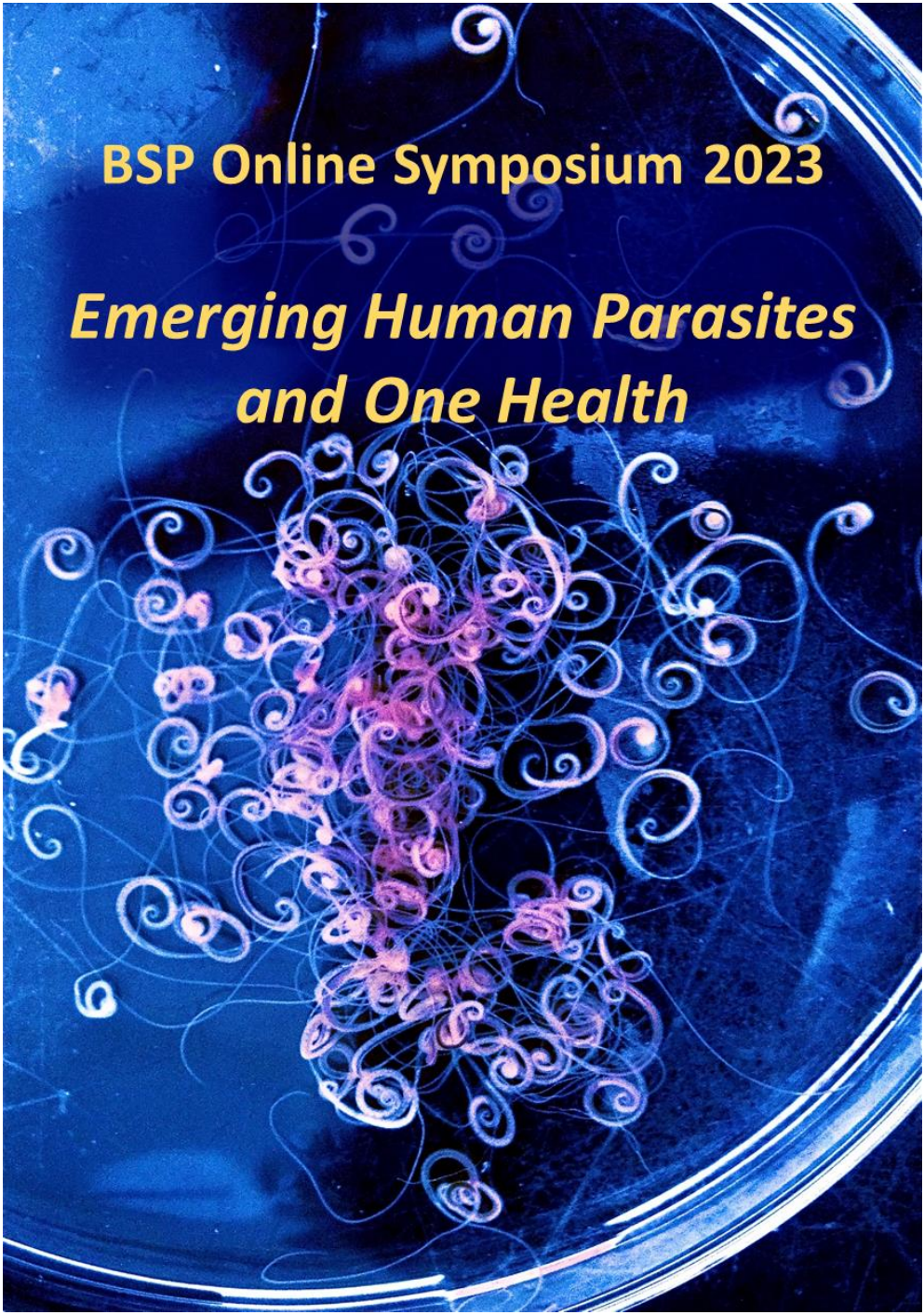
BSP Online Symposium 2023
Emerging Human Parasites and One Health

BSP Online Symposium 2023 - Emerging Human Parasites and One Health

Our fully online symposium reports on world-leading applications of 'One Health' approaches combining human, animal, and environmental health research to combat public health threats posed by emerging zoonotic parasites.

Keynotes Speakers:

- Professor Joanne Webster
- Professor Russell Stothard
- Professor Maria de los Ángeles Peña
- Professor Ana Montoya
- Dr Antonio Peña-Fernández

Recent cross-border parasite outbreaks in Europe involving different emerging human parasites such as *Cryptosporidium* spp. (Pinto *et al*., 2021) and future threats related to globalisation, climate and vector ecology changes (Momčilović *et al*., 2019), for example parasitic infections in non-endemic regions such as *Schistosoma haematobium* in Corsica (France) (Boissier et al., 2016), *Plasmodium falciparum* in Italy (Day, 2017) or *Entamoeba histolytica* in Spain and other developed countries (Escolà-Vergé *et al*., 2017), have highlighted the relevance of appropriate, effective and rapid responses from different animal, environmental and human health professionals and authorities, to tackle these human threats and prevent significant economic loss in food and livestock industries.

Thus, the European Union is funding different joint actions to improve preparedness and response capacities, including at points of entry, to prevent and combat cross-border health threats (Janiec *et al*., 2021), as well as to develop robust public health infrastructures for surveillance, vector control and notification of parasitic diseases (van der Giessen *et al*., 2021). Moreover, the World Health Organization/Europe supports different strategies and partnerships to contribute to the achievement of the Millennium Development Goals related to reduce the burden of vector-borne and parasitic diseases.

The Symposium will showcase international One Health approaches to detect and diagnose parasitic diseases, disrupt disease transmission and highlight novel and effective control strategies including educational interventions. This Symposium will also provide an insight on cutting edge laboratory parasitological techniques and on guidance/tools to address the teaching of medical and veterinary parasitology globally.

Contents

Oral Abstracts

Plenary

4-December-2023, at 09:05 (30 mins)

Global One Health and the WHO NTD 2030 Targets.

Presenter: **Prof Joanne Webster**, *Royal Veterinary College*

We have recently seen the launch of a new WHO Neglected Tropical Diseases (NTD) Roadmap and its Companion document, together with revised Disease Control and Elimination Guidelines and Targets. Across all there is now a clear emphasis on the need to incorporate a One Health approach, recognizing the critical links between human and animal health and the environment. Schistosomiasis, caused by *Schistosoma* spp. trematodes, is a NTD of global medical and veterinary importance, with over 220 million people currently infected as well as untold millions of livestock. Despite decades of mass administration of the anthelmintic praziquantel to, predominantly, school-aged children, the burden of schistosomiasis remains extremely high in certain regions. Whilst animal hosts have long been acknowledged as zoonotic reservoirs across Asia, within Africa or the Americas, in contrast, any zoonotic component of schistosomiasis transmission and its implications for disease control has, until now, been largely ignored. This is true of both *S. mansoni*, but particularly *S. haematobium*, the latter of which was assumed to be an exclusively human infection and thereby (so long as drug resistance does not evolve) amenable to elimination by targeting treatment of humans alone. Here I will focus on the transmission dynamics of *Schistosoma* spp., including notably the emerging risk raised by viable hybridization between *Schistosoma* species of both humans and animals. Such research emphasizes that a truly multi-disciplinary One Health perspective must be implemented in order to achieve the 2030 WHO Roadmap targets of elimination of schistosomiasis as a public health problem and ultimately towards interruption of transmission.

One Health: Helminths

09:45 (15 mins)

The “OneHealth” of helminthiases in context of neglected tropical disease (NTD) control in sub-Saharan Africa.

Presenter: **Prof Russell Stothard**, *Liverpool School of Tropical Medicine*

R Stothard[1];

[1] Liverpool School of Tropical Medicine, UK

Goal 3 of the Sustainable Development Goals (SDGs) is focused upon improving global health and well-being; a key indicator for measuring its progress is access to universal health coverage by 2030. To do so, achieving adequate treatment coverage for those requiring interventions against neglected tropical diseases (NTDs) is considered. Recognising the zoonotic nature of several NTDs, however, the WHO released a companion document to outline and underpin a "OneHealth Approach". The latter fosters an integrated, unifying intervention to balance and optimize the health of people, animals and the environment. In theory, its rationale is sound but in practice its implementation can be problematic. Here, I give an overview of certain nematode and trematode helminths and feature their parasitic diseases, giving particular attention on soil-transmitted helminthiasis and schistosomiasis.

10:00 (15 mins)

Hybridization of UroGenital Schistosomiasis (HUGS): Developing a New One Health Approach in Malawi.

Presenter: **Dr Janelisa Musaya**, Kamuzu University of Health Science, Malawi.

J Musaya [1];
[1] The Malawi Liverpool Wellcome Trust Clinical Research Program, Queen Elizabeth Central Hospital, Kamuzu University of Health Sciences, Blantyre, Malawi.

The discovery of *Schistosoma* hybrids in Mangochi and Nsanje Districts had brought to light a new dimension in the biology of schistosomiasis in Malawi. We believe possible *Schistosoma haematobium*-hybrids are able to be maintained in environmental transmission, cycling through livestock and are still able to infect people and cause disease. To understand our hypothesized casual events, we embarked on a study to assess the presence and viability of hybrids in humans, animals and snails in Mangochi and Nsanje Districts. In humans, we collect urine, stool and blood samples from 1,200 consented participants from each district, aged from 2 to 45 years of age. In animals, we collect stool and adult worms from carcasses at abattoirs. In snails, we collect them from water contact sites and expose them to light and collect emerging cercariae onto FTA cards. All urine and stool samples are inspected for eggs and isolated eggs or miracidia are fixed on FTA cards. All samples on FTA cards plus adult worms are extracted for PCR and DNA sequencing of mitochondrial cox1 and nuclear 18S/ITS regions capable of detecting hybrids and introgressed variants. We will present our preliminary results which so far show the presence of putative hybrids in humans, animals and snails. These findings call

for a renewed One Health approach as the best forward looking intervention to find synergies in the control of schistosomiasis that could make tangible reduction in environmental transmission.

10:15 (15 mins)

A public health study of gastrointestinal parasites in contaminating stool on vehicles from captive olive baboons at Knowsley Safari, UK.

Presenter: **Dr Alexandra Juhász**, *Liverpool School of Tropical Medicine*

As part of their drive through safari experience, Knowsley Safari (KS) offers its visitors a close-up encounter with their colony of olive baboons (*Papio anubis*) from the safety of their vehicles. Exiting vehicles, however, are sometimes contaminated with baboon faeces, posing a small health hazard. Coinciding with an animal welfare check, a coprological survey of baboon stool, both obtained from sleeping areas and cars, was conducted. Faecal material was examined by standard parasitological methods inclusive of: QUIK-CHEK RDT (*Giardia*), Kato-Katz coproscopy (*Trichuris*) and charcoal culture (*Strongyloides*). Across a four-day period, a total of 2,662 vehicles were examined with just under 700 stools obtained. Some 11.4% of vehicles were contaminated with faecal material. Overall prevalence of giardiasis was 37.4%, trichuriasis was 48.0% and strongyloidiasis was 13.7%. Since no faecal cysts of *Giardia* could be seen by microscopy, alongside very low levels of DNA detected by faecal PCR, these RDTs results were judged misleading. Further DNA characterisation confirmed the presence of *Trichuris trichiura* and *Strongyloides fuelleborni*. The latter observation represents this species' most northern report of natural transmission. To minimise any public health risk, a future blanket administration of anthelminthic(s) is recommended, with later coprological inspection(s) to ascertain reinfection levels.

10:30 (15 mins)

Development of short-course azaquinazoline anti-*Wolbachia* drugs for veterinary zoonotic filariasis.

Presenter: **Prof Joseph Turner**, *Liverpool School of Tropical Medicine*

J Turner[1]; JL Dagley[1]; S Hegde[1]; AE Marriott[1]; A Steven[1]; C Fricks[4]; U DiCosty[4]; A Mansour[4]; EJ Campbell[3]; CM Wilson[3]; SA Ward[2]; D Hong[2]; PM O'Neill[1]; A Moorhead[3]; S

McCall[4]; JW McCall[4]; MJ Taylor[1];
[1] Liverpool School of Tropical Medicine, UK; [2] University of Liverpool, UK; [3] University of Georgia, United States; [4] TRS Laboratories Inc, United States

Targeting *Wolbachia* endobacteria within filarial parasites using second generation tetracyclines is a relatively new curative treatment approach for both human and veterinary zoonotic filariasis. Doxycycline alone or in combination with macrocyclic lactones can cure heartworm disease and block transmission of brugian filariasis in dogs or cats. Whilst effective, long treatment durations are required which, along with dysbiosis side-effects and antibiotic stewardship concerns, precludes the widespread use of tetracyclines in companion animals. To address long treatment timeframes targeting *Wolbachia* in filariasis, we have discovered and developed a new azaquinazoline class of anti-*Wolbachia* drug. Unique features of azaquinazolines include the rapid, six-fold superior *Wolbachia* killing kinetic compared with tetracyclines and an exquisite selectivity for *Wolbachia*, with no general broad-spectrum antibiotic activities. A front-running member of this class, AWZ1066S, has demonstrable 5-day curative activity in a range of preclinical filariasis infection models and has been advanced into first-in-human clinical testing.

We utilised AWZ1066S and back-up azaquinazoline analogues in novel immunodeficient mouse models of *Brugia malayi* and *Dirofilaria immitis,* the causative agents of veterinary zoonotic brugian lymphatic filariasis and heartworm disease, respectively. We determined a minimum, two-day per month dosing with AWZ1066S was sufficient to mediate 100% prophylactic efficacy (no adults or microfilariae recovered) whilst once per month exposures mediated 86% efficacy against *B. malayi* infections in mice. We could retrieve *B. malayi* stages 14-35 dpi and determined that initial median anti-*Wolbachia* depletions were 80%, increasing to >99% in day 35 old immature adults following monthly exposures. We then exploited a novel Non-Obese Diabetic (NOD)SCID common gamma chain (NXG) *Dirofilaria immitis* mouse infection model to compare anti-*Wolbachia* efficacy. *D. immitis* 14-day L4 larvae were depleted of *Wolbachia* by an average of 83-90%. We explored four back-up azaquinazolines for *in vivo* efficacy against *D. immitis* and identified equipotent or improved *Wolbachia* depletions compared with AWZ1066S after single day dosing. One candidate, AWZ1023 was selected for a proof-of-concept pilot chemoprophylactic study in dogs and was successful in total prevention of adult heartworm following two monthly one-day treatments.

In conclusion, we provide early proof-of-concept that azaquinazolines targeting nematode *Wolbachia* are a promising new class of drug for the treatment and prevention of veterinary zoonotic filarial infections.

One Health: Protists

4-December-2023, at 11:00 (20 mins)

Toxoplasma gondii epidemiology through a One Health approach.

Presenter: **Prof Ana Montoya**, *Universidad Complutense de Madrid*

Toxoplasmosis is a global zoonotic parasitic disease affecting a wide range of warm-blooded vertebrates (including human beings) in different habitats and regions, from the Arctic to the Tropics, in terrestrial, aquatic, and marine environments. *Toxoplasma gondii* infections in immunocompetent adult hosts is subclinical, however, toxoplasmosis is a significant public health concern for pregnant women and immunosuppressed people. In the epidemiology of toxoplasmosis, felids (domestic and wild) play an important role as they are the only definitive host of the parasite and the main source of contamination by excreting oocysts. The main transmission source are: i) sporulated oocysts infecting environment (water, soil, vegetables and fruits), ii) tissue cysts with bradyzoites in raw or undercooked meat from parasitized animals, iii) pseudocysts with tachyzoites (congenital transmission). In addition, tachyzoites can be acquired through transfusion, organ transplantation, and/or consumption of raw, unpasteurized milk. There are several studies about the prevalence of *T. gondii* infection in cats by serological and coprological analysis, being the cats of highest epidemiological relevance are younger and mainly stray cats with access to intermediate hosts (birds and rodents) or living on farms. These seroprevalence studies are a good indicator of the prevalence of infection and possible environmental contamination. Despite the high seroprevalences reported, oocyst shedding studies reveal a prevalence of less than 1%. In livestock production (sheep, goats, pigs, cattle and poultry), oocysts represent the main source of infection as faeces from cats may contaminate water, soil (pastures fertilized with compost) or stored feed (hay, grain). Extensive livestock farms or farms with poor biosecurity measures (presence of cats) present a higher risk of infection. Also, as in other animal species, the risk of infection increases with age due to continuous exposure to infection. The main source of *T. gondii* contamination in marine environments is likely through freshwater runoff from the land. Marine animals, including mammals, birds, and fish, can become infected by ingesting contaminated water or by consuming infected prey. Filter-feeding marine organisms, such as mussels, clams, and oysters, can concentrate *T. gondii* oocysts from the surrounding water. If humans consume these contaminated seafood items raw or undercooked, there may be a risk of contracting toxoplasmosis. Intermediate hosts (livestock animals, in addition to birds and marine mammals) play an important role in the epidemiology of toxoplasmosis, since infection in humans and carnivores occurs through

ingestion of tissue cysts from intermediate hosts. The main risk of transmission to humans is from raw or undercooked meat (lamb and pork) contaminated with bradyzoite cysts, or vegetables, fruits and water contaminated with sporulated oocysts. In fact, FAO and WHO consider toxoplasmosis to be the fourth most important food-borne parasitic disease in the world.

In conclusion, it's essential to consider toxoplasmosis in all environments, especially in the context of a One Health approach. By understanding the interactions between birds, other animals, and the environment, researchers and public health professionals can develop better strategies for disease surveillance, prevention, and management, ultimately benefiting both wildlife and human health.

11:30 (15 mins)

Observations on the life cycle and transmission of *Dientamoeba fragilis*.

Presenter: **Mr Luke Hall**, *University of Technology Sydney*

Here we discuss the life cycle and mode of transmission of *Dientamoeba fragilis* that is not well understood. Historically, transmission was believed to occur through *Enterobius vermicularis* ova or directly via trophozoites in stool. However, recently evidence for fecal–oral transmission of cysts has emerged. Since cultured trophozoites are vulnerable to extremes of pH, we suspect cysts are the main mode of transmission. Cysts of *D. fragilis* are readily produced by laboratory rodents after oral infection, however their detection in human clinical samples is rare. Additionally, *D. fragilis* was detected in pigs, cats, dogs, budgerigars, rats, goats, cattle, and non-human primates. This highlights the potential for zoonotic transmission, probably through the cyst stage as *E. vermicularis* is a human specific helminth. Zoonosis is unlikely to be the sole source of human infections. Epidemiological analysis of the case prevalence for different age groups reveals dual peaks for children and adults at parental ages which is a common indication of human-to-human transmission. The specificity of current real time PCR diagnostic assays for the detection of *D. fragilis* are unreliable. Cross reactivity with *Pentatrichomonas hominis* and *Tritrichomonas foetus* was observed when testing the assays with DNA from cultures and such assays are not directly transferable from use on human to animal specimens. Further one PCR test has a significant problem with false positive results on human samples. We suggest that the current estimates of *D. fragilis* prevalence are overestimated, and the identification of novel animal hosts without the support of additional (sequence) data is unreliable. We provide further characterisation of *D. fragilis* cysts by electron microscopy.

One Health: Control Strategies

11:45 (30 mins)

Recent advances and strategies to formulate novel drugs to tackle emerging human parasites.

Presenter: **Prof Maria de los Ángeles Peña**,, *Universidad de Alcalá*

Globalisation stands as one of the most influential factors in the introduction and dissemination of animal and human parasites within different ecosystems, such as *Dirofilaria* spp. and *Leishmania* spp. (Semenza *et al*., 2022); in food production systems, including *Cryptosporidium* spp., *Toxoplasma gondii*, *Echinococcus* spp. and *Giardia intestinalis* (Momčilović et al., 2019; Brosseau *et al*., 2023); as well as in drinking water systems, e.g. *Cyclospora cayetanensis*, *Fasciola* spp. and *Fasciolopsis buski* (Singh *et al*., 2014). Furthermore, demographic shifts, migration patterns, and international trade can also contribute to these phenomena. As a result, there is an urgent need for enhancing monitoring, control/prevention and treatment for the eradication of parasites utilising emerging technologies. Simultaneously, the study of suitable animal models for experimenting with new drug candidates is of the upmost importance. Preclinical evaluation must be standardised and compared across various laboratories and researchers. Thus, recent studies propose innovative strategies to gain insights into treating diseases caused by the aforementioned parasites, e.g. leishmaniasis. *Leishmania* possesses unique enzymes and biochemical pathways that are distinct from those found in their mammalian hosts. The biochemical characterisation of these specific enzymes serves as a valuable tool for identifying potential drug targets. Pentavalent antimonials, pentamidine, selenium derivatives and amphotericin B (AmB) are drugs commonly used for treating leishmaniasis (Pacheco *et al*., 2023). Another promising orally administered drug is miltefosine (Sunyoto *et al*., 2018). The combination of liposomal AmB and miltefosine has shown to be effective in treating individuals who have been reinfected (Kumar *et al*., 2023). Miltefosine has also been studied in its liposomal form, demonstrating greater efficacy than the free drug against *Leishmania* promastigotes that were preliminary resistant to conventional miltefosine. In recent years, liposomes and lipid nanoparticles (LNPs) have emerged as excellent drug carriers for treating a wide range of diseases, including leishmaniasis. AmB is an antifungal polyene that acts by selectively binding to ergosterol in the *Leishmania* membrane (Machado *et al*., 2015; Singh *et al*., 2023). Despite its success rate of over 90%, its high toxicity and cost have prompted the development of liposomal formulations, such as AmBisome®, which are less toxic than non-liposomal versions. While AmBisome® is the most effective liposome formulation of AmB, other formulations are available on the market. Amphocyl® colloidal formulation in

Europe, composed of a colloidal dispersion of sodium cholesteryl sulfate, shaped as flat disks (commercialised as Amphotec® in the United States, US), Abelcet® Suspension for Injection, which is an AmB complex with a ribbon-like multilamellar lipid structure consisting of phosphatidylcholine and phosphatidylglycerol (US), Fungisome® (India), and Amphomul® emulsion (India). However, drug administration and toxicity remain challenges with each of these therapeutic alternatives (De Almeida *et al*., 2017).

Liposomal AmB and other drug delivery systems, such as albumin microspheres, niosomes, and nanodiscs, offer sustainable solutions for regions with limited resources. LNPs provide a valuable platform for leishmaniasis treatment and are effective at delivering both hydrophobic and hydrophilic drugs, offering protection for their cargo while reducing toxicity (Etxebeste-Mitxeltorena .., 2021). Numerous studies have explored the use of different drugs, such as diselenide or phthalocyanine, in LNPs.

Furthermore, liposomes have been investigated as adjuvants in vaccine development. For example, Mazumber et al. (2007) have successfully used cationic liposomes as adjuvants to deliver antigens and DNA.

12:15 (15 mins)

Enhancement of solar disinfection of drinking water against the waterborne enteroparasite *Cryptosporidium* by addition of peroxymonosulfate.

Presenter: **Ms Sandra Martín-García**, *Universidade de Santiago de Compostela*

S Martín-García[1]; A Wong[2]; AI Vidal-Varela[3]; S Couso-Pérez[3]; H Gómez-Couso[3];
[1] Universidade de Santiago de Compostela, ; [2] Royal College of Surgeons in Ireland, Ireland; [3] University of Santiago de Compostela, Spain

Nowadays, more than 2000 million people in the world are using contaminated water resources, which leads to a significant risk for infectious diseases transmission. Solar disinfection method (SODIS) is a simple, cheap, and sustainable solution that enhances microbiological quality of drinking water at home level. In the present study, the addition of peroxymonosulfate (PMS) was evaluated to improve the efficacy of SODIS against the enteroprotozoan parasite *Cryptosporidium parvum*, which was chosen by the World Health Organization as the reference pathogen for the waterborne protozoa group in the assessment of household water treatment systems. For this purpose, quartz tubes containing 3 mL of bidistilled water with different concentrations of PMS (0.1, 0.5, 2.5 and 5.0 mM) and/or Fe 2+ (1.8 µM) were contaminated with 2 million oocysts/mL of *C.*

parvum and exposed to simulated solar radiation (40 W/m2 , 290-390 nm) at a temperature of 40 °C. Samples were taken at 1, 2, 4 and 6 hours and the oocyst viability was evaluated by hsp70 mRNA quantification through reverse transcription qPCR, previous 42 °C induction and subsequent mRNA extraction. The results obtained shown a decrease in oocyst viability of ≥4 log reductions with PMS concentrations of ≥0.5 mM after exposure time of 4 hours. In conclusion, addition of PMS enhances and speeds up the inactivation of *Cryptosporidium* oocysts by SODIS procedures/method, providing a high protection against waterborne protozoan diseases.

One Health: Education

4-December-2023, at 13:00 (30 mins)

Recent developments in animal and medical parasitology education.

Presenter: **Dr Antonio Peña-Fernández**, *Universidad de Alcalá* and *De Montfort University*

Outbreaks due to animal and human parasites have significantly increased because of global phenomena including migration, international trade and climate change, which are shifting human-animal interactions, as well as increasing levels of drug resistance in both protozoan and helminth parasites. Strengthening animal and medical parasitology research and education is key to responding to and preventing these events and future parasitic outbreaks. As a result, De Montfort University (DMU, UK) started to build a web-based package named e-Parasitology©, accessible through the DMU website (http://parasitology.dmu.ac.uk/) in 2015, in collaboration with practising National Health Service Biomedical Scientists in England and academics/parasitologists from the Spanish Universities of San Pablo CEU and Miguel Hernández de Elche. The package was initially launched as an open-access resource in 2017 with four virtual modules to aid with the teaching and learning of medical parasitology: 1) theoretical module, which contains e-learning units on common and emerging human parasites; b) virtual laboratory, with units of different laboratory techniques and instruments for biomedical and parasitology research; c) virtual microscope containing a library of real specimens for learning parasitology diagnosis; d) virtual clinical case studies, for promoting acquisition of problem-solving, critical thinking and reflection skills to facilitate the acquisition of diagnostic skills. Multimedia developers (staff and students) and graphic design students were hired to build the different e-Parasitology©'s resources following adult and behavioural theory and gamification pedagogies. Long-term analysis on the effectiveness of e-Parasitology© at the three participating universities have shown that the package could be effective in providing undergraduate/postgraduate students with necessary skills for medical parasitology diagnoses and prevention. As a result, our team has launched two additional resources: 1) an associate e-Parasitology© app, which was originally launched in 2020 with the same resources and tools available in the website (available in Google Store: https://play.google.com/store/apps/details?id=com.DMUHLS.eParasitologyApp&hl=en_US); 2) an e-Parasitology Game Collection© app for Smartphones, specifically designed to learn how to combat malaria through gamification, currently launched as a beta version in 2021 on Android platforms. Recent developments include the development of specific e-learning units for the teaching/learning of animal parasitology in collaboration with EBVS®

European Veterinary Specialists (Complutense University of Madrid, Spain) since March 2020, which demonstrated a strengthening the acquisition of diagnostic and treatment skills for managing important parasitic diseases affecting companion animals (dogs and cats). The main purpose for developing the e-Parasitology© resources was to aid prevention and encourage reversal of the ongoing downtrend numbers of parasitologists graduating from universities in Western countries in conjunction with the lack of appropriate open access/free resources for training future parasitologists needed in developing countries such as in Sierra Leone. Thus, current actions include the following-up of graduated students that have studied using the different e-Parasitology© resources (website and/or associated app) at different participating universities from developed and developing countries, and the potential effect that these resources could have had on their careers. Collection of this feedback/impact will be crucial for updating our virtual resources and developing similar resources.

13:30 (15 mins)

OneZoo Centre for Doctoral Training: Developing future leaders in zoonoses.

Presenter: **Prof Jo Cable**, *Cardiff University*

J Cable[1];
[1] Cardiff University, UK

With >60% of current and emerging human infections being of zoonotic origin and zoonotic pandemics costing the global economy over $60 billion yearly, predicting, detecting and controlling zoonoses represents one of the greatest challenges faced by humanity. These challenges are heightened by a historical lack of interaction among different disciplines studying these diseases, which slows progress on identifying key drivers of zoonoses and in developing pragmatic mitigation strategies. Even within the broad field of biology there are still silos in thinking and approaches, particularly between microbiological and virological fields that are poorly linked to ecological and environmental research. Fields like anthropology and social science are often only weakly integrated into the field of One Health, despite possessing a wealth of potential information and methodological techniques to investigate how human society drives and responds to the emergence and spread of zoonoses. The COVID-19 pandemic is a stark reminder of how a zoonotic pathogen can effectively bring our world to a standstill and cripple the global economy. Prevention is clearly more cost effective than control but remains an elusive goal. One Health/Planetary Health approaches are needed to

determine the drivers of zoonotic emergence and spread, and achieve integrated solutions, grounded in sustainable environmental practice, that create environmental barriers to zoonotic transmission. Here we present our new OneZoo Centre for Doctoral Training (CDT) which aims to equip the next generation of world leading transdisciplinary scientists with a multidisciplinary toolbox bespoke to their project and a global citizenship outlook. Our programme fosters innovative and critical thinking in a transdisciplinary framework, to create scientists that can improve the health and wellbeing of humans and animals in an environmentally sustainable manner that is equitable and cognisant of socioeconomic, political and cultural needs and constraints.

13:45 (15 mins)

Blood, sweat, and faeces: a guide to rearing medically important insects.

Presenter: **Mr Luke Brandner-Garrod**, *London School of Hygiene and Tropical Medicine*

L Brandner-Garrod[1];
[1] London School of Hygiene and Tropical Medicine, UK

Vector borne diseases cause an estimated 700,000 deaths each year. Laboratory based insect colonies are key to understanding vector-pathogen interactions facilitating the development of novel therapeutics and vector control strategies. However, the rearing of insects can be difficult to establish and maintain in the large quantities required for research. This presentation will provide a brief guide to building an insectary, rearing medically important insects, and performing vector-parasite experiments. The focus of which will be on the colonies reared at the London School of Hygiene and Tropical Medicine, including mosquitos (genera: *Anopheles, Aedes, Culex*, and *Toxorhynchites*), bazaar flies (*Musca sorbens*), and triatomine bugs (genera: *Dipetalogaster, Rhodnius*, and *Triatoma*). Highlighting their unique colony conditions and the research utilizing these colonies to combat diseases such as Chagas disease, malaria, and trachoma.

14:00 (15 mins

Introduction of a monitoring training programme for the detection of emerging human microsporidian parasites in farms' pigs from Makeni, Sierra Leone.

Presenter: **Ethel Ukaegbu**, *Department of Public Health, University of Makeni, Makeni*

E Ukaegbu[5]; U Anjum[1]; S Llorens[2]; L Acosta[3]; G Torrado[4]; MD Evans[5]; A Peña-Fernández[4]; [1] Leicester School of Allied Health Sciences, De Montfort University, Sierra Leone; [2] Facultad de Farmacia, Universidad San Pablo CEU, Spain; [3] Área de Parasitología, Departamento de Agroquímica y Medio Ambiente, Universidad Miguel Hernández de Elche, Spain; [4] Departamento de Ciencias Biomédicas, Universidad de Alcalá, Crta, Spain; [5] Department of Public Health, University of Makeni, Sierra Leone

Following the detection of emerging microsporidian spores in faeces from different farm animals collected in April 2019 from different farms across Bombali District, Sierra Leone (SL), De Montfort University (DMU, UK) started the implementation of a multistage project to build medical parasitology capabilities at the University of Makeni (UniMak, SL) to tackle potential future risks due to these opportunistic parasites. Aims: a) to explore the temporal variation of human-related microsporidian parasites in different farms' pigs across Makeni to identify potential risks for the food chain; b) to analyse the effectiveness of our interventions/stages to provide UniMak BSc Public Health students with the appropriate laboratory skills to perform coprological analysis for the detection of these species. UniMak academics introduced novel practicals for the detection of microsporidia in third- and four-year BSc Public Health's modules in 2020/21. Academics followed a blended approach using the Virtual Laboratory freely available in the e-Parasitology package (http://parasitology.dmu.ac.uk/). Students processed thirty-six fresh stool samples from pigs provided by the SL Department of Agriculture and Food Security from a nearby farm (Makeni) in 2021. Students prepared smears in a class II biological safety cabinet and learned to perform modified Trichrome stain for the detection of spores. Moreover, in order to explore temporal variation, further fresh faecal samples were collected from four pigs in Summer 2022. These recent samples were specifically screened for the presence of *Encephalitozoon* spores by immunofluorescent-antibody test using a specific monoclonal antibody (Mab) of murine origin IgG2a, patented as a diagnostic tool for miscrosporidiasis caused by the genus *Encephalitozoon*. Students showed high levels of confidence as soon as they entered the laboratory. They were able to quickly undertake all the different practical steps. A high level of accuracy was observed in students' detection of spores, which were present in 14/36 (38.8%) stool smears monitored and would be in

line with the prevalence of microsporidia detected previously (7/12; 58%) by our group using a SYBR Green real-time PCR on 12 pig stool samples collected in 2019. One of the samples collected in 2022 resulted positive for spores of the genus *Encephalitozoon*. Moreover, structures compatible with *Encephalitozoon* spp. were observed in faeces from another pig. These results would be similar to those observed in 2019, suggesting a moderate circulation of *Encephalitozoon* spp. in the monitored farms, which highlights a potential risk to the Sierra Leonean population, especially those with the immune system compromised, including survivors from the 2014-16 Ebola outbreak. The different stages and strategies used seem to have successfully aided capacity building to prepare future UniMak professionals to tackle microsporidian infections in Sierra Leone, methods and resources that can be easily adopted in other universities owing to their open-access nature. The high prevalence detected of these emerging pathogens in faeces from farm pigs across Makeni suggest a threat to the food chain, which highlights the relevance of our training intervention, as well as the need for implementation of appropriate cleaning and food processing protocols at slaughterhouses, including appropriate management of manure in pig farms to minimise human exposure.

14:15 (15 mins)

Empowering Youth to Combat Neglected Tropical Diseases: Strategies and Success Stories.

Presenter: **Yona Eliud Yangaza**, *NTDs Team Lead, One Health Society Tanzania*

This presentation will focus on the strategies and success stories of empowering youth to combat neglected tropical diseases (NTDs) from One Health Society (youth led NGOs) from Tanzania. Neglected tropical diseases affect over one billion people worldwide, with youth being particularly vulnerable and impoverished communities. This presentation will highlight the importance of engaging youth in NTD control and elimination efforts through education, awareness campaigns, and community-based initiatives. Success stories from ONE HEALTH SOCIETY (youth lead NGOs) of Tanzania will be shared, showcasing the impact of youth-led interventions in reducing the burden of NTDs. The presentation will also explore challenges faced by youth in this field and provide recommendations for effective youth engagement. Overall, this presentation aims to inspire and motivate stakeholders to invest in youth-led initiatives for NTD control and elimination.

Speed talks

4-December-2023, at 14:30 (5 mins)

Assessing Dose-Exposure-Response Relationships of Miltefosine in Adults and Children Using Physiologically-Based Pharmacokinetic Modelling Approach.

Presenter: **Mr Shadrack Madu**, *De Montfort University*

SJ Madu[1]; K Wang[1]; SK Chirumamilla[2]; DB Turner[2]; PG Steel[3]; **M Li**[1];
[1] De Montfort University, UK; [2] Certara UK Limited, Simcyp Division, Sheffield S1 2BJ, UK; [3] Durham University, UK

Objectives: Miltefosine is the first and only oral medication to be successfully utilized as an antileishmanial agent. However, the drug is associated with differences in exposure patterns and cure rates among different population groups e.g. ethnicity and age (i.e., children v adults) in clinical trials. In this work, mechanistic population-based PBPK models have been developed to study the dose-exposure-response relationship of miltefosine in *in silico* clinical trials and evaluate the differences in population groups, particularly children and adults.

Methods: The Simcyp population pharmacokinetics platform was employed to predict miltefosine exposure in plasma and peripheral blood mononuclear cells (PBMCs) in a virtual population under different dosing regimens. The cure rate of a simulation was based on the percentage of number of the individual virtual subjects with $AUC_{d0\text{-}28}$> 535 µg×day/mL in the virtual population.

Results: It is shown that both adult and paediatric PBPK models of miltefosine can be developed to predict the PK data of the clinical trials accurately. There was no significant difference in the predicted dose-exposure-response of the miltefosine treatment for different simulated ethnicities under the same dose regime and the dose-selection strategies determined the clinical outcome of the miltefosine treatment. A lower cure rate of the miltefosine treatment in paediatrics was predicted because a lower exposure of miltefosine was simulated in virtual paediatric in comparison with adult virtual populations when they received the same dose of the treatment.

Conclusions: The mechanistic PBPK model suggested that the higher fraction of unbound miltefosine in plasma was responsible for a higher probability of failure in paediatrics because of the difference in the distribution of plasma proteins between adults and paediatrics. The developed PBPK models could be used to determine an optimal miltefosine dose regime in future clinical trials.

14:35 (5 mins)

Molecular identification of *Acanthamoeba*, *Naegleria fowleri* from various water sources, Egypt.

Presenter: **Dr Rana Elsedawy** , *Faculty of Veterinary Medicine*

R Elseadawy[1]; I Abbas[1]; M Al-Araby[1]; S Abu-Elwafa[1];
[1] Faculty of Veterinary Medicine, Mansoura University, Egypt

In order to identify the prevalence and different genotypes of two neglected waterborne protists (*Acanthamoeba* and *Naegleria fowleri*) in water samples from various sources in Dakahlia governorate, PCR-testing in conjunction with isolate sequencing was carried out. Out of 62 protozoan-suspected samples using microscopic examination, *Acanthamoeba* were molecularly confirmed in 24 (38.7%) samples from various sources including tap water. In addition, *Naegleria* spp. were detected in 6 (9.6%) samples from the Nile, two of them (3.2%) were identified as *N. fowleri*. Sequencing of 20 samples of *Acanthamoeba* was successful; 18 were assigned the genotype T3 and two the T4. Results from the current study were integrated with those from past surveys conducted in Egypt for analysis. *Acanthamoeba* showed the highest mean prevalence (43.03%) with insignificant variations among various water sources. The incidence of finished water in drinking water treatment plants had lower than the raw water, but the filtration efficiency has not exceeded 60.0%. Different genotypes of *Acanthamoeba* were found with the T4 (the highly pathogenic type) was the most significantly identified type. A common T4 haplotype was found circulating in water from Egypt as well as 3 other countries (Tanzania, Rwanda, Uganda) located on the Nile basin. This haplotype included isolates from keratitis-infected patients, which confirms the potential role of water in the epidemiology of AK infecting humans in these countries. The Nile water has the greatest estimated mean prevalence of *Naegleria* spp. at 23.79%. In the current study, occurrence of potentially pathogenic protists with a neglected status, has been confirmed in water from Egypt, which should prompt the authorities to revise the protocols for controlling these pathogens in water.
Keywords: *Acanthamoeba*; *Naegleria fowleri*; Genotype; Water; the Nile; Egypt.

14:40 (5 mins)

Assessment of use of anthelmintic drugs in Bureti sub county, Kericho County in Kenya.

Presenter: **Dr Nathan Kosgei**, *Gene Plus, Veterinary Science Research Institute, Kenya*

N Kosgei[1];
[1] Gene plus, Veterinary Science Research Institute,

Helminths cause significant losses through either a negative impact on production or increased mortality associated more with small ruminants and calves (Enejoh & Suleiman, 2017). Control aims to keep infections low, especially in the young, and decrease associated economic losses. The use of chemotherapeutic anthelmintics has been the primary choice of control due to high efficacies and ease of application. Recent studies, however, demonstrate irregular and haphazard use of these drugs, especially in developing countries. Control of livestock helminths has relied mainly on the use of anthelmintics worldwide. However, recent trends including climate change, intensification of production systems and development of anthelmintic resistance threatens the control of helminths. This therefore means that all stakeholders involved in animal and human health have to be on guard regarding all practices old and new in place for helminth control. This study was undertaken to assess farmer's knowledge, attitudes and practices in regards to anthelmintic use in livestock in Buret sub county. The study was conducted in Tebesonik and Cheboin wards in Bureti sub county, Kericho County, Kenya. Structured questionnaires were administered to a total of 80 farmers through face to face interviews. Information on the farmers' sources of knowledge, types of anthelmintics in use, alternative control strategies, frequencies of deworming, dosing strategies and types of farming systems was gathered through these questionnaires. The demography of the target population was composed of 53% males and 47% females. Majority, 48%, had attained secondary level of education. Access to veterinary services was at 82 %. This comprised the main source of information for them amongst other sources including family members and social media. Synthetic (chemical) anthelmintics, was used by 90% of the population compared to 3 % that used herbal drugs. Extensive, paddock / free range system is still dominantly practiced compared to semi-intensive and intensive systems.

14:45 (5 mins)

Exploring the Dual Role of Dihydroorotate Dehydrogenase in *Toxoplasma gondii*: Beyond Pyrimidine Biosynthesis.

Presenter: **Miss Carolina Urbina Camacho**, *Universidad de los Andes*

C Urbina Camacho[1]; C Barrera Grijalba[1]; BH Zimmermann[1];
[1] Departamento de Ciencias Biologicas, Universidad de los Andes, Bogotá D.C., Colombia

Toxoplasmosis is considered by the Centers of Disease Control and Prevention to be a neglected parasitic disease (Ben-Harari and Connolly, 2019) because of low awareness and underestimation among healthcare professionals, despite high worldwide prevalence of the causative agent, *Toxoplasma gondii*. Limited treatment is available for immunocompromised patients, pregnant women, and those with congenital or chronic infections. In recent years there has been a re-emergence of toxoplasmosis, driven by the increase of drug-resistant strains that can affect immunocompetent individuals and lead to complications including abortions in pregnant women (Montazeri *et al*., 2018), suggesting the infection can pose a threat to a broader population and prompting the need for new anti-infective strategies. The pyrimidine biosynthesis pathway has been previously used as molecular target against related parasites, including *Plasmodium falciparum*. In *T. gondii*, knockouts of the first, fifth or sixth enzymes of this pathway result in uracil-auxotrophic parasites, while elimination of the fourth enzyme (dihydroorotate dehydrogenase, DHODH) results in lack of growth regardless of uracil availability. Replacing DHODH with a catalytically inactive variant restores the auxotrophic phenotype, indicating an additional, unknown role for TgDHODH (Hortua Triana *et al*., 2016). The objective of our project was to explore whether TgDHODH interacts with other proteins in the parasite mitochondrion, since such interactions might provide clues about its pyrimidine-independent function. In humans, DHODH knockouts result in loss of mitochondrial membrane integrity, and the enzyme appears to interact with respiratory complexes I and II (Fang *et al*., 2013). We conducted Blue Native Gel Electrophoresis (BN-PAGE) coupled with in-gel activity assays for DHODH using mitochondrial extracts from tachyzoites, as well as 2D-SDS-PAGE followed by TgDHODH detection using Western Blot with antibodies raised against a recombinantly produced, N-terminally truncated TgDHODH. TgDHODH activity or antibody reaction were observed in bands migrating at much higher molecular weight than expected for the protein alone, both in BN-PAGE and 2D-SDS-PAGE, indicating that the protein could be interacting with one or more proteins in stable complexes. Two high molecular weight bands, both migrating near 700 kDa, were excised and prepared for mass spectrometry analysis. In addition to the TgDHODH domains homologous to class 2 DHODHs from other organisms, TgDHODH possesses a mitochondrial targeting sequence in an unusually long 157 residue N-terminal extension. Previous work shows that this

sequence is proteolytically removed during import, producing a mature enzyme of ~48 kDa (Hortua Triana *et al*., 2012). Extended N-terminal mitochondrial targeting sequences have occasionally been linked to independent functions of the corresponding targeting peptides, which are cleaved from the rest of the protein once this reaches its intended location. We planned to produce the extended 157 residue N-terminal peptide of TgDHODH as a recombinant protein to allow the production of antibodies that could be used to screen for its presence in *T. gondii* mitochondrial complexes. In our preliminary attempts at expression, we were able to detect this recombinant peptide in cell extracts with antihis-tag antibodies, however expression levels were very low, possibly due to the presence of predicted disordered sequences which may have made the sequence susceptible to proteolysis. We plan to express a shorter peptide, encompassing the first 113 residues, to eliminate most of the disordered region. This research was funded by Banco de la República Fundación para la Promoción de la Investigación y la Tecnología #4499, and by the Facultad de Ciencias Universidad de los Andes projects INV-2019-84-1846 and INV-2021-127-2302.

14:50 (5 mins)

New models of heartworm and their application in preventative drug research.

Presenter: **Miss Jessica Dagley***, Liverpool School of Tropical Medicine*

J Dagley[1]; S Hegde[1]; AE Marriott[1]; A Steven[1]; C Fricks[3]; U DiCosty[3]; A Mansour[3]; EJ Campbell[2]; CM Wilson[2]; SA Ward[1]; A Moorhead[2]; S McCall[3]; JW McCall[2]; MJ Taylor[1]; JD Turner[1];
[1] Liverpool School of Tropical Medicine, UK; [2] University of Georgia, United States; [3] TRS Laboratories, UK

Dirofilaria immitis is a filarial nematode causing potentially lethal veterinary heartworm disease in cats and dogs. *Dirofilaria* spp can also cause zoonotic infections and pathologies in humans. Currently, control of heartworm disease relies on chemoprophylaxis with the macrocyclic lactones (ML), however, ML-resistant isolates are increasing and resulting in preventative treatment failure. Heartworm drug research relies on long-term experimental cat and dog studies, with the potential to cause severe welfare issues. Thus, there is a need for alternative refined heartworm models for rapid pre-clinical research to reduce cat and dog experimentation. We discovered lymphopenic mouse strains with ablation of the interleukin-2/7 common gamma chain (NSG/NXG) are susceptible to larval infections of *D.*

immitis. Larvae at 2-5 weeks post-infection were retrieved from subcutaneous and muscle tissues and were morphologically representative of the L4 stage in dogs. Mice did not display any clinical signs of infection. Using single subcutaneous injections of moxidectin, we identified 60-88% reductions in parasites 14-28 days post-infection. Oral oxfendazole dosing on day 1 and 28 also yielded 90% reductions in parasite yields on day 35. Using seven-day oral doxycycline, we demonstrated a 70-90% *Wolbachia* depletion within *D. immitis* L4 in our mouse model. Furthermore, we validated two additional mouse strains as being susceptible to *D. immitis* infection, C.B-17 SCID and Rag2/Il2rg, improving the commercial availability of mice as heartworm models and working towards the reduction and replacement of cats and dogs in heartworm preventative drug research.

Workshop

4-December-2023, at 15:45 (60 mins)

Practical guidance, tools and experience from the field to respond to biological incidents.

Presenter: **Dr Antonio Peña-Fernández**, *Universidad de Alcalá* and *De Montfort University*

Relevant guidance and tools to combat biological incidents or attacks is becoming increasingly relevant due to current global health threats such as the war in Israel-Gaza. Attacks involving biological substances, although rare, can affect large groups of a population and affect multiple countries simultaneously. Biological substances are used as they can spread quickly in the environment without being easily detected. Moreover, non-intentional events involving these substances can also challenge healthcare systems, resulting in high morbidity and mortality levels in the absence of appropriate preparedness and resources, highlighting the importance of having appropriate capacity, capabilities and trained personnel, to quickly respond to these events. Thus, technical guidance and resources should be available as part of the biological preparedness that all governments should establish, so first responders can tailor robust recovery strategies to minimise the number of people exposed and remediate the environment to avoid future exposures to the hazards released. The aim of this workshop is to introduce participants to available guidance and methods in the UK Recovery Handbook for Biological Incidents (UKRHBI; Pottage *et al*., 2015) for tailoring effective protection, decontamination and remediation strategies to respond to these events, which has been developed by Public Health England (now UK Health Security Agency) in collaboration with other UK Government Departments, Agencies and local authorities. The UKRHBI provides a series of protection and recovery options to decontaminate biological hazards in the environment; the user is able to select options using up-to-date information about the physiological characteristics and the life cycle of the pathogen. Participants will use the UKRHBI to develop a basic protection and environmental recovery/restoration plan to protect human health following an outbreak of infection due to *Cyclospora cayetanensis*. The workshop will cover: Relevance of biological incidents. Introduction to the PHE guidance (UKRHBI) and tool (Recovery Record Form). Attendees will be given a workbook and data so they can easily complete the scenario proposed.

Posters

Poster 1: Exertion of Malaria Prophylaxis by Bioactive Constituents of a Medicinal Apocynaceae Plant.

Presenter: **Dr Chidiebere Otuu**, *Federal University Oye-Ekiti*

CA Otuu[1]; RN Obiezue[2]; SS Eke[3]; H Usman-Yamman[4]; IC Ekuma[5]; EO Udeh[6]; AA Otuu[7];
[1] University of Nigeria, Nsukka, Enugu State, Nigeria; [2] University of Nigeria Nsukka, Enugu State, Nigeria; [3] Airforce Institute of Technology Kaduna, Kaduna State, Nigeria; [4] Newgate University Minna, Niger State, Nigeria; [5] Alex Ekwueme Federal University Teaching Hospital Abakaliki, Ebonyi State, Nigeria; [6] Center for Integrated Health Programs, Abuja, Nigeria; [7] West African Postgraduate College of Pharmacy, Yaba, Lagos State, Nigeria

One of the strategies employed in malaria control and possible elimination efforts is the prevention of establishment of infection of the malaria parasite using prophylactic antimalarial drugs. This strategy has been negatively affected with the development of resistance of the malaria parasite to the currently used antimalarial drugs. This development has increased the urgent need for new antimalarial compounds with prophylactic properties against malaria. This study was thus carried out to evaluate the malaria prophylactic potential of bioactive compounds from extracts of *Alstonia boonei*, a medicinal plant used locally for malaria treatment in Nigeria and other African countries. The bioactive compounds were identified by Gas Chromatography – Mass Spectrometer analysis technique and then subjected to antimalarial tests to determine their prophylactic activity in mice experimentally infected with *Plasmodium berghei*. Results from the tests showed that bioactive compounds possess significant dose – dependent prophylactic property at $p < 0.05$ at the doses used. From these findings it was concluded that the plant is a potential source of prophylactic antimalarial molecules and should be further researched on for antimalarial drug development.
Keywords: Exertion, malaria, prophylaxis, bioactive constituents, drug development

Poster 2: Detection of zoonotical microsporidia *Encephalitozoon intestinalis* in giant anteater *Myrmecophaga tridactyla* (LINNAEUS, 1758) from Brazil.

Presenter: **Mrs Beatriz Carvalho**, *Universidade Paulista - UNIP*

BR Carvalho[1]; RS Araújo[2]; D Kluyber[3]; BL Araújo[4]; MA Lallo[4];
[1] Universidade Paulista - UNIP, ; [2] CETESB-Companhia Ambiental do Estado de São Paulo, Brazil; [3] 3ICAS - nstituto de Conservação de Animais Silvestres, Brazil; [4] Programa de Patologia Ambiental e Experimental, Universidade Paulista (UNIP), Brazil

Microsporidia are opportunistic, emergent fungi that infect an exceptionally diverse group of hosts, including vertebrates and invertebrates. Among the more than 1,500 species identified, 17 were described as potential zoonoses in immunosuppressed and/or immunocompetent vertebrates. These pathogens have been identified in several domestic and wild mammals, however they have not yet been described in individuals of the order Xenarthra. This order of mammals includes animals that harbor and transmit various pathogens, acting as important sources of infection for the spread of some zoonoses. The aim of this study was to analyze the occurrence of zoonotic microsporidia *Encephalitozoon cuniculi, E. intestinalis,* and *Enterocytozoon bieneusi* in the feces of giant armadillos (*Priodontes maximus*) and giant anteaters (*Myrmecophaga tridactyla*) monitored on highways by the Giant Armadillo Conservation Program . Forty-four fecal samples were subjected to thermal shock (cold and heat) and then subjected to DNA extraction with the QIAamp fast DNA stool mini kit (Qiagen), according to the manufacturer's recommendations. The DNA was amplified by polymerase chain reaction (PCR) with generic primers and the product generated from this reaction was submitted to Nested PCR with specific primers: EBIEF1/EBIER1 for *E. bieneusi*; ECUNF/ECUNR for *E. cuniculi* and SINTF/SINTR for *E. intestinalis*. Of the 5 samples collected from *Priodontes maximus* - giant armadillo, none was positive for microsporidia. However, of the 38 samples of *Myrmecophaga tridactyla* - giant anteater analyzed, one was positive for *E. intestinalis*, one of the most described zoonotic species of microporidioses. It was an anteater cub, male and with no report of diarrhea, corresponding to the first report of microsporidiosis by *E. intestinalis* in a giant anteater.

Poster 3: Prevalence of gastrointestinal helminth parasites of trade cattle in Aguata and Orumba South Local Government Areas, Southeastern Nigeria.

Presenter: **Dr Chukwunonso Obi**, *University of Nigeria, Nsukka*

CF Obi[1]; OJ Ezubelu[2]; MC Akata[2];
[1] University of Nigeria, Nsukka Nigeria, Nigeria; [2] Federal College of Education (Technical), Umunze, Nigeria

Gastrointestinal helminth parasites (GHPs) constitute a major impediment to livestock production in the tropics. The prevalence of gastrointestinal helminth parasites of trade cattle were investigated in Aguata and Orumba South Local Government Areas (LGA), Southeastern Nigeria. Fecal samples were collected per rectum from 210 randomly selected cattle [Aguata LGA (n=140) and Orumba South LGA (n=70)] over a three-month period. The sex, breed and body condition scores of the cattle were noted. The samples were individually subjected to floatation and sedimentation techniques. The overall prevalence of GHPs was 57.6% (95% CI = 0.509–0.643). Of the 140 cattle screened at Aguata LGA, 74 (52.9%; 95% CI = 0.446–0.609) were positive for gastrointestinal helminth ova while 47 (67.1%; 95% CI = 0.555–0.77) were positive out of the 70 cattle screened at Orumba South LGA. A variety of gastrointestinal helminth ova were detected including strongyles,
strongyloids, *Toxocara*, *Fasciola*, *Schistosoma*, *Moniezia* and *Paramphistomum* ova. Four zoonotic helminth ova were detected in the study area. Strongyle eggs were the most prevalent eggs detected in single infections followed by *Fasciola* eggs. Mixed infections were more common than single infection. Body condition score was significantly associated ($p<0.0001$) with the prevalence of GHPs of cattle in the study area while sex and breed were not. It was therefore concluded that trade cattle in Aguata and Orumba South LGAs, Southeastern Nigeria were affected by variety of GHPs including zoonotic helminths. Thus, routine anthelmintic treatment, good management practices and public enlightenment on the zoonotic importance of GHPs is highly essential.

Poster 4: Molecular identification of *Acanthamoeba*, *Naegleria fowleri* from various water sources, Egypt.

Presenter: **Dr Rana Elsedawy** , *Faculty of Veterinary Medicine*

R Elseadawy[1]; I Abbas[1]; M Al-Araby[1]; S Abu-Elwafa[1];
[1] Faculty of Veterinary Medicine, Mansoura University, Egypt

In order to identify the prevalence and different genotypes of two neglected waterborne protists (*Acanthamoeba* and *Naegleria fowleri*) in water samples from various sources in Dakahlia governorate, PCR-testing in conjunction with isolate sequencing was carried out. Out of 62 protozoan-suspected samples using microscopic examination, *Acanthamoeba* were molecularly confirmed in 24 (38.7%) samples from various sources including tap water. In addition, *Naegleria* spp. were detected in 6 (9.6%) samples from the Nile, two of them (3.2%) were identified as *N. fowleri*. Sequencing of 20 samples of *Acanthamoeba* was successful; 18 were assigned the genotype T3 and two the T4. Results from the current

study were integrated with those from past surveys conducted in Egypt for analysis. *Acanthamoeba* showed the highest mean prevalence (43.03%) with insignificant variations among various water sources. The incidence of finished water in drinking water treatment plants had lower than the raw water, but the filtration efficiency has not exceeded 60.0%. Different genotypes of Acanthamoeba were found with the T4 (the highly pathogenic type) was the most significantly identified type. A common T4 haplotype was found circulating in water from Egypt as well as 3 other countries (Tanzania, Rwanda, Uganda) located on the Nile basin. This haplotype included isolates from keratitis-infected patients, which confirms the potential role of water in the epidemiology of AK infecting humans in these countries. The Nile water has the greatest estimated mean prevalence of *Naegleria* spp. at 23.79%. In the current study, occurrence of potentially pathogenic protists with a neglected status, has been confirmed in water from Egypt, which should prompt the authorities to revise the protocols for controlling these pathogens in water.
Keywords: *Acanthamoeba*; *Naegleria fowleri*; Genotype; Water; the Nile; Egypt.

Poster 5: Assessing Dose-Exposure-Response Relationships of Miltefosine in Adults and Children Using Physiologically-Based Pharmacokinetic Modelling Approach.

Presenter: **Mr Shadrack Madu**, *De Montfort University*

SJ Madu[1]; K Wang[1]; SK Chirumamilla[2]; DB Turner[2]; PG Steel[3]; **M Li**[1];
[1] De Montfort University, UK; [2] Certara UK Limited, Simcyp Division, Sheffield S1 2BJ, UK; [3] Durham University, UK

Objectives: Miltefosine is the first and only oral medication to be successfully utilized as an antileishmanial agent. However, the drug is associated with differences in exposure patterns and cure rates among different population groups e.g. ethnicity and age (i.e., children v adults) in clinical trials. In this work, mechanistic population-based PBPK models have been developed to study the dose-exposure-response relationship of miltefosine in *in silico* clinical trials and evaluate the differences in population groups, particularly children and adults.

Methods: The Simcyp population pharmacokinetics platform was employed to predict miltefosine exposure in plasma and peripheral blood mononuclear cells (PBMCs) in a virtual population under different dosing regimens. The cure rate of a simulation was based on the percentage of number of the individual virtual subjects with $AUC_{d0\text{-}28}$> 535 µg×day/mL in the virtual population.

Results: It is shown that both adult and paediatric PBPK models of miltefosine can be developed to predict the PK data of the clinical trials accurately. There was no significant difference in the predicted dose-exposure-response of the miltefosine treatment for different simulated ethnicities under the same dose regime and the dose-selection strategies determined the clinical outcome of the miltefosine treatment. A lower cure rate of the miltefosine treatment in paediatrics was predicted because a lower exposure of miltefosine was simulated in virtual paediatric in comparison with adult virtual populations when they received the same dose of the treatment.

Conclusions: The mechanistic PBPK model suggested that the higher fraction of unbound miltefosine in plasma was responsible for a higher probability of failure in paediatrics because of the difference in the distribution of plasma proteins between adults and paediatrics. The developed PBPK models could be used to determine an optimal miltefosine dose regime in future clinical trials.

Poster 6: New models of heartworm and their application in preventative drug research.

Presenter: **Miss Jessica Dagley**, *Liverpool School of Tropical Medicine*

J Dagley[1]; S Hegde[1]; AE Marriott[1]; A Steven[1]; C Fricks[3]; U DiCosty[3]; A Mansour[3]; EJ Campbell[2]; CM Wilson[2]; SA Ward[1]; A Moorhead[2]; S McCall[3]; JW McCall[2]; MJ Taylor[1]; JD Turner[1];

[1] Liverpool School of Tropical Medicine, UK; [2] University of Georgia, United States; [3] TRS Laboratories, UK

Dirofilaria immitis is a filarial nematode causing potentially lethal veterinary heartworm disease in cats and dogs. *Dirofilaria* spp can also cause zoonotic infections and pathologies in humans. Currently, control of heartworm disease relies on chemoprophylaxis with the macrocyclic lactones (ML), however, ML-resistant isolates are increasing and resulting in preventative treatment failure. Heartworm drug research relies on long-term experimental cat and dog studies, with the potential to cause severe welfare issues. Thus, there is a need for alternative refined heartworm models for rapid pre-clinical research to reduce cat and dog experimentation. We discovered lymphopenic mouse strains with ablation of the interleukin-2/7 common gamma chain (NSG/NXG) are susceptible to larval infections of *D. immitis*. Larvae at 2-5 weeks post-infection were retrieved from subcutaneous and muscle tissues and were morphologically representative of the L4 stage in dogs. Mice did not display any clinical signs of infection. Using single subcutaneous injections of moxidectin, we identified 60-88% reductions in parasites 14-28 days post-infection. Oral oxfendazole

dosing on day 1 and 28 also yielded 90% reductions in parasite yields on day 35. Using seven-day oral doxycycline, we demonstrated a 70-90% *Wolbachia* depletion within *D. immitis* L4 in our mouse model. Furthermore, we validated two additional mouse strains as being susceptible to *D. immitis* infection, C.B-17 SCID and Rag2/Il2rg, improving the commercial availability of mice as heartworm models and working towards the reduction and replacement of cats and dogs in heartworm preventative drug research.

Poster 7: Assessment of use of anthelmintic drugs in Bureti sub county, Kericho County in Kenya.

Presenter: **Dr Nathan Kosgei**, *Gene plus, Veterinary science research institute*

N Kosgei[1];
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the population compared to 3 % that used herbal drugs. Extensive, paddock / free range system is still dominantly practiced compared to semi intensive and intensive systems.

Poster 8: Exploring the Dual Role of Dihydroorotate Dehydrogenase in *Toxoplasma gondii*: Beyond Pyrimidine Biosynthesis.

Presenter: **Miss Carolina Urbina Camacho**, *Universidad de los Andes*

C Urbina Camacho[1]; C Barrera Grijalba[1]; BH Zimmermann[1];
[1] Departamento de Ciencias Biologicas, Universidad de los Andes, Bogotá D.C., Colombia

Toxoplasmosis is considered by the Centers of Disease Control and Prevention to be a neglected parasitic disease (Ben-Harari and Connolly, 2019) because of low awareness and underestimation among healthcare professionals, despite high worldwide prevalence of the causative agent, *Toxoplasma gondii*. Limited treatment is available for immunocompromised patients, pregnant women, and those with congenital or chronic infections. In recent years there has been a re-emergence of toxoplasmosis, driven by the increase of drug-resistant strains that can affect immunocompetent individuals and lead to complications including abortions in pregnant women (Montazeri *et al*., 2018), suggesting the infection can pose a threat to a broader population and prompting the need for new anti-infective strategies. The pyrimidine biosynthesis pathway has been previously used as molecular target against related parasites, including *Plasmodium falciparum*. In *T. gondii*, knockouts of the first, fifth or sixth enzymes of this pathway result in uracil-auxotrophic parasites, while elimination of the fourth enzyme (dihydroorotate dehydrogenase, DHODH) results in lack of growth regardless of uracil availability. Replacing DHODH with a catalytically inactive variant restores the auxotrophic phenotype, indicating an additional, unknown role for TgDHODH (Hortua Triana *et al*., 2016). The objective of our project was to explore whether TgDHODH interacts with other proteins in the parasite mitochondrion, since such interactions might provide clues about its pyrimidine-independent function. In humans, DHODH knockouts result in loss of mitochondrial membrane integrity, and the enzyme appears to interact with respiratory complexes I and II (Fang *et al*., 2013). We conducted Blue Native Gel Electrophoresis (BN-PAGE) coupled with in-gel activity assays for DHODH using mitochondrial extracts from tachyzoites, as well as 2D-SDS-PAGE followed by TgDHODH detection using Western Blot with antibodies raised against a recombinantly produced, N-terminally truncated TgDHODH. TgDHODH activity or antibody reaction were observed in bands migrating at much higher molecular weight than expected for the protein alone, both in BN-PAGE and 2D-SDS-PAGE, indicating that the

protein could be interacting with one or more proteins in stable complexes. Two high molecular weight bands, both migrating near 700 kDa, were excised and prepared for mass spectrometry analysis. In addition to the TgDHODH domains homologous to class 2 DHODHs from other organisms, TgDHODH possesses a mitochondrial targeting sequence in an unusually long 157 residue N-terminal extension. Previous work shows that this sequence is proteolytically removed during import, producing a mature enzyme of ~48 kDa (Hortua Triana *et al.*, 2012). Extended N-terminal mitochondrial targeting sequences have occasionally been linked to independent functions of the corresponding targeting peptides, which are cleaved from the rest of the protein once this reaches its intended location. We planned to produce the extended 157 residue N-terminal peptide of TgDHODH as a recombinant protein to allow the production of antibodies that could be used to screen for its presence in *T. gondii* mitochondrial complexes. In our preliminary attempts at expression, we were able to detect this recombinant peptide in cell extracts with antihis-tag antibodies, however expression levels were very low, possibly due to the presence of predicted disordered sequences which may have made the sequence susceptible to proteolysis. We plan to express a shorter peptide, encompassing the first 113 residues, to eliminate most of the disordered region. This research was funded by Banco de la República Fundación para la Promoción de la Investigación y la Tecnología #4499, and by the Facultad de Ciencias Universidad de los Andes projects INV-2019-84-1846 and INV-2021-127-2302.

Poster 9: Prevalence of intestinal parasites and associated risk factors in El-Shokria village Al-Gazira state

Presenter: **Mr Ahmed Hassabo**, *Sudan*

Intestinal parasitic infection represents a major health problem in sub-Saharan Africa as the prevalence count in the region is as high as 90% in central Sudan with socioeconomic consequences therefore the study aimed to assess the species, prevalence of intestinal parasite and the associated risk factor in a representative sample in El-Shokria village at Al Gazira state. This is a cross-sectional study: Stool samples were collected from 398 villagers. Study subjects were selected using random sampling method. Data were gathered through direct interview using a pretested questionnaire. The collected stool samples were examined microscopically for the presence of the egg, cyst, larvae and trophozoites of intestinal parasites using direct saline smear and formal ether concentration methods. Data entry and analysis were done using SPSS program. Out of the 398 study subjects, 194 (48.7%) were found to be infected with intestinal parasites. Most of the cases were single infection 160 (82.4%) and only 24 (12.3%) were double infection

Giardia lamblia with *Hymenolepis nana*. The prevalence of intestinal parasites was high in age group 0-12 years compared to other age groups. The predominant intestinal parasite was *Entamoeba histolytica* (47.4%) followed by *Giardia lamblia* (44.35), *Hymenolepis nana* (18.5%) and *Enterobius vermicularis* (2.0%). This study suggests intensive health education on personal hygiene and environmental sanitation is needed. Implementing of the formal ether technique in the diagnostic lab will increase sensitivity.

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