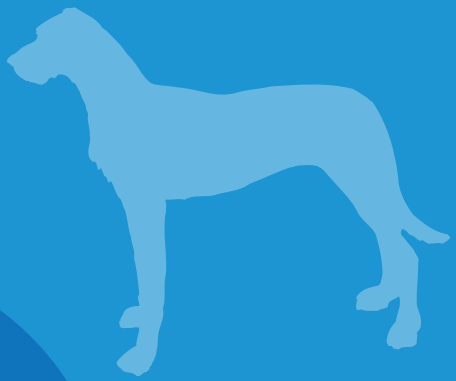
LONDON CENTRE FOR NEGLECTED TROPICAL DISEASE RESEARCH

AN INNOVATIVE RESEARCH COLLABORATION:

SELECTED RESEARCH HIGHLIGHTS 2023



@NTDResearch

Contents

Director's note

It gives me great pleasure to write my first Director's note as we celebrate together the 10th anniversary of the London Centre for Neglected Tropical Disease Research (LCNTDR). This 10th anniversary comes at a particularly exciting time for the centre, as we welcome new members from both the UK and endemic settings, a new and expanded Executive Board, and a broader focus across the neglected tropical and zoonotic diseases.

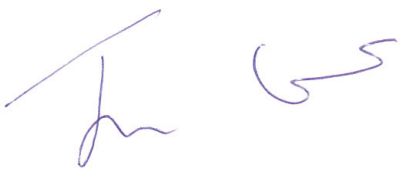
As we mark World NTD day on 30th January, several key international developments in the NTD sphere are worthy of particular mention. Notably, the World Health Organization's Department of Control of NTDs has recently published a further important companion document to its flagship NTD 2021-2030 road map entitled *'Ending the neglect to attain the Sustainable Development Goals: a rationale for continued investment in tackling neglected tropical diseases 2021–2030'.* This calls for continued mobilisation of resources to support the implementation of cost-effective interventions to both consolidate the hard-won gains of recent years and to accelerate progress toward the 2030 targets. Such efforts should help ultimately reduce the burden of disease for millions of people worldwide.

We also continue to see great progress in acknowledging NTDs from a One Health perspective – where so often it is the same diseases that disproportionately affect the livestock of the same impoverished populations, causing profound economic losses and untold suffering to the animals and accentuating the poverty cycle. Likewise the critical roles of animals in maintaining transmission despite control efforts is increasingly appreciated.

This growing recognition is evident in the publication of the WHO NTD road map's One Health companion document published in 2021 and the One Health Joint Plan of Action, published by the quadripartite in 2022. It is such One Health approaches, in partnership with multifactorial environmental, behavioural and WASH interventions, that will underpin the elimination and eradication of NTDs.

Of course, all such developments raise new challenges for sustainable financing – which comes at an especially painful time as NTD funding has been so severely curtailed throughout the UK and international sphere. These cuts are particularly perplexing in light of the COVID -19 pandemic, which serves as a stark reminder of the direct and indirect global impact zoonotic diseases can inflict, and the importance of multifactorial research and disease control approaches.

The research conducted by members of the LCNTDR thereby remain as pertinent as ever, as we all strive toward achieving these ambitious 2030 targets for such critically important diseases. This booklet represents a snapshot of some of our recent studies from across this last year. I am extremely proud of all the work achieved by researchers at the centre, and am excited by the work to come through the centre's expanded membership and focus. I very much look forward to working with you all to achieve continued successes and impact in the year and years ahead. Thank you.



Professor Joanne P. Webster FMedSci

MVLA-ompA sequencing of ocular *Chlamydia trachomatis* in a trachoma-endemic population in Amhara, Ethiopia

Anna Harte, Harry Pickering, Martin J. Holland – London School of Hygiene & Tropical Medicine

With: Ambahun Chernet, Eshetu Sata, Mulat Zerihun, Andrew W. Nute, Mahteme Haile, Taye Zeru, Zerihun Tadesse, E. Kelly Callahan, and Scott D. Nash

Background

Trachoma caused by *Chlamydia trachomatis (Ct)*, is the leading infectious cause of blindness worldwide. The World Health Organization recommends the use of mass drug administration (MDA) using the antibiotic azithromycin for treatment of the active stage of *Ct* infection. To investigate the molecular epidemiology of trachoma in the context of MDA and investigate transmission dynamics, the identification of *Ct* genotypes is required. The majority of epidemiological studies focus on the *Ct* major outer membrane protein ompA for genotype differentiation, however this has been shown to be of limited use. The aim of our study was to apply a genotyping system [multiple loci variable number tandem repeat analysis combined with ompA (MLVA-ompA)] which has not previously been evaluated in trachoma endemic populations and determine the discriminatory power for trachoma molecular epidemiology.

Methods

Ocular swabs were collected pre- and post-MDA between 2011-2017 from four trachoma-endemic zones located in the Amhara region of Ethiopia (North Gondar, South Gondar, East Gojam and Waghemra), with age, gender, woreda (village) and the clinical signs of trachoma recorded for each participant. DNA was further purified from a population representative sub-sample of 300 participants with highest *Ct* PCR loads, and genotyped by MLVA-ompA, which is based on sanger sequencing of ompA sequences and three loci (CT1299, CT1291, and CT1335) within the *Ct* genome containing a variable number of tandem repeats (VNTR). This method was selected as VNTRs have a higher rate of mutations since DNA polymerase is prone to error over repeated nucleotide regions. This could therefore provide a useful identifier of novel strains for small scale, local epidemiological studies. Variants were called by aligning sequences within each VNTR and manually counting the number of repeated nucleotides. Discriminatory power was calculated using Simpson's index of diversity. Mixed-effects models were used to determine if any genotypes were associated with the clinical signs of trachoma, adjusting for age, gender and village, as well as investigating the effects of MDA on genotype diversity.

Results and discussion

The typeability of each VNTR and ompA individually were above 80%, however, there were consistent difficulties with sequencing CT1299, with samples exceeding 14C repeats, demonstrating evidence of polymerase slippage and PCR stutter. Due to a small proportion of dissimilarity between the samples, the overall typeability of MVLA-ompA for this study was 64.6%, however, the discriminatory power was high (0.967), surpassing the 0.95 threshold recommended for epidemiological studies. A total of 61 MVLA-ompA sequence types (ST) were identified from 26 woredas. The minimum spanning tree shown in the image shows the relationship and distribution of each ST with woreda. There were no significant associations between ST and clinical signs of trachoma. There was an observable switch in ST types over time, with a higher proportion of serovar A after the additional rounds of MDA (91%) compared to before (46%), although ST diversity did not change. Genotyping ocular serovars using MLVA-ompA provides an efficient technique relative to the gold-standard discriminatory power of whole genome sequencing, and can therefore provide valuable information to elimination programmes on the local epidemiology of trachoma.

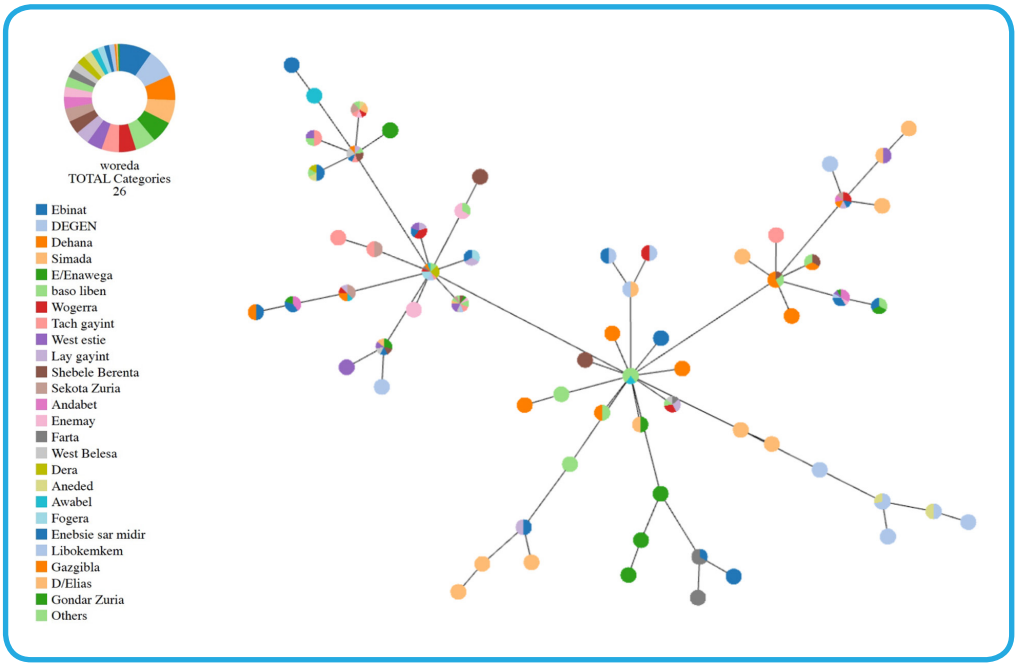


Figure: Minimum spanning tree of MLVA-ompA strains types and districts (woreda) in Amhara (2011-2017).

Quantifying the interconnectedness between poverty, health access, and rabies prevalence and mortality

Emma Taylor, Katy George, Emily Johnson, Hannah Whitelegg, Joaquin M. Prada, Daniel L. Horton – University of Surrey

Background

Rabies is classified as a neglected zoonotic disease by the World Health Organization (WHO) and has the highest case-fatality rate of any infectious disease, at nearly 100%. Dog mediated rabies is responsible for 99% of human rabies cases, with susceptible individuals becoming exposed to the virus through the bite of an infected dog. Without prompt administration of post-exposure prophylaxes (PEP), and once symptoms have developed, exposure to rabies virus causes an acute, progressive fatal encephalitis. The global goal set by the WHO, the World Organisation for Animal Health, and the Food and Agriculture Organization to eliminate dog-mediated human rabies deaths by 2030, has undoubtedly been a catalyst for many countries to evaluate existing dog rabies control programmes. In addition, the 2030 agenda for sustainable development details a blueprint for global targets to benefit both people and the planet. Rabies is recognised as a disease of poverty, but the links between economic development and achieving rabies elimination are poorly quantified. Providing evidence for these links is vital for disease prioritisation and control.

Methods

The relationship between health care access, poverty, and death rate as a result of rabies were evaluated, with separate indicators that can be used at country-level. These were: total Gross Domestic Product (GDP), and current health expenditure as a percentage of the total gross domestic product (% GDP) as an indicator of economic growth; and a metric of poverty assessing the extent and intensity of deprivation experienced at the individual level (Multidimensional Poverty Index, MPI).

Results and discussion

NTDs are important indicators for the health of communities, and act as benchmarks for disparities in health care access. There was no evidence for a relationship between GDP or the proportion of GDP spent on health care in a country and the probability of receiving lifesaving PEP. In contrast, there was a significant relationship between PEP access and poverty at the individual level (MPI). By exploring separate metrics related to wealth, economic development, and poverty, we demonstrate that countries experiencing higher levels of poverty report a higher death rate as a result of rabies, and poorer health access, when compared to wealthier, more developed countries. Our results provide clear evidence that economic growth alone (as measured by GDP and expenditure on healthcare), is not a good predictor, and will not be enough to meet the 2030 goal of the elimination of dog mediated human rabies deaths. Focus should therefore also be directed to cost-effective and sustainable control programmes, including education and ensuring universal health care access particularly to the poorest communities. Indeed, other important development indications relevant to rabies control such as education are also needed. In addition, investment in control in the reservoir is needed with methods such as mass dog vaccination, coupled with reliable real world data on dog demographics to inform dog vaccination, if we are to achieve SDG's and the 2030 rabies elimination goals.

The University of Surrey is a member of the United Against Rabies Forum, committed to working together to end human deaths from dog-mediated rabies.

Figure: Free-roaming dog. Africa. Vaccinating dogs is the most cost-effective way of eliminating human rabies, long term.

Ongoing Zika virus transmission in Brazil

Laith Yakob – London School of Hygiene & Tropical Medicine

Background

Zika virus (ZIKV) is a flavivirus transmitted through the bite of mosquitoes, principally Aedes aegypti. ZIKV was declared a Public Health Emergency of International Concern (PHEIC) by the World Health Organization (WHO) on 1 February 2016, by which time, autochthonous ZIKV transmission had been reported across Central and Latin America. The PHEIC was prompted by the reporting two months earlier of a suspected link between ZIKV infection during pregnancy and subsequent disabilities in babies, most notably microcephaly.

From 2017 to 2021, infection rates decreased substantially, however, ZIKV did not disappear from the region. Over 150,000 cases were reported in the Americas from 2017 up until September 2021. ZIKV's large epidemic potential coupled with its persistence in this region meant ZIKV remains in the WHO list of priority diseases for research and development in emergency contexts. This study explored post-PHEIC ZIKV transmission in Brazil. Objectives included i) to analyse post-PHEIC infection trends in Brazil, and ii) to identify consistent hotspots for ongoing transmission for intervention targeting.

Methods

Data on new confirmed and suspected ZIKV cases during the post-PHEIC period (2017-2020) were used to train and validate Seasonal Autoregressive Integrated Moving Average (SARIMA) models for Brazil at the regional level. We applied a data filter to 5,570 municipalities in Brazil, and included in our analysis those that have reported ZIKV infections consistently every year since 2017. We generated a kernel density plot for these areas of consistent transmission and weighted the data by minimum annual rate of infection, standardized to local municipality population. We identified centroid locations of all municipalities that reported ZIKV cases after 2016 and the hotspots of sustained transmission during the post-PHEIC period, in which >40 cases were reported per 100,000 population consistently each year. We generated contours from kernel density estimates weighted by the minimum annual rate of infection standardized to local municipality population.

Results and discussion

The SARIMA models demonstrated very good predictive accuracy: 4643 (95%CI 309–19,831) confirmed cases were forecasted for 2021, and, 4092 confirmed cases were subsequently reported for that year by the Ministry of Health, Brazil. Consistency-weighted ZIKV hotspots were observed in Roraima and Tocantins (North region), Alagoas (Northeast), Rio de Janeiro (Southeast), Paraná (South) and the border between Rondônia (Northern) and Mato Grosso (Central-West).

Accurate and timely forecasts for ZIKV infections can guide decision-making on medical countermeasures, allowing them to be used in more effective ways. Specifically, they enable anticipating resource requirements, refining situational awareness and monitoring control efforts. Complementing our forecasts with maps of standardised ZIKV cases weighted by inter-annual transmission consistency had two purposes. First, it allowed for the spatial targeting of vector management to prioritise the worst affected populations. Second, identifying areas of consistent transmission is of particular value to inform site selection for seroprevalence studies and intervention trials.

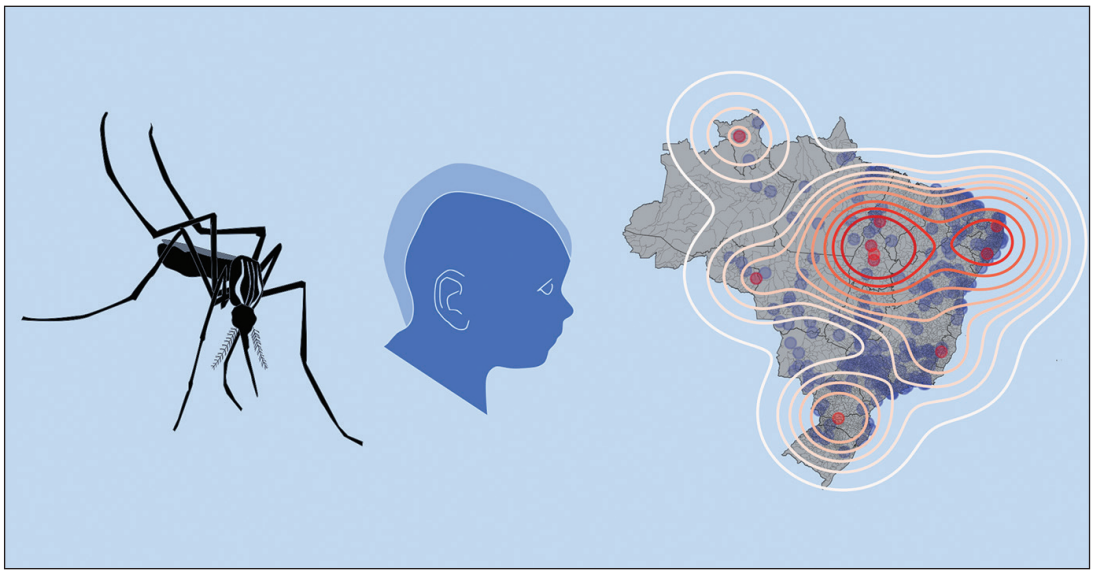


Figure: *Aedes aegypti* (left); Congenital Zika Syndrome (middle); and, Consistency Weighted Kernel Density Plots of Post-PHEIC Zika Cases in Brazil.

Ending the neglect of cystic echinococcosis by integrating mathematical modelling, economic analysis, and social sciences

Mahbod Entezami, Jo Widdicombe, Daniel Jackson, Victor del Rio Vilas, Joaquín M Prada – University of Surrey

Maria-Gloria Basáñez – Imperial College London

With: Adriano Casulli, M Graciela Céspedes, Cesar Gavidia, Edmundo Larrieu, Guillermo B Mújica, Martina Nocerino, T Verónica Poggio, Laura Rinaldi, Katherina A Vizcaychipi

Background

Cystic Echinococcosis (CE) is a zoonotic neglected tropical disease (zNTD), caused by the cestode parasite *Echinococcus granulosus sensu lato*. It has a global distribution, but most commonly affects pastoral and rural communities. It is endemic in many regions worldwide and causes a substantial socioeconomic burden. Dogs act as the definitive host, while livestock, mainly sheep, are the intermediate hosts. In livestock, CE causes a loss of productivity (most notably through liver condemnation, weight loss, lower reproductive capacity, lower wool and milk production). Humans act as dead-end hosts and do not contribute to onward transmission; however, morbidity and mortality are present in untreated patients. Effective methods of surveillance and control are available, such as the EG95 vaccination for sheep and anthelmintics for dogs. In humans, infection can be treated early with anthelmintics, but could require surgical intervention due to complications later in life if undetected.

Nevertheless, little epidemiological data is available for many endemic regions. As one of the truly more neglected NTDs, often very little resources are allocated to the prevention of CE. Some countries have no formal control or surveillance programmes in place. The WHO 2030 goal for CE is to increase the number of countries with intensified control in hyperendemic areas.

Methods

A wide array of mathematical modelling tools can be developed to maximise the value of the limited data available. Integrating mathematical modelling with other disciplines, such as economic analysis and social sciences, is key to evaluate the implications of alternative interventions in different settings.

We work with collaborators across several endemic countries to better understand the impact of CE through the analysis of data collected by routine national surveillance programmes and other sources of information. For example, in central-southern Italy, using routine surveillance abattoir data across several livestock species, we evaluated the spatial distribution of CE by implementing a geostatistical mathematical model (see figure). In Argentina, a new ELISA diagnostic for sheep was recently developed, and we evaluated its performance with a semi-mechanistic Bayesian Latent Class Analysis model. Moreover, we recently reviewed the literature to evaluate the information available on cost and cost effectiveness of CE control interventions targeting its zoonotic hosts, and we found little published data. With complex mechanistic mathematical models in development, that can integrate the limited data available across disciplines, alternative, context specific, control strategies for the prevention of CE can be assessed.

Results and discussion

Although different interventions to control CE are available, and elimination has been achieved in insular geographies such as Iceland, New Zealand and Tasmania, CE remains endemic in many regions. This can be explained in part by the lack of resources allocated to combating the disease. Our research aims to address gaps in literature, maximising the value of the information available, and using evidence-based research to provide a portfolio of cost-effective and socially accepted surveillance and control strategies for CE.

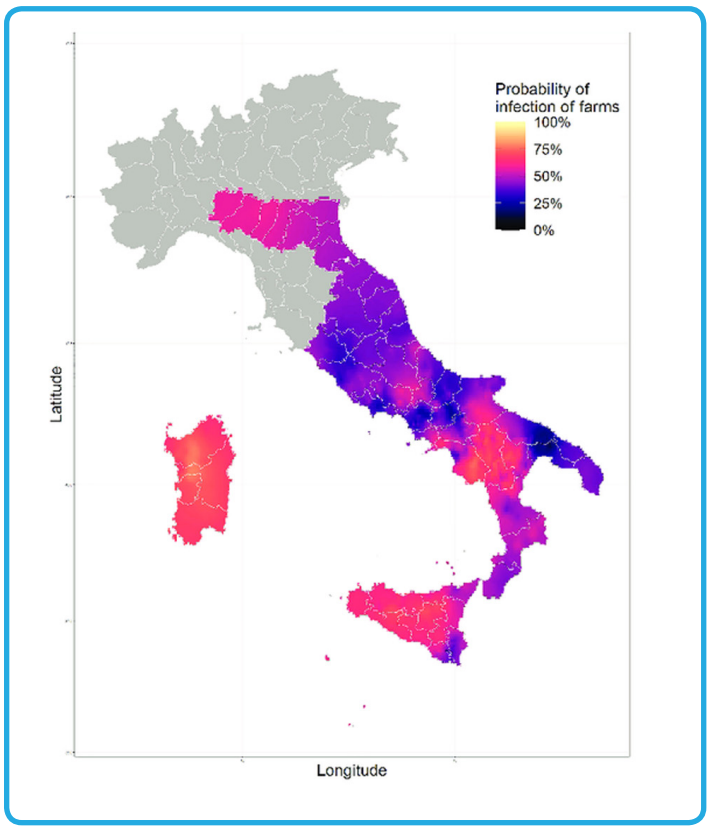


Figure: The probability of infection across ruminant farms in Italy.

Investigating the molecular epidemiology of *Schistosoma* species in Eswatini

Felix Llanwarne – London School of Tropical Medicine & Hygiene and Natural History Museum

Aidan Emery, Bonnie Webster, David Rollinson, Fiona Allen – Natural History Museum

With: Precious Dlamini

Background

Schistosoma haematobium is highly prevalent in sub-Saharan Africa (SSA), and uniquely causes urogenital schistosomiasis, displays unexplained genetic homogeneity and readily hybridises with closely related species. Eswatini (formerly Swaziland) is endemic for human schistosomiasis, but its *Schistosoma* populations have not previously been genetically characterised. Eswatini is also endemic for bovine schistosomiasis, caused by *Schistosoma mattheei*, a species of high veterinary importance and of possible human medical importance, being reported as a potential zoonotic species. It has been shown to readily hybridise with *S. haematobium*, with *S. haematobium-mattheei* hybrids recovered from human hosts in other co-endemic countries.

Methods

Human parasitological and malacological surveys were conducted in May 2019 in five known transmission localities from all four administrative areas of Eswatini (Hhohho, Manzini, Shiselweni and Lubombo). *Schistosoma* eggs were obtained, by sedimentation, from urine samples from school-aged children. Eggs were hatched in fresh water and individual miracidia preserved on Whatman FTA cards for DNA analysis. Additionally, *Bulinus* and *Biomphalaria* snails were collected and induced to shed cercariae, which were preserved for molecular analysis. Partial fragments of the mitochondrial cytochrome oxidase subunit I (*cox1*) and the NADH-dehydrogenase subunit 5 (*nad5*) genes were sequenced from individual miracidia and cercariae to confirm species identification and to investigate genetic diversity. The nuclear internal transcribed spacer 1 (ITS1) was sequenced to confirm identify and to detect any inter-species hybridisation.

Results

19 children were found to be infected and miracidia were collected from recovered eggs. From the 148 *S. haematobium* miracidia molecularly analysed, there was no evidence of inter-species hybridisation with all miracidia presenting *S. haematobium* genetic profiles. However, miracidial cox1 haplotype diversity was higher than that observed in others endemic African mainland areas with 11 unique haplotypes identified from just a small subset of samples collected from the 19 infected individuals. This is in stark contrast to the one predominant *cox1* haplotype seen throughout continental African strains. Individual *Bulinus* snails were found emitting both *S. haematobium* and *S. mattheei* cercariae, confirming co-endemic transmission but no inter-species hybridisation was found. Additionally, *Biomphalaria* snails were found to be shedding *S. mansoni*, showing local transmission of human intestinal schistosomiasis.

Discussion

The high genetic diversity of *S. haematobium* in Eswatini is unique compared to what is observed across other parts of Africa. This may suggest that schistosomes being transmitted in Eswatini have experienced a different evolutionary past to the rest of the continent. For example, different origins of parasite spread into the African continent, not being subjected to the same selection pressures such as mass drug administration, or not undergoing the same species mixing due to a restricted freedom of movement. Additionally, the identification of *Bulinus* snails transmitting *S. mattheei* confirms co-endemicity of both species presenting risk of hybridisation/zoonoses. However, no evidence that this was occurring was observed. Surprisingly, *S. mansoni* transmission was detected creating further need for both intestinal and urogenital schistosomiasis monitoring and control in Estwatini. This work further highlights the utility of molecular epidemiological investigations of *Schistosoma* populations to determine and understand schistosomiasis transmission.

Figure: Human water contact site where *Bulinus* were found shedding *S. haematobium* and *S. mattheei.*

Evolution of Tetraspanin antigens in the zoonotic Asian blood fluke *Schistosoma japonicum*

Daniel A. J. Parsons – Kingston University and Natural History Museum
Anthony J. Walker – Kingston University
Aidan M. Emery – Natural History Museum
Joanne P. Webster – Royal Veterinary College

With: Scott P. Lawton

Background

Despite recent successful control efforts in China, zoonotic schistosomiasis caused by *Schistosoma japonicum* remains a threat to human and animal health in the region. This has prompted calls for a novel integrated control strategy, with a human/livestock-focused anti-schistosome vaccine as a core element, particularly for endemic regions nearing elimination, such as China, where parasite re-emergence presents an enduring risk.

The development of transmission-blocking anti-schistosome vaccines, and immunisation attempts utilising various antigens in non-human mammalian hosts, have yielded mixed success, with some studies highlighting genetic and antigenic variation in schistosome antigen coding-genes (ACGs) as possible confounders of vaccine efficacy. Thus, robust selection of target ACGs is of paramount importance, particularly when considering the genetic diversity and antigenic variability of potential candidates. Tetraspanins (TSPs), a family of tegument-surface antigens in schistosomes which function at the host-parasite interface, and therefore interact directly with the host's immune system, are considered promising vaccine candidates. Yet, experimental evaluation of TSPs to date has demonstrated variable efficacy between vaccine studies.

The main aims of this work were to:

1. Determine the extent of TSP diversity in *Schistosoma japonicum* (SjTSPs);
2. Identify the extent to which TSPs reported as promising antigens differ geographically;
3. Measure the potential impact of any identified variation on the structural, functional and antigenic properties of each SjTSP protein;
4. Evaluate the evolutionary selection pressures that could lead to the measured variation.

Methods

SjTSP sequences, derived from parasite populations sampled from seven provinces across China, were identified and extracted by baiting published *S. japonicum* short-read NGS data from the International Nucleotide Sequence Database Collaboration. Seven SjTSPs (-1, -2, -8, -13, -14, -23, -25) were analysed using *in silico* methods, employing a phylogeographic and evolutionary approach, to determine SjTSP genetic diversity, geographical variability, and predict the impact of selection pressures on variation in antigen protein structure, function, and antigenic propensity.

Results and discussion:

27 SjTSPs were identified across three subfamilies, highlighting the diversity of the TSP gene family in *S. japonicum.* Considerable variation was demonstrated for several SjTSPs between geographical regions, revealing that episodic, diversifying positive selection pressures promote amino acid variability in the large extracellular loop (LEL) domain of several SjTSPs. Accumulating polymorphisms in the LEL domain of SjTSP-2, -8 and -23 led to alterations in their structural, functional, and antibody-binding characteristics. This variation was predicted to impact host antibody recognition and thus reduce the host's ability to respond to *S. japonicum* infection. Such variability, therefore, appears to represent a mechanism utilised by *S. japonicum* to evade host immunity, and may reflect adaptive evolution of key schistosome TSPs as an evolutionary response to selective pressures from the host immune system. Whilst the genetic and antigenic variability amongst these SjTSPs is predicted to represent a challenge to vaccine development, it was uniquely demonstrated that genetic and antigenic conservation amongst SjTSP-1, -13 and -14 from the different parasite populations in China may highlight their potentially utility as efficacious vaccine candidate antigens, signifying a possible future route of investigation with potential implications for control of African schistosomes.

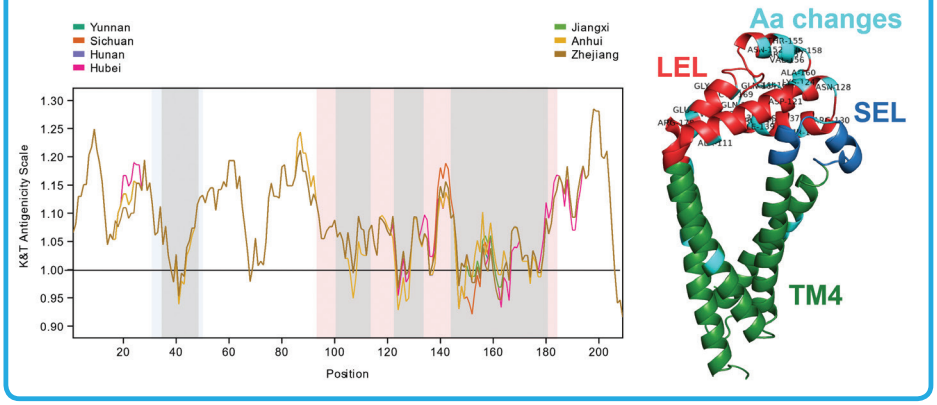


Figure:
Key domains of SjTSP-2 and identified amino acid changes:

LEL = Large extracellular loop,

SEL = Small extracellular loop,

TM4 = four transmembrane helices.
Grey boxes overlayed onto antigenicity profile of SjTSP-2 denote predicted antibody binding-sites.

Assessing the food-borne risks of *Toxocara* spp. infection in support of public health and food quality assurance

Sara R. Healy, Martha Betson, Joaquin M. Prada – University of Surrey

With: Eric R. Morgan

Background

Human toxocariasis is a neglected tropical disease, which is global in distribution and has a significant impact on public health worldwide. The infection can lead to several serious conditions in humans, including allergic, ophthalmic and neurological disorders such as epilepsy. It is caused by the common roundworm species *Toxocara canis* and *Toxocara cati*, with humans becoming accidentally infected via the ingestion of eggs or larvae. *Toxocara* eggs are deposited on the ground when infected dogs, cats and foxes defecate, with the eggs contaminating crops, grazing pastures, and subsequently food animals. However, transmission of *Toxocara* to humans via food consumption has received relatively little attention in the literature.

This study aimed to quantify the transmission of *Toxocara* from the environment to the final food product in order to evaluate the risk to the consumer and the potential impact of any control interventions. This knowledge can then be utilised to inform food safety policy and, ultimately, protect public health.

Methods

For the first time in the UK, field studies have been conducted in the south of England to assess the presence of *Toxocara* on vegetable produce utilising microscopic and molecular analysis. The focus has been on leafy vegetables, as their 'folded' structure has been shown to harbour more soil and thus potentially more parasitic eggs. Lettuce *(Lactuca sativa)* grown in community gardens and spinach *(Spinacia oleracea)* grown on commercial farms were selected, as these vegetables can be consumed without peeling or cooking and could pose more of a risk if ingested unwashed.

Results and discussion

Toxocara sp. eggs were detected on both lettuce and spinach samples tested. The prevalence of *Toxocara* eggs on lettuce crops grown in community gardens was found to be 6.5%. Questionnaire data obtained from garden plot holders revealed that 88% of respondents had seen a definitive host species (such as a dog or a fox) or its faeces on their site. Field-grown spinach sampled from commercial farms was found to have a prevalence of *Toxocara sp.* eggs ranging from 7.4% up to 45.5 %. In this study, *Toxocara* was isolated from the crops grown at every farm sampled; *T. canis* was the most common species detected (93.3% of positive samples) but *T. cati* was also found to be present (6.7% of positive samples).

These findings highlight the importance of effective public health measures, including hand hygiene and vegetable washing, to reduce the risk of *Toxocara* transmission to humans via the cultivation and consumption of vegetables. In addition, improving farm biosecurity to minimise farmland access by definitive host species would decrease the risk of parasitic contamination of soil and crops at their point of origin. Further research is now required to evaluate how food consumption habits shape the epidemiology of toxocariasis, and whether any risks to consumer safety warrant intervention measures to protect public health.

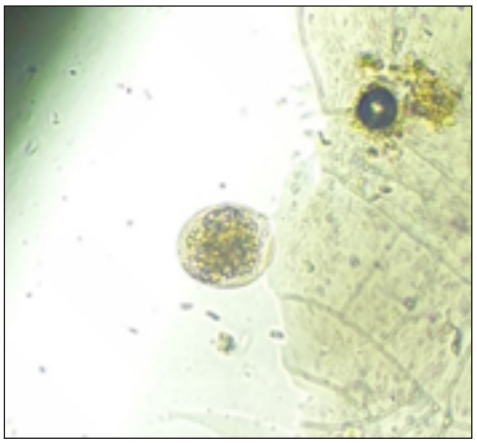


Figure: *Toxocara* egg which was recovered from lettuce grown in a UK community garden

Investigating the role of *Chlamydia*-specific antibodies in ocular infection and disease progression

Rebecca Sarsam, Anna Harte, Harry Pickering, Tamsyn Derrick, Amber Barton, Tara Mtuy, Robin L. Bailey, David CW Mabey, Matthew J. Burton and Martin J. Holland – London School of Hygiene & Tropical Medicine

With: Athumani Ramadhani, Elias Mafuru, Patrick Massae, Kelvin Mbuya, William Makupa

Background

Trachoma, the world's leading infectious cause of blindness, is a public health problem in 42 countries, and is estimated to have caused 1.9 million cases of blindness. The introduction of the 'SAFE strategy' for trachoma elimination by the World Health Organization in 1993 (Surgery, Antibiotics, Facial cleanliness and Environmental improvement) has resulted in a 92% reduction in the number of people at risk worldwide.

Currently, 15 countries have been validated by the WHO as having eliminated trachoma as a public health problem. However, trachoma remains prevalent in poverty-stricken and rural areas of many countries, especially in sub-Saharan Africa, and for eradication rather than elimination as a public health problem, an effective vaccine is likely to be necessary. Frequency and severity of *C. trachomatis* infection decreases with age, one explanation being that partial protective immunity develops with repeated exposure. However, the immune correlates of protection from scarring trachoma are poorly understood, especially relating to antibody correlates of protection, hindering efforts to develop an effective vaccine. Immunity is also likely to be serovar-specific, so an effective vaccine would likely need to target both serovar A and serovar B of *Chlamydia trachomatis.*

Methods

448 children aged 6-10 years old from three villages in northern Tanzania were observed longitudinally between 2012-2016 with eye examinations and Ct PCR tests at three-monthly intervals to determine infection prevalence and scarring progression. Annual mass drug administration (MDA) using azithromycin was given three times in two of the villages and four times in the other. From this group, 91 children with known scarring progression status were selected for serum sampling between November 2019-January 2020 to include a higher proportion of scarring progressors compared to the full cohort; 38% experienced scarring progression and over 90% experienced at least one *C. trachomatis* infection (93% serovar B infections before the first round of MDA opposed to 85% serovar A in the years subsequent to MDA).

Serum samples were tested using ELISA to determine the concentration and avidity of IgG to *Chlamydia*-specific antigens: elementary bodies (EBs) and recombinant major outer membrane proteins (MOMPs) of serovar A and serovar B, with avidity expressed as the proportion of antibody remaining bound to the plate after washes with 8M urea compared to PBST washes.

Results and discussion

Binary logistic regression was performed to determine factors affecting scarring progression, adjusting for age, sex, village and concentration and avidity of IgG to EB-A, EB-B, MOMP-A and MOMP-B. The main finding of this study was that increased avidity of anti-MOMP-A-specific serum IgG was significantly negatively associated with scarring progression at 4-years follow-up ($p<0.005$). No other factor was associated with scarring progression. Further research is needed to investigate the mechanism by which high-avidity anti-MOMP-A IgG may protect from scarring, and to investigate the role of IgA in the protective immune response to ocular *C. trachomatis* infections.

A limitation of the study was that serum was collected three years after scarring progressor status was determined using conjunctival photographs; however, based on nurse-assessed scarring field grades, 7.1% of non-progressors in 2016 had further scarring when serum was sampled (compared to 37.1% of scarring progressors with further progression).

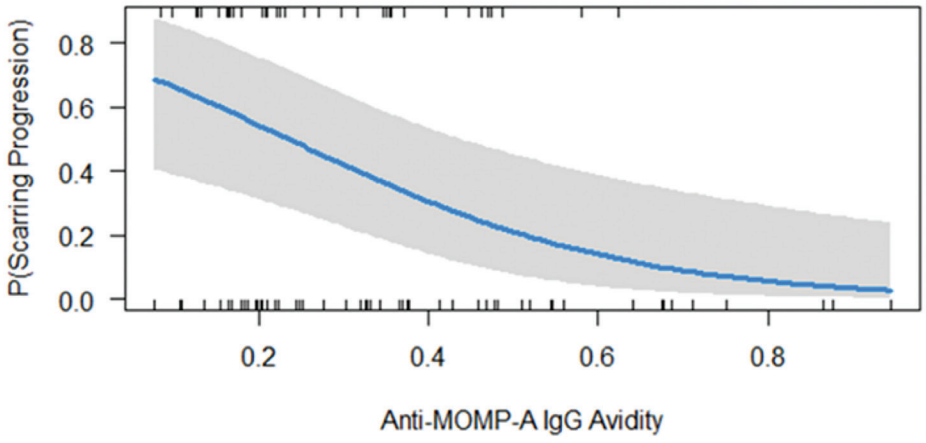


Figure: Increased anti-MOMP-A serum IgG avidity was significantly negatively associated with presence of scarring progression at four-year follow-up ($p<0.005$).

Parasites and childhood stunting – a mechanistic interplay with nutrition, anaemia, gut health, microbiota, and epigenetics

Isobel L. Gabain, Joanne P. Webster – Royal Veterinary College

With: Anouschka S. Ramsteijn

Background

Childhood stunting is a serious public health problem, which in its physical form is defined by the World Health Organization (WHO) as falling at least two standard deviations below the height-for-age WHO Child Growth Standards median. In 2020, an estimated 149.2 million children under five years of age were stunted globally. Physical stunting can also be accompanied by sub-optimal neurocognitive development and reductions in intellectual capacity. As a result, stunting pervasively hinders developmental potential and human capital of entire communities and countries. Having been identified as a major global health priority, the World Health Assembly targets aim to reduce childhood stunting by 40% between 2010 and 2025, although current trends are falling significantly short of this target and hence further research to understand the typology of childhood stunting are imperative.

Helminthic and parasitic protozoal infections are extremely common, particularly in low-resource settings where sanitation is poor, and are frequently cited as significant contributing factors to childhood stunting. However, empirical evidence definitely associating parasites which childhood stunting is currently either inconsistent or lacking, particularly from key risk groups such as pregnant women and infants.

We reviewed evidence within the literature to help elucidate potential mechanistic pathways that may be predicted to connect parasitic infection to childhood stunting.

Results and discussion

A range of key potential pathways leading from parasitic infection of mother and/or baby to childhood stunting were identified (see figure). Certain parasite species may contribute to malnutrition via interruption of normal absorption and digestion of macro- and micronutrients required for growth, for example, by reducing appetite. Alternatively, or additively, parasites may cause diarrhoeal illness and/or contribute to environmental enteric dysfunction (EED), an incompletely defined syndrome of inflammation, reduced absorptive capacity, and reduced barrier function in the small intestine. The composition of the gut microbiome (the collection of microorganisms residing in the gastrointestinal tract, dominated by bacteria) may be influenced by parasites, for example by inducing immature microbiota profiles, resulting in gut dysbiosis and nutritional interruption. Local gut inflammation, characteristic of EED, has been shown to trigger systemic inflammation. This elevated immune activation may lead to an energetic trade-off, allocating resources towards immune function and away from linear growth. Parasitic infection can also cause blood loss and/or destruction, thus causing and/or contributing to anaemia (a deficiency of red blood cells), which may be causative in the pathway to stunting by causing hypoxic conditions within the host. Finally, although current data are scarce, epigenetic changes (modifications of gene expression rather than alteration of the genetic code itself) resulting from parasitic infection (including that of the mother) may influence subsequent childhood growth.

No single pathway has, however, been unquestionably implicated in the pathway to stunting and stunting likely results from a geographically dependent culmination of interacting factors. Longitudinal studies are therefore needed to further elucidate the role of parasites in childhood stunting. One such London Centre for NTD Research-associated study, the Action Against Stunting Hub (AASH), is following cohorts of mothers and babies in India, Indonesia, and Senegal. Data collected assess a range of health and nutritional indications, including determination of the prevalence of key parasitic infections of mother and child throughout the first 1000 days of life (from conception to two years of age).

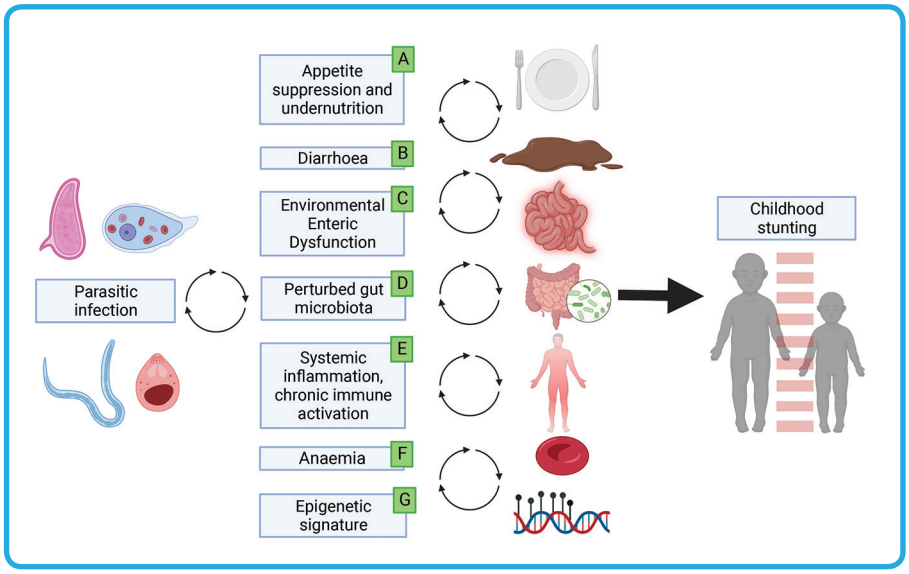


Figure: Overview of potential key pathways linking parasitic infection to childhood stunting. There is no singular, simple linear trajectory from parasite infection to stunting; there are many potential pathways and cycles at play.

Buruli-RifDACC: Evaluation of the efficacy and cost-effectiveness of high-dose vs. standard-dose rifampicin on outcomes in *Mycobacterium ulcerans* disease: a randomised controlled trial in Ghana

Yaw Ampem Amoako, Jacob Novignon, Richard Odame Phillips – Kwame Nkrumah University of Science and Technology

Iris Mosweu, Ruth Canter, Charles Opondo, Elizabeth Allen, Catherine Pitt, Stephen L. Walker, Michael Marks – London School of Hygiene & Tropical Medicine

With Abigail Agbanyo, Lucy Owusu, Joseph Tuffour, Adwoa Asante-Poku, Yohannes Hailemichael, Dorothy Yeboah-Manu

Background

Buruli ulcer (BU), a necrotising skin condition caused by *Mycobacterium ulcerans* (MU) is endemic in tropical Africa and can lead to disfiguring ulcers and permanent disability. The 2030 World Health Organization (WHO) road map for neglected tropical diseases (NTDs) has advocated for major scaling up of diagnosis and management to facilitate elimination of BU related disability. Current treatment is with daily oral rifampicin (10mg/kg dose) and clarithromycin (15mg/kg dose) for eight weeks combined with standard gauze wound dressings. Secondary infections and paradoxical reactions may cause variability in healing rates, and additional antibiotics may be prescribed, leading to a risk of antimicrobial resistance. Wound dressings with antimicrobial properties may help to improve outcomes. A clinical trial is underway to investigate whether combining high-dose rifampicin and Dialkylcarbamoyl Chloride (DACC) dressings may significantly improve time to clearance of viable mycobacteria, reduce paradoxical reactions and secondary infections, and improve outcomes when compared to DACC dressings plus standard dose. Alongside the trial, an economic evaluation will compare the financial and economic costs and outcomes of these two BU treatment strategies from a societal perspective.

Methods

This is a phase three individual, multi-center randomised controlled trial currently recruiting participants in three endemic districts in Ghana- Ga West Municipal Hospital, Pakro Health Centre and Wassa Akropong Municipal Hospital (target sample size: 112 individuals). Consented participants are randomly assigned to either the intervention group (HR+DACC) receiving daily high-dose oral rifampicin (20mg/kg) and oral clarithromycin (15mg/kg) for four weeks, with DACC-coated dressing applied to the wound and changed every 48 hours until the lesion heals, or the control group (SR+DACC) receiving the WHO-recommended daily oral rifampicin (10mg/kg) and oral clarithromycin (15mg/kg) for eight weeks, with DACC-coated dressing applied to the wound and changed every 48 hours until the lesion heals.

At each of the 15 scheduled visits following randomisation, data on participant demographic, clinical, cost and health-related quality of life outcomes are obtained along with lesion photograph and measurement taken by the Silhouette Wound Imaging System (ARANZ, Wellington, New Zealand), Visual Analogue Scale (VAS) and pain measurements.

Swab or fine needle aspirate (FNA) samples are taken at weeks 0, 2, 4, 6, 8, 12 and 16 (if lesions have not healed), to evaluate the persistence of viable MU during treatment as well as standard microbiological testing when secondary infection is suspected.

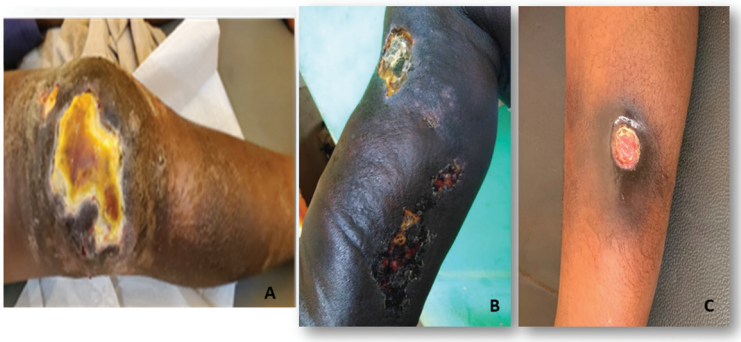


Figure: Clinical forms of Buruli ulcer disease. Oedema with ulceration (A), Plaque with ulceration (B) and Ulcer (C)

Results and discussion

The trial is expected to evaluate the mean time to clearance of viable *Mycobacterium ulcerans*, the proportion of patients with healed wound at 20 weeks, reoccurrence of BU, incidence of paradoxical reactions, incidence of secondary infection and cost-effectiveness of the intervention.

The findings from this trial could lead to a change in how BU is treated. A shorter but more efficacious regimen would lead to improved treatment outcomes and better adherence and potentially substantial financial and economic savings for both patients and the health system.

Investigating the molecular epidemiology of *Schistosoma mansoni*

John Archer – Natural History Museum

Schistosomiasis is a neglected tropical disease (NTD) caused by infection with parasitic blood fluke trematodes of the genus *Schistosoma* that can lead to debilitating morbidity and mortality. Whilst it is estimated that over 230 million people are currently infected globally, over 90% of schistosomiasis cases occur within sub-Saharan Africa; primarily in rural areas lacking adequate water, sanitation and hygiene (WASH) infrastructure. Of these, approximately one-third are caused by *Schistosoma mansoni*, the causative parasite of the disease known as intestinal schistosomiasis.

Routine methods used to diagnose intestinal schistosomiasis, such as faecal-egg microscopy (checking for eggs passed in the faeces using a microscope), are extremely unreliable; particularly when assessing individuals harbouring low-intensity infections. In addition, alternative methods of diagnosis such as immunological rapid diagnostic tests can also be unreliable. Improved and highly sensitive diagnostic tools that can be carried out at the point-of-care in resource-poor schistosomiasis-endemic settings are therefore needed for successful and impactful disease control and transmission monitoring. Such diagnostic tools are also required to reach elimination targets set out by the World Health Organization (WHO) in areas where the burden of infection has been significantly reduced as a result of ongoing disease control efforts.

Figure: Collection of freshwater snail intermediate hosts of Schistosoma spp. parasites, Lake Malawi, Mangochi district, Malawi, July 2022.

As part of my PhD, I am working to investigate the molecular epidemiology of *Schistosoma mansoni* along the southern shoreline of Lake Malawi, Mangochi District, Malawi, following a recently discovered outbreak of intestinal schistosomiasis in this area. This involves, in part, measuring the prevalence of infection within the community using highly sensitive molecular diagnostic methods, such as real-time polymerase chain reaction (PCR).

Whilst sensitive and reliable, PCR, as well as the essential preliminary steps of extracting DNA from clinical samples (such as faeces), can only be carried out using robust and sophisticated laboratory infrastructure seldom available in schistosomiasis-endemic areas. It is for this reason that highly portable and rapid methods of molecular diagnosis, such as recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP) are currently being developed, optimised, and validated for diagnosing infection with *S. mansoni*. In addition, highly portable and rapid methods of extracting DNA from clinical samples are also under development.

Further to assessing the prevalence of intestinal schistosomiasis within the community using PCR, I therefore also hope to further develop, optimise, and validate a recently developed RPA assay that can be used to detect *S. mansoni*-specific DNA within DNA extracted from faecal samples. To do this, the same DNA extracts used with PCR will also be used with RPA, allowing a direct performance comparison between each assay. In addition, I also aim to further develop methods that can be used to rapidly extract DNA from faecal samples in resource-poor settings (e.g., lacking electricity or refrigeration facilities). These extraction methods will be tested using PCR initially, and then also with RPA in the hope of progressing a protocol that can be used to rapidly extract DNA from faeces and screen that DNA for *S. mansoni* infection in schistosomiasis-endemic areas.

To do this requires the generous provision of faecal material from members of the community in Mangochi and so when I was awarded the LCNTDR travel grant I was extremely pleased, as it meant I had the opportunity to visit this area and support in collecting these samples myself. As schistosomiasis predominantly afflicts school-aged children, faecal samples were requested from 220 children attending four different primary schools located in close proximity to the lake's southern shoreline.A number of rapid diagnostic tests were carried out using these faecal samples, such as faecal-occult blood assays that assess the degree of blood in the stool (used as a marker of intestinal pathology), and a subset (~5 grams) of these samples were safely stored in ethanol and transported back to the UK for molecular diagnostic testing.

Advancing the environmental monitoring of schistosomes using fish faecal xenomonitoring

Zikmund Bartonicek – Natural History Museum

My PhD project at the Natural History Museum and University College London focuses on advancing the environmental monitoring of schistosomiasis using molecular methods. As part of the project, we have tested whether we can use juvenile fish to help us detect schistosome larvae in water.

Schistosomes, the parasites causing the disease schistosomiasis, have a complex lifecycle, relying on intermediate freshwater snails, which release cercariae, the human-infecting larvae, into the water. People (or animals) then get infected when they come in contact with the water, where cercariae are waiting to burrow through their skin. Once infected, the worms migrate into their bloodstream, pair up, and start laying eggs. In the case of *Schistosoma mansoni,* the worms then live and lay eggs around the intestines. Once the eggs are laid, they make their way into the intestines and get passed with faeces. In areas with insufficient sanitation, the faeces with eggs will then reach water, where the eggs will hatch into larvae that then infect the freshwater snails, completing the lifecycle.

To identify sites where people get infected with schistosomes, we usually collect the freshwater snails responsible for releasing the cercariae into the environment at water contact sites where transmission is suspected. In the case of *S. mansoni,* these are the snails of the genus *Biomphalaria*. Once we collect the snails, we expose them to light in water pots and check if the cercariae are emerging. This method is relatively simple; however, it can be tricky to find the snails, and not all infected snails release the cercariae when checked. To address this shortcoming, my PhD project aims to improve the molecular methods of detecting the parasite life stages living in the environment (cercariae and miracidia) using environmental DNA (eDNA) and fish faecal xenomonitoring (FFX).

Firstly, we have tested some aspects of the production and persistence of the parasite eDNA and how to best capture it in the field. Subsequently, we developed and tried a new method of monitoring schistosomes – using fish faeces. The fish faecal xenomonitoring approach uses juvenile Nile tilapia (*Oreochromis niloticus*) as a natural sampler of schistosome larvae. As the larvae are a natural part of zooplankton, we hypothesised that fish consume the larvae and then accumulate parasite DNA in their faeces. Testing the faeces using molecular methods, such as qPCR, should help us detect the parasite in the environment. Indeed the experiments we have done in the lab were very promising – in some cases, up to 100% of all the ish that were offered cercariae consumed them and had traces of *Schistosoma mansoni* DNA in their faeces.

After the promising lab results, I was lucky to obtain additional funding from the London Centre for NTD Research and the Fisheries Society of the British Isles to test this new method in the field and compare it against the traditional malacological surveys and the eDNA approach. I travelled to Uganda and spent three weeks on Lake Albert's shores along with researchers from Ugandan Vector Control Division and Dr Amaya Bustinduy's Praziquantel in Preschool Children (PiP) project. We visited multiple sites, collected water samples for eDNA analysis, snails for traditional malacology, and caught young Nile tilapias. After collections, we took all the samples back to the research camp in Bugoigo, where we checked the snails for emergent trematode larvae. Then, we put the fish into aquaria, fed them, and kept them overnight. In the morning, we collected all the faeces and released the fish back to the lake while storing the faeces in ethanol for later molecular analysis.

Figure: The shores of Lake Albert can be very tranquil and photogenic, but a dangerous parasite hides in the water.

Although the work has not yet been published, one thing is clear: the fish consume schistosome larvae, and their faeces could be used as a monitoring method. And not only that – the fish could also work as a natural protection against schistosome transmission, removing some of the infectious larvae from the environment, showing how important healthy ecosystems can be for human health.

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- **Acting as an NTD knowledge base for disseminating innovative and evidence-based information for policy and programme formulation;**
- **Providing a neutral coordinating platform for partner collaboration on NTD control and prevention efforts;**
- **Carrying out research on new approaches to the study of the geography, transmission dynamics and control of NTDs, with a particular focus on integrated diagnosis and mapping and integrated control of more than one NTD.**

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The London Centre for Neglected Tropical Disease Research is a collaboration between C. K. Tedam University of Technology and Applied Sciences, Ghana, the Ethiopian Public Health Institute, Imperial College London, Kingston University, Kwame Nkrumah University of Science and Technology, London School of Economics and Political Science, London School of Hygiene & Tropical Medicine, Natural History Museum, Royal Veterinary College, St Georges University of London, UK Neqas Parasitology, University of Greenwich, University of Papua New Guinea, and University of Surrey.