DISPERSION OF PERI-MEDITERRANEAN CYPRINOIDS USING HOST-SPECIFIC DACTYLOGYRUS PARASITES AS A HELPFUL GUIDE

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Introduction: Monogeneans and their fish hosts represent one of the best models for studying host-parasite evolutionary relationships using cophylogenetic approach. These parasites developed remarkably high host specificity, where each host species often harbour its own host-specific monogenean species. Herein, we focused on the host-parasite system of Dactylogyrus (gill ectoparasites) and their cyprinoid hosts in the peri-Mediterranean region.

Methods: Dactylogyrus parasites were collected from cyprinoid fish hosts in peri-Mediterranean region. For the phylogenetic analyses, four genetic markers were used to assess the evolutionary history in Dactylogyrus. The complete mtDNA cytochrome b gene was sequenced for the cyprinoids. For the cophylogenetic analyses, dual-based approach was employed: distance-based and event-based methods.

Results: Fifteen new species were described in the peri-Mediterranean region. The phylogenetic analyses divided Dactylogyrus species into four divergent lineages. Although the relationships within lineages were not always fully resolved, subsequent mapping of the haptoral characters into phylogeny helped to shed more light on diversification processes. Cophylogenetic methods revealed a strong coevolutionary structure between phylogenies of Dactylogyrus and their respective cyprinoid hosts in a peri-Mediterranean area, with host switch as a common coevolutionary event.

Conclusions: The overall diversity of Dactylogyrus appears to be underexplored, even in the regions like Mediterranean Europe or Anatolia. Our data suggest, continental bridges connecting southern Europe and North Africa played a crucial role in the dispersion of cyprinoids, and also affected the distribution of their host-specific gill parasites.

Keywords: Monogenea, phylogeny, host specificity, Cophylogeny

P567 / #709

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

GETTING INTO FISH BRAINS: INFECTION AND TRANSCRIPTOMICS OF A BEHAVIORAL MANIPULATIVE TREMATODE

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Introduction: Parasites usually impact on their hosts at different levels, sometimes altering their behavior to enhance transmission. We studied the trematode Cardiocephaloides longicollis, whose metacercariae infect the brain of a variety of fish species, including farmed gilthead seabream Sparus aurata. We aim to identify and describe the altered behavior of the fish and relate this to concurrent changes in gene expression and protein profiles.

Methods: Control and experimentally infected fish were used in different experiments to examine various aspects of behavior, including vertical distribution, escape behavior and grouping, accounting for infection intensity and maturity. Brain tissue and cerebrospinal fluid were collected for RNA-seq and protein analyses. Differentially expressed genes were analyzed in early and mature infection using DESeq2.

Results: The presence of C. longicollis metacercariae impairs host anti-predator and escape behavior, as infected fish are captured more frequently than control fish, and group formation is disrupted for longer. Differentially expressed genes and proteins were identified when comparing control and infected fish and in early and mature infections. These were identified as components linked to behavioral changes in other organisms, as well as to infection and immune evasion strategies.

Conclusions: Transcriptomic and proteomic analyses aid elucidation of the physiological basis of infection-induced changes in host behavior, filling an important gap in understanding the molecular basis of fish behavioral responses to digenean trematodes.

Keywords: Trematode, Fish, Transcriptomics, behavior changes

P568 / #409

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

DESCRIPTION OF A NEW SPECIES OF LECITHOCLADIUM (DIGENEA) FROM SCOMBER SCOMBRUS (TELEOSTEI) OFF SWEDEN: TOWARDS RESOLUTION OF LECITHOCLADIUM EXCISUM-LIKE SPECIES COMPLEX

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Introduction: The trematode Lecithocladium excisum is a parasite of the intestine and stomach of marine teleosts. First described from Scomber scombrus from the Mediterranean coast of Italy, it was subsequently reported on various hosts worldwide. This could indicate an euryxeny or that several cryptic species exist. This is an attempt to investigate this problem.

Methods: Fish were collected from Sweden and southern Mediterranean. Trematodes were stained and mounted for morphological examination. Genetic sequences were generated from hologenophores for internal transcribed spacer (ITS2), large (28S) ribosomal subunit and cytochrome c oxidase subunit 1 (COI). Cladograms were inferred using neighbour-joining and maximum likelihood methods. Genetic distances were estimated with MEGA7.

Results: A comparison of Lecithocladium n. sp. from Sweden with published descriptions and museum specimens of L. excisum did not yield clear morphological difference. Genetic divergence among Lecithocladium n. sp. from Sweden and L. excisum was 10-11% for COI, and 1-2% for ITS2. Divergence for COI among Lecithocladium n. sp. from Sweden and L. excisum from the Mediterranean was low (0-2%). Both 28S and COI trees placed Lecithocladium n. sp. from Sweden and L. excisum from the Mediterranean in different clades.

Conclusions: Reciprocal monophyly in phylogenetic tree and genetic divergences support that hemurids from the North Sea and those from the Mediterranean are different. The species from Sweden is therefore described as Lecithocladium n. sp. Differential diagnoses with Lecithocladium species from the North Sea and from scombrids are provided. These results suggest that a molecular study of L. excisum-like specimens from various localities may reveal additional cryptic biodiversity.

Keywords: Cryptic, New species, Digenea, North Sea, Integrative taxonomy

P569 / #1140

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

THE AMOEBIC GILL DISEASE IN SALMO SALAR: A TRIPARTITE ENDOSYMBIOSIS OF A PARAMOEBAE WITH AN AFLAGELLATE KINETOPLASTID AND INTRACELLULAR BACTERIA

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Introduction: Amoebic gill disease (AGD), caused by various strains of Neoparamoeba perurans, is a worldwide disease of salmonids aquaculture associated with sustained and intensive year-on-year losses (≥20%). N. perurans hosts in the vicinity of its nucleus an endosymbiotic aflagelate Kinetoplastid called Perkinsela spp known as Perkinsela-like organism (PLO) or ihctyobodo necator related organism (IRO), similar to kinetoplastid pathogens of human and domestic livestock (Trypanosoma brucei sp., T. cruzi, Leishmania sp.). As well as a Kinetoplastid, the N. perurans cytoplasm is also home to many intracellular microbial communities. This project aims to understand the functional associations between the host and its endosymbionts (Kinetoplastid and intracellular bacteria) by identifying genomic signatures of their metabolic interdependencies

Methods: Omics data (metagenomics and transcriptomics) around ~ 1 Terra were generated with short and long reads sequencing technologies, using Illumina and Nanopore respectively.

Results: The preliminary results showed 10 complete genomes of intracellular vibrios and halophilic bacterial. The annotations of the host and its kinetoplastid genomes are under investigation, with a particular focus on the mobile genes, pathogenicity islands, metabolic genes and genes associated with water osmoregulation and salinity fluctuations.

Conclusions: The intracellular bacteria and the kinetoplastid may contribute to the pathogenicity of the paramoeba. The increasing temperature in the summer with AGD outbreaks of highest score and mortality rate in salmonids farm settings would be implying potential changes in the molecular crosstalk between the paramoeba and their partners, the intracellular bacteria and kinetoplastid

Keywords: Ameobic Gill Disease, Intracellular bacteria, omics, Aflagelate kinetoplastid, endosymbiosis

P570 / #206

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

PARASITES OF DESERT FISH COPTODON GUINEENSIS: LIFE IN AN EXTREME ENVIRONMENT

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Introduction: Sebkhat Imlili (Western Sahara) is a vulnerable ecosystem of ecological and social importance. This salt flat is a shallow depression approximately 13 km long characterized by the presence of over 160 permanent pockets of hypersaline water generated in the Holocene and that are inhabited by a variety of organisms considered to be relics of the past, including invertebrates and the cichlid fish, Coptodon guineensis.

Methods: Parasites were surveyed in December 2018 (winter), and April (spring), July (summer), and October (fall) of 2019 to 1) identify parasites, and 2) determine a possible seasonality in infection patterns. Identification was carried out morphologically and molecularly (ITS rRNA gene) for the acanthocephalan and molecularly (partial 28S rRNA gene) for the digeneans.

Results: Over 60% of the fish were infected by at least one of three parasites: an adult acanthocephalan (Acanthosentis sp.) in the intestine and two digeneans (one heterophyid and one unidentified) with metacercariae in kidney, spleen, liver, and mesenteries. The acanthocephalan and the heterophyid metacercariae were found throughout the sampling periods but the unidentified metacercariae were present only in the summer and fall, reflecting a possible severe pathological impact of this parasite, which was particularly abundant in the spleen of the fish

Conclusions: Given the general life cycles of these parasites, their presence lends insight into the fish's diet, the current use of this sebkha by piscivores, and provides a window into the past relative to this unique environment. Supported by the Fulbright MENA program, the Department of Biology (College of Charleston), and the Institute of Research and Development. The Association of Nature-Initiative facilitated field work.

Keywords: hypersaline, salt flat, Cichlidae, Acanthocephala, Heterophyidae

P571 / #601

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

HIGHLY VARIABLE GENE ARRANGEMENTS IN MYXOZOAN MITOCHONDRIAL DNA

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Introduction: Mitochondrial (mt) gene order is generally well conserved within metazoans, including anthozoan and medusozoan cnidarians. Myxozoans, highly reduced cnidarian parasites, have been found to have fast evolution of their mt genomes, leading to problematic annotations of mt genes, as evidenced by the identification of only two protein-coding genes in Myxobolus squamalis. Gene order can only be documented in closely related Kudoa species with identical arrangements of their mt genes. Here we present high variability in the gene order of three newly obtained myxozoan mt genomes.

Methods: Prior to mtDNA extraction, the mitochondrial fraction was isolated from the three myxozoans Myxidium lieberkuehni, Nephrocystidium pickii and Zschokkella nova. Long reads of mtDNA were sequenced using OxfordNanopore technology. The reads were assembled into circular mtDNA molecules. Specific primers were designed to obtain long overlapping PCR products covering the entire mtDNA molecule. The products were sequenced using Barcode-Tagged Sequencing on an Illumina sequencer and mapped to mtDNA to correct nanopore sequencing inaccuracies.

Results: We identified four mitochondrial protein-coding genes (cox1, cox2, nadh1, cytB) and 12S and 16S ribosomal RNA genes in three myxosporean species. The close position of two rRNA genes was the only common feature for all mtDNAs. The order of protein-coding genes was different even in the very closely related species M. lieberkuehni and N. pickii.

Conclusions: In the metazoans, mtDNA gene rearrangements might have acted to rearrange the tRNAs. In the myxozoans, all tRNAs were found to be absent from the mt genome, so the reason for the translocations of their mt genes remains unknown. Foundation: Czech Science Foundation (21-29370S).

Keywords: mtDNA, myxozoa, gene order, Nanopore sequencing

P572 / #1338

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

PARASITE CONTRIBUTION TO HOST DIVERGENCE: INSIGHTS FROM LAKE VICTORIA CICHLIDS.

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Introduction: Parasite-driven selection could be a major contributor to ecological speciation. Studies investigating the role of parasites in host diversification have begun to accumulate. However, it is still unclear at what stage of the speciation process parasite-mediated divergent selection acts, and to what extent it actually contributes to speciation, especially in the context of adaptive radiation. The adaptive radiation of cichlid fish in Lake Victoria provides a good system to study the role of parasites at different stages of host speciation.

Methods: We analysed the macroparasite infection of 4 replicates of sympatric blue and red Pundamilia pairs, that vary in their age of speciation and extent of genetic differentiation.

Results: Sympatric host species differed in parasite community composition and in the infection levels of some of these parasite taxa. Most infection differences were consistent between sampling years, indicating temporal consistency in parasite-mediated divergent selection. Infection differentiation increased linearly with genetic differentiation. However, infection differences were significant only for the old and most genetically differentiated Pundamilia species pair.

Conclusions: A certain amount of genetic differentiation (driven by factors other than parasites) may be needed for parasite-mediated divergent selection to act and to lead to significant species differences in infection. This suggests that parasites may contribute to host differentiation after speciation, but do not initiate host speciation.

Keywords: adaptive radiation, Cichlidae, Monogenea, diversification, parasite-mediated selection

P573 / #292

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

GENE EXPRESSION PROFILING OF TWO FISH HELMINTHS THROUGHOUT THEIR COMPLEX LIFE CYCLES. ARE THE PARASITE'S LIFE STAGES GENETICALLY DECOUPLED?

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Introduction: Complex life cycles are a widespread but challenging lifestyle among parasites. Parasites with complex life cycles infect multiple hosts in succession. Therefore, they are adapted to exploit different ecological niches but using the information encoded in a single genome. Are many genes expressed in all hosts, such as when parasite stages exhibit similar functions, e.g. during growth in intermediate and definitive hosts? Or are divergent gene sets expressed in different hosts? The adaptive decoupling hypothesis suggests that the different stages in a complex life cycle should be genetically, and thus evolutionarily, independent, such that selection on parasite traits in one host doesn't affect traits in other hosts.

Methods: We are testing this hypothesis using a tapeworm (Schistocephalus solidus) and a nematode (Camallanus lacustris). Both species have 3-host life cycles and both were sampled throughout their complex life cycles for transcriptomic sequencing (55 transcriptomes from 10 S. solidus stages and 25 transcriptomes from 6 C. lacustris stages). Gene expression analysis will identify genes that are differentially expressed between hosts (e.g. 1st host vs 2nd host), between parasite functions (e.g. invasion vs growth), and between hosts and functions (e.g. growth in 1st host vs growth in 2nd host).

Results: The more the gene expression is divergent between stages, even stages with similar functions, the more the decoupling is supported. Further, the types of differentially expressed genes, such as splice isoforms or paralogous genes, may hint at the mechanisms driving decoupling.

Conclusions: By comparing parasite gene expression across a complex life cycle, our results will provide insight on the mechanisms that regulate such a successful lifestyle.

Keywords: Adaptive decoupling, Complex life cycles, Helminths, Transcriptomic

P574 / #1405

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

THE IDENTITY OF A LITTLE-KNOWN GROUP OF LECANICEPHALIDEAN TAPEWORMS PARASITIZING COWTAIL RAYS IN THE INDO-PACIFIC REGION

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Introduction: Since its description in 1979, the lecanicephalidean genus *Flapocephalus* has received little attention. The type species, *F. trygonis*, was described from a cowtail ray (genus *Pastinachus*) from the west coast of India as possessing a unique apical organ morphology in the form of two flap-like semicircles; proglottid anatomy remains essentially unknown. Phylogenetic analysis of molecular sequence data placed specimens consistent with *Flapocephalus* as members of the Polypocephalidae. The goals of this study were to fully characterize the genus, and asses its diversity and host associations.

Methods: Specimens of *Flapocephalus* from a total of 23 cowtail rays representing three of the five known species of *Pastinachus*, as well as an undescribed species, collected throughout the Indo-Pacific region, were examined with light and scanning electron microscopy.

Results: *Flapocephalus trygonis* was found parasitizing specimens of *P. ater* from Sri Lanka and was redescribed. Additionally, at least four new species of Flapocephalus were discovered collectively parasitizing *P. ater, P. gracilicaudus*, and *P. solocirostris* from off Australia, Borneo, Madagascar, and the Solomon Islands. Species range in size from a few millimeters to those that are several centimeters in total length, and, in addition to size, can be distinguished by the arrangement of testes and vitelline follicles.

Conclusions: *Flapocephalus* was confirmed as a distinct genus. The genus is more morphologically diverse and speciose than anticipated. Members appear to exclusively parasitize cowtail rays, likely exhibit slightly relaxed host specificity. Interestingly, Flapocephalus is morphologically at odds with members of the Polypocephalidae. This work was supported by NSF grants 1921404 & 1921411.

Keywords: Cestodes, cowtail ray, morphology, Diversity

P575 / #923

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

SCOLEX MORPHOLOGY VERSUS PROGLOTTID ANATOMY: DISCOVERING PHYLOGENETIC SIGNAL IN A POORLY KNOWN GROUP OF ELASMOBRANCH TAPEWORMS

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Introduction: This work aims to expand understanding of the “tetraphyllidean” group Clade 4 as part of an effort to resolve the non-monophyletic order “Tetraphyllidea.” Newfound diversity in Clade 4 proves to be an excellent system to investigate the evolutionary intrigue surrounding phylogenetic signal of proglottid anatomy versus scolex morphology.

Methods: Specimens were prepared for light microscopy and scanning electron microscopy. Sequence data were generated for multiple orthogroups for 15 members of the clade as part of a larger project. The phylogenetic relationships for Clade 4 were extracted from an ASTRAL tree of the larger analysis.

Results: Examination of previous global collections of batoids yielded cestodes from 13 species in the elasmobranch families Dasyatidae, Glaucostegidae, Myliobatidae, Pristidae, and Rhinidae. Morphological and phylogenetic analyses revealed 15 undescribed species. These taxa grouped robustly with representatives of the genus Pithophorus (Southwell 1925) included in the analysis. Morphology and tree topology suggest three subclades within Clade 4. When conflicting proglottid anatomies and scolex morphologies seen in these specimens were mapped on the tree, proglottid anatomy was congruent with topology.

Conclusions: The monophyly and morphological diversity of Clade 4 suggest it is a candidate for establishment as a new order. Further investigation into additional host species is required for a more comprehensive understanding. Proglottid anatomy, rather than scolex morphology, was found to reflect the phylogenetic relationships of this group. We thank Kirsten Jensen, Elizabeth Jockusch, and Jill Wegrzyn for assistance with sequencing and phylogenetic analysis. This work was supported by NSF grants 1921404 & 1921411.

Keywords: evolution, cestode, classification

P576 / #1178

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

HIDDEN MYXOZOAN DIVERSITY: DETECTION IN SEDIMENT AND WATER BY EDNA METABARCODING APPROACH

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Introduction: Monitoring of parasitic diversity in fish is essential for fish aquaculture. Myxozoans are economically important microscopic fish endoparasites that include more than 2600 species, but their numbers seem to be greatly underestimated. A classical approach to study their diversity is invasive dissection of fish hosts followed by their microscopic and molecular screening. We have recently demonstrated that eDNA metabarcoding of sediment samples containing myxozoan spores is an effective tool for exploring myxosporean diversity without the need for host examination. Our objective was to apply and prove our metabarcoding approach to assess and monitor myxozoan diversity along the entire Malše River (Czech Republic).

Methods: We collected sediment and water samples from 14 sites along about 80 km of the Malše River. DNA was extracted using a commercial soil kit. The V4 region of the SSU rDNA was amplified by PCR with myxozoan specific and barcoded primer sets and prepared for amplicon Illumina sequencing. The obtained metabarcoding data were bioinformatically analyzed.

Results: We detected more than 80 myxosporean OTUs (>3% dissimilarity) along the Malše River with the number of myxosporean species increasing from the source to the lower part of the river. We were able to demonstrate the correlation of myxozoan diversity with the availability of their specific host species and the impact of fishpond aquaculture associated with the Malše River on myxozoan communities.

Conclusions: We proved that myxozoan diversity is still largely unexplored and that our methodological eDNA approach is suitable for myxozoan diversity assessment and monitoring.
Funding: Czech Science Foundation (#29-28399X)

Keywords: phylogeny, eDNA, metabarcoding, myxozoan diversity

P577 / #1154

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

A NEW LOOK ON MOLECULAR PHYLOGENY OF OLIGONCHOINEA (POLYOPISTHOCOTYLEA: MONOGENEA): 20 YEARS AFTER MOLLARET ET AL. 2000

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Introduction: Phylogenetic relationships of Oligonchoinea were early proposed based on morphology and cladistic, but results do not reached consensus, even at high taxonomic levels. Since the pioneering studies of molecular phylogeny of Monogenea, phylogeny of Oligonchoinea has not been resolved. Here, we analyzed the phylogeny of Oligonchoinea based on SSU rDNA, LSU rDNA and concatenated genes (SSU rDNA+LSUr DNA+cox1)

Methods: We sampled marine fishes searching for Oligonchoinea in gills and oral cavities of the hosts. Specimens were preserved in 70% and 96% ethanol for morphological and molecular analysis. Phylogenetic trees were constructed for SSU rDNA, LSU rDNA and concatenated genes (SSU rDNA+LSU rDNA+Cox1), including 171 sequences from 157 species, belonging to the three orders of Polyophistocotylea parasitizing fishes. Models of nucleotide substitution were evaluated for each data partition independently, using IQ-TREE and Mr Bayes.

Results: Data included 171 concatenated sequences from 157 species, analysis based on BI and ML generated trees with similar topology but nodes in ML tree often exhibited lower support than BI. Chimaericolidea and Diclybothriidae are basal to Oligonchoinea, whereas Mazocraeidea is sister to the remainder Oligonchoinea parasitizing Teleostei. Well supported eight clades were identified: Hexabothriidae, Mazocraeinea; Gastrocotylinea, Plectanocotylidae, Discocotylinea, Microcotylinea, Diclidophoridae and Anthocotylidae.

Conclusions: Even though, our study presents higher resolution than previous studies, further phylogenetic studies of this group using more informative molecular markers (e.g. the whole mitochondrial genome) are needed to resolve the true evolutionary history of Oligonchoinea

Keywords: phylogeny, LSU rDNA gene, cox1 gene, SSU rDNA gene, concatenated gene

P578 / #1395

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

UNDERAPPRECIATED DIVERSITY AND COMPLEXITY IN A POTENTIALLY NOVEL ORDER OF CESTODES

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Introduction: The genus Caulobothrium remains one of the more poorly known genera of cestodes that parasitize elasmobranchs. New collections from stingrays in Australia, Ecuador, India, Malaysian Borneo, Mexico, Mozambique, and the Solomon Islands yielded what appears to be additional material of this genus. The aims of this study were to investigate the morphological diversity and phylogenetic relationships of the species discovered in these hosts.

Methods: Cestode specimens preserved in formalin from each host species were prepared for and examined with light and scanning electron microscopy. Sequence data for the D1–D3 region of the 28S rDNA gene for the subset of these species for which material preserved in ethanol was available were generated and a Maximum Likelihood analysis was conducted to assess their phylogenetic relationships.

Results: This material was found to include as many as 15 new species of Caulobothrium exhibiting a wide array of morphological and anatomical features. In addition to substantially extending the geographic distribution of the genus, these taxa expanded the hosts of Caulobothrium to include species in the genera Himantura, Maculabatis, Rhinoptera, Urobatis, and Urotrygon as well as additional species of Myliobatis and Pastinachus. The resulting phylogenetic tree provides multiple instances of species parasitizing the same host species that appear to be only distantly related.

Conclusions: This work suggests that the ten described members of the genus represent only a small portion of the morphological diversity, host associations, and geographic distribution of this genus overall. We thank Kirsten Jensen for assistance with the collection of specimens. This work was supported by NSF grants 1921404 & 1921411.

Keywords: cestode, Diversity, stingrays, phylogeny

P579 / #2078

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

MARINE MAMMAL PARASITES: PHYSICAL ASPECTS IN A CHALLENGING ENVIRONMENT

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Introduction: Marine mammals host a great variety of endo- and ectoparasites, which are adapted to their hosts in a co-evolutionary arms race. However, only little is known about the ecology of marine mammal arthropod parasites, and even less about the physical aspects of their life in such a challenging environment.

Methods: We hypothesized that the exoskeleton and the cuticular structures of seal lice (*Echinophthirius horridus*), whale lice (*Isocyamus deltobranchium*), and naso-pharyngeal mites (*Halarachne halichoeri*) have evolved by adapting their morphology and material properties to survive on diving wildlife. By using µ-CT and Cryo-SEM we characterized anatomical specializations in these parasites for attachment, which enable their fixation to their hosts during dives, currents, turbulence, and social interactions. Furthermore, we determined adaptations to their lifestyle in material composition of the cuticle, spiracles and ventral spines by histology and various imaging techniques, such as CLSM and TEM. Additionally, we performed mechanical characterization of the parasites' attachment abilities depending on the properties of the host surface properties.

Results: We were able to detect first indications for convergent solutions to the challenges connected with living on marine hosts and numerous structure-function relationships in the parasite attachment structures.

Conclusions: Some results appear promising for transferring functional solutions from biological systems to materials science and engineering.

P580 / #131

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

A LONG-TERM STUDY OF PARASITE COMMUNITIES IN BOOPS BOOPS (TELEOSTEI: SPARIDAE) AFTER THE PRESTIGE OIL-SPILL

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Introduction: In November 2002, the oil-tanker Prestige sank over the Galician Bank and contaminated virtually all types of marine habitat. It has been proved that pollution affects parasite populations and communities, both directly and through effects on intermediate and final hosts. Previous studies on parasite communities in Boops boops carried out during 2001−2006 revealed directional trends of parasite community alteration with no full support for community recovery from pollution. A long-term comparative study on this host-parasite system was carried out to assess the recovery of the ecosystem.

Methods: Four seasonal samples from two locations were collected in 2014−2015 (n=120) and compared with data obtained previously (n=208).

Results: Analysis based on abundance data showed an effect of ‘time sequence’ and ‘season’ factors on infracommunity composition. Monoxenous species revealed a certain tendency to recover; however, the infection levels of some heteroxenous species suggest alterations that may be associated to different anthropogenic impacts preventing the recovery of parasite communities to the pre-spill situation.

Conclusions: The structure of parasite communities found in 2005−2006 can no longer be considered baseline data. Thus, it is relevant to continue conducting periodic sampling of this host-parasite system which has proven to be a good sentinel to detect changes in marine ecosystems.
This study was funded by the Program for Special Actions of the University of Valencia, the Spanish Government (MICIN/FEDER PID2019-110730RB-I00, MCIN/AEI/10.13039/501100011033) and the Valencian Regional Government (AICO/2021/279, GVA-THINKINAZUL/2021/029).

Keywords: North East Atlantic, Multivariate analysis, Bioindication

P581 / #200

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

THE EFFECTS OF TEMPERATURE AND LARVAL AGE ON THE SWIMMING BEHAVIOUR OF THE LARVAE OF SPARICOTYLE CHRYSOPHRII FROM MEDITERRANEAN GILTHEAD SEABREAM

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Introduction: Swimming behavior of free-living stages is a key aspect for host-finding and thus, parasite transmission, but it remains poorly understood for many parasitic species, especially monogeneans. The aim of this study was to assess the swimming behavior of Sparicotyle chrysophrii regarding larval age and water temperature.

Methods: Three experimental studies were used to test the effects of different ages (4h-old and 24h-old) and temperatures (14ºC-26ºC) on the swimming periods, speed and vertical movements of S. chrysophrii.

Results: Swimming parameters of S. chrysophrii were significantly and negatively affected by larval age, especially at high temperatures at which swimming duration, speed and covered distances were the lowest. By contrast, temperature about 18ºC promotes swimming of infective stages, regardless of larval age, while at 22ºC is still optimal for vertical swimming.

Conclusions: The role of larval swimming in parasite transmission is limited to the first 12h post-hatching, but can be determinant, especially in wild environments where host-encounter is challenging. Transmission of S. chrysophrii is seasonally modulated in the Mediterranean: active host-finding is maximum in spring and autumn whereas in summer, when temperatures reach 26ºC, it may depend on chance encounters resulting from passive dispersion. Funded by MICIN/FEDER PID2019-110730RB-I00, AICO/2021/279 and GVA-THINKINAZUL/2021/029

Keywords: Oncomiracidia, Environment, Platyhelminthes, Longevity

P582 / #909

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

THE WORM'S JOURNEY: POPULATION STRUCTURE IN MONOGENEAN GENERALIST SPECIES

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Introduction: Microsatellite-based approaches are commonly used for modern population genetic studies; however, they are weakly applied in parasites' population studies. Dactylogyrus vistulae is a genetically diverse generalist species evidenced from many phylogenetically divergent cyprinoid hosts. Thus, it represents a suitable candidate monogenean species for such studies. Herein, we investigated the genetic diversity of this generalist monogenean using microsatellite markers.

Methods: The D. vistulae specimens were collected from 15 cyprinoid host species in the following countries: Albania, Bosnia, Croatia, the Czech Republic, Greece, and Italy. We used a set of 24 polymorphic microsatellites to examine population structure and genetic variability. Population structure was investigated utilizing Bayesian clustering analyses.

Results: According to the clustering analyses, all investigated specimens were divided into four groups. Among these, multiple subpopulations were detected, and the overall structure indicated that the D. vistulae intraspecific genetic variability is linked to the diversification of its cyprinoid hosts. Generally, each population exhibited low genetic variability and contained a high proportion of homozygotes.

Conclusions: In the southern Balkans, D. vistulae was highly genetically diverse, suggesting that this region might represent a diversification centre of this species in Europe. Our results also imply that the species historically spread from the south to the north in the Balkans and later into central Europe.

Keywords: Cyprinoidei, historical dispersion, population genetics

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Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

COPHYLOSPACE OF MONOGENOIDEANS AND MARINE CATFISH (SILURIFORMES: ARIIDAE) FROM BRAZILIAN THE COAST

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Introduction: Due to their high specificity, fish-monogenoidean systems provide an interesting model to study historical associations of hosts and parasites. The occurrence of cophylogenetic signal (high agreement between host and parasite phylogeny) is often interpreted as evidence of cospeciation. However, it may also arise from other adaptive and non-adaptive processes. The Cophylospace Framework (Blasco-Costa et al. 2021) is aimed at enhancing the explanatory power of cophylogenetic analysis. We applied such approach to establish whether the relationship between monogenoideans and catfish can be explained by cospeciation processes.

Methods: Cophylospace requires evaluation of morphological and phylogenetic data. Molecular data of host and parasite species were used for phylogenetic reconstruction. We used anchor morphology based on Procrustes coordinates to evaluate whether closely related hosts are associated with morphologically similar parasites. Likewise, to test the association between parasite phylogeny and host morphology, we produced a distance matrix based on Marceniuk et al. (2012). The relationship between phylogenies and between phylogeny and morphology was assessed with PACo.

Results: PACo revealed a cophylogenetic signal $m^2 = 0.797$; the interaction of host phylogeny with morphology of parasite $m^2 = 0.759$ and $m^2 = 0.818$ and interaction of parasite phylogeny with host morphology $m^2 = 0.697$.

Conclusions: Our results indicate that cospeciation is not a major force accounting for parasite diversification. Rather host traits seem to influence speciation of the parasites. Funded by São Paulo Research Foundation (BEPE-FAPESP#2021/07380-0) and Ministry of Science and Innovation of Spain (PID2019-104908GB-I00).

Keywords: PACo, Host-parasite associations, cophylogenetic

P584 / #417

Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

INTRASPECIFIC DIVERSIFICATION AND MITONUCLEAR DISCORDANCE IN THE MONOGENEAN PARASITE DOLICIRROPLECTANUM LACUSTRE, CO-INTRODUCED WITH THE INVASIVE NILE PERCH

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Introduction: Parasites typically speciate faster, and become more species-rich than their hosts. This especially holds in large and long-lived hosts. Most African lates perches (Latidae), some of the largest freshwater fishes, host only a single monogenean flatworm: Dolicirroplectanum lacustre. This parasite has 'failed to diverge', but displays high morphological variability, with two morphotypes identified. The Nile perch (Lates niloticus) is a notorious invasive species. The introductions of Nile perch from lakes Albert and Turkana into lakes and rivers in the Lake Victoria region led to the impoverishment of the trophic food webs, particularly well documented in Lake Victoria. Along with the introductions of the Nile perch, its parasites were co-introduced.

Methods: To investigate the pattern of parasite co-introduction, we studied the intraspecific diversity of D. lacustre from Nile perch in Lake Albert and Lake Victoria by assessing morphological and genetic differentiation.

Results: A single morphotype is suggested to be co-introduced in Lake Victoria. Based on our results, we reported reduced genetic and morphological diversity in Lake Victoria. The diversification in the COI mitochondrial gene portion was directly linked with the morphotypes, while the nuclear gene portions indicated conspecificity.

Conclusions: Mitonuclear discordance within the morphotypes of D. lacustre indicates an incomplete reproductive barrier between the morphotypes. The reduced genetic and morphological diversity in Lake Victoria potentially resulted from a founder effect.

Keywords: Monogenea, Parasite co-introduction, Phenotypic plasticity, Sympatric speciation, COI

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Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

LONG-TERM CHANGES IN PARASITE COMMUNITIES OF AN INTENSELY EXPLOITED COASTAL FISH: EVIDENCE OF OVERFISHING?

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Introduction: Cynoscion guatucupa is one of the main resources of coastal fisheries in Argentina. During the last decades, changes in its abundance and population structure have been detected, due mostly to fishery pressures. Indeed, fishery reduces fish density and the complexity of food webs, having a correlate on the parasite assemblages they harbor. Aim: to assess if the composition and structure of parasite assemblages have changed in different cohorts along the last three decades.

Methods: 80 fish caught at different latitudes in the Argentine Sea in 2018 and 2019 (3 samples, TL: 30, 35 and 45 cm, respectively) were examined, and the structure and composition of their parasite infracommunities were compared with those of 197 fish from the same regions caught in 1993-1994 (5 samples) by multivariate methods.

Results: 19 parasitic taxa were found and 9 long-lived parasites were selected for analyses. Infracommunity diversity did not vary across samples, although their structure and composition were significantly different between periods, but not between samples of the same period. That result was related to an increase of abundance for some species and to a decrease of others. Changes were observed also for some adult parasites, specific of this host.

Conclusions: It is difficult to identify the causes of the observed changes, especially because this fish is targeted in a multispecific fishery that affects the entire community. Most of the changes seems related to species having elesmobranchs as definitive hosts. Intermediate dates should be necessary to asses if changes are unidirectional or not, however the value of parasites to monitor temporal variability of exploited populations is confirmed.

Keywords: parasite communities, Temporal variability, Cynoscion guatucupa, Overfishing

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Topic: *AS04 Parasites of fish / AS04.2 Evolution and ecology*

PARASITES OF THE ARGENTINE SHORTFIN SQUID ILLEX ARGENTINUS AS BIOLOGICAL TAGS FOR STOCK DISCRIMINATION: ADVANTAGES AND LIMITATIONS

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Introduction: The Argentine shortfin squid (I. argentinus) sustains a major fishery in the southwestern Atlantic. Its lifespan lasts around one year and its recruitment and density largely depend on environmental conditions. Four stocks are recognized with the Southpatagonic (SPS) and Summer Spawning (SSS) Stocks, overlapping their distributions in northern Patagonian waters during summer. Aim: to assess the value of parasites of I. argentinus as indicators of stock structure

Methods: A total of 187 squids (5 samples) were caught at the region where SPS and SSS overlaps, measured, assigned to each stock according their gonadal maturity index and examined for parasites. A sample of 41 squids from a third stock (Bonaerensis-northpatagonic-BNPS) was included in the analyses. The structure of parasite infracommunities was compared across samples and across stocks by mean of multivariate methods.

Results: Thirteen parasitic taxa were found. Infracommunities were similar among the 5 samples of SPS-SSS, but significantly different from BNPS. When grouped by stock, assemblages of SPS-SSS were similar each other and different from BNPS.

Conclusions: Since most parasites, especially those numerically dominant, were short-lived and trophically transmitted, they can only indicate recent feeding history. Therefore, they were unable to discriminate squids of two stocks mixing for a period. Those squids geographically distant and living in different oceanographic conditions were clearly discriminated. Squid parasites are suitable indicators for stock assessment of spatially discrete unities, however, its usefulness for discrimination of temporarily mixing stocks is limited by the transitory condition of the parasite communities.
Acknowledgement: Grant PICT 2019-3376

Keywords: Shortfin squid, stock assessment, biological tags, Fishery

P587 / #802

Topic: *AS04 Parasites of fish / AS04.3 Host-parasite interactions*

SMOLSTATIN, A STRUCTURALLY UNIQUE CYSTEINE PROTEASE INHIBITOR OF THE MYXOZOAN PARASITE SPHAEROSPORA MOLNARI

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Introduction: Parasite-driven cystatins, besides the housekeeping regulation of inner protein degradation, are recognized as essential molecules involved in host-parasite interactions. These protease inhibitors have been used as effective therapeutic targets against various parasitic infections. Myxozoa (Cnidaria), the evolutionary ancient fish parasites, possess phylogenetically distinct cystatins of a stefin type with an amino acid structure like atypical stefins of flukes. Here, we biochemically and structurally characterize a stefin (Smolstatin) of Sphaerospora molnari, a myxozoan pathogen of common carp Cyprinus carpio. Further, we address protein abundance and localization in parasite intrapiscine developmental stages.

Methods: Smolstatin was produced as an active recombinant protein against which we raised the polyclonal antibodies. Biochemical properties were determined by inhibitory activity assays and protein abundance in parasite stages was assessed by western blotting. Localization was performed by immunofluorescence and immunogold methods. Protein was crystallized under different conditions and data was collected at the BESSY-II synchrotron. The crystal structure was solved at 1.99 Å resolution.

Results: The native protein was highly abundant in the sporogonic parasite stages and localized within the ER and membrane-bound vesicles in the vicinity of the blood stage plasma membrane. Recombinant Smolstatin effectively inhibited enzymatic activities of cathepsins L, H, K and S. The protein crystallized as a domain-swapped dimer.

Conclusions: Smolstatin is an evolutionary and structurally unique protein whose role in host-parasite interactions is currently being elucidated. Funded by Czech Science Foundation (21-16565S, 19-28399X).

Keywords: myxozoa, Host-parasite interaction, common carp, protein structure, cystatin

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Topic: *AS04 Parasites of fish / AS04.3 Host-parasite interactions*

ASSESSMENT OF THE SPATIAL DISTRIBUTION OF POLYCHLORINATED BIPHENYLS USING FISH AND THEIR PARASITES IN A HEAVILY POLLUTED AREA IN EASTERN SLOVAKIA

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Introduction: Zemplín region belongs to the most contaminated hot-spots by polychlorinated biphenyls (PCBs) for a long time not only in Slovakia, but also on the global scale. The spatial distribution of PCBs from the former PCB-producing chemical plant, in the Zemplínska Šírava water reservoir and in the Bodrog River basin, using fish (Silurus glanis) and their parasites (Glanitaenia osculata), was studied.

Methods: The levels of 6 indicator PCB congeners were determined in fish muscle, liver, intestine, and cestodes by GC-ECD chromatography.

Results: In fish, the highest PCB concentrations were mostly measured in muscle, followed by intestine and liver at all localities. Concentrations of ∑PCBs above the limits set by European regulations were detected in the muscle tissue of catfish at all sites except in the Bodrog River, which is furthest away from the source of contamination. The highest amounts of ΣPCBs (125 $ng.g^{-1}$ wet weight) were found in wells catfish from the Zemplínska Šírava reservoir, followed by the Laborec, Latorica and Bodrog rivers. For the first time, the ability of G. osculata to accumulate higher amounts of PCBs compared to fish matrices was confirmed.

Conclusions: PCB concentrations in fish decreased with increasing distance from the chemical plant. The PCB amounts in fish meat above the limit values indicate that the high risk to aquatic organisms and humans in this region still exists. It was confirmed that the fish cestode G. osculata can be used for biomonitoring of water pollution due to its high sensitivity to PCBs. The study was supported by the Slovak Research and Development Agency, No APVV-18-0467 and Grant Agency of the Ministry of Education of the Slovak Republic and Slovak Academy of Sciences (VEGA), No 2/0126/20.

Keywords: Fish, Cestodes, Pollution, Polychlorinated biphenyls

P589 / #953

Topic: *AS04 Parasites of fish / AS04.3 Host-parasite interactions*

AB-NORMAL ERYTHROCYTES IN PROLIFERATIVE KIDNEY DISEASE (PKD) – RAINBOW TROUT (ONCORHYNCHUS MYKISS) INFECTED BY TETRACAPSULOIDES BRYOSALMONAE HARBOR IGM+ RED BLOOD CELLS

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Introduction: The myxozoan Tetracapsuloides bryosalmonae causes proliferative kidney disease – a deadly/virulent disease of salmonid fishes, notably of the commercially farmed rainbow trout Oncorhynchus mykiss. In order to understand the causes and consequences of the disease, we studied the immune response towards the parasite. The kidney swelling associated with PKD can be explained in part by underlying changes such as B lymphocyte proliferation. While studying this population during a seasonal outbreak of PKD, we unexpectedly detected the B cell marker IgM on red blood cells (RBCs) of infected commercially farmed rainbow trout. Here, we studied the nature of this IgM and this ab-normal cell population.

Methods: We verified the presence of surface IgM via parallel approaches: flow cytometry, microscopy, and mass spectrometry. We also profiled the transcriptome of this peculiar population by microarray analysis.

Results: In the fish we tested, we measured up to 100% of RBCs being IgM$^+$. This phenotype has not been described before in healthy fishes nor in fishes suffering from disease. The absence of self-reactive and hemolytic Igs in the plasma of PKD fish and the stability of the phenotype lasting at least 6 days in culture, are evidence that the IgM is not bound to an antigen on the erythrocyte surface. In addition to IgM, we transcriptomically profiled changes in RBC metabolism, adhesion, and responses to cytokines and inflammation.

Conclusions: Overall, this marker represents an opportunity to build on the knowledge we already have on the B cell response in PKD. What antigens and receptors do the antibodies bind to? Potentially, this form of IgM may be a novel marker of PKD which may inform us about how to test for and treat the disease.

Keywords: Proliferative kidney disease (PKD), Erythrocytes, Immunoglobulin (Ig), antibody, Red blood cells

P590 / #623

Topic: *AS04 Parasites of fish / AS04.3 Host-parasite interactions*

NO LONGER A STRANGE: INFECTION LEVELS, HISTOLOGY AND GENETICS OF CLESTOBOTHRIUM CRASSICEPS (CESTODA: BOTHRIOCEPHALIDEA) FROM EUROPEAN HAKE REVEALED IN THE NW MEDITERRANEAN

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Introduction: The cestode Clestobothrium crassiceps infects its type host, the European hake Merluccius merluccius, across a wide geographical range. However, several features are still unknown for this parasite, especially in the Mediterranean area. The aim of this study is to provide, for the first time, quantitative descriptors of C. crassiceps populations infecting M. merluccius from the NW Mediterranean, as well as a histological assessment of its attachment to the host and a genetic characterization.

Methods: Hakes from the continental shelf off Barcelona (NW Mediterranean) were captured seasonally in 2007 (n=82) and in Summer 2019 (n=21). On board, they were measured, weighed and frozen for further parasitological analysis. Some individuals were fixed in buffered formalin for histological studies.

Results: Parasite abundance increased with host size. Overall, prevalence and abundance reached 31.7% and 0.7±1.7 in 2007, and 71.4% and 1±0.8 in 2019 samples. Seasonally, values increased in Spring and decreased in Autumn, and also increased in 2019 vs. 2007 summer samples. In histological sections, fish intestinal epithelium can be recognized inside the muscular grooves of the two bothria of the scolex. Although no clear detrimental effects to the intestinal mucosa were observed, some slight alterations in the mucosa surrounding the attached parasite were noticed. New genetic data were obtained and used for comparison with those available from the North Sea.

Conclusions: Infection descriptors of C. crassiceps on European hake seem to be influenced by host ontogeny and diet shifts. This parasite does not seem to induce important harmful effects to the health of its host.

Keywords: Clestobothrium, Bothriocephalidae, Cestoda, Merluccius, Hake

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Topic: *AS04 Parasites of fish / AS04.3 Host-parasite interactions*

MORTALITY THRESHOLDS FOR ATLANTIC SALMON WITH SALMON LICE INFECTIONS

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Introduction: The salmon louse is a marine ectoparasite of salmonid fish. In Norway, aquaculture production of salmonids is mainly in open net-pens giving this parasite access to large number of hosts. Production is regulated through permissions of maximum allowable biomass issued by the governmental authorities. To ensure sustainability, environmental indicators are considered, and at present, the abundance of salmon lice on wild salmon hosts is considered the most critical indicator due to the negative effects on wild migrating Atlantic salmon post smolt. This risk estimate uses dose-dependent mortality associated with the number of salmon louse per fish. Relatively little data support the risk limits currently in use.

Methods: The goal of the present study was to determine mortality thresholds for Atlantic salmon using a laboratory set-up which resembled conditions in nature: small, newly-smoltified fish were infected with a wide range of intensities, subjected to a simulated migration, and followed as the infection developed. Fish were closely monitored to provide information on mortality and infection parameters (time and louse intensity).

Results: Mortality did not occur before development of preadults and was closely correlated with lice intensity, whereas migration had no effects on the survival of the fish. Blood plasma parameters were similar among infection intensities before mortalities began to occur, and in surviving fish. Mortality thresholds were calculated for lice intensities both at the chalimus stages and for the mobile stages.

Conclusions: Validated mortality limits will provide better management tools and contribute to the protection of wild Atlantic salmon.

Keywords: salmon louse, Atlantic salmon, survival, swimming

P592 / #66

Topic: *AS04 Parasites of fish / AS04.3 Host-parasite interactions*

TREMATODE TRANSMISSION BETWEEN SNAIL AND FISH IN DANISH FRESHWATER LAKES

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Introduction: Trematodes have a heteroxenous life cycle and use molluscs as first intermediate host, different metazoans may act as second intermediate host and vertebrates as definitive host. The transmission of different species is interrelated with the occurrence of their hosts. The present analysis connects the different hosts and describes their relative importance in the ecosystem.

Methods: All snail and fish data were adopted from studies conducted in two Danish freshwater lakes, Lyngby Sø (55° 46' 27.4866", 12° 29' 10.2726") and Bromme Lillesø (55° 28' 52.2402", 11° 30' 51.5736"). All trematode species were identified by PCR and sequencing of DNA regions comprising 18S (partial) - ITS1- 5.8S- ITS2- 28S (partial) regions.

Results: The trematode species Posthodiplostomum cuticola was recovered both in snail and fish hosts in Lyngby Sø. Species Tylodelphys clavata and Diplostomum mergi were transmitted between snail and fish hosts in Bromme Lillesø.

Conclusions: The differential occurrence of trematodes in the two lakes reflects ecological differences. The abiotic and biotic elements of the two freshwater systems are highlighted and discussed.

Keywords: Trematode, snail, transmission, freshwater fish, metacercariae

P593 / #71

Topic: *AS04 Parasites of fish / AS04.3 Host-parasite interactions*

TREMATODE DIVERSITY REFLECTING THE COMMUNITY STRUCTURE OF DANISH FRESHWATER SYSTEMS: MOLECULAR CLUES

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Introduction: Digenean trematodes are parasitic platyhelminths that use several hosts in their life cycles and are thereby embedded in various ecosystems affected by local environmental conditions. Their presence in a habitat will reflect the presence of different host species and, as such, they can serve as ecological indicators. Only limited information on the occurrence of trematodes and their link to other trophic levels in the Danish freshwater ecosystems is currently available. Therefore, the main aim of the present study was to increase our knowledge in this field.

Methods: Snails were sampled from 21 freshwater lakes in Denmark, following which shedding procedures were performed, cercariae were recovered and the released parasites were identified using molecular tools (PCR and sequencing).

Results: A total of 5657 snail hosts belonging to ten species were identified, revealing a highly diverse parasite fauna comprising 22 trematode species. The overall trematode prevalence was 12.6%, but large variations occurred between host species. The snail host Lymnaea stagnalis showed the highest prevalence and also exhibited the highest diversity, accounting for 47.6% of the species richness.

Conclusions: This survey contributes updated information on parasite-host relations and compatibility and may assist in describing the ecological structure of the investigated Danish freshwater ecosystems.

Disclosure: This work has been published in Journal "Parasites & Vectors" with open access (https://doi.org/10.1186/s13071-020-04536-x).

Keywords: Freshwater lakes, Trematode, Snails, Diversity, Molecular identification

P594 / #2077

Topic: *AS04 Parasites of fish / AS04.3 Host-parasite interactions*

MARINE MAMMALS AND ARTHROPODS: HOST-PARASITE RELATIONSHIPS IN THE MARINE ENVIRONMENT

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Introduction: Adaptation of cetaceans and pinnipeds to marine life has also caused a coevolution of their parasite fauna in the new environment, leading to new host-parasite relationships of marine intervertebrates.

Methods: Three of these parasites can be found in the North- and Baltic Sea. *Isocyamus spp*. are ectoparasitic crustaceans living on cetaceans, *Echinophthirius horridus* are blood sucking lice found on seals, and *Halarachne halichoeri* are endoparasitic mites found in the respiratory system of seals. Knowledge about the biology and ecology of these parasites is scarce. The goal of this study is to investigate functional adaptations of different stages of parasites and their role in the life cycle of different taxa. In addition, their potential as vectors for heartworm filaria and impact on host health will be evaluated. Therefore, a unique sample set of archived parasites has been collected from pinnipeds and cetaceans stranded along the North- and Baltic Sea coast. The parasites were screened for viral and bacterial pathogens to understand their role in vector-borne diseases. Moreover, histopathology was used to characterize the lesions caused by the parasites investigating reduced vitality of the host and adaption of parasites to marine life.

Results: The marine habitat has put special pressures on the ability of these parasites to cling on to surfaces and to recruit new hosts. We will characterize their recruitment of hosts as well as their prevalence within different age groups providing information about possible ways of transmission and conditions needed for a successful reproduction of the parasites.

Conclusions: This study provides essential information on the host-parasite relationship just as important veterinary aspects.

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Topic: *AS04 Parasites of fish / AS04.3 Host-parasite interactions*

DO PARASITISED AND NON-PARASITISED FISH RESPOND DIFFERENTLY TO A POTENTIAL PREDATOR AND BAITED HOOK?

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Introduction: Parasite infection can significantly alter fish behaviour, including changes in predator avoidance behaviour and neophobia towards novel foods. Here, we present results on the link between infection with parasites and taking more risks with predators and neophobia in juvenile spangled perch (Leiopotherapon unicolor).

Methods: Fish response to a large territorial fish-eating Murray cod was examined in the laboratory. A separate test examined fish response to a novel baited hook. Fish were then dissected and examined for parasites and the links between parasite infection and behavioural responses in the two tests examined.

Results: Parasite infection had no effect on the amount of time spent in the predator stimulus side. However, fish infected with protozoa or camallanid nematodes showed more avoidance of the predator stimulus side than unifected. On average, fish took 71 minutes to bite the hook and 13% of fish failed to bite the baited hook after a total of 240 minutes of presentation in the home tanks. Fish infected with camallanids and protozoa bit the hook significantly sooner after its introduction than fish not infected with these parasites. Fish infected with camallanids also bit the hook more times than uninfected fish.

Conclusions: Our finding indicates that fish can learn to avoid locations where predators were previously observed. Our results also point to potential relationships between parasite infection and risk-taking behaviour, with further experimentation required to confirm the causal links between parasitism and risk averse and neophobic behaviour.

Keywords: Behaviour, predator avoidance, neophobia

P596 / #179

Topic: *AS04 Parasites of fish / AS04.3 Host-parasite interactions*

"SEEK AND YOU SHALL FIND": SPECIES DIVERSITY OF ACANTHOBOTHRIUM (CESTODA: ONCHOPROTEOCEPHALIDEA) IN DOMINANT NEAR-SHORE SKATES (RAJIDAE) IN WESTERN SOUTH AFRICA.

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Introduction: The spotted skate, Raja straeleni Poll and the white skate, Rostroraja alba (Lacépède), are frequently encountered along the western South African coast. Both species are heavily affected by anthropogenic impacts which cause drastic declines in skate populations. As a result, R. alba has been declared an endangered species in the IUCN´s Red List of Threatened Species.

Methods: As part of a larger study on elasmobranch affiliates in southern Africa, both species were screened.

Results: Seven new species of Acanthrobothrium van Beneden, 1849, were recovered from these previously unexplored hosts; four of these affiliate species were encountered in the intestinal tracts of R. straeleni, while three additional species were found in R. alba.

Conclusions: Given the devastating state of elasmobranchs worldwide, it becomes apparent to also address the conservation status of their affiliated species and intimate interrelationships. Currently, environmental conditions caused by anthropogenic pressures have direct impacts on this host-affiliate system, increasing risks of extinction. Yet, our knowledge on the actual species diversity is limited, with merely 9 % of elasmobranchs examined for cestodes in southern Africa. Extensive studies on these organisms and their hosts implementing multisource approaches are needed to provide a better understanding on the intimate nature of host-affiliate systems that may lead to the preservation of threatened host species together with their unique fauna of affiliate species and an alteration of future conservation practices. The work presented has been published in Folia Parasitologica (doi: 10.14411/fp.2020.036) and the International Journal of Parasitology: Parasites and Wildlife (doi: 10.1016/j.ijppaw.2021.12.010).

Keywords: Elasmobranchs, Taxonomy, Affiliate species, Conservation biology

P597 / #184

Topic: *AS04 Parasites of fish / AS04.3 Host-parasite interactions*

UNIQUE DIVERSITY OF PARASITES OF THE EVIL-EYE PUFFERFISH, AMBLYRHYNCHOTES HONKENII BLOCH, 1785 OF SOUTH AFRICA.

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Introduction: The Evil-eye pufferfish, Amblyrhynchotes honkenii Bloch, 1785 is a marine and brackish fish species commonly found in the Indo-West Pacific Ocean from South Africa to China. As these fish are extremely poisonous, which attributes to the unique diversity of parasite species infecting these host species. Despite their importance as key components of natural ecosystems, marine parasites remain dramatically understudied. Marine parasites are rarely studied in South Africa, and as such there is little information available on the effects these parasites may have on their hosts and the surrounding environment. The only parasites currently known from this host is the digenean Opistholebes cotylophorus Ozaki, 1935, the acanthocephalan, Rhadinorhynchus gerbera Lisitsyna, Kudlai, Cribb and Smit, 2019, the isopod, Cinusa tetrodonis (Schioedte and Meinert, 1884), the fish bloodparasite, Haemogregarina koppiensis Smit and Davies 2001, and the monogenean, Heterobothrium victorwepeneri Acosta and Smit, 2021, all described from the coast of South Africa.

Methods: As part of a larger study on the role of Marine Protected Areas in the conservation of marine fish parasites, the Evil-eye pufferfish was screened.

Results: This resulted in the discovery of both external and internal parasites, including copepod, digenean, cestode, nematode, isopod, myxozoan and monogenean parasite groups.

Conclusions: The discovery of these parasites can successfully establish detailed knowledge of the nature of the parasite fauna of A. honkenii. These parasites could then be used as bioindicators to determine the success of conservation interventions as well as the extent of chemical pollution in Marine Protected Areas for South Africa's unique biodiversity.

Keywords: Marine parasites, Marine Protected Areas, Taxonomy

P598 / #408

Topic: *AS04 Parasites of fish / AS04.4 Parasitic diseases in aquaculture*

HOLOGENOMIC APPROACH TO UNDERSTANDING CESTODE INFECTIONS IN FARMED ATLANTIC SALMON

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Introduction: Cestode infections impact human and animal populations. Eubothrium contributes to health and economic burdens in Atlantic salmon aquaculture. There is growing evidence that both host and parasite microbes interact with the host to influence disease outcomes. We used hologenomics to determine how cestode and salmon microbiota affect host phenotype.

Methods: Genomes, transcriptomes, metabolomes and metagenomes were generated from the guts of 460 harvest-aged salmon at a Norwegian seafarm and integrated with multi-omic factor analysis. Cestode infection levels in the gut were scored and metagenomes were generated from cestode tissue.

Results: Salmon weight significantly decreased as the level of cestode infection increased. Both cestode and salmon microbiota were dominated by mycoplasma species. However, the prevalent phylotype differed phylogenetically and functionally between cestodes and salmon. Salmon microbiota were perturbed during cestode infection, with decreased abundance of the salmon mycoplasma phylotype and increased abundance of other mycoplasmas, including those detected in the cestode microbiota. Preliminary multi-omic analysis suggests that the shift in the salmon gut microbiota was associated with salmon genomic variation and accompanied by changes in salmon gut metabolic and gene expression profiles.

Conclusions: Our results indicate that cestode infection is associated with salmon gut dysbiosis, which correlates with shifts in host functional profiles. Future work will determine the biological significance of these changes. Such hologenomic approaches will further our understanding of host-parasite interactions and reveal novel targets to improve fish welfare and sustainable aquaculture practices.

Disclosure: Co-author Harald Sveier is employed at Lerøy Seafood Group, who produce, market and sell the farmed Atlantic salmon used in this study. Lerøy Seafood Group provided partial funding for this study. All other authors declare no competing interests.

Keywords: tapeworm, multi-omic, Microbiome

P599 / #1115

Topic: *AS04 Parasites of fish / AS04.4 Parasitic diseases in aquaculture*

MONOGENEAN INFECTIONS THREATENING THE DIVERSIFICATION OF MEDITERRANEAN MARICULTURE

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Introduction: Mediterranean aquaculture is currently suffering a lack of diversification in fish species production due to several zootechnical and health-related issues; among the latter, parasitic infections by Monogeneans are important limiting factors.

Methods: During 2020-21, monogenan infections were observed in sea caged greater amberjack (Seriola dumerili) and common dentex (Dentex dentex), and in tank-reared meagre (Argyrosomus regius) broodstock from Adriatic Sea. Parasites were identified by morphology and associated lesions were studied by histology.

Results: The following monogeneans were identified: Zeuxapta seriolae (Microcotylidae) in the greater amberjack, with high intensity related to severe gill anaemia; specimens referable to Microcotyle erythrini species complex (Microcotylidae) in the common dentex at low intensity; Ktariella polyorchis (Calceostomatidae) and Diplectanum sciaenae (Diplectanidae) respectively on skin and gills of meagre, the former affecting broodstock with high intensity and mortality.

Conclusions: The species Z. seriolae and D. sciaenae have already been reported in wild and farmed S. dumerili and A. regius, respectively, while K. polyorchis and M. erythrini species complex have been reported only from wild fish. The finding of these monoxenous parasites in aquaculture facilities raises concern for their rapid spread in the farming environment and highlight a possible bottleneck for the diversification of Mediterranean aquaculture.

Keywords: Monogenea, farmed fish, Aquaculture, Mediterranean sea

P600 / #853

Topic: *AS04 Parasites of fish / AS04.4 Parasitic diseases in aquaculture*

IDENTIFICATION OF NEW AMOEBAE STRAINS IN FARMED RAINBOW TROUT (ONCORHYNCHUS MYKISS) AFFECTED BY NODULAR GILL DISEASE (NGD)

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Introduction: The Nodular Gill Disease (NGD) is an emerging disease caused by amoebic infestations in farms of freshwater salmonids, resulting in huge economic losses. Nevertheless, unambiguous identification of the pathogenic amoebae has not yet been achieved. The aim was to identify the amoebae species involved in periodic NGD episodes in 2 rainbow trout (Oncorhynchus mykiss) farms in Northern Italy between February and April 2021.

Methods: Four episodes were monitored and a total of 88 fish were euthanized and their gills evaluated by macroscopic, microscopic, and histological examination, with the assessment of 6-grade lesion scores. A portion of the second left gill arch from each animal was put on non-nutrient agar (NNA) Petri dishes for amoebae isolation, cultivation, and identification with SSU rRNA sequencing.

Results: Histology confirmed moderate to severe NGD-related lesions and mild to moderate amoebae infestation. The presence of parasitic amoebae was significantly correlated with lesions severity. Light microscopy of cultured amoebae strains and SSU rDNA analysis revealed infection of Naegleria sp. strain GERK, two amoebae strains from Hartmannelidae, vannelid amoebae from the genus Ripella, and cercozoan amoeba Rosculus.

Conclusions: The results highlight the utility of histopathology and SSU rDNA sequencing in NGD evaluation. The identification of known and new amoebae highlights the multi-etiological origin of this pathology, although the pathogenic role of each amoeba remains undefined.

Keywords: amoebae, Nodular Gill Disease (NGD), Rainbow trout

P601 / #1204

Topic: *AS04 Parasites of fish / AS04.4 Parasitic diseases in aquaculture*

EXPERIMENTAL INFECTION OF BENEDENIA HUMBOLDTI ON SERIOLA LALANDI, FROM SOUTHEASTERN PACIFIC, EXPOSED TO A NON- PHARMACOLOGICAL PRODUCT.

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Introduction: The monogenean B. seriolae have been reported as the main parasitic problems of farmed kingfish. These flatworms can reduce fish appetite in heavy infections, slower growth and, in extreme cases, can cause death to the host if left untreated. Therefore, it is important look alternatives non-pharmacological products to control this parasite in farms. The objective of this study was to evaluate the infection dynamics of B. humboldti exposed to extract to olive oil (HT).

Methods: The experiment considered 3 treatments and a control (with two replicates per treatment and control). The treatments (T) were: food with the HT at 0.05% (T1) and 0.5% (T2); and with praziquantel (T3). Each pond containing 20 fish (65.2- 116g) were infested with 500 oncomiracidium. Infestation was monitored weekly, recording number of parasites per fish at 14,21,28,35,42,49 and 56 post-infestation days (dpi). GLM were used to analyze the infestation between treatments.

Results: Up to 33 dpi parasite loads were low in fish (3- 8 mean parasite per fish and with no evidence of juvenile stages). In this period, there was no effect of the treatments on the parasite abundances ($p>0.05$). However, from 40 dpi juvenile stages began to appear due to parasite reproduction. Juveniles increased from 24 mean juveniles per fish to over 75 mean juveniles per fish at the end of the experiment. The parasite load varied significantly among treatments, with a lower load in fish feed with high HT (0.5%) and praziquantel ($P<0.05$).

Conclusions: The fish feed with HT 0.5% showed lower parasite load during the experimental period suggesting a positive effect of HT-0.5% against this parasite. However, this difference in parasite load (47 dpi) could be explained because some fish in control group had infections up to 205 parasites per fish.

Disclosure: This study was financed by project FONDEF IDEA ID 16110453 of the Chilean Government. Food with HT was manufactured by SPAROS I&D (www.sparos.pt)

Keywords: Monogenea, Hidroxytyrosol (HT), Chile, Seriola lalandi, Infestation

P602 / #55

Topic: *AS04 Parasites of fish / AS04.4 Parasitic diseases in aquaculture*

QUANTITATIVE TRAIT LOCI (QTL) ASSOCIATED WITH RESISTANCE OF RAINBOW TROUT ONCORHYNCHUS MYKISS AGAINST THE PARASITIC CILIATE ICHTHYOPHTHIRIUS MULTIFILIIS

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Introduction: The parasitic ciliate Ichthyophthirius multifiliis has a low host specificity eliciting white spot disease (WSD) in a wide range of freshwater fishes worldwide. The parasite targets both wild and cultured fish but the huge economic impact of the protozoan is associated with mortality, morbidity and treatment in aquacultural enterprises.

Methods: Applying the DNA typing system Affymetrix® and characterizing the genome of the individual fish by use of 57,501 single nucleotide polymorphisms (SNP) and their location on the rainbow trout chromosomes, we have genetically characterized rainbow trout with different levels of natural resistance towards WSD. Quantitative trait loci (QTL) which can be used for the selection of breeders with specific markers for resistance are reported. Expression of immune-relevant genes in rainbow trout organs following exposure to I. multifiliis at different time points was assessed by quantitative reverse transcription PCR (qPCR)

Results: We found a significant association between resistance towards I. multifiliis infection and SNP markers located on the two specific rainbow trout chromosomes Omy 16 and Omy 17. The fish exposed to the infection showed a significant elevation of expression of immune relevant genes related to both innate and adaptive responses.

Conclusions: The study has described a QTL for resistance in rainbow trout against WSD. The specific genes associated with resistance are still being investigated. In this context it should be noted that trout surviving the infection showed high expression levels of genes encoding IgT, T-cell receptor TCRβ, complement C3, cathelicidins 1 and 2 and serum amyloid A SAA, suggesting these genes to be associated with protection.

Keywords: selective breeding, susceptibility, disease, quantitative trait loci, resistance

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Topic: *AS04 Parasites of fish / AS04.4 Parasitic diseases in aquaculture*

A COMPARATIVE PARASITOLOGICAL STUDY OF WILD AND AQUACULTURED FISH IN DANISH MARINE WATERS

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Introduction: Fishborne zoonotic helminths place half a billion people at risk worldwide. Freeze regulations may secure killing of infective larvae in fish meat, but the EU commission regulation (EU) No. 1276/2011 may exempt aquaculture products from pre-treatment, if epidemiological data show absence of zoonotic parasites in the products. In this study, we present results from a comparative parasitological investigation of Danish aquacultured rainbow trout and wild fish (Atlantic cod).

Methods: We compared rainbow trout and wild Atlantic cod. We sampled 180 cultured rainbow trout (Oncorhynchus mykiss) in 18 freshwater farms and 170 rainbow trout from 17 net cage based mariculture farms in Denmark. For comparison we sampled ten wild Atlantic cod (G. morhua) in the Sound (Øresund).

Results: Five different species of nematodes (Anisakis simplex, Contracaecum osculatum, Pseudoterranova decipiens, Hysterothylacium aduncum, Cucullanus cirratus), three species of trematodes (Lepidapedon elongatum, Cryptocotyle lingua, Hemiurus communis), one copepod (Lernaeocera branchialis) and one myxozoan (Cnidaria) (Myxobolus aeglefini) were recovered from wild cod. No zoonotic parasites were found in aquacultured fish. Recorded parasites in these fish were: Diplostomum spp., Echinorhynchus truttae and Eubothrium crassum.

Conclusions: The absence of zoonotic parasites in Danish aquacultured may be explained by practice of using heat treated feed pellets, filtering of farm-water, sheltering farms from animals and locating net cages with safe distance from mammalian host populations. The highly infected wild cod indicate the existence of zoonotic life cycles in Danish marine waters, where presence marine mammals should be considered a risk factor.

Keywords: Zoonosis, Fish, Aquaculture

P604 / #92

Topic: *AS04 Parasites of fish / AS04.4 Parasitic diseases in aquaculture*

VANELLA AMOEBAE FROM RAINBOW TROUT: IDENTIFICATION, CULTURE AND CONTROL

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Introduction: Farming of freshwater rainbow trout is challenged by a range of gill infecting parasites such as flagellates, ciliates and amoebae. Infections may be associated with economic losses and reduced animal welfare. Correct diagnosis and control measures are highly needed.

Methods: We conducted a survey of Danish rainbow trout farms, isolated amoebae on non-nutrient agar moistened by Neff's amoebae saline. We then diagnosed a series of amoebae. We finally conducted experimental in vitro testing of biocides for their effect on Vanella amoebae.

Results: We found amoebae from all six farms within the genera Trinema and Vanella and hartmannellid amoebae. We established a monoculture of Vannella sp. and performed a dose-response study of the compounds sodium chloride (NaCl), hydrogen peroxide, peracetic acid, formalin, extracts of garlic and oregano and a Pseudomonas H6 surfactant (SPH6). Biocide concentrations found lethal to the amoebae were for NaCl (7.5 mg/ml), hydrogen peroxide (100 µg/ml), peracetic acid (0.03 µg/ml), formaldehyde (25 µg/ml), 16.90 mg/ml (garlic), 17.90 mg/ml (oregano), and the Pseudomonas H6 surfactant (250 µg/ml).

Conclusions: All compounds showed antiparasitic properties, but as several biocides are questioned due to carcinogenicity or allergenicity it is recommended to elaborate on the use of sustainable compounds including natural surfactants such as Pseudomonas H6.

Keywords: treatment, biocides, amoebae

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Topic: *AS04 Parasites of fish / AS04.4 Parasitic diseases in aquaculture*

A NOVEL SEVERE MICROSPORIDIASIS IN CULTURED ATLANTIC BLUEFIN TUNA (THUNNUS THYNNUS L.)

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Introduction: The Atlantic bluefin tuna (ABT, Thunnus thynnus) is one of the most promising new species in Mediterranean aquaculture, due to its large size, fast growth, and high market value. Thus, disease control is crucial to prevent and reduce mortality and monetary losses. One of the main issues for the management of fish cultures is disease control, and new fish cultures, as that of the ABT, are often subjected to new diseases related to unknown pathological agents.

Methods: A new microsporidian species is described from farmed bluefin tunas from the Spanish Mediterranean, based on molecular and morphological evidence.

Results: This new pathogen is described in a juvenile associated with a highly severe pathology of the visceral cavity. Whitish xenomas of this microsporidian species were mostly located at the caecal mass ranged from 0.2 to 7.5 mm. Light and transmission electron microscopy of the spores revealed mature spores with an average size of 2.2 × 3.9 µm in size and a polar filament with 13–14 coils arranged in one single layer. Phylogenetic analysis clustered this species with the Glugea spp. clade.

Conclusions: The morphological characteristics and molecular comparison confirm that this is a novel microsporidian species. The direct life cycle and the severe pathologies observed makes this parasite a hard risk for bluefin tuna cultures. Projects: MINECO/FEDER PID2019-110730RB-I00 co-funded by MCIN/AEI/10.13039/501100011033 by “ERDF A way of making Europe” by the EU; AICO/2021/279; and GVA-THINKINAZUL/2021/029 (with NextGenerationEU funds).

Keywords: Xenoma, Pathology, Microsporidia, Glugea sp.

P606 / #1141

Topic: *AS04 Parasites of fish / AS04.4 Parasitic diseases in aquaculture*

THE NON-NATIVE CARP PARASITE KHAWIA JAPONENSIS (CESTODA): NEW DATA ON ITS DISTRIBUTION IN CENTRAL EUROPEAN COUNTRIES

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Introduction: The Asian tapeworm Khawia japonensis was originally described from the common carp (Cyprinus carpio) in Japan and has since been found in several other localities in Asia and the USA. In Europe, K. japonensis was first found in a carp from northern Italy (in 2011). In a relatively short time, the tapeworm appeared in a fish-breeding farm in Slovakia (2015), and the third occurrence of this parasite in Europe was reported from fish ponds in the southern Czech Republic.

Methods: A routine ichthyoparasitological survey of fish cestodes in several farmed fish ponds in Central European countries (Hungary, Poland and Slovakia), including the heavily polluted river system in eastern Slovakia, confirmed the new distribution of this Asian parasite.

Results: Tapeworms found in cultured carp and free-living fish populations showed species-specific characteristics of K. japonensis and comparison with specimens from Asia and Italy revealed no obvious differences. The highest prevalence (47%) of K. japonensis was found in a carp breeding facility in eastern Slovakia and the highest intensity of infection (8 parasites per fish) was found in a carp from the Laborec River. Macroscopic observation of the carp gut did not reveal any obvious pathological lesions. However, histopathological examinations showed signs of chronic inflammation of the intestinal wall in the areas that had come into contact with the parasite.

Conclusions: The recent spread of this parasite in several large European river basins (Danube, Elbe, and Vistula) indicates its great ability to establish in new regions and represents another example of the anthropogenic introduction of fish pathogens. The study was supported by the Slovak Research and Development Agency, No APVV-18-0467 and Grant Agency VEGA, No 2/0126/20

Keywords: Aquaculture, tapeworm, Carp, biological invasions

P607 / #194

Topic: *AS04 Parasites of fish / AS04.4 Parasitic diseases in aquaculture*

CARDICOLA MEDITERRANEUS (TREMATODA, APOROCOTYLIDAE): EFFECTS OF A NEW SPECIES INFECTING SPARUS AURATA IN THE WESTERN MEDITERRANEAN SEA

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Introduction: Cardicola species are blood flukes causing epizootic infections in aquaculture. The recent description of C. mediterraneus has introduced a new pathogen of Sparus aurata out of the known C. aurata, the first species reported in this host. Pathological effects of C. aurata have been previously described although the impact of the new species has not been assessed to date. The aim of the study was to analyse infection patterns of both species in the Mediterranean and provide insight into their diagnosis and potential pathological effects.

Methods: The circulatory system of 280 gilthead sea breams off the Mediterranean was examined for eggs and adults of Cardicola spp. and morphological, molecular and pathological analyses were performed.

Results: The seasonal infection pattern was similar for both species increasing the number of eggs from autumn to summer. Both species are clearly distinguishable by the morphology of adults and eggs which affects eggs distribution into the gill vessels and related damages

Conclusions: The occurrence of mixed infections in gilthead sea bream may hamper treatment design and application; thus current data discriminating Cardicola spp. should be considered to improve the infection management in fish farms. Funded by MICIN/FEDER PID2019-110730RB-I00, AICO/2021/279 and GVA-THINKINAZUL/2021/029.

Keywords: gilthead seabream, Taxonomy, disease, Platyhelminthes, Aporocotylidae

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Topic: *AS04 Parasites of fish / AS04.4 Parasitic diseases in aquaculture*

UNUSUAL CLINICAL MANIFESTATION OF DERMOCYSTIDIUM INFECTION IN COMMON CARP (CYPRINUS CARPIO L)

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Introduction: In common carp, Dermocystidium koi induces pathological lesions manifested mainly as cysts on the skin, fins, or gills, occasionally in the internal organs. In typical skin infections develop small blisters in the epidermis, up to the size of a lens or bean, which sometimes affect the hypodermis. In the present work, an unusual form of the infection could be observed in 3-year-old common carp where a large, tumor-like cyst formed under the skin intruded deeply in the red muscle.

Methods: After gross examination under microscopes and taking photo-documentation, small pieces were excised for further histological and molecular studies.

Results: In the cross-section of cyst consisting of a web of hyphae filled with typical D. koi spores surrounded by more or less damaged muscle tissue, two regions (pale and dark) were distinguishable. To confirm the observations comparative studies were also performed using a sample from infection manifested in typical skin cysts on common carp. In the molecular analysis of 18S rDNA sequences obtained the pathogen from tissue samples of the typical and atypical infections were identical and showed high similarity to the sequences of D. anguillae and D. salmonis.

Conclusions: The observed morphological traits, despite the unusual manifestation and involvement of muscle tissue, suggested the presence of the Dermocystidium koi in the found severe pathological lesion. The manifestation in the giant-cyst form of infection has hitherto been unknown to the scientific literature. Currently, the scarce nucleic acid data and incomplete phylogenetic characterization of the given taxonomic group make clear molecular taxonomic identification of the detected pathogen questionable. We thank for the carp samples to Gábor Nagy and Géza Simonics

Keywords: giant cyst, atypical skin infection, Mesomycetozoea, common carp, Dermocystidium koi

P609 / #90

Topic: *AS04 Parasites of fish / AS04.4 Parasitic diseases in aquaculture*

A NOVEL MICROSPORIDIAN DISEASE IN FARMED SALMONIDS: MULTI-HOST TRANSMISSION UTILIZING FISH AND CRUSTACEAN HOSTS

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Introduction: A microsporidian disease of rainbow trout was newly confirmed in Japan. As the experimental infection by feeding common prawn Palaemon paucidens was succeeded, its involvement in the transmission of the microsporidian (Microsporidium sp. RBT: MsRBT) was strongly suggested. To elucidate the transmission route of the disease, P. paucidens was investigated for microsporidian infection.

Methods: We collected P. paucidens from Lake Biwa in 2019 and 2020. Prawns showing opaque muscle were examined by wet mount, and the nucleotide sequence of the small subunit ribosomal DNA was determined when microsporidians were found. Transmission electron microscopy (TEM) was performed for MsRBT and the microsporidians from P. paucidens.

Results: Four microsporidians (Type 1-4) were found from P. paucidens. The pairwise nucleotide identity between MsRBT and Type 1-4 was 100% (Type 1), 84% (Type 2), 41% (Type 3) and 84% (Type 4), indicating MsRBT and Type 1are conspecific. However, intriguingly, the spore morphology and the mode of development in fish and prawn were strikingly different. Only Type 1 spores possessed hair-like external appendages, which is a typical character of the genus Inodosporus Overstreet and Weidner, 1974 (crustacean microsporidians). TEM observation also indicated that Type 1 belongs to the genus Inodosporus, while MsRBT shows characteristics of the genus Kabatana Lom, Dyková and Tonguthai, 2000 (fish microsporidians). In the molecular phylogeny, Type 1 was placed within a clade comprising Kabatana spp. and Inodosporus octosporus.

Conclusions: This study indicates MsRBT (= Type 1) is a novel species in the genus Inodosporus which has a multi-host life cycle utilizing fish and crustacean hosts and different modes of development in each host.

Keywords: life cycle, Salmonid, Crustacean, phylogeny, Microsporidia

P610 / #198

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

MORPHOLOGICAL CHARACTERIZATION OF A 3RD STAGE LARVA GENETICALLY IDENTIFIED AS PSEUDOTERRANOVA SP. CF. CETICOLA (NEMATODA: ANISAKIDAE) FROM MESOPELAGIC FISHES OFF NW AFRICA

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Introduction: The genus Pseudoterranova includes parasite species of cetaceans and pinnipeds. The third stage larva (L3) of seal-infecting species occur in intermediate or paratenic fish hosts. This study firstly describes a Pseudoterranova L3 from meso/bathypelagic fishes off Macaronesia.

Methods: L3 were morphologically and genetically studied by light microscopy and sequencing of the mtDNA cox2 and entire ITS rDNA genes.

Results: Pseudoterranova sp. L3 occurred mainly in the viscera of Bolinichthys indicus, Chaulodius danae, Eupharynx pelecanoides, Diaphus rafinesquii, D. mollis, Diretmus argenteus and Maulisia argipalla. A prominent characteristic is a circumoral ridge extending from the ventral boring tooth. An anterior excretory pore, intestinal caecum and absence of ventricular appendix are characters shared with Pseudoterranova spp. The shape of the tail: conical, long, pointed and lacking mucron are characters shared with P. kogiae and P. ceticola from kogiid whales. Phylogenetic analysis based on cox2 suggests that the anisakid herein identified as Pseudoterranova sp. cf. ceticola is distinct from but most closely related to P. ceticola from Kogia breviceps. Results based on cox2 and ITS sequences suggest that it is closely related to a clade formed by Anisakis paggiae, A. brevispiculata and A. physeteris, parasites of cetaceans, and not with Pseudoterranova spp. parasitizing pinnipeds.

Conclusions: The morphology of Pseudoterranova sp. L3 belonging to a species infecting cetaceans is described for the first time. L3 differ from those of the pinniped-infecting species in several characters. Likely, the parasite completes its life cycle in the mesopelagic realm, with mesopelagic fish as 2nd intermediate or paratenic hosts and kogiids as final host.

Keywords: anisakid, pseudoterranova, mesopelagic fish, Parasite, cetacean

P611 / #864

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

PREVALENCE AND ABUNDANCE OF LARVAL ANISAKIS SPP. IN THE ARGENTINE SHORTFIN SQUID ILLEX ARGENTINUS: STOCK, AGE, SIZE AND COHORT AS SOURCES OF VARIABILITY

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Introduction: Illex argentinus is a semelparous squid, with a lifespan lasting about 1 year and non-overlapping generations. Squids perform a latitudinal/bathymetric migratory cycle along which they grow, mature, visit different oceanographic regions and changes its diet. Its recruitment and population size are largely dependent on oceanographic conditions and, consequently, variable temporally. All such sources of variability are expected to have an effect on trophically transmitted parasites, especially for larval Anisakis that can persist for long periods and could be indicative of such changes. Aim: to evaluate the temporal variability of larval Anisakis spp. in relation to habitat, size and date of capture of I. argentinus.

Methods: A total of 430 squids caught at different latitudes and dates in 2019-2021 were examined for Anisakis and their length and maturity index were determined. Samples were identified according to stock, geographical origin, date of capture and squid size. Parasites were counted and prevalence and mean abundance were calculated for each sample.

Results: Larvae of Anisakis spp. (n= 270) were found encysted in viscera of 111 squids, mostly in the stomach. No larvae were found in the mantle. Both prevalence and mean abundance varied across stocks, increasing in southern ones, and cohorts. Burdens increased also with squid size and age. Cohorts of the same stock also differed in their loads between consecutive years.

Conclusions: Despite some patterns were found, such as increasing loads southwards and in larger/older hosts, the nature of this parasitism seems as dynamic and unpredictable as that of populations of I. argentinus, being largely dependent on the feeding behaviour of this host in each region it inhabits along its migratory cycle. PICT 2019-3376

Keywords: anisakis, South-western Atlantic, Illex argentinus

P611a / #876

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

BATHYMETRIC DISTRIBUTION OF LARVAL ANISAKIS ACROSS NINE SPECIES OF SKATES IN THE SOUTHWESTERN ATLANTIC

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Introduction: Host size and trophic level are known determinants of abundance for many parasite species. For larval Anisakis in teleost fish, depth has often been shown as an additional relevant driver of parasite burdens, which increase in deeper waters. In the Argentine sea, temperature decreases along the bathymetric gradient due to both, the depth itself and the presence of subantarctic waters (Malvinas current) flowing northwards along the continental slope. These waters have been related to higher loads of larval Anisakis in several teleost species and some elasmobranchs. Aim: to evaluate the drivers of distribution of larval Anisakis spp. across skate species along a bathymetric gradient in the southwestern Atlantic.

Methods: A total of 379 skates (2 Rajidae and 7 Arhynchobatidae), belonging to 9 species, were measured and examined for larval Anisakis. The prevalence (P) and mean abundance (MA) were calculated for each species and distance based linear models were fitted on Euclidean distance of these indices, with depth, trophic level and mean length as explanatory variables.

Results: Marginal tests showed that only depth was significantly related to both, P ($P<0.01$) and MA ($P<0.05$), representing this variable alone the best models (lowest Akaike information criterion) for both descriptors, explaining 80.5 % and 32.9% of the total variation, respectively.

Conclusions: The distribution of larval Anisakis is highly influenced by depth, although, in this case, depth could be, at least partially, a surrogate of water temperature. However, the demersal-benthic habits of skates permit hypothesize that depth itself has a role in parasite distribution. Specific identification of these larvae using molecular tools surely will reinforce the observed patterns. PICT 2018-1981

Keywords: Elasmobranchs, anisakis, Argentine sea

P614 / #622

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

ULTRASTRUCTURAL AND PHYLOGENETIC ANALYSIS OF A HENNEGUYA SP. (MYXOZOA) FOUND IN THE MARINE FISH ARCHOSARGUS PROBATOCEPHALUS (TELEOSTEI), FROM BRAZILIAN WATERS

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Introduction: Myxozoans are aquatic endoparasites that use vertebrates as temporary hosts. They have been widely reported from different geographic areas, being one of the most important parasitic groups infecting vital organs of freshwater and marine fishes.

Methods: In this study, specimens of the teleost fish Archosargus probatocephalus (Sparidae) were collected from Brazilian waters. Macroscopic analysis revealed myxozoan cysts in the gill filaments. Isolated myxospores were photographed using a light microscope, and infected fragments were processed for transmission electron microscopy and molecular studies. The SSU rDNA gene sequence obtained was used for phylogenetic analyses.

Results: Cysts spherical to ellipsoidal, containing numerous myxospores. Myxospores measured ± 21.3 µm in total length and had 2 equal tapering tails extending from the posterior end. Two equal polar capsules were located at the anterior end, showing 4-5 coils of the polar filament. The SSU rDNA sequence was 1992 bp long and presented highest similarity to Henneguya cynoscioni and H. lagunensis, both from marine perciform fishes.

Conclusions: The morphometry, ultrastructural aspects, host specificity and site of infection of this species were compared to the data available for other Henneguya. Molecular analysis confirmed the novelty of the observations, suggesting this as a potential new species. Phylogenetic analyses agreed with previously published cladograms, with the new sequence clustering within the clade of marine Myxobolidae, alongside congeners that infect fishes of the order Perciformes.
The authors acknowledge funding from: FCT under the project PTDC/BIA-BMA/6363/2020; the Foundation A. Almeida; CAPES Brazil; Alagoas State Research Support Foundation (FAPEAL).

Keywords: Fish, Parasite, myxozoa, Henneguya

P615 / #1404

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

AN INTEGRATIVE TAXONOMIC INVESTIGATION TO THE DIVERSITY OF METAZOAN PARASITES OF TAKIFUGU NIPHOBLES (JORDAN & SNYDER) (TELEOSTEI: TETRAODONTINAE) FROM THE STRAIT OF KOREA

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Introduction: The grass puffer fish, Takifugu niphobles (Jordan & Snyder, 1901) (Teleostei: Tetraodontinae), is known for its hight levels of toxic tetrodotoxin accumulation through the food chain and is one of the most valuable commercial fish species in Asia. Despite being widely recognised for its great delicacy still very little is known about its parasite diversity. We carried out a pilot study assessing the metazoan parasite diversity in a wild population of the grass puffer fish from the Strait of Korea employing an integrative taxonomic approach.

Methods: A total of 30 T. niphobles was sampled at the Korea Strait off Busan in January 2022. The metazoan parasites recovered were characterized both morphologically and molecularly. We obtained partial sequences of the mitochondrial cox1 and 28S rRNA genes for molecular identification of representative isolates.

Results: Combined molecular and morphological analyses confirmed presence of a total of 11 metazoan parasites and a fauna dominated by heteroxenous species. We identified 7 species of digenean trematodes of which two were larval metacercariae using birds as definitive host. The monoxenic parasites comprised a single monogenean and three parasitic copepod species.

Conclusions: The present study provides the first comprehensive characterisation of the metazoan parasites in T. niphoblesfrom off Korea and will serve as a baseline for future exploration of the costal Western North Pacific ecosystem. Our study is the first to report a species of Diplostomum using a tetraodontid fish as an intermediate host.

Keywords: North West Pacific, Integrative taxonomy, Grass puffer, metazoan parasites, Takifugu niphobles

P616 / #868

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

PARASITES OF EUROPEAN ANCHOVY AND MEDITERRANEAN HORSE MACKEREL IN NW MEDITERRANEAN SEA, WITH EMPHASIS ON THOSE POTENTIALLY TRANSMISSIBLE TO HUMANS

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Introduction: European anchovy (Engraulis encrasicolus) and Mediterranean horse mackerel (Trachurus mediterraneus) are pelagic fish species with an outstanding commercial value and also a key component of pelagic ecosystems in the Mediterranean Sea. The aim of this study was to describe their parasite communities, with focus on detecting the potential presence of zoonotic species.

Methods: European anchovies and Mediterranean horse mackerels were obtained from aboard commercial fishing vessels with purse-seine fishing gear in three different localities off the Catalan Coast (NW Mediterranean) during 2019. External surfaces and oral cavity were checked macroscopically for ectoparasites and the rest of organs, including gills, stomach, caeca, intestine, gonads, spleen, brain, body cavity and muscle were carefully inspected for endoparasites under stereomicroscope. Parasites were identified to the lowest possible taxonomic level and molecular analyses were performed when needed. Parasite prevalence, mean abundance, richness and Brillouin diversity indices were calculated.

Results: More than ten different taxa belonging to digeneans, monogeneans, cestodes and nematodes were identified in both species. The digenean Aphanurus virgula and the nematodes Hysterothylacium aduncum and H. fabri were common in both fish species. No zoonotic parasites, such as Anisakis spp. or Contracaecum spp. were identified.

Conclusions: Our results confirm that the parasite community of these two species in the Catalan coast does not include zoonotic parasites, which gives an added value for the fishing, commercialization and human consumption of the two analysed species.

Keywords: parasite communities, European anchovy, Mediterranean horse mackerel, Zoonotic parasites

P617 / #518

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

DACTYLOSOMA IN SHARKS, AND THE ROAD TOWARDS A CLEARER UNDERSTANDING OF FISH HAEMOGREGARINE PHYLOGENETICS

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Introduction: Haemogregarines are obligate endoparasitic intracellular protozoa found in the blood of a range of vertebrates. Many of these are described based on a single developmental stage only, with many of these descriptions lacking accompanying molecular data. As such, knowledge on the phylogenetic placement and taxonomy of many of these is deficient, and this is particularly true for those of fishes, and especially sharks. The aim therefore is to address this through the increased molecular characterization of taxa and their addition to phylogenetic analyses.

Methods: Blood was collected from sharks from which thin blood smears were prepared for morphological description of parasite stages, and a portion fixed in 96% ethanol for molecular characterisation using fragments of the 18S rRNA gene.

Results: Of the 98 individuals of four species examined, 87% were infected with two morphotypes of an unnamed species of haemogregarine. Both morphotypes were observed in Haploblepharus edwardsii and Haploblepharus pictus with a prevalence of 91-100% (morphotype A) and 38-53% (morphotype B). Only morphotype A was observed in Poroderma africanum and Poroderma pantherinum with a prevalence of 88% and 57%, respectively. Phylogenetic analyses placed both morphotypes in the Dactylosoma clade, with a 1% divergence between these morphotypes.

Conclusions: As such this represents the first Dactylosoma discovered in sharks, as well as the first molecularly characterized from fishes, a valuable contribution to this research field.

Keywords: Sharks, Haemogregarine, phylogeny, Dactylosoma, Haploblepharus

P618 / #204

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

INFECTION OF THE TRIPLETAIL, LOBOTES SURINAMENSIS (TELEOSTEI: LOBOTIDAE), BY BRAIN METACERCARIAE, CARDIOCEPHALOIDES MEDIOCONIGER (DIGENEA: STRIGEIDAE): A CASE STUDY

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Introduction: In January 2020, specimens of tripletail, Lobotes surinamensis, caught off the coast of South Carolina (SC) USA and maintained in aquaria in clean water displayed a profoundly altered swimming behavior.

Methods: Fish were found to be infected in their cerebellum by metacercariae of a digenean we identified via ITS2 region rRNA gene sequencing as the strigeid, Cardiocephaloides medioconiger. Histology also revealed metacercariae in the optic tectum and the tectal ventricle.

Results: This parasite uses larid birds for definitive hosts, and gastropods (species yet to be verified) and fish as first and second intermediate hosts, respectively. Issues associated with infection by Cardiocephaloides metacercariae in fish include their low host specificity and the alteration of the fish behavior when they encyst in the brain or eyes, leading to higher predation by birds. Consequently, transmission of Cardiocephaloides is enhanced in areas of high fisheries activities, including aquaculture settings. To our knowledge, fish infected by Cardiocephaloides metacercariae have been reported only twice, in the 1960s, from the northern Atlantic coast of the USA, including in the brain and eyes of the grey mullet, Mugil cephalus, and the silverside, Menidia menidia.

Conclusions: Tripletail is a new report as a second intermediate host for this species and our findings represent new infection localities in the southeastern USA. The finding of C. medioconiger in tripletail is significant because this fish is a promising candidate for extensive aquaculture in the USA; thus, if left unmonitored, infection by C. medioconiger may remain unrecognized and thereby jeopardize production as well as propagate to other fish in neighboring natural ecosystems and aquacultural impoundments.

Keywords: swimming behavior, aquaculture candidate, histology, Strigeidae, Optic tectum

P619 / #1244

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

TOWARDS AN IN-SITU NON-LETHAL RAPID TEST TO ACCURATELY DETECT THE PRESENCE OF THE NEMATODE PARASITE, ANGUILLICOLOIDES CRASSUS, IN EUROPEAN EEL, ANGUILLA ANGUILLA

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Introduction: Anguillicoloides crassus is an invasive nematode parasite of the European eel, Anguilla anguilla, and possibly one of the primary drivers of eel population collapse. The presence of the parasite has been shown to impact many features of eel physiology and life history. Early detection of the parasite is vital to limit the spread of A. crassus, and to assess its potential impact on spawning biomass. However, until recently, accurate diagnosis of infection could only be achieved via necropsy. To support A. anguilla fisheries management in the context of A. crassus we developed a rapid, non-lethal, minimally invasive and in-situ DNA-based method to infer the presence of the parasite in the swim bladder

Methods: Screening of 131 wild eels was undertaken between 2017 and 2019 in Ireland and the UK to validate the procedure. DNA extractions and PCR were conducted using both a Qiagen Stool kit at Glasgow University and in situ using Whatman qualitative filter paper No. 1 and a miniPCR DNA Discovery System. Primers were specifically designed to target the cytochrome oxidase mtDNA gene region and in situ extraction and amplification takes approximately 3h for up to 16 individuals

Results: Our in situ diagnostic procedure demonstrated Positive Predictive Values at 96% and Negative Predictive Values at 87% by comparison to necropsy data. Our method could be a valuable tool in the hands of fisheries managers to enable infection control and help protect this iconic but critically endangered species.

Conclusions: Our test offers managers the opportunity to engage in infection control by assessing the disease status of adult eels before allowing transfers between river systems and it represents an important contribution to the conservation and management of this critically endangered species.

Keywords: non-invasive, eDNA, detection, red list IUCN, NEMATODE

P620 / #63

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

EYE FLUKE EFFECTS ON DANISH FRESHWATER FISH: FIELD AND EXPERIMENTAL INVESTIGATIONS

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Introduction: Eye flukes in fish are common in freshwater lakes. Fish become infected by the penetration of cercariae released from freshwater snails, and high infection pressures may be associated with mortalities in a Danish lake.

Methods: Examination of two other freshwater lakes, combined with laboratory study, supported the notion. We investigated 77 freshwater fish from two lakes and the infection level suggested the occurrence of a high cercarial infection pressure in the Danish lakes. Cercariae of the prevalent species Diplostomum pseudospathaceum were used to infect zebrafish Danio rerio for the elucidation of short-term effects on the fish host.

Results: Dominant genera were Tylodelphys and Diplostomum covering a range of species identified by PCR and sequencing of the 18S (partial)-ITS1-5.8S-ITS2-28S (partial) of the rDNA. Zebrafish did not display abnormal behaviour when exposed to 200–400 cercariae, but a dosage of 600 and 1,000 cercariae/fish proved lethal. When fish were exposed to sublethal dosages, 19 out of 27 immune genes were significantly regulated and three genes encoding cytokine (IL 4/13B, IL-6 and IL-8) were upregulated at 3 hr post-infection (hpi), whereas others were downregulated especially at a later time point.

Conclusions: We suggest that direct massive cercarial penetration of fish surfaces may be detrimental and may represent a threat to fish populations.

Disclosure: This work has been published in the "Journal of Fish Diseases" with open access (DOI: 10.1111/jfd.13496).

Keywords: pathogenicity, Diplostomidae, eye fluke, freshwater fish, Immune response

P621 / #152

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

METACESTODES OF ELASMOBRANCH TAPEWORMS IN THE SOUTHERN AFRICAN ENDEMIC INTERTIDAL KLIPFISH, CLINUS SUPERCILIOSUS – DIC, SEM AND MOLECULAR DATA

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Introduction: Endoparasitic tapeworms (Cestoda) infect many ray-finned and bony fishes, with various elasmobranchs as definitive hosts. Research on their diversity, host associations and distribution, specifically in South Africa, are scarce. This study investigates the cestodes infecting Clinus superciliosus from South Africa by using an integrative taxonomy approach.

Methods: Clinus superciliosus (n = 93) obtained from various localities along the South African coast were subjected to a full parasitological examination. Cestodes recovered were fixed and preserved in the field following standard protocols. The cestode stages and identification, to the lowest possible taxonomic level, were determined by differential interference contrast and scanning electron microscopy, as well as sequencing of the partial 28S rDNA gene region.

Results: Based on morphology and sequence data, the two taxa found were identified as Nybelinia sp. (Trypanorhyncha) (prevalence 13%) and Acanthobothrium sp. (Onchoproteocephalidea) (prevalence 19%). These taxa were found in the body cavity and intestine of C. superciliosus, respectively.

Conclusions: This study provides morphological and molecular support for the role of clinid fish as paratenic hosts in the life cycle of elasmobranch cestodes. However, there are limited molecular studies on the cestodes of elasmobranchs in South Africa, and even more so on larval cestodes of teleost fish. Thus, by obtaining molecular data from both larval stages of cestodes, future studies on the life-cycles of these species will be facilitated.

Keywords: Tentacullariidae, Onchobothriidae, Intermediate host, 28S rDNA, Marine parasites

P622 / #977

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

WHAT ABOUT METAZOAN PARASITES IN JUVENILES OF THE STARRY SMOOTH-HOUND MUSTELUS ASTERIAS (CARCHARHINIFORMES TRIAKIDAE), A COASTAL SHARK SPECIES NEAR THREATENED IN EUROPE?

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Introduction: Mustelus asterias is a near threatened species in Europe but commercially exploited and whose parasitofauna is largely unknown. We address this knowledge gap and investigate the potential impact of parasites on host condition and their use as bioindicators.

Methods: We dissected 20 immature sharks (13 males and 7 females) from the English Channel for the search of metazoan parasites. We used two body condition indices, Hepato-Somatic Index and Fulton's K, as proxies of host fitness.

Results: Twelve metazoan taxa were recorded in the whole sampling: one trematode, six cestodes and two nematodes trophically transmitted; one monogenean, one copepod and one myxosporean on gills. All the sharks were parasitized by one to six taxa, with a mean abundance of 30.5 ± 21.4 parasites per fish (myxosporeans not included). The three major taxa were in decreasing order: the nematode Acanthocheilus rotundatus (prevalence: 75%, Confidence Interval 53-89%), the cestode Eutetrarhynchus sp. (70%, CI 48-85%), and the monogenean Erpocotyle laevis (60%, CI 39-78%). The gill copepod Kroyeria lineata and the gut nematode Proleptus obtusus were identified as significant pathogens. Parasite community differed between males and females despite their immature stage, suggesting early spatial sex-segregation, with E. laevis, Eutetrarhynchus sp. and Anthobothrium sp. proposed as biological tags.

Conclusions: Metazoan parasites can induce host fitness loss and give information on diet ecology and stock discrimination. We recommend incorporating parasitology in further research to improve shark conservation and management. This abstract is based on our work accepted for publication on 15 February 2022 in Aquatic Living Resources.

Keywords: triakid shark, host fitness loss, sex discrimination, metazoan parasites, biological tags

P623 / #143

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

ANISAKIS TYPICA (NEMATODA: ANISAKIDAE) IN COMMERCIAL FISHES FROM SOUTH-WEST INDIAN OCEAN: NEW INSIGHTS INTO THE GENETIC DIVERSITY AND INFECTION SITE

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Introduction: Anisakis typica is a nematode parasite of whales widely distributed in temperate and tropical waters worldwide. 3rd-stage larvae of A. typica generally occur around the viscera and sporadically in the flesh of various paratenic fish host species. In this study we investigated the infection sites and genetic diversity of A. typica infecting commercial fishes from the SW Indian Ocean.

Methods: 20 largehead hairtail and 72 brushtooth lizardfish caught in the SW Indian Ocean, were inspected for anisakid nematodes by UV-press. Worms (N=168) were identified by sequence analysis of the ITS rDNA region and cox2 mtDNA gene.

Results: Phylogenetic analyses of the obtained gene sequences indicated the existence of two closely related but distinct phylogenetic lineages forming two subclades. The first subclade comprised one undescribed species indicated as Anisakis sp. 1 along with A. typica var. indonesiensis. The second subclade comprised A. typica, including specimens from its type locality (central Atlantic Ocean). Both genotypes identified in this study were detected in viscera and flesh of both fish hosts.

Conclusions: This study demonstrated the existence of two distinct genotypes of A. typica, probably representing two sibling species. The occurrence of these genotypes in sympatry and syntopy strengthens the hypothesis that they are not conspecific. The ability of both A. typica genotypes to migrate into the flesh may indicate a possible health risk when consuming raw or undercooked infected fish, although human infections with A. typica have not yet been reported.

Keywords: Anisakis typica, South-West Indian Ocean, Epidemiology, Food Safety, genetic diversity

P624 / #343

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

DRIVERS OF K. THYRSITES-INDUCED 'SOFT FLESH' IN NORTHEAST ATLANTIC MACKEREL

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Introduction: Northeast Atlantic (NEA) mackerel (Scomber scombrus) represents an economically important target for the Norwegian pelagic fishing industry. Despite its commercial significance, little is known about the infections with the myxosporean parasite Kudoa thyrsites (Cnidaria, Myxozoa). The parasite infects the skeletal and cardiac muscle of mackerel and heavy infections in the flesh are associated with post-mortem myoliquefaction, commonly known as 'soft flesh'. This condition may reduce the quality of the fish fillet and the marketability of the fish product, resulting in both economic losses to the seafood industry and loss of consumer confidence. In this study, we investigated potential drivers of K. thyrsites-induced 'soft flesh' in NEA mackerel, with special focus on the fish caught during the main Norwegian fishing season in autumn, which represent the economically most valuable target for the Norwegian pelagic fishery industry.

Methods: Fish were examined for 'soft flesh' by texture testing and microscopy 48 hours after thawing. Prior to examination, the fish host body size (weight and length), sex and age were recorded.

Results: Preliminary analyses indicate that the K. thyrsites-induced 'soft flesh' in mackerel is influenced by fish host body size, age and sex.

Conclusions: Preliminary findings support the hypothesis that the probability of a mackerel being infected with K. thyrsites is mainly related to age rather than body size and sex. K. thyrsites infections may accumulate in the fish host over time, and thus older and more infected mackerel may also be at greater risk of developing 'soft flesh'.

Keywords: Kudoa thyrsites, myxozoa, Northeast Atlantic mackerel, 'soft flesh', Drivers

P625 / #1364

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

MORPHOLOGICAL AND MOLECULAR DATA REVEALS THE FIRST RECORD OF A CO-INVADING CHONOPELTIS (BRANCHIURA) IN THE WESTERN CAPE, SOUTH AFRICA

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Introduction: To date, Chonopeltis australis has only been collected in South Africa's Orange-Vaal River system, from Labeo capensis, L. umbratus and juveniles from Labeobarbus aeneus. The only other Chonopeltis species known south of the Orange-Vaal River System is C. minutus, collected from Sedercypris calidus, S. erubescens and Pseudobarbus burgi from the Twee, Tra Tra and Groot Berg Rivers, respectively.

Methods: During fish surveys in the Groot River (Gourits River System, Western Cape, South Africa), branchiuran material was collected from the external surfaces of Pseudobarbus tenuis. An integrated approach using morphological and molecular techniques were used to establish the identity of the specimens.

Results: The specimens collected were morphologically identified as Chonopeltis australis and were 100% similar to C. australis from the Vaal River based on the molecular results of the partial 18S sequences.

Conclusions: This record confirms the translocation and co-invasion of C. australis to a different river system, via the Orange Fish-Sunday River Inter-basin water Transfer scheme, and the occurrence on a new host, the slender redfin P. tenuis. As species of Chonopeltis are known to be host specific, recording one as a co-invader is unique and a first for this genus.

Keywords: freshwater fish parasite, Chonopeltis australis, Branchiura, alien parasite

P626 / #755

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

MORPHOLOGICAL AND MOLECULAR EVIDENCE OF A NEW SPECIES OF GONOCERCA MANTER, 1925 (HEMIUROIDEA: GONOCERCIDAE) FROM THE PACIFIC COD OFF KOREA

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Introduction: Gonocerca is a genus of hemiuroid trematode parasitsing in various marine fishes as definitive host and consists of 14 valid species. The Pacific cod (Gadus macrocephalus) is one of the important commercial fish in Korea. Despite the gadiforms are known as one of the major hosts of Gonocerca, it has never been found in Pacific cod with a wide distribution across the arctic and Pacific oceans. The aim of the present study was focused to find Gonocerca species from the Pacific cod, and to report a putative novel species.

Methods: A total of 35 cod fishes was purchased from a fish market on the southeast coast of Korean peninsula (Pohang and Gadeok) from June 2021 to January 2022. Specimens were characterized both morphologically and molecularly. Total genomic DNA was extracted from 6 isolates, and the partial 28S rDNA sequences were newly-generated. Phylogenetic analysis was carried out under both Bayesian inference and maximum likelihood algorithm based on the available sequences of four Gonocerca spp. (G. phycidis, G. crassa, G. oshoro and G. muraenolepisi) in GenBank to inform on species identification and relationship among Gonocerca spp.

Results: Total 37 individuals were recovered from the host. The phylogenetic analyses indicated our specimens were clearly made a distinct clade with above four Gonocerca species. Gonocerca oshoro found to be closely-related to our specimens, although it showed significant morphological differences. Gonocerca crassa was the most resemble species with our specimens, but G. crassa could be distinguished by smaller seminal vesicle and the presence of genital cone, and molecular evidence.

Conclusions: Integrated molecular and morphological analyses confirmed the presence a distinct species of Gonocerca, a putative new to science.

Keywords: Gonocerca, Gadus macrocephalus, Pacific cod, Gonocercidae, Republic of Korea

P627 / #1365

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

ENTEROGYRUS SPECIES IN THE STOMACH OF CICHLIDS FROM SOUTH AFRICA

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Introduction: Most monogeneans are ectoparasites but Enterogyrus species have adapted to an endoparasitic lifestyle and are found in the stomach of fish. Four species of Cichlidae, Chetia flaviventris, Coptodon rendalli, Oreochromis mossambicus and Tilapia sparrmanii were examined for the presence of stomach monogeneans from four localities situated in different river systems across three provinces of South Africa (Limpopo, Northern Cape and North-West).

Methods: Monogenean parasites found in the stomach mucosa were fixed in ammonium picrate-glycerine on microscope slides and examined and measured using an Olympus BX50 Nomarski Differential Contrast microscope.

Results: Five Enterogyrus spp. were identified from the different hosts. Enterogyrus malmbergi was found from O. mossambicus and T. sparrmanii and represents new host and geographical records. Enterogyrus coronatus was recorded from C. flaviventris, C. rendalii and T. sparrmanii from three localities and all of these represent new host records and contributes to new knowledge on the wide geographic range of E. coronatus within South Africa. Enterogyrus mashegoi and E. cichlidarum are reported from new localities. Tilapia sparrmanii represents new host records for E. cichlidarum and E. multispiralis.

Conclusions: More cichlid hosts from the remainder six provinces should be examined to fully comprehend the geographical range of these endoparasites. This work is based on research supported by the South African Research Chairs Initiative of the Department of Science and Innovation and National Research Foundation of South Africa (Grant No 101054).

Keywords: Oreochromis mossambicus, Tilapia sparrmanii, Chetia flaviventris, Coptodon rendalli, Monogeneans

P628 / #1738

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

ASSESSMENT OF MORPHOLOGICAL MOLECULAR DIVERSITY OF MYXOZOAN PARASITES IN THE GULF OF GABES, IN TUNISIA.

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Introduction: Myxosporeans are abundant and diverse group of parasites. Some species are considered biomarkers of host species amd others are biomarkers of a specific ecosystem environement. Despite the importance of the ecological and economic fisheries sector of the Gulf of Gabes in Southeastern coast of Tunisia, no previous studies on myxosporean infection were performed in this semi-closed system

Methods: We presented our parasitological survey of myxosporean parasites in the Gulf of Gabes for a periode between 2010 and 2021. For this purpose we have examined more than 2200 teleost fishes belonging to 7 genera for myxosporeans infections using morphological and molecular tools.

Results: We reported 11 species of parasites distributed in two orders and six genera: *Ceratomyxa*, *Ellipsomyxa*, *Kudoa*, *Coccomyxa, Sigmomyxa* and *Zschokkella*. Morphological and molecular characterization based on the 18S rDNA sequences allowed as to identify five new species, *C. tunisiensis*, *C. ghannouchensis*, *C. pilchardi*, *E. kalthoumi* and *Z. belonae* and six species already reported in the same host species elsewhere. These are *C. pallida* , *E. mugilis* , *C. truncata* , *Coccomyxa morovi* , *C. belonae* and *S. sphaerica* . The ultra-structural features of some stages of the sporogenesis of *C. tunisiensis* and *K. azevedoi* have been described.

Conclusions: The phylogenetic positions of these myxosporidia has been established and shows high affinity with species reported from American waters. The study of the epidemiological dynamics of the different species of myxosporidia mentioned in this work shows that the prevalence of some species varies according to season and size of host fish.

Keywords: Myxosporea, Mediterranean, Geabes Gulf

P629 / #1744

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

MYXOZOAN BIODIVERSITY ON SAUDI ARABAIAN COASTS

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Introduction: Saudi Arabia is located at the crossroads of Europe, Asia and Africa. The coasts of the Kingdom of Saudi Arabia include in the west the Red Sea, and in the east, the Arabian Gulf. Till 2013, no Myxosporean species have been reported in fishes from Saudi Arabia coasts either in the Arabian Gulf or Red Sea.

Methods: We have explored large number fish species of economic interest for myxosporean infections using morphological and molecular tools based on the 18S rDNA subunit.

Results: 15 new species have been identified belonging to 5 genera. Among them 6 species belong to *Ceratomyxa* genus., and 5 species are of *Kudoa* genus. One *Ortholinea*, one *Auerbachia* and one *Myxobolus* species were also identified. From the Arabian Gulf we have identified five *Ceratomyxa* species, including *C. sultani* from *Upeneus margarethae, C. azevedoi* from *Lutjanus ehrenbergii*, *C. mehlhorni* from *Gnathanodon speciosus*, *C.husseini* from *Cephalopholis hemistiktos* and *C.a hamour* from *Epinephelus coioides*, and one *Kudoa*; *K. quraishii* from the Indian mackerel *Rastrelliger kanagurta*. From the Red Sea, seven neww species including three Kudoa; K. crenimugilis, *K. saudiensis and K. barracudai* from *Crenimugil crenilabis*, *Rastrelliger kanagurta* and *Sphyraena putnama*e respectively, one Ceratomyxa, *C. bohar* from *Lutjanus bohar*, one *Ortholina, O. saudii* from *Siganus rivulatus*, one *Auerbachia, A. maamouni* from *Gnathanodon speciosus* and one *Myxobolus*, *M. allami* from *Sparidentex hasta*. Full morphological and molecular characterization were performed for all these species

Conclusions: Both Red Sea and Arabian Gulf show relative interesting richness in myxozoan infections. Molecular data did not foun any clustering of these species to specific clade based on host species or geographic locality

Keywords: Myxosporea, Arabian Gulf, Red Sea, Saudi Arabia

P630 / #1411

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

MYXASSIGN - A DATABASE FOR THE TAXONOMIC ASSIGNMENT OF MYXOZOA IN METABARCODING DATA CREATED WITH THE EUKREF PIPELINE

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Introduction: The power of metabarcoding, the simultaneous sequencing of all taxa from an environmental sample, is dependent on the correct taxonomic annotation of the resulting data. This identification, however, is dependent on the availability of complete and correct reference databases. Myxozoa is a unique lineage of highly reduced endoparasitic Cnidaria with a complex life cycle. They comprise about 25% of all Cnidaria yet their diversity is not well known, and they are underrepresented in reference databases. The aim of the current study is to create a database for the taxonomic assignment of Myxozoa in metabarcoding data with then intention to gain more knowledge about the myxozoan diversity and their role in microplankton.

Methods: The database was built using the EukRef pipeline, which searches GenBank for SSU sequences starting form an input dataset. Retained sequences (> 500bp) are downloaded including their metadata and curated by updating or correcting the metadata, especially the taxonomy, based on available literature.

Results: The resulting database consists of over 1600 SSU sequences and 800 species of Myxozoa. Compared to the PR2 database, the best available one containing metazoan taxa, this represents and increase of 50% and 30%, respectively, and over 40% of taxonomic assignment in a tested dataset.

Conclusions: This curated database improves the taxonomic annotation of reads in metabarcoding studies and will hopefully increase the annotation accuracy for Myxozoa. These results show that correct and complete databases are crucial for the accurate annotation of metabarcoding data and hope that the inclusion of this database in to the PR2 database will increase the visibility and Myxozoa and our understanding of their diversity and distribution in the future.

Keywords: EukRef, metabarcoding, taxonomic assignment, myxozoa

P631 / #205

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

THE ROLE OF INVASIVE GOBIES FOR TRANSMISSION OF ACANTHOCEPHALANS OF THE GENUS POMPHORHYNCHUS

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Introduction: Ponto-Caspian gobies became highly abundant in many regions outside their native distribution range (e.g. Rhine River system). In newly invaded habitats, the parasite communities of gobies are characterized by a lower species richness compared to their native range. However, acanthocephalans of the genus *Pomphorhynchus* are highly abundant in gobies, in which they do not become mature and mostly remain encapsulated in the abdominal cavity as preadults. Thus, gobiids could either represent a dead-end host leading to a decline of the *Pomphorhynchus* sp. population (dilution effect), or act as a paratenic host increasing the infection rates in local host community (spill back).

Methods: To determine the importance of gobiids for one or the other process mentioned, we conducted two infection experiments using smaller and larger individuals of an appropriate definitive host (chub, *Squalius cephalus*), which were infected with preadults of *P. bosniacus* collected from the abdominal cavity of *Neogobius melanostomus*.

Results: The results showed that preadults can develop and mature in the definitive host with mean recovery rates of 17.9 % in smaller and 27.0 % in larger chubs. Maximum recovery rates were 50.0 % and 70.0 %, while no infection was observed in 38.0 % and 20.0 % for smaller and larger chubs, respectively.

Conclusions: Our study clearly demonstrated that gobies serve as a paratenic host for acanthocephalans of genus *Pomphorhynchus* and thus spill back the infection into the local fish community. However, comparisons with previous experimental studies conducted with cystacanths from intermediate gammarid hosts showed that the preadults have significantly lower recovery rates than cystacanths.

Keywords: Pomphorhynchus sp., spill back, paratenic host, Gobies

P632 / #585

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

COMPARISON OF THE DIET AND PARASITE FAUNA OF THE WHITING AND COD FROM THE SOUTHERN BALTIC SEA

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Introduction: Whiting (Merlangius merlangus) and cod (Gadus morhua) are demersal fish representing Gadidae family. They share similar ecological niche and range of occurrence in the North-East Atlantic. Both species are reported in the southern Baltic Sea catches. The diet and parasite fauna of cod is well known, but there was a lack of basic information about the whiting from this area. The aim of the presented studies was to compare the diet composition and parasite fauna of both species in the Polish EEZ, southern Baltic Sea.

Methods: The samples were collected during scientific cruises in February and April 2016. In total 447 cods and 91 whitings were analysed. The diet composition of fish was determined based on stomach content analysis. All found organisms were identified to the lowest possible taxonomic level on the base of anatomo-morphological features. The parasitological analysis were focused on the presence of nematodes in the liver. Parasites were identified to the genus level based on anatomo-morphological characteristics.

Results: In the diet of cod the dominant fish species were sprat and herring (or Clupeidea), Gobidae; while invertebrates were represented by Crangon crangon and Gammarus sp. The most abundant fish prey eaten by whiting was sprat (found in 60.71% of stomachs) and the only one representative of the invertebrates was Mysis mixta (21.43% of stomachs). The prevalence and intensity of infection with Anisakidae nematodes was higher in cod (40,04 % and up to 268 parasites per fish), than in whiting (16.48% and 1-29).

Conclusions: The diet of cod was more diverse compared with the diet of whiting. The livers of cod were more intensively infected compared with livers of whiting. Greater diversity of the diet may influence the level of infection with parasitic nematodes.

Keywords: whiting, Cod, Baltic

P633 / #1164

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

HYBRID PSEUDOTERRANOVA DECIPIENS X PSEUDOTERRANOVA KRABBEI AMONG ANISAKID NEMATODES IN THE MUSCLE TISSUE OF COD (GADUS MORHUA) FROM THE NORWEGIAN SEA

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Introduction: The Norwegian Sea has been an important and intensively exploited fishing ground of Atlantic cod (Gadus morhua) since many years. However, the occurrence of zoonotic nematodes of the genera Anisakis and Pseudoterranova in cod muscle tissue arouse the food safety and human health concerns. The aim of our studies was to explore the presence, intensity of infection and distribution of the nematodes of the different genera of Anisakidae in the muscle tissue of cod from the Norwegian Sea.

Methods: Samples were collected during commercial surveys in March 2017 in fishing areas FAO IIa1 (n = 50) and FAO IIa2 (n = 56). After ichthyological analysis, the unskinned flesh of each fish was divided into three parts - anterior ventral (belly flaps), dorsal fillet and caudal fillet - and examined using a white-light transilluminator. All parasites found were collected and identified to the genus level and subsample was identified using molecular methods (ITS-1 rDNA).

Results: Higher prevalence of infection with Anisakis than with Pseudoterranova in the musculature of cod from both fishing areas was found. In FAO IIa1, a lower prevalence of infection with Pseudoterranova was recorded (14%) than in FAO IIa2 (~39%), whereas the opposite was found with Anisakis (88% and ~55%, respectively). In FAO IIa2 a hybrid of Pseudoterranova decipiens x Pseudoterranova krabbei was detected.

Conclusions: The two parasite genera were distributed differently in cod muscle tissue: most Anisakis larvae were present in the belly flaps (predominantly the left side), while Pseudoterranova spp. were dispersed with descending frequency in belly flaps, dorsal fillet and caudal fillet. Despite the relatively small sample subjected to genetic analysis, the presence of a hybrid P. decipiens x P. krabbei was detected.

Keywords: anisakis, pseudoterranova, Atlantic cod

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Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

SPRAT AS PRESUMABLE SOURCE OF SALMON (SALMO SALAR) INFECTION WITH CONTRACAECUM OSCULATUM

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Introduction: In the Baltic Sea, the Atlantic salmon (Salmo salar) is on the top of the trophic pyramid. It is also important species for fisheries, willingly chosen by consumers. The occurrence of zoonotic nematodes arouse the food safety and human health concerns. The level of Baltic salmon infection with Anisakidae parasites was unknown. Diet of fish is not only the source of nutrients, but may be also the way of infection with parasites. The aim of our studies was to evaluate the level of Baltic salmon infection with Anisakidae liver nematodes and determine the diet of fish from Polish sea waters.

Methods: Samples have been collected during 2020. Standard ichthyological analyses of 120 fish were performed and livers were frozen for further parasitological investigation. Thawed livers were digested in artificial digestive juice. All parasites were collected and identified on the basis of anatomo-morphological features. A subsample of parasites have been identified using molecular methods. Generalized linear models (GLMs) were applied to analyse the prevalence of cod infection with Contracaecum osculatum with respect to various biological and spatial parameters.

Results: C. osculatum nematode parasites have been detected in 13.33% of investigated fish. The modeled intensity of infection was significantly correlated with the length of the fish. Diet composition was studied on the basis of stomach content analysis. Among food items the most abundant were fish: sprat Sprattus sprattus and three-spined stickleback Gasterosteus aculeatus, while invertebrates were represented only by Mysis mixta.

Conclusions: Baltic Sea sprat have been previously found infected with Contracaecum osculatum, therefor it is probably the main source of salmon infection with that nematode parasite.

Keywords: Baltic Sea, Contracaecum osculatum, Atlantic salmon

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Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

A NEW SPECIES OF TIDDERGASILUS (COPEPODA, ERGASILIDAE) FROM THE GILLS OF ASTYANAX LACUSTRIS (LÜTKEN, 1875) (OSTEICHTHYES, CHARACIDAE) IN BRAZIL

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Introduction: The copepod Family Ergasilidae is one of the most specious parasitic families from the Order Clyclopoida with over 260 species described worldwide. In Brazil, over 70 species from 19 different genera have already been reported/described. Currently, the monotypic genus Tiddergasilus is one of 29 valid genera of Ergasilidae and the records of Tiddergailus iheringi (type-species) are restricted to a single region and fish species in Brazil.

Methods: During the survey of the parasitic fauna of fishes from Pardo River, municipality of Botucatu, São Paulo State, Brazil (22°59'59.76"S; 48°22'37.38"W), we found some ergasilids parasitizing the gills of Astyanax lacustris. Morphological analysis of the copepods indicated that they represent a putative new species of Tiddergasilus, which is herein described. Twenty specimens of A. lacustris were sampled. In laboratory, copepods were carefully removed from gills using fine needles and then stored in vials with 70% ethanol. For morphological identification, copepods were cleared in lactic acid and then mounted (whole or dissected) in Hoyer's medium.

Results: The new copepod was identified as a member of Tiddergasilus by having: antennule 6-segmented, maxillary basis ornamented with multiple spinules, and second and third legs both with 3-segmented endopod. Regarding its congener, the new copepod can be distinguished by the morphology of claw, ornamentation of legs, and by the oligomerization of fourth leg and abdomen.

Conclusions: The present study contributes to improving the knowledge about ergasilids in Brazil through the description of a new species parasitizing the gills of A. lacustris. The authors thanks Fundação de Amparo à Pesquisa do Estado de São Paulo for the finacial support (FAPESP 2019/26831-2).

Keywords: Cyclopoida, Freshwater, Ergasilidae

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Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

IMPORTANCE OF INTEGRATIVE TAXONOMY IN DIFFERENTIATING CRYPTIC DIVERSITY – AN EXAMPLE OF THE TRYPANORHYNCH CESTODE SUBGENUS GRILLOTIA

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Introduction: In recent years, parasite taxonomy has shifted towards more integrative approaches in species identification and classification.

Methods: Recent applications of molecular genetics to identify species has uncovered numerous cryptic parasite species or genetically distinct lineages of morphologically indistinguishable species.

Results: However, genetic data by itself may not be sufficient for confining species, as different methods may produce different results. A combination of modern genetics and traditional morphological methodologies is widely thought of as the most effective approach in taxonomic research. However, even the combination of these methodologies might not reveal unambiguous results. Members of the trypanorhynch subgenus Grillotia are morphologically very similar, only presenting slight deviations in tentacular armature patterns. In addition, molecular sequence data based on ribosomal markers did not reveal genetic variations. This greatly hinders definitive species identifications.

Conclusions: Integrative taxonomy implementing various sources of data (morphology, modern genetics, biogeography, host-parasite associations etc.) might be the solution to differentiate taxa not only within the Grillotia subgenus but the entirety of morphologically similar species and species complexes.

Keywords: trypanorhynch, Cestodes, subgenus Grillotia, Integrative taxonomy, cryptic diversity

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Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

STUDY OF THE PARASITIC FAUNA OF THE SARDINE, SARDINA PILCHARDUS (WALBAUM, 1792) FROM THE PORTUGUESE COAST

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Introduction: In the scope of SARDINHA2020 project, a parasitological study was carried out at 11 points along the Portuguese coast, aiming to 1) identify the parasitic fauna; 2) compare the infection levels in study areas; 3) recognise the parasite main infection sites in the hosts´ tissues and 4) update information on the prevalence of Anisakis larvae as a helpful tool to evaluate the risk of fishery products to human consumers.

Methods: A total of 356 sardines were analysed and parasites were collected from different organs. Peptide muscle digestion of groups of sardines from each sampling area was used.

Results: A total of 15 different parasite taxa have been recorded from Myxozoa, Apicomplexa, Monogenea, Cestoda, Digenea, Nematoda and Acanthocephala.

Conclusions: Species diversity was higher in the Digenea group with seven species and spatial distribution variability and diversity were also highlighted. A notable feature is the residual presence of the zoonotic Anisakis larvae, which will keep fish consumer's confidence. Acknowledgements The authors are grateful to colleagues from the Pelago, Iberas Juvesar/18 and MPDO Campaigns for their assistance to obtain the samples. This research was funded by SARDINHA2020 MAR2020-01-04-02-FEAMP-0009 and SANAQUA MAR-02.05.01-FEAMP-0004 projects through national funds. Thanks also are due to FCT/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/2020+LA/P/0094/2020) and CIIMAR (UIDB/04423/2020 and UIDP/04423/2020).

Keywords: sardine, Sardina pilchardus, Northeast Atlantic, Parasites, anisakis

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Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

ASSESSMENT OF THE PARASITE FAUNA OF BATOID RAYS IN ICELAND: NEW APPROACHES AND FUTURE IMPLICATIONS

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Introduction: Fifteen species of batoid rays occur in Icelandic waters. However, little is known about their parasites. A total of 28 parasite species were recorded from merely three species of skates, corresponding to almost 70 % of the known parasite fauna of Icelandic cartilaginous fishes. Given that only 20 % of skate species have been observed for parasites, a vast majority of parasites remains to be discovered. Investigations on the parasite fauna of the remaining batoid rays are required to rectify the sparse records, build on existing host-parasite data and contribute to the current knowledge of Icelandic marine biodiversity.

Methods: Samples from a broad array of batoid species will be obtained during the Marine and Freshwater Research Institute´s spring and autumn groundfish surveys. The parasite diversity (blood-, ecto-, endoparasites) will be assessed using integrative taxonomy approaches (morphology, molecular systematics, biogeography, ecology).

Results: This project will positively contribute to efforts on the Icelandic biodiversity assessment by increasing the number of known species. The data will reveal insights on host-parasite relationships, processes of diversification, faunal differences and the biogeographical dispersal of hosts and parasites in distinct marine ecoregions in the northern Atlantic Ocean.

Conclusions: This project will enhance our knowledge on marine parasites and the history of co-evolution of marine apex predators and associated parasites in Iceland. The scientific program will provide the baseline for future transnational research collaborations, leading to new foundations in interconnected disciplines and paving the way for future scientific discovery in Iceland and beyond.

Keywords: Chondrichthyan fishes, marine biodiversity, northern Atlantic Ocean, Integrative taxonomy, biogeography

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Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

THE ´HIDDEN´ PARASITE DIVERSITY OF LAMNIFORM AND CARCHARHINIFORM SHARKS IN KWAZULU-NATAL, SOUTH AFRICA

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Introduction: South Africa´s bather protection program was implemented to reduce the risks of unprovoked shark attacks. The KwaZulu-Natal Sharks Board (KZNSB) currently deploys safety gear (gill-nets, baited drumlines) at 37 beaches, thereby trying to minimise environmental impacts. Apart from invaluable biological data on captured marine animals, parasite samples are obtained from a wide array of cartilaginous fishes. Given the paucity of parasitological information available, the aim of this project is to gather parasite data from marine apex predators in South Africa.

Methods: The safety gear of the KZNSB is primarily aimed at large mackerel (Lamniformes) and ground sharks (Carcharhiniformes). While live animals are released, deceased specimens are transported to the KZNSB facilities and frozen prior to animal dissection and scientific research. For parasitological studies, specimens are obtained from internal organs and body surfaces and fixed for subsequent morphological and molecular methods.

Results: Apart from parasitic copepods with almost 100 reported species, information on other dominant parasite groups (i.e. endoparasitic platyhelminths) infecting cartilaginous fishes in South Africa is sparse. The large-scale, collaborative research project will assess the full spectrum of parasitic organisms and reveal the ‘hidden’ parasite fauna of lamniform and carcharhinid sharks in eastern South Africa.

Conclusions: A large proportion of the parasite fauna infecting cartilaginous fishes in South Africa is currently unknown. Future studies on this neglected faunal aspect will not only increase and build upon the largely incomplete knowledge of marine parasites but also expand the number of known species, many of which still awaiting scientific discovery.

Keywords: Cartilaginous fishes, southwestern Indian Ocean, southern Africa, neglected fauna, marine biodiversity

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Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

OCCURRENCE OF JAINUS SPP. (DACTYLOGYRIDAE) IN FISHES FROM UPPER PARANAPANEMA RIVER, SÃO PAULO STATE, BRAZIL WITH THE DESCRIPTION OF TWO NEW SPECIES

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Introduction: Brazil has one of the greatest diversity of wild freshwater fishes with more than 2,000 species. This study aims to describe two new species of Jainus in fishes from the Paranapanema River in Brazil.

Methods: The study was conducted in the Taquari River, Upper Paranapanema River basin, located in the southwest of the State of São Paulo (23º12'17" S; 49º13'19" W). Seventeen fish species were collected and the nasal cavity and gills were examined for monogeneans. The parasites were collected with the aid of a stereomicroscope. Specimens were preserved in 70% alcohol and also prepared in Hoyer, Gray & Wess or Glycerin with Ammonium Picrate (GAP) media for the study of sclerotized structures; and some specimens were stained with Gomori's Trichrome and mounted on permanent slides with Canadian balm for the study of internal organs.

Results: Two new species of Jainus were identified. Jainus sp.1 is similar to J. amazonensis in the morphology of the ventral anchor and both have an accessory piece articulated to the cirrus base in the MCO. However, the copulatory complex formed by two subunits in Jainus sp.1 differs from other congeners. Jainus sp.2 resembles J. leporini and J. piava in having a thin, delicate ventral bar in the shape of a "W" and the tip of the accessory piece in the shape of a "toucan beak". It differs from all other congeners in having a simple vagina and an accessory piece formed by a proximal stem not articulated to the cirrus. Besides, we provide a redescription of Jainus piava.

Conclusions: The present study contributes to reducing the discrepancy in studies on diversity, taxonomy, biogeography and systematics, increasing the known richness of monogeneans in the Upper Rio Paranapanema basin and also in the Neotropical region (Financial Support: Fapesp 2020/05412-9 and CNPq).

Keywords: Taxonomy, Monogenea, New species, Jainus, biodiversity

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Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

DESCRIPTION OF A NEW SPECIES OF CHARACITHECIUM (DACTYLOGYRIDAE) PARASITES OF OLIGOSARCUS PARANENSIS FROM THE UPPER PARANAPANEMA RIVER, SÃO PAULO STATE, BRAZIL

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Introduction: Dactylogyridae is the most abundant family in freshwater fishes in South America. To date, nine species of Characithecium have been recorded in association with characids. This study aims to describe a new Characithecium species in Oligosarcus paranensis from Brazil.

Methods: The study was conducted in the Taquari River, Upper Paranapanema River basin, located in the southwest of the State of São Paulo, Brazil (23º12'17" S; 49º13'19" W). Sixty O. paranensis specimens were collected and examined for monogeneans. The parasites were collected with the aid of a stereomicroscope. Specimens were preserved in 70% alcohol and also prepared in Hoyer, Gray & Wess or Glycerin with Ammonium Picrate (GAP) media for the study of sclerotized structures; and some specimens were stained with Gomori's Trichrome and mounted on permanent slides with Canadian balm for the study of internal organs.

Results: A new species of Characithecium is identified. The new species has a mid-ventral vagina, ventral bar with posteromedial process, and morphology of the male copulatory complex with the cirrus base fused to the accessory piece. The vagina is a tube with clockwise turn and seminal receptacle posterior to the vaginal pore. The posteromedial process of the ventral bar is long. The accessory piece is composed of 2 subunits, dorsal pincer-shaped subunit and ventral rod-shaped subunit with 2 extensions, junction point of the cirrus base with the accessory piece with lateral expansion. These characteristics differ this new species from the more related congeners, C. costaricensis and C. triprolatum.

Conclusions: This study contributes to increasing the Characithecium spp. in the Upper Rio Paranapanema basin and also in the Neotropical region (Financial support: Fapesp 2020/05412-9 and CNPq).

Keywords: New species, Characithecium, Taxonomy, biodiversity

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Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

TOWARDS A MOLECULAR PHYLOGENY OF THE FISH PARASITIC FAMILY CYMOTHOIDAE LEACH, 1818 (CYMOTHOOIDEA: ISOPODA).

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Introduction: Isopods of the Cymothoidae are diverse, remarkably large fish parasites that are known to infect the skin, mouth, and gills of their hosts. To work towards a complete, accurate, and reproducible molecular phylogeny for this family, this presentation aims to: i) use cymothoids from Moreton Bay, Australia and the southern coast of South Africa as a case study to provide best practice guidelines for generating a morphological and molecular cymothoid dataset for future phylogenetic work, and ii) determine the most suitable set of gene fragments for cymothoid species delineation and phylogenies.

Methods: To achieve this, novel sequences of the partial mitochondrial genes cytochrome c oxidase subunit 1 (COI) and 16S, and the partial ribosomal gene 18S were generated for 25 cymothoid taxa.

Results: Phylogenetic relationships for COI and 16S showed a pattern congruent with the morphological classification of the genera Anilocra, Ceratothoa, Cinusa, Cymothoa, Mothocya, and Nerocila, in which congeneric species clustered together in supported clades. However, species of Elthusa did not cluster together in the COI analyses, which corroborates morphological evidence that this genus should be split into at least two. The 18S tree depicted two main clades: one clustering together two freshwater species from Brazil, and the other uniting 12 marine species.

Conclusions: This is the first study to compare the phylogenetic relationships of cymothoid species using the most current taxonomic classifications available.

Keywords: marine fish parasites, phylogenetic relationships, 18S ribosomal gene, 16S mitochondrial gene, cytochrome c oxidase subunit 1

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SILVER CATFISH ACTING AS DEFINITIVE HOST FOR TREMATODE, CESTODE AND NEMATODE WORMS

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Introduction: Schilbe intermedius (Silver catfish) is a freshwater fish species from Schilbeidae and occurs abundantly in most dams and river systems in the Limpopo Province of South Africa.

Methods: Parasitological surveys were conducted between 2009 and 2018 at five different localities within the Limpopo River System. The hosts (n = 485) were collected using gill nets and conventional fishing gear and examined for adult endoparasites. All parasites removed were fixed and preserved according to standard methods for each group.

Results: Five species, belonging to three different parasite groups were collected during this study. A digenean, only recorded from Nwanedi-Luphephe Dam, was unknown to science and described as Emoleptalea nwanedi. Adult cestodes were recovered from five fish during the study. Schyzocotyle acheilognathi was recorded from a single host from respectively Loskop Dam and Flag Boshielo Dam, having one worm each. Kirstenella gordoni was recorded from two hosts at Flag Boshielo Dam with each host infested by respectively two and three specimens of this parasite and one host from Phalaborwa Barrage infested by one individual of this species. The adult nematode, Paracamallanus cyathopharynx was present at all localities and Procamallanus laeviconchus was only recorded from Phalaborwa Barrage.

Conclusions: Besides the trematode E. nwanedi described as a new species, the alien cestode S. acheilognathi and K. gordoni are first records for this South African host and the latter, the first record from South Africa. Paracamallanus cyathopharynx was documented for S. intermedius before, but records were limited to the Kruger National Park and this is the first record of P. laeviconchus from this host in South Africa.

Keywords: Fish, Helminths, endoparasite, South Africa

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Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

PARASITE COMMUNITIES IN THE SO-IUY MULLET, PLANILIZA HAEMATOCHEILUS (TEMMINCK & SCHLEGEL, 1845) (TELEOSTEI: MUGILIDAE), FROM THE WESTERN NORTH PACIFIC

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Introduction: Parasites are recognised as important mediators in biological invasions.The so-iuy mullet, Planiliza haematocheilus (Temminck & Schlegel, 1845), is a native to the Western North Pacific and introduced in the Black Sea and Sea of Azov. The establishment success and expansion of the so-iuy mullet has been linked with a sharp decline and replacement of the native mugilid species. We carried out a pilot study assessing the diversity and composition of metazoan parasite communities of the so-iuy mullet at its native southernmost distributional range.

Methods: A total of 30 P. haematocheilus was sampled at the Korea Strait off Busan in January 2022. All metazoan parasites recovered were characterized morphologically and molecularly. Partial sequences of both, the mitochondrial cox1 gene and 28S rRNA were used for molecular identification of representative isolates.

Results: Combined molecular and morphological analyses revealed a parasite fauna dominated by heteroxenous species. We recovered a total of 12 macroparasites; of these, 6 were digeneans, 2 acanthocephalans, 1 nematode, and 1 parasitic arthropod. We recovered lower species richness in comparison with both, the northern regions of the native distributional range of the so-iuy mullet, and across its introduced range in the Eastern Mediterranean.

Conclusions: This is the first known assessment of the metazoan parasites in P. haematocheilus from its southernmost natural distributional range in the Western North Pacific. Our study provides important information that will help: (i) construct a lage-scale framework on host-parasite dynamics across times and geographical scales, and (ii) address further broad questions on the role of parasites in biological invasions.

Keywords: Northwest Pacific, Korea Strait, parasite communities, so-iuy mullet, Planiliza haematocheilus

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PREVALENCE AND PHYLOGENETIC AFFILIATION OF ICHTHYOPHONUS SP. IN ATLANTIC MACKEREL, SCOMBER SCOMBRUS

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Introduction: Ichthyophonus spp. are cosmopolitan parasites causing proliferative, systemic disease in marine and freshwater fish. At present, two species have been formally described, Ichthyophonus hoferi, and I. irregularis. However, genetic studies suggest a far greater species diversity within the genus. Northeast Atlantic (NEA) mackerel are known to be susceptible to Ichthyophonus sp. infections, but the prevalence has not been extensively monitored. The present study details observations of Ichthyophonus sp. infections in NEA mackerel from the North Atlantic and explores the phylogenetic relationship with Ichthyophonus spp. from other fish hosts.

Methods: Mackerel were sampled during research cruises and from commercial catches in the North-, Norwegian and Greenland Seas in 2019, 2020 and 2021. Visceral organs were examined macroscopically for visible signs of granulomatous infections, and sub-samples of fish were examined microscopically for the presence of resting spores or hyphae. Additionally, the parasite's 18S rRNA and 28S rRNA gene sequences were comparatively analyzed.

Results: Macroscopic and microscopic observations indicated 32-100 % prevalence of Ichthyophonus sp. in individual mackerel shoals. Histological examination, tissue transplant cultures and hyphal growth pattern, along with phylogenetic analysis of the obtained gene sequence, suggest that Ichthyophonus sp. infecting NEA mackerel is distinct from I. hoferi infecting herring.

Conclusions: The prevalence of Ichthyophonus in NEA mackerel is high, and morphological examinations and genetic analyses indicates that it may be a novel species.

Keywords: histology, ichthyophonus, mackerel, Prevalence, phylogeny

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Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

LONG REPRODUCTIVE PERIOD MAY SUPPORT EXPANSION OF NON-NATIVE PARASITIC COPEPOD NEOERGASILUS JAPONICUS IN EUROPEAN WATER BODIES

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Introduction: of free-living species often lead to co-introduction of their parasites. While some parasites remain to infect their original hosts, other may switch to local host species. Consequent effects of these new host-parasite interactions vary in dependence on the host competence to the new parasite. Neoergasilus japonicus (Copepoda) is an Asian fish parasitic copepod, currently spreading in Europe and North America, exhibiting high plasticity in host preference.

Methods: During the period between March - November 2021, we compared prevalence, abundance and maturity status of the three copepod parasites, including two native (Ergasilus sieboldi, Paraergasilus longidigitus) and one non-native (N. japonicus) species.

Results: All three species preferred different part of the fish host body, limiting the space competition between the parasites. Fish nasal cavity parasitizing P. longidigitus showed one clear peak with highest prevalence in March-April and occurrence of gravid females between April-June, while no parasites were observed between July-September. Gill preferring parasite E. sieboldi was found in almost all months, with overall prevalence up to 50% found in June and significant occurrence of gravid females found between March-July. N. japonicus infected fish fins continuously in all months with prevalence ranging between 18-64%, showing the highest abundance among the copepod species. High number of gravid females was observed between April and September.

Conclusions: Our data show that non-native parasite predominates in community of parasitic copepod in the locality, possibly as a result of long reproductive period compared to related native species.

Keywords: Neoergasilus japonicus, non-native Copepod, Host-parasite interactions

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ARE SEA LICE INFESTATIONS LINKED TO ENDOPARASITE BIOMASS IN ATLANTIC SALMON?

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Introduction: Co-infections involving multiple parasite species are common, with co-infecting parasites capable of modifying the burdens of one another. Atlantic salmon parasite research is heavily focused on parasitic copepods acquired during sea migrations; Lepeophtheirus salmonis and Caligus elongatus. However, juvenile salmon acquire freshwater parasites during their first 2-3 years of life before migrating to sea. The present study investigated the potential influence of endoparasite biomass on the number of sea lice acquired by Atlantic salmon.

Methods: Parasite communities were assessed from recently migrated Atlantic salmon of three Norwegian fjords, from which the potential influence of endoparasite biomass, host length and condition on sea lice infestations was evaluated.

Results: Atlantic salmon were infected by 5 – 10 endoparasite taxa of freshwater origin, and 77-90% of the fish were infected with sea lice with abundance ranging from 1.97-11.47 lice/fish. Sea lice infestations were positively influenced by endoparasite biomass and host length in two out of three fjords. Sea lice infestations were also modified by the combined influences of endoparasite biomass, host length and condition.

Conclusions: Our study indicates that endoparasite biomass may influence sea lice infestations on Atlantic salmon. Furthermore, we highlight the importance of considering co-infecting parasites when evaluating sea lice infestation dynamics.

Keywords: Atlantic salmon, co-infection, Sea lice, Norway

P648 / #159

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

DIGENEAN DIVERSITY OF THE MARINE FISH HOST DIPLODUS CAPENSIS (SPARIDAE) FROM SOUTH AFRICA

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Introduction: The Cape white seabream, Diplodus capensis (Smith, 1844), is endemic to the coast of southern Africa. Although D. capensis is a common shore angling fish, its parasite fauna remains unknown. In fact, the number of currently known parasites from this biodiversity-rich coastline is believed to be merely a fraction of the true parasite community. Thus, our study focused on exploring the diversity of digenean trematodes of this fish species from a large portion of its distribution.

Methods: We collected a total of 39 D. capensis from four sites located within the Eastern and Western Cape provinces in South Africa: Chintsa, Tsitsikamma National Park, De Hoop Nature Reserve and Witsand.

Results: A total of 87% of these fish were infected with digenean trematodes. Morphological and molecular analyses based on the 28S, ITS and COI genes/regions revealed that D. capensis serves as intermediate and definitive host for nine species of digenean trematodes from six families: Acanthocolpidae Lühe, 1906, Bucephalidae Poche, 1907, Fellodistomidae Nicoll, 1909, Lecithasteridae Odhner, 1905, Opecoelidae Ozaki, 1925 and Zoogonidae Odhner, 1902.

Conclusions: Not only are these novel host records for the nine digenean species, but many of these species are likely new to science. We emphasise the further need for the use of a combined approach incorporating both morphological and molecular methods, which promise more reliable estimates of digenean diversity in marine fishes of the unique coastal areas in South Africa.

Keywords: Marine, Genetics, morphology, Parasitology, Digenea

P649 / #214

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

CHARACTERISING LERNAEOPODID COPEPODS OF THE MARINE FISHES DIPLODUS CAPENSIS (SMITH, 1844) AND RHABDOSARGUS GLOBICEPS (VALENCIENNES, 1830) FROM SOUTH AFRICA

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Introduction: Marine parasites of the subclass Copepoda have not been extensively studied along the South African coast, and molecular data of these organisms are extremely scarce. This is also true for the copepod family Lernaeopodidae. Various species from this family found along the South African coast, have undergone taxonomic reorganisation in the past. Thus, this study aimed to explore the diversity of parasitic copepods hosted by two marine fishes of the family Sparidae Rafinesque, 1818: Diplodus capensis (Smith, 1844) and Rhabdosargus globiceps (Valenciennes, 1830); and to employ an integrative taxonomic approach to identify these parasitic organisms.

Methods: Fish were collected from five localities within the Eastern and Western Cape provinces in South Africa: Chintsa, Tsitsikamma National Park, De Hoop Nature Reserve, Witsand and Langebaan. These fishes were found to host what initially looked like two copepod species – one found on the gill arch and the other found on the gill filament.

Results: Genetic analyses of the COI and 18S genes revealed that the species found on the gill filaments, although sharing morphological resemblances, were unique for each fish species. Genetic characterisation of specimens found on the gill arches is still ongoing; however preliminary morphological results suggest that they are a single species. Thus these fishes are hosts to a minimum of three lernaeopodid species on the gills.

Conclusions: This study thus provides valuable molecular data on parasitic copepod species. Furthermore, it highlights the lack and necessity for molecular data of these parasites and the need for further explorative studies that employ an integrative taxonomic approach, by using both molecular and morphological identification techniques.

Keywords: Parasitology, Marine, Characterization, Copepoda, Lernaeopodidae

P650 / #483

Topic: *AS04 Parasites of fish / AS04.5 Parasites of wild fish populations*

DESCRIPTION OF HENNEGUYA SP., A GILL PARASITE OF THE PSALIDODON BOCKMANNI, BASED ON MORPHOLOGICAL AND MOLECULAR EVIDENCE

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Introduction: Myxozoans are mandatory parasitic metazoans that diverged from their free-living cnidarian ancestors. Despite its potential to cause economic impacts on some species, there is still much to be explored on the life cycle, the great diversity of species and evolution. In this study, Henneguya sp. parasitizing the gills of Psalidodon bockmanni, a characiform fish, was reported based on the myxospore morphology and ssrDNA sequence.

Methods: Fifty specimens of P. bockmanni were collected from October to December 2020 in the Pardo River, Brazil. All organs of the fish were analyzed. Some plasmodia found were collected and freshly examined between slide and coverslip, photographed and measured, while other plasmodia were separated for molecular analysis. Phylogenetic analysis was used to compare the new Henneguya species with genetically similar species.

Results: Plasmodia were observed in the gills with a 14% prevalence. Myxospores measuring: 12.4 ± 0.9 µm in length, 7.2 ± 0.4 µm in tail length, 19.7 ± 0.9 µm in total length, 5.4 ± 0.3 µm in width, 4.4 ± 0.4 µm in thickness. Two polar capsules measuring: 4.7 ± 0.3 µm in length and 1.5 ± 0.2 µm in wide. A sequence of the ssrDNA gene from Henneguya sp. of 1930-bp was obtained. The species that most resembled Henneguya sp. was Myxobolus ovarium. Phylogenetic analysis showed that Henneguya sp. groups in a clade formed by species that parasitize Characiformes from Brazil. These data supported the diagnosis of the parasites as distinct and novel species.

Conclusions: Using molecular and morphological characterization, this species was identified as a putative new species of the genus Henneguya. The study contributes to the knowledge of myxozoan biodiversity in Brazil. Henneguya sp. is the first myxozoan reported parasitizing P. bockmanni.
"The authors thank FAPESP for funding (Process 2019/19060-0)."

Keywords: biodiversity, Brazil, Pardo River, phylogeny, Taxonomy

P651 / #1606

Topic: *AS04 Parasites of fish / AS04.6 Other studies related to parasites of fish*

FISH PARASITES IN THE AUSTRALIAN HELMINTHOLOGICAL COLLECTION AT THE SOUTH AUSTRALIAN MUSEUM

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Introduction: The renowned Australian Helminthological Collection (*AHC*) at the South Australian Museum in Adelaide, Australia is a resource available to researchers and students worldwide who are studying fish parasites. It is a highly active collection with ~800 new registrations/year and many loans of material provided annually to external clients.

Methods: The collection, which contains specimens preserved in ethanol and on microscope slides, is stored in a well-managed purpose-built facility. Specimen and host data is carefully entered into the Museum's Collections' Management database EMu. This data is publicly available for reference.

Results: There are currently ~45,500 items in *AHC* including 8000 helminth (Acanthocephala, Cestoda, Digenea, Monogenea, Nematoda and Trematoda) items, from host species of Actinopteri and Elasmobranchii. Some of this fish parasite material is only identified to Order or higher and therefore it is likely that we hold new species yet to be described. While many of our parasite items are from hosts collected in Australian waters, we also have material from a diversity fish collected from 48 other countries.

Conclusions: The *AHC* is a secure and well-managed depository for type and voucher specimens. If you wish to borrow from or donate specimens to this prestigious collection please contact the Senior Collection Manager: Dr Leslie Chisholm, Science Centre, South Australian Museum, North Terrace, Adelaide 5000, South Australia (leslie.chisholm@samuseum.sa.gov.au).

Keywords: Australian Helminthological Collection, Fish parasites, Diversity

P652 / #993

Topic: *AS04 Parasites of fish / AS04.6 Other studies related to parasites of fish*

CO-INFECTION OF THE MARINE TEREBELLID ENOPLOBRANCHUS SANGUINEUS BY THE BLOOD FLUKE, CARDICOLA LANGELI, AND A MYXOZOAN, SINUOLINEA SP.

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Introduction: Enoplobranchus sanguineus is a marine terebellid polychaete known to be an intermediate host for several aporocotylid digeneans (blood flukes) in South Carolina, USA. Herein we report a case of co-infection in the hemocoel of a specimen of E. sanguineus by the aporocotylid Cardicola langeli and myxozoan actinospores identified as Sinuolinea sp

Methods: Identification of the host was verified using COI mtDNA sequencing. Infection by the aporocotylid was detected molecularly via partial 28S rRNA gene sequencing and by the myxozoan via partial 18S rRNA gene sequencing.

Results: The aporocotylid was identified as Cardicola langeli; no sporocysts or cercariae were found, indicating a very recent infection of the annelid. The species C. langeli infects the cardiovascular system of the sheepshead Archosargus probatocephalus and, to our knowledge, has thus far only been reported from the Gulf of Mexico. Pansporocysts of the myxozoan contained immature or mature actinospores that were determined to be a new saccimyxon. Partial 18S rRNA gene sequence was 89% similar to Sinuolinea capsularis.

Conclusions: Our finding indicates the presence of Cardicola langeli on the South Carolina coast (western North Atlantic), and allows the reconstruction of its life cycle. This is the second record of a myxozoan infection in a marine polychaete in North America.

Keywords: actinospore, saccimyxon, polychaete, Aporocotylidae, life cycle

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Topic: *AS04 Parasites of fish / AS04.6 Other studies related to parasites of fish*

DE NOVO GENOME AND TRANSCRIPTOME SEQUENCING PROJECT OF MYXOBOLUS PSEUDODISPAR (CNIDARIA, MYXOZOA), A COMMON MYXOZOAN OF CYPRINIDS

Edit Eszterbauer[1], Rashmi Sukumaran[2], László Orbán[3], Gyula Szabó[1]
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Introduction: Most of the microscopic myxozoan species (Cnidaria, Myxozoa) infect vertebrate hosts without clinical signs, however, some species cause typical symptoms and significant mortality among fish. The whole genome of low virulence myxozoan species has not been available up to now. To be able to perform virulence-related genomic comparisons, we aimed to obtain the de novo whole genome assembly of the common, low virulence, myxozoan parasite of cyprinids, Myxobolus pseudodispar.

Methods: DNA sequencing was performed with single-molecule real-time (SMRT) technology on PacBio Sequel new generation sequencing system. Besides, Illumina paired-end sequencing was performed using Truseq Nano DNA Prep Kit (insert size 550 bp) on Illumina Novaseq 6000 instrument. To aid genome annotation, the transcriptomes of three parasite developmental stages were sequenced: the parasite's early stages in fish, sporogonic stages in fish, and sporogonic stages in annelid hosts.

Results: The descriptive statistics indicate reliable and good quality assembly (e.g. No. of contigs: 4677, N50: 37218; largest contig: 368003 bp). The estimated genome size is approximately 143 Mb. It is one of the largest genome size among myxozoan parasite (the largest one detected so far is 170 Mb, whereas the smallest is around 22 Mb). The GC content is rather low (~30%), similar to other members of this parasite group.

Conclusions: The genome characteristics and gene distribution analysis is in progress, and virulence-related gene orthogroups are to be identified. The study was funded by the National Research, Development and Innovation Office, Hungary (Grant No. NN140345).

Keywords: fish parasite, genome assembly, PacBio, Illumina, RNAseq

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Topic: *AS04 Parasites of fish / AS04.6 Other studies related to parasites of fish*

TRACING THE STAGE-SPECIFIC PARASITIC MECHANISMS OF SPHAEROSPORA MOLNARI (MYXOZOA, CNIDARIA) IN COMMON CARP

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Introduction: Sphaerospora molnari is a myxozoan parasite infecting common carp in central Europe. It develops mature spores in the gills of fish and causing respiratory damage. Before reaching the gills of the host, S. molnari undergoes presporogonic development in the blood and liver of the host. We used S. molnari as a model to understand genetic mechanisms that parasite uses to successfully invade and proliferate within the host.

Methods: The blood, gills, and liver of infected fish were sampled and sequenced for transcriptomic analyses. Parasite reads were host-filtered using their respective reference genomes. We studied the differentially expressed genes in each life-cycle stage/tissue using the statistical analysis method DESeq2 and functional enrichment analysis.

Results: While the most common functional gene groups in the gill stages were related to cellular differentiation and cytoskeletal rearrangement, blood and liver stages gene groups were related to parasite feeding and immune evasion strategies. We identified homologs of these genes in other parasitic organisms (e.g., Plasmodium, Giardia, Trypanosoma), that are essential for their successful survival in their hosts, and proposed a list of “pathogenicity-related” gene families.

Conclusions: We have uncovered genes that are critical for each developmental stage of S. molnari, suggesting potential candidates for disease control in myxozoans. Acknowledgments: Czech Science Foundation (20-30321Y, 19-28399X).

Keywords: Transcriptomics, parasitic strategies, myxozoa, common carp

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Topic: *AS04 Parasites of fish / AS04.6 Other studies related to parasites of fish*

RNA INTERFERENCE IN „MICRO-JELLYFISH" PARASITE SPHAEROSPORA MOLNARI (CNIDARIA: MYXOZOA)

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Introduction: RNA interference (RNAi) represents an effective approach to suppress gene expression and monitor gene regulation in organisms. Despite the wide application of RNAi in free-living cnidarians, research in endoparasitic cnidarians, Myxozoa, is limited.

Methods: We introduced the RNAi method by soaking dsRNA and successfully suppressed gene expression using in vitro cultures of the common carp infecting Sphaerospora molnari. We selected two S. molnari genes for actin and produced dsRNA. We applied the soaking strategy of dsRNA to induce RNAi silencing using different concentrations and incubation times. The downregulation of gene expression after silencing was measured by real-time PCR. We also tested the cell entry of dsRNA by labelling dsRNA and performed confocal microscopy.

Results: We established an optimal workflow for the generation of dsRNA and induction of RNAi interference in vitro myxozoan culture of Sphaerospora molnari. We observed a relatively rapid suppression of gene expression after 30 minutes lasting up to 8 hours of 80% and 50% over the next 24/48 hours. Despite testing two genes, we successfully silenced the gene expression of only one gene. Immunolabeling of dsRNA revealed the ubiquity of dsRNA in all endogenously produced cells within the cell-in-cell multicellular blood stages of S. molnari.

Conclusions: The soaking approach of dsRNA entry to the cell is a low-cost, effective, and constitutive method. In addition to the beneficial advances in functional research in Myxozoa, this method opens new venues for studying either potential therapeutic targets or for drug discovery in these economically important fish parasites. This work was supported by the Czech Science Foundation [20-30321Y to A. Kosakyan, 19–28399X to A. Holzer].

Keywords: myxozoa, RNA interference, Sphaerospora molnari

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Topic: *AS04 Parasites of fish / AS04.6 Other studies related to parasites of fish*

BEYOND COMPLEXITY TO REDUCTION: NOTCH SIGNALLING PATHWAY IN PARASITIC CNIDARIANS, MYXOZOA

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Introduction: Notch signalling is a conserved ancient mechanism of metazoan origin, having a crucial role in cell fate, growth, and division. Given the role in neurogenesis, stinging cell maturation and tentacle development of Notch in free-living cnidarians, the function in highly reduced cnidarians, Myxozoa, is unexplored. Encouraging this, we aimed to understand the context and role of Notch signalling in such modified organisms.

Methods: To track the core components of Notch signalling, we performed a genome-wide survey of Notch pathway genes in myxozoan and free-living cnidarians. By ascertaining the role of the Notch pathway, we performed inhibition/activation assays of proteolytic complex (γ-secretase) and Notch receptor, using two myxozoan species (Sphaerospora molnari, Myxidium lieberkuehni). The assays were evaluated by gene expression of primary Notch targets or stinging cell-related minicollagen. We also performed immunolabelling of the Notch receptor in S. molnari proliferative stages.

Results: We revealed a substantial reduction of the Notch pathway genes in Myxozoa, as a unique characteristic in multicellular organisms. Myxozoa lack some of the core components of Notch signalling, including nuclear coactivators/corepressors, primary targets (HES, HEY genes), etc. Nevertheless, the inhibition/activation assays showed downregulation of putative primary targets in Myxozoa. By confocal microscopy we detected the Notch receptor in primary cells of S. molnari cell-in-cell life stages.

Conclusions: The study provides interesting insight into reductions of the complex Notch pathway in Myxozoans and sheds light on Notch signalling during early metazoan evolution. This study was funded by the Czech Science Foundation (19-25536Y).

Keywords: Notch signalling pathway, Function, myxozoa, reduction, loss

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Topic: *AS04 Parasites of fish / AS04.6 Other studies related to parasites of fish*

PARVATREMA SP. (DIGENEA: GYMNOPHALLIDAE) IN NATURAL POPULATION OF MYTILUS GALLOPROVINCIALIS FROM NORTH WESTERN ADRIATIC COAST

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Introduction: Parvatrema (Gymnophallidae) is a trematode infecting waterfowl at their adult stage and several species of clams and mussels in larval stages. Mytilus galloprovincialis is a widespread bivalve in the Adriatic Sea, both in natural and farmed populations. Recently, Parvatrema sp. was detected in M. galloprovincialis in a lagoon of Croatia and its presence was associated with the decline of the host population.

Methods: After the presence of visible pearls was reported in a natural population of M. galloprovincialis along the Northwestern coast of the Adriatic Sea, samples were collected in seven localities between June 2020-September 2021 to evaluate the etiology of the finding. Parasitological investigations, including morphological and molecular analyses, and histopathology were carried out on infected mussels.

Results: Metacercariae of the genus Parvatrema were morphologically identified in 75.3% of the examined specimens (119/158) with an interval of prevalence 0-100% among localities. The intensity of infection ranged from 1 to 3700 parasites/mussel and involved mostly the mantle, with parasites surrounded by a variable numbers of conchiolin layers. The pairwise comparison of the sequenced ITS region, revealed a 96.8% identity of our isolate with Parvatrema duboisi. The phylogenetic analysis demonstrated the independent clustering of the obtained sequences compared to other available Parvatrema species.

Conclusions: In addition to be a disease affecting mussels health, the presence of pearls implies a loss in the commercial value of the infected mussels. Several issues, such as identification of the involved parasite species, ecological and epidemiological aspects still need to be further investigated.

Keywords: metacercaria, Parvatrema, Mytilus galloprovincialis, Mediterranean sea, pearl

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Topic: *AS04 Parasites of fish / AS04.6 Other studies related to parasites of fish*

INFECTION DYNAMICS AND METAL ACCUMULATION EFFICIENCY OF ENDEMIC ACANTHOCEPHALAN DENTRITRUNCUS TRUTTAE IN THE KRKA RIVER, CROATIA

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Introduction: Dentritruncus truttae is an acanthocephalan species endemic to limited areas in Croatia, Italy, and Bosnia and Herzegovina. Since these parasites are characterised as effective metal accumulators, we estimated for the first time application of D. truttae as bioindicator of metal exposure. Therefore, metal distribution, seasonal variation and accumulation were compared in D. truttae and its host Salmo trutta from the Krka River in order to evaluate their sensitivity and indications of metal exposure in the moderately contaminated watercourse.

Methods: Sampling was conducted in autumn 2015, spring 2016 and autumn and spring 2021 at Krka River source (KRS) as reference site, and location near the Town of Knin (KRK) impacted by industrial and municipal wastewaters. Molecular analysis confirmed species D. truttae and its taxonomic classification in the family Illiosentidae. Metals were measured by HR ICP-MS in acid-digested acanthocephalans and fish intestine, and their ratio was used to calculate bioconcentration factors (BCFs = C[parasite]/C[host intestine]), as possible indication of exposure duration.

Results: Parasite prevalence ranged 92-100% at KRS and 80-97% at KRK. Fish and acanthocephalans showed higher levels of most elements at KRK compared to KRS, while metal accumulation was more efficient in acanthocephalans than fish. That is especially valid for Cd, Cu, Mn, Pb or Sr, resulting in higher BCFs and therefore, reflecting their higher input into Krka River watercourse in recent times.

Conclusions: D. truttae showed more effective metal accumulation than fish and even significant differences in metal accumulation between two sites, confirming this parasite as a promising bioindicator species sensitive even to low environmental metal concentrations.

Keywords: Intestinal Parasites, brown trout, trace elements, bioconcentration factors

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Topic: *AS04 Parasites of fish / AS04.6 Other studies related to parasites of fish*

"HAECKELIZE", DIGITAL CLASSICAL SCIENTIFIC DRAWINGS

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Introduction: During the last third of the 20th century new morphological features are included in parasite descriptions. Previously unobserved aspects of the external and internal anatomy have also been described, thanks to SEM, TEM and improvements in light microscopy (such as DIC or confocal). Higher resolution has allowed descriptions to be increasingly detailed, with photographs and videos completing the information provided by museum type specimens. At the same time that the technological improvement happened, researchers began to use "software" for the representation of new species, instead of the conventional ink drawings. Sometimes digital drawings are simplistic, using simple geometric figures that ignore the real profiles and do not provide correct 3D information on the structures and organs and their arrangement. Volume is often represented by digital automated gradients, or drawing the contours with digital scatter brushes.

Methods: This study presents "Haeckelize", a method designed to make the scientific drawing of microorganisms accessible, using protocols adaptable to different types of software (from PowerPoint® to specialized program packages).

Results: These protocols allow automated drawing of contours, volumes and transparencies in a realistic way, which is necessary to adequately represent the specimens.

Conclusions: Despite the "automation" of these computerized drawings, the realism or the correct schematization of these drawings continues to depend on the ability of the authors, even on their artistic concerns. Projects: MINECO/FEDER PID2019-110730RB-I00 co-funded by MCIN/AEI/10.13039/501100011033 by "ERDF A way of making Europe" by the EU; AICO/2021/279; and GVA-THINKINAZUL/2021/029 (with NextGenerationEU funds).

Keywords: Taxonomy, Digital drawing, Scientific represntation, New protocol

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Topic: *AS04 Parasites of fish / AS04.6 Other studies related to parasites of fish*

POTENTIAL OF USING CONFOCAL LASER SCANNING MICROSCOPY FOR TAXONOMICAL INVESTIGATIONS ON THE EXAMPLE OF TRYPANORHYNCHA (CESTODA) FROM BALINESE WATERS

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Introduction: The parasite fauna of *Neotrygon caeruliopunctata*, *Rhinobatos jimbaranensis* and *R. penggali* from Bali was studied (Neitemeier-Duventester et al. 2022 a, b). Light microscopy (LM) and scanning electrode microscopy (SEM) are the standardized methods for the examination of fish parasites. During these studies the Confocal Laser Scanning Microscope (CLSM) was also used.

Methods: The parsites of the three ray species were examined with a LM, for this they were stained with Mayer-Schuberg's acetic carmine staining and mounted in Canada balsam. Furthermore, samples were examined with the SEM. In addition, the samples prepared for the LM were examined with a CLSM.

Results: Within the presented study 9 species of Trypanorhyncha were found yet. During the study of Trypanorhyncha, all three microscopic techniques were used. Especially the hook patterns of the Trypanorhyncha could be shown in higher detail and it was also possible to visualize the bothrial pits.

Conclusions: The use of the CLSM allows detailed studies of the morphological characteristics of flatworms. It is possible to use the samples prepared for the LM for the CLSM without any further preparation. Through the 3-D representation of the features, a precise description and identification is possible. The CLSM offers the advantage of visualizing several planes at the same time and creating 3-D images. Another advantage of the CLSM is the ability to visualize internal structures, while the SEM can only visualize the surface ultra-structures. In conclusion, the use of CLSM is a complementation to existing microscopy techniques but should not replace them. In addition, it can establish itself as a standard technique for morphological examinations.

Keywords: Neotrygon caeruliopunctata, Rhinobatos jimbaranensis, R. penggali, Mayer-Schuberg's acetic carmine

P661 / #1106

Topic: *AS04 Parasites of fish / AS04.6 Other studies related to parasites of fish*

RELATIONSHIP BETWEEN PRESENCE OF PARASITES, HISTOPATHOLOGY IN GILLS AND MUSCLE MELANISATION OF SAND FLATHEAD FROM DECEITFUL COVE IN THE TAMAR ESTUARY (TASMANIA, AUSTRALIA)

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Introduction: Muscle melanisation is the presence of dark pigments in skeletal muscle of the fish which can have a range of causes including infections, a side effect of vaccination or excessive metal concentrations. Muscle melanisation has been reported in farmed and wild fish, including wild southern sand flathead, Platycephalus bassensis, which is popular with Tasmanian recreational fishers. However, in the case of sand flathead, the cause of muscle melanisation is unclear.

Methods: In this study, sand flathead were collected from Deceitful Cove in the Tamar Estuary, Tasmania, Australia where the fish had higher proportion of gross muscle melanisation compared to other sites within the Tamar Estuary. The fish were scored from 0-3 for melanisation based on the percentage surface areas of melanised muscle. Gill samples were fixed, processed, paraffin-embedded, sectioned at 4 µm, and stained with haematoxylin and eosin (H&E) for histological assessment.

Results: Monogenean gill flukes were found in gill filaments of all fish. The number of monogeneans in gills of melanised fish was significantly higher than in non-melanised fish. Histopathological investigations showed that the severity score of gill telangiectasia was significantly greater in the highest score melanised fish. The total number of gill mucous cells/interlamellar unit (ILU) was significantly greater in the melanised fish than those in non-melanised fish. The number of monogeneans, mucous cells/ILU, and melanomacrophage centres in the gill sections were positively correlated with percentage areas of melanised muscle.

Conclusions: Overall, the results provide further insight into the relationship between parasites and histopathology in gills and areas of blackened muscle underpinning muscle melanisation.

Keywords: gill histology, Tasmanian flathead, gill mucous cells, melanisation in muscle, Monogeneans

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Topic: *AS04 Parasites of fish / AS04.6 Other studies related to parasites of fish*

CHARACTERIZING EARLY ENCYSTMENT IN SPIRONUCLEUS: NOVEL TECHNIQUES, A NEW STRUCTURE, STAGING FOR TESTING TREATMENTS

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Introduction: Testing novel life-cycle interrupting strategies, including those targeting encystment, requires detailed knowledge of morphological changes in the parasites. Encystment is poorly understood in Spironucleus, therefore we sought to develop a novel suite of light microscopy techniques, combine them with ultrastructure, and document key stages of encystment.

Methods: Spironucleus vortens (ATCC 50386 from Angelfish, Pterophylum scalare) were cultured axenically in modified liver digest, yeast extract, iron (LYI) medium, (22^{o}C, in dark). We observed live swimming and encysting cells from old cultures, unstained and fluorescently stained with NucBlue Live Ready Probes Reagent (nucleus), and ViaFluor (cytoskeleton). We also employed scanning electron microscopy.

Results: In early encystment, six anterior flagella beating on cell surface were covered by flexible cyst wall; in maturation, two posterior flagella lay exterior to cyst. NucBlue showed: a pair of nuclei, ⇒ two adjacent pairs of nuclei (after longitudinal binary fission),⇒ two distant pairs of nuclei (movement within cyst). ViaFluor showed three pairs of microtubular bands, and lateral and counter-crossing elements. In swimming and encysting trophozoites, SEM revealed a novel ring structure, “linea globuli”, around emergence of each anterior flagellum.

Conclusions: By our novel application of fluorescent stains, along with SEM, we have described four key stages of S. vortens encystment for the first time. The staging scheme can be used to monitor the effects of potential life-cycle interrupting treatments. Two intriguing questions remain: (i) what is the fate of the posterior flagella after early encystment (retracted, deconstructed, detached?), and (ii) what is the role of the “linea globuli”?

Keywords: encystment, Spironucleus, staging

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NEW DATA ON PARASITES AND FEEDING HABITS OF HALOBATRACHUS DIDACTYLUS AND POMADASYS INCISUS (TELEOSTEI) IN THE WESTERN MEDITERRANEAN SEA

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Introduction: The Strait of Gibraltar is a natural corridor for Atlantic species that enter the Mediterranean Sea, as the Lusitanian toadfish (Halobatrachus didactylus) and the bastard grunt (Pomadasys incisus). The biological and parasitological information about these species is scarce. This work provides the first data on their parasite fauna and diet at the Spanish Coast.

Methods: Halobatrachus didactylus (N = 3) and P. incisus (N = 14) were fished at the Spanish Coast. Parasitological and diet analyses of the fishes were carried out.

Results: Parasites from four different taxa were found in the toadfish: 2 cestodes (Grillotia sp. and Nybelinia sp.) and nematodes (Contracaecum sp. and Hysterothylacium reliquens). In the case of the grunt, we collected 4 digeneans (Acanthocolpidae gen. sp., Aephnidiogenes cf. barbarus, Didymozoidae gen. sp. and Genolopa sp.), 1 cestode, (Tetraphyllidea gen. sp.), 2 monogeneans (Dicrumenia sp. and Intracotyle hannibali), 1 leech (Trachelobdella lubrica) and 2 crustaceans (Parabrachiella sp. and Gnathia sp).

Conclusions: Four new parasite citations are added for H. didactylus and 7 for the P. incisus (three new species for science). Regarding the diet, 12 different taxa have been identified for H. didactylus and 22 for P. incisus. Differences with the diet described in other regions have been observed. The presence of larval and heteroxenous adult parasites in H. didactylus points to an intermediate role at the food web, while the absence of adults in P. incisus points to more basal position. Projects: MINECO/FEDER PID2019-110730RB-I00 co-funded by MCIN/AEI/10.13039/501100011033 by “ERDF A way of making Europe” by the EU; AICO/2021/279; and GVA-THINKINAZUL/2021/029 (with NextGenerationEU funds).

Keywords: Lusitanian Toadfish, Bastard grunt, Parasites, Preys, Ecobiology

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Topic: *AS04 Parasites of fish / AS04.6 Other studies related to parasites of fish*

EXPLORING NEW WAYS FOR THE DIAGNOSIS OF ANISAKID LARVAE

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Introduction: Anisakiasis is a well-known human pathology produced by Anisakis spp. (Nematoda, Ascaridoidea). However, another ascaridoid species (e.g. Anisakidae: Pseudoterranova spp., Contracaecum spp.; Raphidascaridae: Hysterothylacium spp.) produce similar pathologies ("anisakidosis" for anisakids). The diagnosies of this disease is increasing due to the consumption of raw or semi-cooked fish and the improvement of clinical diagnosis; nevertheless, clinical diagnostic is generally unspecific. The aim of present study is to look for new morphological tools, helpful to identify larvae stages.

Methods: Different model ascaridoid species were selected: Hystherothylacium sp., from frogfish (Halobatrachus didactylus); and Anisakis (type I and II) and Contracaecum sp. from blue whiting (Micromesistius poutassou). The specimens were fixed in 4% formaldehyde; some parasites were dissected and processed for scanning electron microscope (SEM).

Results: The SEM study revealed some previously undescribed morphological and topographic structures, regarding: i) longitudinal grooves at ventriculum and ventricular appendix, ii) ventriculum arrangement of Anisakis spp., iii) tridimensional disposition of the intestinal caecum and iv) distal globular regions at the ventricular appendix of Hysterothylacium spp.

Conclusions: These characters, not visible under light microscopy, could be used to identify and differentiate between species that traditionally have been considerate morphologically indistinguishable, supporting genetic markers. Projects: MINECO/FEDER PID2019-110730RB-I00 co-funded by MCIN/AEI/10.13039/501100011033 by "ERDF A way of making Europe" by the EU; AICO/2021/279; and GVA-THINKINAZUL/2021/029 (with NextGenerationEU funds).

Keywords: Anisakidae, Raphidascarididae, Diagnostic tool, Ultraestructure

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DEVELOPMENT MEETS TAXONOMY; POST-LARVAL DEVELOPMENT OF SCIENACOTYLE PANCERII

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Introduction: Sciaenacotyle pancerii is a pathogenic microcotylid infecting the Mediterranean meagre. Despite being relevant for diagnosis and infection assessment, morphological data about this monogenean is still limited to taxonomical descriptions. The aim of this study was to analyse the post-larval development of S. pancerii, focusing on the attachment structures.

Methods: 114 S. pancerii specimens were examined to describe the main developmental events and the morphological variability throughout post-larval development.

Results: Post-larval development of S. pancerii is characterized by size increment, acquisition of haptoral clamps, bifurcation of the gut, loss of the larval haptor, protandrous development of the genitalia and development of the haptoral asymmetry in mature stages. The size and number of most morphological variables increase with the clamp pair number, even after parasite maturity.

Conclusions: Developmental data of S. pancerii should be integrated into parasite diagnosis as size ranges of most taxonomic structures increase after maturity. The exceptional haptoral features (i.e., a large number of clamps and asymmetry) and attachment strategy support the close phylogenetic relationship between microcotylids and heteraxinids. The chronology of the development of the haptoral asymmetry can be a useful tool to discriminate among mazocraeid families. Funded by MICIN/FEDER PID2019-110730RB-I00, AICO/2021/279 and GVA-THINKINAZUL/2021/029.

Keywords: haptor, Monogenea, growth