

1) A novel facile non-invasive method for diagnosis of *Onchocerca volvulus* antigen in human urine samples

LUM AMBE^{1,2,*}, ELISABETH LIMUNGA¹, CLARISSE MBAH², NGWEWONDO ADELA², NDUMU ERIC¹, MARTHA NGOE¹, BERTRAND SONE¹, GÜNTER LOCHNIT³, JULIUS TACHU¹, SAMUEL WANJI⁴, ANJA TAUBERT⁵, CARLOS HERMOSILLA⁵ AND FAUSTIN KAMENA^{1,*}

¹LABORATORY FOR MOLECULAR PARASITOLOGY, DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY, UNIVERSITY OF BUEA, BUEA P.O. BOX 63, CAMEROON · ²CENTRE FOR RESEARCH ON HEALTH AND PRIORITY PATHOLOGIES, INSTITUTE OF MEDICAL RESEARCH AND MEDICINAL PLANTS STUDIES (IMPM), YAOUNDE, P.O. BOX 13033, CAMEROON · ³PROTEIN ANALYTICS, INSTITUTE OF BIOCHEMISTRY, FACULTY OF MEDICINE, JUSTUS LIEBIG UNIVERSITY GIESSEN, 35392 GIESSEN GERMANY · ⁴DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY, FACULTY OF SCIENCE, UNIVERSITY OF BUEA, BUEA, P.O. BOX 63, CAMEROON · ⁵BIOMEDICAL RESEARCH CENTER SELTERSBERG (BFS), INSTITUTE OF PARASITOLOGY, JUSTUS LIEBIG UNIVERSITY GIESSEN, 35392 GIESSEN, GERMANY

Despite several decades of mass drug administration and elimination-related activities, human onchocerciasis still represents a major parasitic threat in endemic regions. Among the challenges encountered by the elimination program is the lack of a suitable diagnostic tool that is accurate and non-invasive. Currently used methods are either invasive or not suitable for monitoring large numbers of patients. Herein, we describe the identification and characterization of *Onchocerca volvulus* heat shock protein 70 (OvHSP70) as a novel diagnostic biomarker for human onchocerciasis, which can directly be detected in urine samples of infected patients. This nematode-specific antigen was identified through LC-MS after differential SDS-PAGE using urine-derived protein extracts from *O. volvulus*-infected patients in Cameroon. Polyclonal antibodies generated in rabbits after cloning and expression of OvHSP70 in *Escherichia coli* reliably differentiated between urine samples from infected- and uninfected patients in a hypoendemic area of human onchocerciasis. These results provide an excellent basis for further development of a non-invasive and scalable diagnostic assay for human onchocerciasis using urine samples. Such a urine-based diagnostic assay will be of major importance for the elimination program of human onchocerciasis in endemic countries.

2) Utility of Recombinant *Schistosoma bovis* 22.6 kDa Antigen for Diagnosis of Human Urogenital Schistosomiasis by Enzyme-Linked Immunosorbent Assay

¹ANUMUDU, C.I., ¹OLA, A.B., ²EFENOVWE, M., ¹AWOBODE, O., ¹EBUH, R., ¹AKPABIO C., ¹ANTHONY, C., ²ADEKEYE, T.A., ²AWOBODE H.O., ^{3,4}TRELIS, M., ^{3,4}MARCILLA, A., ⁵OLEAGA, A.

¹CELLULAR PARASITOLOGY PROGRAMME, DEPARTMENT OF ZOOLOGY, UNIVERSITY OF IBADAN, NIGERIA; ²PARASITOLOGY UNIT, DEPARTMENT OF ZOOLOGY, UNIVERSITY OF IBADAN, NIGERIA; ³ PARASITES AND HEALTH RESEARCH GROUP, DEPARTMENT OF PHARMACY AND PHARMACEUTICAL TECHNOLOGY AND PARASITOLOGY, FACULTY OF PHARMACY, UNIVERSITÄT DE VALENCIA, SPAIN; ⁴JOINT UNIT OF ENDOCRINOLOGY, NUTRITION AND CLINIC DIETETICS, IIS LA FE, VALENCIA, SPAIN; ⁵ANIMAL PARASITOLOGY, INSTITUTE OF NATURAL RESOURCES AND AGROBIOLOGY, SPANISH NATIONAL RESEARCH COUNCIL (IRNASA, CSIC), SALAMANCA, SPAIN

In tropical and subtropical regions, flatworms of the genus *Schistosoma* cause schistosomiasis, a severe medical and veterinary disease. Hybridization between the human (*Schistosoma haematobium*) and animal (*Schistosoma bovis*) schistosome is a topic of major importance for global health and disease control. *S. haematobium* and *S. bovis* hybrids exhibit zoonotic potential and human infection risk. *S. bovis* antigens have been used in seroepidemiological surveillance of ruminant livestock in connection to a schistosomiasis outbreak in Europe. We here investigated the effectiveness of an enzyme-linked immunosorbent assay diagnostic based on the recombinant *S. bovis* antigens (rSb 22.6 kDa) for human urogenital schistosomiasis in Ogun State, Nigeria. Samples from 559 individuals (290 adults and 269 children), comprising 235 males and 313 females, from Ibese, Imeko, Orile-Igbooro, Ijoun and Eggua communities, were used for this study. Urine samples were screened for anti-schistosomal antibodies by recombinant rSb22.6 kDa ELISA. Urine was examined for *S. haematobium* eggs by microscopy and urinalysis was done with chemical reagent strips. The *S. haematobium* prevalence by microscopy was 13.9%. A positive antibody response to *S. bovis* antigen was found in 299 (54.1%) individuals, of which 165 (29.8%), 265 (47.9%), and 205 (37.1%) samples had haematuria, leukocyturia and proteinuria, respectively. Diagnostic values of microscopy and rSb 22.6 kDa-ELISA differed significantly ($P < 0.05$), and there was a correlation between the data obtained from clinical findings and the ELISA test. 221 negative individuals by microscopy (39.96%), were detected as positive by the rSb 22.6 kDa-ELISA. The assay had a pooled sensitivity of 45.7%, specificity of 41.7%, accuracy of 43% in the diagnosis of urogenital schistosomiasis, indicating its potential as a diagnostic tool for urogenital schistosomiasis caused by *S. haematobium*.

3) The impact of regenerative grazing strategies on defence against nematode infection in sheep

PHOEBE A.C. BEAL^{1,2}, FIONA KENYON¹, JADE M. DUNCAN¹, YOLANDA CORRIPIO-MIYAR¹ AND ADAM D. HAYWARD¹

¹ MOREDUN RESEARCH INSTITUTE; ² THE UNIVERSITY OF EDINBURGH

Productivity loss caused by gastrointestinal nematodes (GIN) is a major problem in the livestock industry. Management of GIN has relied on anthelmintic drugs, but the evolution of anthelmintic resistance means that this is unsustainable. Consequently, there has been a shift towards control strategies that rely on boosting the natural defences of the animals, including regenerative grazing strategies that can reduce parasite exposure and improve nutritional status. The impacts of such strategies on resistance (reducing GIN through immune-mediated killing) and tolerance (maintaining performance in the face of increasing GIN burden) of infection are, however, unknown. We explored how two regenerative grazing strategies impact cell-mediated and humoral immune responses, GIN burden, and tolerance of infection in grazing lambs. 120 weaned lambs were maintained under 4 treatments in a 2x2 design. Lambs were kept on one of two 'pasture' treatments, either grazing on traditional grass, or 'improved' pasture containing bioactive plant species. They were also kept on one of two 'grazing' treatments: lambs were either kept on the same pasture throughout the season (set-stocked) or rotationally-grazed around a paddock of the same area. Every two weeks for 12 weeks, we monitored weight gain, GIN faecal egg count, and immunological parameters including GIN-specific antibody responses and cytokine levels to estimate productivity and defence against infection. Faecal samples were also analysed for intestinal microbial diversity using 16S rRNA sequencing. Statistical analysis using mixed-effects models and random regression models focus on (1) estimating differences between treatment groups in productivity, resistance, and tolerance of infection; (2) estimating between-individual variation in these parameters; (3) determining how these traits are associated with humoral and T cell-mediated immune responses. These results will provide the first quantitative insight into how regenerative grazing impacts defence against infection in ruminant livestock and how it can mitigate the impact of GIN.

4) *Hpb* regulates epithelial cellular junction

SHIRA BEN-SIMON¹, IRAH L. KING^{2,3,4}, DANIELLE KARO-ATAR⁵

¹ AZRIELI FACULTY OF MEDICINE, BAR-ILAN UNIVERSITY, SAFED, ISRAEL; ² DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY, MEAKINS-CHRISTIE LABORATORIES, RESEARCH INSTITUTE OF MCGILL UNIVERSITY HEALTH CENTRE, MONTREAL, QUEBEC, CANADA; ³ MCGILL INTERDISCIPLINARY INITIATIVE IN INFECTION AND IMMUNITY, MONTREAL, QUEBEC, CANADA; ⁴ MCGILL REGENERATIVE MEDICINE NETWORK, MONTREAL, QUEBEC, CANADA; ⁵ DEPARTMENT OF PHARMACOLOGY AND CLINICAL BIOCHEMISTRY, BEN-GURION UNIVERSITY OF THE NEGEV, BEER-SHEVA, ISRAEL

Enteric helminths are a unique example of highly invasive yet truly tolerable parasites, promoting mechanism of host defense favoring tissue adaptation and repair over parasite expulsion. These intestinal worms form intimate connections with the intestinal epithelium, a frontline effector of barrier immunity and integrity. The epithelium is one of the most highly regenerative tissues in our body thanks to the function of intestinal stem cells (ISC). Multipotent ISC undergo extensive transcriptional reprogramming in response to injury to promote rapid regeneration of the tissue and maintain barrier integrity. We have recently identified the expansion of an intestinal stem cell population, named revival stem cells (revSC), following infection with the enteric parasitic nematode *Heligmosomoides polygyrus bakeri* (*Hpb*). RevSC are a damage-induced, fetal-like population that is critical for the regeneration of the gut following diverse insults. Additionally, we showed counter-regulation of the intestinal stem cell compartment by helminth secreted proteins and the host type 2 immune response, a critical effector of host resistance to helminth infection. This tug-of-war between helminths and type 2 immunity suggest a possible mechanism promoting the chronicity of infection while enabling tissue recovery. Disease tolerance is a defense strategy to infectious disease that is aimed to prioritize tissue damage control over pathogen load. While this strategy promotes host fitness and survival, it also leads to chronic infection. Thus, in this work we hypothesize that *helminths promote the regenerative capacity of the intestine to subvert host resistance yet maintain disease tolerance*. Our preliminary results identified aberrant expression of cellular junctions including tight and gap junctions as well as integrins and wound-healing associated genes in *Hpb*-stimulated small intestinal organoids. This data indicate that *Hpb* regulates cell-cell interactions in the ISC compartment and suggest that helminths might directly regulate barrier integrity as a mechanism of disease tolerance.

5) Control of Schistosomiasis Soil Transmitted Helminths and Trachoma in Ethiopia - A comprehensive community and government intervention model

ZVI BENTWICH^{1,2}, MICHAL BRUCK ¹, and RACHEL GOLAN^{1,2}

NALA ¹& BEN-GURION UNIVERSITY of the Negev, ISRAEL, ², ISRAEL

Schistosomiasis, soil transmitted helminths (STH) and Trachoma, are still major public health challenges in Ethiopia and other developing countries. Starting in 2008 we developed a comprehensive model of intervention, for schistosomiasis, and later for STH and Trachoma, covering most parts of Ethiopia. The main objectives of this model were to attain the commitment of the community, the school system and the health authorities, in reaching health behavioral change, improved inter-sectorial coordination and cooperation between all stakeholders. Fifteen years later, when evaluating the outcomes of the schistosomiasis intervention, we found that infection rate in the city of Mekele where we started our program, decreased by more than 90% and remained below 2%, correlating with changes in knowledge and behavior of the school children, and reflecting adoption of improved health behaviors. In all other regions, where this approach and model have been applied, similar results were obtained with all three major diseases. Since inter-sectorial coordination, is a major factor determining the success of the intervention we developed a toolkit for inter-sectorial coordination that has now been adopted by the Federal Ministry of Health and applied throughout the country. Additional targets of our program were-a) preschool children as messengers of behavioral change b) the adult poorly educated population especially in less developed environments c) the control and provision accessible water through the Wash on Wheels program. The overall results obtained from the intervention, demonstrate the long-sustained success of the intervention model, combining behavioral change with community commitment, improved sanitation, intersectoral coordination and better control of etiologic factors of disease, for control and elimination of schistosomiasis STH and Trachoma. These results may form the basis for similar programs that may be applied in all other developing countries having similar problems.

6) Antibody neutralisation of an extracellular parasitic Argonaute protein blocks cell uptake and serves as a vaccine strategy against nematode infection

KYRIAKI NEOPHYTOU¹, ISAAC MARTÍNEZ-UGALDE², JOSE R BERMÚDEZ-BARRIENTOS¹, ELAINE ROBERTSON¹, YVONNE HARCUS¹, CHANEL NAAR¹, RUBY WHITE¹, RICK MAIZELS³, RAFFI AROIAN⁴, DAN PRICE⁵, MIKE J EVANS¹, ALASDAIR J. NISBET⁵, CEI ABREU-GOODGER²,
AMY H BUCK¹ *

1 INSTITUTE OF IMMUNOLOGY AND INFECTION RESEARCH, SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF EDINBURGH, EH9 3FL, UK; 2 INSTITUTE OF ECOLOGY AND EVOLUTION, SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF EDINBURGH, EH9 3FL, UK; 3 SCHOOL OF INFECTION & IMMUNITY, UNIVERSITY OF GLASGOW, UK; UMASS CHAN MEDICAL SCHOOL, PROGRAM IN MOLECULAR MEDICINE, WORCESTER, MA 01604, UNITED STATES; 5 DEPARTMENT OF VACCINES AND DIAGNOSTICS, MOREDUN RESEARCH INSTITUTE, EDINBURGH, EH26 0PZ, UK

Argonautes are ancient proteins with well characterised roles in gene regulation and genome defence but with poorly understood functions outside of cells. Extracellular Argonautes have been identified across plant and animal species but their composition within or outside of extracellular vesicles and capacity for trafficking to other cells is not clear. Nematodes have evolved a suite of Argonaute proteins and we previously identified one specific extracellular Argonaute, termed “exWAGO”, that is highly conserved in parasitic nematodes. Here we use the rodent-infective model *Heligmosomoides bakeri* to show definitively that parasites release two distinct forms of exWAGO bound to small RNAs (sRNAs) derived from different TE families. The non-vesicular form is more abundant than the vesicular form, and is selectively internalised by epithelial cells *in vitro*. Administration of recombinant exWAGO as a vaccine confers partial protection against subsequent infection with *H. bakeri* larvae and generates antibodies that block exWAGO uptake into cells. Finally, we show that exWAGO is secreted across Clade V nematodes infecting humans and livestock where it shows conserved properties of binding 22-23G sRNAs.

7) High molecular weight DNA extraction for genome assembly in filarial nematodes

LINDSEY CANTIN¹, JEREMY FOSTER¹

¹BIOCHEMISTRY AND MICROBIOLOGY DIVISION, NEW ENGLAND BIOLABS, IPSWICH, MA, UNITED STATES

Filariasis is a disease caused by parasitic nematodes, which can infect a variety of reptiles, birds, and mammals including humans, leading to significant morbidity. High quality reference genomes are valuable tools to study the biology and evolution of these worms and can help elucidate mechanisms of drug resistance, pathogenesis and identification of new drug targets. Genome assembly is drastically improved using long read sequencing technologies but quality high molecular weight (HMW) DNA can be difficult to obtain using standard methods, due to undigestible fibrous cuticle proteins. Here, we present a novel HMW DNA extraction method in *Dirofilaria immitis* (*D. immitis*) by incorporating a nuclei isolation step prior to whole cell lysis. Intact DNA is protected by the nuclear membrane while difficult to digest proteins and cell debris are removed through filtration. HMW *D. immitis* DNA extracted with a standard sodium dodecyl sulphate lysis buffer and DNA extracted using the nuclei isolation protocol were sequenced on individual Oxford Nanopore (ONT) flow cells using the Ultra Long DNA sequencing kit. Reads were assembled into separate assemblies using Canu. The N50 of the sequencing reads was 81 kb for the nuclei method, with many reads over 1 Mb, compared to 69 kb for the standard method, with no reads over 1 Mb. The resulting nuclei genome assembly had a contig N50 of 13 Mb, with 32 total contigs. The standard method had a contig N50 of 8.4 Mbs and 63 contigs. Both assemblies contained complete *Wolbachia* and mitochondrial chromosomes. The nuclei isolation method run on a single ONT flow cell resulted in a more accurate and complete genome compared to recently published *D. immitis* genomes that used standard DNA extraction and sequencing methods. These results show the importance of the DNA extraction method for genome sequencing and assembly of nematodes.

8) Developing a medium-throughput screen for juvenile *Schistosoma mansoni*

KAITLYN COTTON¹, SARAH COBB¹, JAMES J. COLLINS III¹

¹DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER, USA

Schistosomiasis infects over 240 million individuals globally, causing severe morbidity and up to 200,000 deaths annually. Despite this, praziquantel (PZQ) remains the only available drug to treat schistosomiasis. PZQ has some significant drawbacks, most notably, PZQ has limited efficacy against juvenile schistosomes, highlighting the need for novel anthelmintic drugs to treat schistosomiasis. To address this issue, we have developed a medium-throughput screen capable of testing tens of thousands of compounds against juvenile schistosomes. In this screen, drug-treated juvenile parasites are stained with viability dyes to label live and dead cells to assess drug efficacy against juvenile worms. Using a high content imager to capture timelapse acquisitions, drug hits are identified by presence of dead cells & absence parasite mobility. Efforts are currently underway to semi-automate this process through the use of IN Carta image analysis software. Currently, around 200 compounds can be screened an hour, allowing for quick and efficient screening of compound libraries. Furthermore, this pipeline can be adapted & applied to future studies with other parasitic helminths.

9) ***Heligmosomoides polygyrus* extracellular vesicles as modulators of the host immune system and platforms for multivalent vaccines**

LI AN COWLEY¹, KYLE CUNNINGHAM¹, CLAIRE CIANCIA¹, RICK MAIZELS¹

¹WELLCOME CENTRE FOR INTEGRATIVE PARASITOLOGY, UNIVERSITY OF GLASGOW, UK

Parasites such as *Heligmosomoides polygyrus* release EVs that, *in vitro*, enter host macrophages and compromise their function, while vaccination with EVs confers protection against helminth infection in mouse models. This suggests that *H. polygyrus* EVs (or their components) can be used as an effective vaccine; we therefore investigated their effect on the immune system *in vivo*. Upon EV injection, M1-associated responses in the peritoneal cavity are reduced along with M2 markers such as Arg1 in LPMs/SPMs; together these data suggest that EVs inhibit both classical and alternative activation of peritoneal macrophages *in vivo*. Furthermore, there are indications that EVs inhibit the type 2 immune response, responsible for helminth clearance, through downregulation of the co-stimulatory molecule OX40L on peritoneal macrophages (leading to reduced Th2 activation) and downregulation of MHC-II (reducing antigen presentation). In the bone marrow, EVs downregulate the co-stimulatory molecule CD80, as opposed to OX40L, suggesting that EV action differs across organs but to the same effect. Contributions to a reduced Th2 response may also originate from the MLN where cytokines which induce other T cell subsets (IL-12, IL-6, IL-10) are upregulated, suggesting that Th1 and Treg cells outgrow Th2 cells. Furthermore, in hope of developing a pan-species vaccine, we have identified the EV surface proteins responsible for eliciting strong antibody titres and elucidated the domains most highly conserved across Clade V nematodes. Vaccination with these domains protects against *H. polygyrus* infection with varying degrees of success, with higher protection observed for antigen combinations. Thus, we have identified specific outcomes resulting from exposure to EVs along with a number of promising vaccine candidates which act synergistically. Studies are now under way to determine whether immunisation with candidate antigens is able to prevent inhibition of the Th2 response, as this may represent the mechanism of action of the vaccine.

10) Breaking boundaries: *in vitro* maintenance of *Strongyloides stercoralis* life cycle

MICHELA DEIANA¹, NATALIA TIBERTI¹, MONICA DEGANI¹, ELEONORA RIZZI¹, ELISABETTA VEZZELLI¹, SIMONE MALAGO¹, FRANCESCA TAMAROZZI¹, ZENO BISOFFI¹, CHIARA PIUBELLI¹, DORA BUONFRATE¹

¹ DEPARTMENT OF INFECTIOUS TROPICAL DISEASES AND MICROBIOLOGY, IRCCS SACRO CUORE DON CALABRIA HOSPITAL, NEGRAR, VERONA, ITALY

Laboratory maintenance of *Strongyloides stercoralis* is important to comprehensively study its biology and pathogenesis. Currently, it relies on animal models, but an *in vitro* system could represent a relevant advancement for providing clonally-derived populations at different parasite stages, thus simplifying strongyloidiasis studies. Here, we describe our steps towards the implementation of an innovative *in vitro* culturing system, employing intestinal human epithelial cells (CaCo-2) to maintain *S. stercoralis* throughout its life cycle from infective larvae (iL3) derived from patients. First, we examined a 2D *in vitro* system, coupled with a specific growth medium. Co-culturing iL3 with CaCo-2 induced phenotypical changes over time. Notably, after one month we observed elongation and thickening of the parasite body, development of intestine and shortening of the esophagus, suggesting a potential development into adult stage. However, after six months of co-culturing we did not observe any further morphological changes, so we switched to a 3D *in vitro* model, that might simulate better the *in vivo* environment. For the 3D system, we used an agarose scaffold coated with collagen, seeded with CaCo-2 cells. iL3 larvae were seeded directly onto the scaffold, and after one week resulted in the same phenotypic changes observed in the 2D system. Moreover, after one month some larvae showed maturation of genital traits and spherical structure in correspondence of oviducts. To confirm this *in vitro* progression towards the development of a parasitic female, we are currently evaluating by qPCR the expression of specific genes indicative of sexual development. If this assay will confirm the effective *in vitro* development into parasitic female worms, we will further induce *in vitro* sexual development using hormonal stimuli.

11) *In vitro* antischistosomal activity of some medicinal plant extracts against *Schistosoma mansoni* parasites

DONGMO NMS¹, ITOE SU¹, ITOE FA¹, NTIECHE D², TSOUH FPV⁴, NOUBISSI PA³, FEKAM BF²

¹UNIVERSITY OF BUEA, FACULTY OF SCIENCE, DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY, PO BOX: 63 BUEA, CAMEROON, ²UNIVERSITY OF YAOUNDÉ 1, FACULTY OF SCIENCE, DEPARTMENT OF BIOCHEMISTRY, LABORATORY FOR PHYTOBIOCHEMISTRY AND MEDICINAL PLANTS STUDIES, PO BOX: 812 YAOUNDE, ANTIMICROBIAL AND BIOCONTROL AGENTS UNIT, CAMEROON, ³UNIVERSITY OF BUEA, FACULTY OF SCIENCE, DEPARTMENT OF ANIMAL BIOLOGY AND CONSERVATION, PO BOX 63: BUEA, CAMEROON, ⁴UNIVERSITY OF BAMENDA, FACULTY OF SCIENCE, DEPARTMENT OF BIOCHEMISTRY PO BOX: 39 BAMBILI

Schistosomiasis is a chronic disease that affects millions of people around the world particularly low-income countries, with over 90% of the total schistosomiasis cases resulting to high rates of morbidity occurring in Africa. Previous studies reported anti-schistosomal activity of several medicinal plant extracts against *S. mansoni*, providing a basis for the use of traditional herbal remedies to treat symptoms of the disease. This study evaluates the cercaricidal, antioxidant, anti-inflammatory activities as well as the cytotoxicity and phytochemical composition of avocado, turmeric and garlic extracts against *S. mansoni*. Cercaricidal tests revealed significantly high anti-cercariae activity of plant extracts even at sublethal concentrations with LC₅₀ values ranging from 7.671 to 56.8 µg/mL. Results from antioxidant activity of plant extracts revealed that methanol extracts of avocado bark and turmeric have the most potent antioxidant scavenging activities, with SC50 values ranging from 91.47 ± 5.83 to 138.75 ± 5.73 µg/mL. Results from anti-inflammatory analysis showed that plant extracts exhibited potent anti-inflammatory activity by inhibiting protein denaturation. At test concentration of 125 µg/mL, garlic, turmeric, avocado root, bark, stem and leaves extracts showed 33.89 ± 2.21, 108.26 ± 5.18, 93.37 ± 1.47, 108.18 ± 3.49, 93.96 ± 1.25 and 118.15 ± 8.37 % inhibitions respectively while the standard Diclofenac sodium produced a 24.67 ± 0.01 % inhibition. Plant extracts also showed low toxicity on kidney Vero cells and murine macrophage cell lines with mean inhibitory concentrations (IC₅₀) ranging between 132.15 and 549.3 µg/mL. Analysis of the phytochemical composition of plant extracts showed high amounts of saponins, flavonoids, cardiac glycosides and alkaloids which can be associated with the high antioxidant scavenging activity of plant extracts. These results indicate the efficacy of medicinal plant used as traditional remedies in disease endemic regions in combating the devastating effect of *S. mansoni* infection.

12) Establishing an *in vitro* culture method and genetic approaches to investigate the infection biology of the parasitic nematode *Teladorsagia circumcincta*

SAMUEL DUNCAN¹, ROBERT BRINZER¹, DANIEL PRICE², THOMAS MCNEILLY², COLLETTE BRITTON¹

¹ THE UNIVERSITY OF GLASGOW, SCHOOL OF BIODIVERSITY, ONE HEALTH AND VETERINARY MEDICINE, URQUHART BUILDING, GLASGOW G61 1QH, ² MOREDUN RESEARCH INSTITUTE, PENTLANDS SCIENCE PARK, BUSH LOAN, PENICUIK, MIDLOTHIAN EH26 0PZ

The Trichostrongylid scour worms *Trichostrongylus* and *Teladorsagia* infect the gastrointestinal tract of their ruminant hosts, causing tissue damage and significant weight loss. Some livestock acquire protective immunity to scour worms, indicating that vaccination is a promising therapeutic approach. The precise mechanisms of immunity are poorly understood and development of an effective vaccine to protect livestock is needed. Effective vaccine design requires knowledge of how scour worms establish infection and mediate host-parasite interactions during infection. Ability to culture larval stages as they develop, together with the application of reverse genetics approaches such as RNA interference (RNAi) or CRISPR-Cas9 should aid vaccine design. We aim to better understand the biology of scour worms by modulating expression of genes encoding putative virulence factors and genes essential for worm development. We have developed a culture system to grow parasitic stages of *Teladorsagia circumcincta in vitro*. Infective L3 larvae were exsheathed and grown in medium formulated from egg yolk, resulting in ~30% of xL3 molting and developing to L4 stage. L4 larvae that have undergone ecdysis are viable *in vitro* for over 4 weeks, and display a significant increase in worm length. We observed sexual dimorphism of L4 stages, with the development of male or female tail morphology indicative of differentiation to late-stage L4. Initial studies using ovine abomasal organoids to co-culture *T. circumcincta* larvae will also be discussed. Using our *in vitro* larval culture system, we aim to modulate the expression of putative parasite virulence factors and determine any phenotypic effects. These initial advances in our ability to culture *T. circumcincta in vitro* hold promise for application of genetic manipulation and ultimately to elucidate the role of parasitic virulence factors in establishing infection. This knowledge will contribute to our understanding of disease progression and antigen discovery for the development of a scour worm vaccine.

13) Persistent transmission of onchocerciasis in Southwest Ethiopia, 2001 to 2021

**SINDEW FELEKE^{1,2}, SHANNON HEDTKE¹, EMILY HENDRICKSON¹, MILLICENT OPOKU¹,
WARWICK GRANT¹.**

¹DEPARTMENT OF ENVIRONMENT & GENETICS, LA TROBE UNIVERSITY, MELBOURNE,
AUSTRALIA, ²ETHIOPIAN PUBLIC HEALTH INSTITUTE (EPHI), ETHIOPIA

Onchocerciasis is a debilitating skin and eye disease transmitted by blackflies. More than 240 million people are at risk worldwide, 99% of whom are Africans. In Ethiopia, 25 million people remain at risk. Mass drug administration using ivermectin drug (MDAi) was started in 2001 in the hyper- (>40% nodule prevalence) and meso-endemic (20-40%) districts of Keffa and Sheka zones in southwest Ethiopia, and later expanded to include 285 districts. The initial MDAi was administered annually from 2001 to 2012, then biannually from 2013. According to WHO guidelines, this regime should result in interruption of transmission after 12 years. We report the 2001 to 2021 MDAi impact on transmission using data on MDAi coverage and epidemiological and entomological surveillances carried out at multiple time points. The MDAi coverage was generally well above the recommended threshold (80%), although lower coverage was reported in the beginning. Serology analysis by Ov-16 ELISA of dried blood spots from children aged 5-10 years and entomological surveillance using O-150 PCR on pools of *Simulium* blackfly heads failed to meet the WHO transmission interruption thresholds (<0.05% Ov16 ELISA and <0.1% fly pools PCR positive, respectively). In order to investigate the role of vector movement as a possible cause for this low but persistent transmission, we conducted whole genome sequencing and population genetic analysis of blackflies collected in Keffa and Sheka zones and surrounding endemic. The analysis showed movement of flies between endemic areas across a large geographical range, and implied that transmission zone boundaries defined by vector movement do not correspond to the boundaries currently used for program implementation and monitoring. The analysis thus supports the hypothesis that vector movement may contribute to the failure to interrupt transmission in some implementation districts. Furthermore, defining the true transmission zone boundaries and implementing MDAi within these boundaries is recommended.

14) Evaluating the real-world efficacy of novel helminth vaccines

OLIVIA FLEMING¹, ELAINE ROBERTSON², KYRIAKI NEOPHYTOU², ROWAN BANCROFT¹,
FRANCES BLOW², SIMON BABAYAN³, AMY PEDERSEN¹, AMY BUCK²

*INSTITUTE OF ECOLOGY AND EVOLUTION, UNIVERSITY OF EDINBURGH*¹, *INSTITUTE OF IMMUNOLOGY AND INFECTION RESEARCH, UNIVERSITY OF EDINBURGH*², *SCHOOL OF BIODIVERSITY, ONE HEALTH AND VETERINARY MEDICINE, UNIVERSITY OF GLASGOW*³

Gastrointestinal helminths are estimated to affect over 1.5 billion people and have a huge economic and social burden. Mass drug administration programmes are deployed to control soil-transmitted helminth infections in endemic regions. However, multidrug anthelmintic resistance has been evident in the veterinary field for decades and there is a clear need for new anthelmintic vaccines. These vaccines will eventually be administered to varied hosts experiencing varied environmental conditions. It is therefore of great interest to know how certain environmental variables, e.g. diet quality, could alter the efficacy of these vaccines. We intend to trial promising vaccine candidates in wild and laboratory-reared wood mice (*Apodemus sylvaticus*), which are naturally infected with *Heligmosomoides polygyrus*. We show here that *Heligmosomoides bakeri* excretory-secretory (HES) products effectively protect against *H. polygyrus* in laboratory-reared wood mice. We present our plans to trial HES, or another promising candidate, in the field. In the wild wood mouse model, providing a supplemented diet can reduce worm burdens, and reduce IgG1 antibody responses to vaccination. Hence, we intend to employ diet perturbations to reveal how these effects interact and whether diet supplementation could be provided pre-vaccination for livestock to boost vaccine efficacy (or conversely, whether this would reduce efficacy). In the same experiment, we can assess how, in response to vaccination, the assemblage of parasites infecting a host, and levels of stress hormones (corticosterone) change. Our work makes an exciting contribution towards the goal of effective helminth vaccination.

15) Identification of novel anti-schistosomal starting points within Merck KGaA's mini library

JOSEPHINE FORDE-THOMAS¹, HUW SUMMERS², PAUL REES², MARY EVANS¹, ZIADA KIWANUKA¹, SVEN LINDEMANN³, KARL HOFFMANN¹

¹ THE DEPARTMENT OF LIFE SCIENCES, ABERYSTWYTH UNIVERSITY, UK ²BIOMEDICAL ENGINEERING DEPARTMENT, SWANSEA UNIVERSITY, UK ³MERCK KGaA, DARMSTADT, GERMANY

Schistosoma mansoni is a major contributing species to the infectious human disease schistosomiasis, which affects in excess of 240 million people worldwide. A single chemotherapeutic agent, praziquantel (PZQ), is currently used for the control of this disease. However, PZQ is ineffective against juvenile worms, necessitating repeated treatment and raising concerns around the development of resistance. New drugs are urgently needed for the sustainable control of this disease and strategies to objectively assess compound suitability utilising medium- and high-throughput platforms are essential to identify new chemical starting points. As part of an open innovation initiative with Merck KGaA, we screened the 'Mini Library' (77 compounds), using 'Roboworm', a custom-built, automated high-content imaging platform that enables the objective repositioning of existing drugs and the identification of new compounds as next generation anthelmintics. We identified 6 compounds as hits (7.8% hit rate) on larval stage schistosomula at 72h. Hit compounds were then screened against adult worms using a newly developed, quantitative method of assessing worm motility. From these screens, four chemically related lipid-kinase inhibitors were identified. Target validation is underway and further characterisation of these inhibitors is on-going.

16) New onset refractory status epilepticus induced by intracerebroventricular injection of *Taenia solium* oncospheres in mice.

NCHIA GREENFIELD FUOH.

DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY, UNIVERSITY OF BUEA, CAMEROON.

Drug management of neurocysticercosis and temporal lobe epilepsy faces significant therapeutic challenges despite the substantial progress made. This study was undertaken to provide a pharmacological basis of new onset refractory status epilepticus induced by intracerebroventricular injection of *Taenia solium* oncospheres in mice. The *Taenia solium* parasite used in this research was characterized by histologic analysis and PCR. Four groups of six mice each were used. The negative control group was injected intracerebroventricularly with 50 μ L of *Taenia* oncospheres. The two positive control groups received sodium valproate (300 mg/kg) and praziquantel (50mg/kg) respectively and normal group of mice received 0.9% NaCl (10ml/kg) without parasite. Behavioural parameters were tested using the score of convulsions, the T-maze test, the Object recognition test and the Open field test to assess seizure severity and memory test. Biochemical analysis was carried out on the brain and serum. Malondialdehyde, glutathione, superoxide dismutase, and nitric oxide index were recorded to determine the level of oxidative stress. GABAergic and cholinergic neurotransmissions were addressed by measuring Gamma amino butyric acid (GABA), GABA transaminase, acetyl cholinesterase, butyryl cholinesterase and Brain-derived neurotrophic factor (BDNF). Also, inflammatory biomarkers (TNF alpha, interleukin-1 β , interleukin-6, interleukin-10, and interferon gamma) were evaluated to give an insight on inflammation. The results obtained revealed that the *Taenia solium* injection significantly increased seizures severity in mice. Treatment with sodium valproate and praziquantel definitely restored the normal state. In addition, these drugs reduced the latency to the entry into the discriminated arms of the T-maze by 25,33%, Rearing by 74,05%, faecal boli by 95,65%, Malondialdehyde by 62,75%, nitric oxide by 47,52%, GABA-T by 60,83%, acetyl cholinesterase by 26,62%, butyryl cholinesterase by 31,75%, TNF α by 59,88%, INF γ by 69,87%, Interleukin 1 β by 34,17%, Interleukin 6 by 44,15%, and Interleukin 10 by 51,17%. While the treatments increased the time spend in the chosen arm by 10, 74%, the recognition index by 51,75%, crossing by 221,53%, grooming by 95,25%, glutathione by 300%, superoxide dismutase by 40,84%, catalase by 81,25%, GABA by 54,2% and BDNF by 83,51. Therefore, administration of sodium valproate and praziquantel significantly antagonised the deleterious effect of the intracerebroventricular injection of *Taenia solium* oncospheres in mice, by reduction of seizures severity, improvement of memory in mice, alterations of the oxidative stress, restoration of GABAergic cholinergic neurotransmission and also relieved neurodegeneration and inflammation