Multidisciplinary studies of schistosomiasis and HIV on the shoreline of Lake Malawi: A longitudinal cohort study of male genital schistosomiasis (MGS) among fishermen in Mangochi District.

'Thesis submitted in accordance with the requirements of the University of Liverpool for the Degree of Doctor in Philosophy (Tropical Medicine)

by Dr. Sekeleghe Amos Njelwike Kayuni.'

14th February 2020.

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Declaration

This thesis is my original work and has not been presented for a Degree in any other University or Institution.

Sekeleghe Amos Njelwike Kayuni

Signed: Date: 14.02.2020

We confirm that the work reported in this thesis was carried out by the candidate and has been submitted for the examination with our approval as University supervisors.

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Dedication

To my family and people of Malawi

Acknowledgements

This has been a long adventurous journey which would not have been possible if it was not for the love of God Almighty, family and many friends near and far, which I am very grateful for.

I am grateful for the tremendous care, support, advice and encouragement from my supervisors Professor J. Russell Stothard and Dr. E. James LaCourse at Liverpool School of Tropical Medicine (LSTM) during the entire period which enabled me to gain much knowledge and research expertise. Also, to the members of my Progress Assessment Panel, Professors Sally Theobald and Bertie Squire, for their critical advice and support during my studies.

Furthermore, much appreciation to Commonwealth Scholarship Commission for providing a full-time doctoral scholarship and baseline fieldwork research of my multidisciplinary longitudinal cohort studies in Malawi; LSTM for hosting me and support during the entire doctoral period; British Society for Parasitology (BSP) for the 1- and 3-months follow-up research fieldwork; LSTM MSc Education Fund for the 6-month follow-up research fieldwork; World Friendship Charity Fund for the 12-month follow-up research fieldwork; African Research on NTD (ARNTD) for Small grants program (SGP II) award to conduct HIV viral load analyses; and Burrough-Wellcome Collaborative Travel Award from Skaggs School of Pharmacy & Pharmaceutical Sciences, UC San Diego, USA for laboratory training and analyses of research semen samples using real-time polymerase chain reaction (PCR) at Elizabeth-TweeSteed Hospital (ETZ) – Tilburg, Netherlands.

I am also thankful to the many collaborators in the longitudinal research studies: Mr Peter Makaula of Research for Health, Environment and Development (RHED) – Malawi; Professors Fanuel Lampiao, Johnstone Kumwenda and Khumbo Kalua of College of Medicine - Malawi; Dr Sam Kampondeni of Mpingwe Private Clinic – Malawi; Professor Peter D.C. Leutscher of Aalborg University – Denmark, Dr Jaco Verweij and team at ETZ - Tilburg, Netherlands; Professors Lisette van Lieshout, Govert van Dam, Paul Corstjens, Drs Claudia de Dood and Ptyjse Hoekstra of Leiden University Medical centre (LUMC) – Leiden, Netherlands; Professor Annamaria Geretti, Dr Apostolos Beloukas and Mr Adam Abdullahi from Institute of Infection and Global Health in University of

Liverpool – United Kingdom (UK); Dr Elizabeth Joekes of Radiology Department at Royal Liverpool Hospital – UK; and Dr Jutta Reinhard-Rupp of Merck plc – Germany for the overwhelming support, training, donations and diagnostic expertise in making the studies successful.

Furthermore, my appreciation to the Mr. Lazarus Juziwelo, Programme Manager of National Schistosomiasis and STH Control in Malawi Ministry of Health; Dr Henry Chibowa Jnr, Director of Health Services, Drs Stalin Nzinkana, Deborah Kamlongera – Mzikamanda, James Kumwenda Jnr and entire Management team of Mangochi District Health Services; Head and staff of Laboratory department at Mangochi District Hospital; District ART coordinators, in-charge and staff of Tikondane clinic; in-charges and staff of Billy Riordan Memorial Clinic, Monkey-Bay Community Hospital, Nkope and Koche Health Centres; Dr Lumbua Katenda, Mr Anwar and staff of Mangochi LMJ clinic; technicians: Messrs Bright Mainga, Pilirani Mkambeni, Patrick Hussein, Mkonazi Nkhoma, Matthews Elias and Boniface Injesi; local community health workers: Ambali Makochera, Alfred Kachikowa, Rodgers Wengawenga, Michael Tsatawe, Chikondi Mtsindula, Mambo Amin, Elias Matemba, Austin Kaluwa, Justin Mndala, Promise Mwawa, Dickson Tabu, George Matiki, Alfred Mdoka and Charles Katandi; Brother Henry Chagoma and staff of Montfort Mission Guest House; all traditional leaders in the hosting fishing communities and beach committee members; and all the fishermen who participated for their enthusiasm and support during the study.

Also, to colleagues at LSTM for their additional support: Mr Mohammad Alhabri; Dr Hajri Al-Shehri; Dr Suzy Campbell; Ms. Grace Macklin, Joanna Fawcett, Alexandra Shaw, Charlotte Condemine, Rosie Christiansen, Josie Heskett; Mr Angus O'Ferrall and Hamish Baxter.

Finally, but not least, my dear wife Sandy, our children, my parents and siblings for your amazing support, prayers, endurance and patience in my long absence away from home and studying and working hard to complete this important research.

Abbreviations

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
CAA	Circulating anodic antigen
CCA	Circulating cathodic antigen
Ct	Threshold cycle
DALYs	Disability adjusted life years
ELISA	Enzyme-linked immunosorbent assay
FGS	Female genital schistosomiasis
GU	Genitourinary
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus type 1
KAP	Knowledge, attitudes and practice
LMICs	Low-and-middle income countries
LSTM	Liverpool School of Tropical Medicine
MDA	Mass drug administration
MGS	Male genital schistosomiasis
MSM	Men having sex with men
NHSRC	National Health Sciences Research Committee
NTD	Neglected tropical disease
PC	Preventive chemotherapy
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
POC	Point-of-care
PSA	Prostatic specific antigen
PSAC	Pre-school aged children

PVPP	Polyvinylpolypyrrolidone
PZQ	Praziquantel
REC	Research Ethics Committee
SAC	School aged children
SSA	sub-Saharan Africa
UCP-LF	Up-converting phosphor-lateral flow assay
UGS	Urogenital schistosomiasis
UK	United Kingdom
UoL	University of Liverpool
VL	Viral load
WHO	World Health Organization

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Abstract

Male genital schistosomiasis (MGS) is a specific chronic manifestation of schistosomiasis associated with schistosome eggs and related pathologies in the genital system of men inhabiting or visiting endemic areas. Despite description of the first recognised MGS patient by Madden in 1911, the epidemiology, diagnostic testing and case management of MGS are not well described owing to limited research and diminishing focus over several decades. Furthermore, as the human immunodeficiency virus (HIV) epidemic expands across sub-Saharan Africa (SSA), there is renewed interest in MGS owing to plausible but as of yet under-explored interactions with HIV.

To shed new light on MGS, a longitudinal cohort study was conducted among fishermen along the southern shoreline of Lake Malawi, an endemic area in SSA to investigate the prevalence of MGS, its associated knowledge, attitudes and practices (KAP), and determine the potential increase of viral shedding in semen of HIV-positive men with MGS. A systematic review conducted prior to the onset of the research fieldwork describing the MGS epidemiology, clinico-pathological manifestations, diagnostic techniques and treatment outlined and informed the current approach to the body of research presented here.

Fishermen aged 18+ years were recruited into the study after providing informed written consent and individual questionnaires were administered to assess their KAP associated with MGS. Thereafter, participants submitted urine, semen, and blood for point-of-care (POC) field parasitological tests, and later laboratory-based molecular polymerase chain reaction (PCR), and HIV VL analyses. In addition, transabdominal and scrotal ultrasonography to assess the pathological effects of MGS on their genital organs were performed. Praziquantel therapy was provided to all participants, together with the follow-up diagnoses and treatment dates after 1, 3, 6 and 12-months' intervals.

376 fishermen (320 HIV-negative and 56 HIV-positive on Antiretroviral therapy (ART)) aged between 18 and 70 years (median: 30.0 years), were recruited into the study, and had questionnaires interviews. At baseline, prevalence of UGS (*S. haematobium* eggs in urine) was

17.1% (n = 210, median: 2.3 per 10 ml, range: 0.1 – 186.0), 3.8% had a positive point-of-care circulating cathodic antigen (POC-CCA) indicative of intestinal *S. mansoni* infection, while MGS prevalence was 10.4% by semen microscopy (n = 114, median: 5.0, range: 0.1 – 30.0) and 26.5% by real-time PCR (n = 65, Ct value range: 18.8 – 36.6). More participants (66.7%) with schistosome eggs in semen were observed to not have any eggs in their urine. 6.9% of participants (n = 130) were observed to have pathological lesions in their genital organs on ultrasonography. For 15 HIV-MGS cases and 16 HIV-only controls who submitted paired blood and semen samples during the longitudinal study, more cases than controls had detectable and quantifiable VL, which regressed with PZQ. Similarly, the results of the diagnostic tests improved, with negative schistosome egg, real-time PCR in semen and pathological resolution on follow-up.

In conclusion, MGS has been observed, via parasitological, molecular and radiological examinations, to be common in local male inhabitants (fishermen) of endemic areas along the south shoreline of Lake Malawi in the SSA region and shown to respond to standard PZQ treatment. Improving availability and accessibility for all people in these areas to PZQ, diagnostic tools for MGS, and combined HIV and schistosomiasis control interventions are advocated to reduce morbidity and improve the lives and reproductive health of men, their partners and communities in endemic areas.

Chapter 1: General overview

1.1. Introduction

Schistosomiasis, also known as Bilharzia, is a prevalent parasitic disease with the highest burden among 17 recognised neglected tropical diseases (NTDs) (Christinet *et al.*, 2016) and endemic in 78 low-and-middle income countries (LMICs) of tropical and subtropical regions. This freshwater snail-borne disease affects over 200 million people globally, 85% in sub-Saharan Africa (SSA) with 20 million suffering from the severe urogenital and intestinal complications of the disease (Engels *et al.*, 2002; WHO, 2018b). Each year, 200,000 people are estimated to die annually from the disease (van der Werf *et al.*, 2003; Colley *et al.*, 2014).

Urogenital schistosomiasis (UGS) caused by *Schistosoma haematobium* is responsible for most of the global schistosomiasis burden, infecting over 112 million people annually (WHO, 2013b) and causing chronic urinary bladder calcification, carcinoma, obstructive uropathy, renal hydronephrosis and genital complications (Butterworth *et al.*, 2013; Squire and Stothard, 2014). However, much emphasis of UGS has been on urinary pathology, overlooking the genital complications especially in men. Male genital schistosomiasis (MGS) is a specific gender manifestation of schistosomiasis, associated with presence of schistosome eggs and pathologies in the genital fluids and organs, which was first described in 1911 (Madden, 1911).

Despite several case reports and research studies describing MGS since the first case description (Gelfand *et al.*, 1970; Corachan *et al.*, 1994; Leutscher *et al.*, 2000), there has been little knowledge on its current burden and morbidity among local inhabitants in endemic areas of SSA. In the last decade, UGS has gained much attention in SSA for morbidity control through the preventive chemotherapy programmes with mass drug administration (MDA) of Praziquantel (PZQ), a mainstay pyrazinoisoquinole antihelminth for schistosomiasis (Knopp *et al.*, 2013).

In addition, the epidemic of Human immunodeficiency virus (HIV) infection in SSA has been observed to overlap with that of schistosomiasis (Ndeffo Mbah *et al.*, 2013), highlighting the possible interactions between the two diseases. While women with female genital schistosomiasis (FGS) have shown to have an increased risk of HIV acquisition (Kjetland *et al.*, 2006; Jourdan *et al.*, 2011), men

whose semen harbours *Schistosoma* eggs have been observed with increased levels of inflammatory cells and immunological mediators associated with HIV infection, highlighting the possible risk to HIV acquisition and transmission (Leutscher *et al.*, 2005; Leutscher *et al.*, 2008b). Reduction of viral load in semen have been demonstrated after PZQ treatment in HIV positive men coinfected with UGS (Midzi *et al.*, 2017), which suggest the need for further research studies to explore the role of PZQ treatment as one of the HIV control interventions in schistosomiasis-endemic areas.

1.2. Justification and rationale of the present study

Malawi (Figure 1) is a land-locked country in SSA where both urogenital *S. haematobium* and intestinal *S. mansoni* are prevalent and focally distributed along lakes, rivers and other water bodies (Teesdale and Chitsulo, 1985; Makaula *et al.*, 2014). The shorelines along Lake Malawi, third largest lake in Africa and a renowned tourist destination (NSO and ICF, 2011), has high prevalence of *S. haematobium* infection, with evidence of frequent re-infection episodes especially in children (Madsen *et al.*, 2011). Very little is known about the burden of MGS in these schistosomiasis-endemic areas in SSA.

Furthermore, Malawi has a generalised HIV epidemic whose prevalence is high among adults aged 15 – 49 years, estimated at 10.0% in 2016 (Ministry of Health, 2017), and the communities on the shoreline of Lake Malawi like Mangochi district have higher prevalence at 11.8% in 2014, compared to other non-endemic areas in the country (NSO and ICF, 2011; UNAIDS, 2014). Fishermen are among the high-risk occupational groups in Malawi with higher HIV prevalence, which was 11.5% in 2014 (NSO, 2014; NAC, 2015), despite the many control efforts to reduce HIV incidence and mortality.

The current control interventions of schistosomiasis and HIV, running parallel in dual-epidemic regions, are also leaving out sexually-active men, women and girls who are also at high-risk of both conditions, thereby slowing down efforts of arresting the burden of both diseases (Hotez, Fenwick and Kjetland, 2009; Rollinson *et al.*, 2013; Stothard, Bustinduy and Montresor, 2014; Stecher *et al.*, 2015). The multidisciplinary research studies on MGS test the hypotheses that PZQ

treatment reduces the prevalence and morbidity of MGS as well as the potential risk of HIV transmission through viral load shedding in semen of adult fishermen living in schistosomiasis-endemic areas along Lake Malawi.

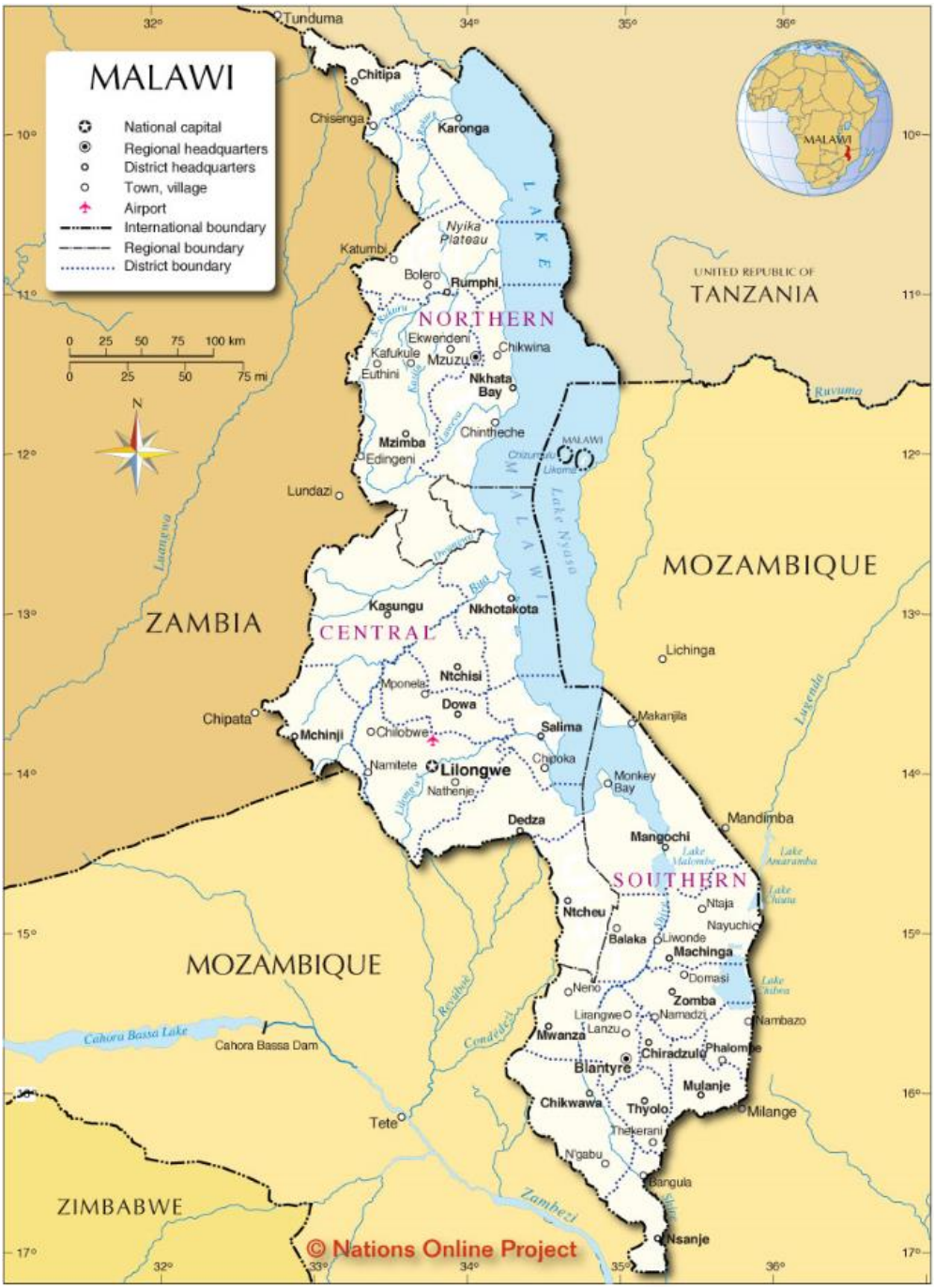


Figure 1: Map of Malawi showing Mangochi District in the Southern region and Lake Malawi

Image courtesy of

https://www.nationsonline.org/oneworld/map/malawi_map.htm

1.3. My research aspirations for the study

In order to determine the current prevalence and morbidity of MGS in a schistosomiasis-endemic area in SSA, I conducted multidisciplinary cohort research studies among high-risk population of local fishermen along south shoreline of Lake Malawi in Mangochi district. The cohort studies were further set to evaluate the extent of relationship between MGS and HIV-1 VL through potential increase of viral shedding in semen of HIV-positive men with MGS in these fishing communities.

1.4. Context of the study

The research study was conducted among local fishermen aged 18 years and above, dwelling in specific fishing villages (communities) identified and selected along the south shoreline of Lake Malawi in Mangochi district, which is endemic for schistosomiasis. Fishermen just like children and women, have high frequency of contact with infested lake water when carrying out their daily household and income-generating activities, posing themselves at huge risk to schistosomiasis infection, and its complications like MGS. Hence, selection of this population in order to investigate MGS and its potential impact of HIV infection.

As misconceptions or cultural perceptions regarding the nature of the research including semen collection were anticipated to affect implementation of the research, adequate awareness and sensitisation meetings with local traditional leaders, healthcare personnel and individual fishermen were conducted to describe the research objectives and address their concerns, using the experiences and lessons from recent studies with similar methodology in the district (Kipandula and Lampiao, 2015). All participants were offered PZQ treatment in accordance with the national guidelines.

1.5. Thesis layout

The chapters of this PhD thesis have been presented in the following structure:

Chapter 1 General overview: Provides the general overview of the thesis, rationale, research aspirations and context of the MGS cohort studies.

Chapter 2 General Introduction: Provides the background knowledge of urogenital schistosomiasis, current literature review on MGS, HIV co-infection, existing gaps, specific aims and objectives of the MGS cohort studies.

Chapter 3 Systematic review on MGS: Provides the updated systematic review of MGS, describing the current epidemiology, clinico-pathological descriptions, diagnostic techniques, treatments and management of MGS.

Chapter 4 Description of the Cohort studies: Provides the general description of the fishermen cohort recruited at baseline and followed up at 1-, 3-, 6- and 12-months' time-points of the study as well as the methodology used for data collection.

Chapter 5 Baseline prevalence of MGS and associated knowledge, attitudes and practices: Provides the baseline prevalence of MGS in the cohort and describes the knowledge, attitudes and practices associated with MGS.

Chapter 6 Molecular diagnosis of MGS and progression after treatment: Provides the results of the novel diagnosis of MGS using advanced molecular technique, real-time PCR on semen and compared to the traditional semen microscopy and urine filtration. It also reports on the results of the study follow-up at 1-, 3-, 6- and 12-months' time-points after PZQ treatment.

Chapter 7 Morbidity of MGS described by ultrasonography: Provides the results of the transabdominal and scrotal ultrasonography conducted to describe the morbidity of MGS and subsequent regression after PZQ treatment during the follow-up at 1-, 3-, 6- and 12-months' time-points.

Chapter 8 Seminal HIV-1 RNA detection among HIV-positive in men with MGS: Provides the results of the novel pilot HIV-1 RNA detection in semen and blood plasma among men with MGS and HIV co-infection compared with HIV-positive men without MGS, and followed up at all time-points of the cohort studies.

Chapter 9 General Discussion: Provides discussion on the results of the MGS longitudinal cohort studies in Malawi, implications to the schistosomiasis control in Malawi and region, possible recommendations and future work on MGS.

Chapter 2: General introduction

2.1. Summary

This chapter provides detailed background knowledge on schistosomiasis, with emphasis on urogenital schistosomiasis (UGS), its life cycle, clinic-pathological features, diagnosis, treatment, control and prevention. In addition, one of the complications particularly in men, male genital schistosomiasis (MGS) is further described, highlighting the current literature review, HIV co-infection, existing gaps, and research focus, with specific aims and objectives of the multidisciplinary MGS cohort studies.

The section on the diagnosis of schistosomiasis was published in Parasitology journal (https://doi.org/10.1017/S0031182019000969) as cited:

Kayuni S.A., Corstjens P.L., LaCourse E.J., Bartlett K.E., Fawcett J., Shaw A., Makaula P., Lampiao F., Juziwelo L., de Dood C.J., Hoekstra P.T., Verweij J.J., Leutscher P.D.C., van Dam G.J., van Lieshout L. and Stothard J.R. (2009). **How can schistosome circulating antigen assays be best applied for diagnosing male genital schistosomiasis (MGS): an appraisal using exemplar MGS cases from a longitudinal cohort study among fishermen on the south shoreline of Lake Malawi.** *Parasitology*, Volume 146, Issue 14, pages 1785-95. (Received: 20 February 2019, revised: 30 June 2019, accepted: 3 July 2019, published online: 27 August 2019).

My contribution to this manuscript is that I conducted the literature review on MGS and the research fieldwork along south shoreline of Lake Malawi. Thereafter, I wrote the manuscript and made all the changes suggested by the co-authors and journal referees.

2.2. Background

Schistosomiasis is among five of the most common NTDs which include: lymphatic filariasis (LF), onchocerciasis, soil-transmitted helminths (STH) and trachoma (Hotez *et al.*, 2007). These diseases affect poor people in LMICs and contribute to significant morbidity and considerable mortality in the world. Schistosomiasis is a parasitic disease which remains a major public health concern as children, young women and other vulnerable populations are disproportionately affected in most rural and deprived urban localities. Such areas have low socio-economic status, poor sanitation, inadequate access to clean and safe potable water, with limited medical resources, consequently depriving them of normal development and potential (Figure 2) (Molyneux, Hopkins and Zagaria, 2004; Tchuente, 2012).

Furthermore, significant proportion of infected children and adults usually harbour more than one parasite, making polyparasitism a norm in the developing world, as such the control strategies on NTDs should focus on integrated approach for cost-effectiveness and streamlined efficiency. However, the highest priority of the international health community is on the 'big three' diseases, HIV/AIDS, tuberculosis and malaria, while burden of NTDs is estimated to be a quarter of that of HIV and half of malaria, resulting in less attention on huge morbidity of schistosomiasis and other NTDs, compared to the big three's higher mortality (Molyneux, Hotez and Fenwick, 2005).

Figure 2: Women, children and men in their daily activities along south Lake Malawi shores

Photo credit: Mohammad H. Alharbi, LSTM, U.K. November 2017.

Schistosomiasis is caused by digenetic trematodes (schistosomes) of *Schistosoma* genus and remains one of the commonest NTDs in Africa region (Engels *et al.*, 2002; Hotez, Fenwick and Kjetland, 2009). In 1851, Theodor Bilharz first reported and described these human parasites after discovering adult schistosome worms in post-mortems conducted in Egypt (Ross *et al.*, 2002; Squire and Stothard, 2014). This trematode was initially termed as *Distomum haematobium* and later changed to *Schistosoma haematobium* (Rollinson *et al.*, 1997; Rollinson, 2009). He did not realise presence of *Schistosoma mansoni* worms in the post-mortems too. Schistosome eggs were recovered in Chinese and Egyptian mummies demonstrating the presence of infection in early civilisation.

Schistosomes belong to Phylum Platyhelminthes (flatworms), Class Trematoda (flukes / schistosomes) (Figure 3), with 24 species of schistosomes currently recognised, six of which cause disease in humans namely, *S. haematobium, S. mansoni, S. japonicum, S. intercalatum, S. mekongi* and *S. guineensis.* Some species have particular veterinary importance, namely *S. bovis, S. mattheii* and *S. curassoni,* however, hybridization between animal and human species has been reported, highlighting potential emergence of zoonotic infections (Huyse *et al.*, 2009; Leger and Webster, 2017; Webster *et al.*, 2019). Interestingly, *S. japonicum* has highest zoonotic potential, infecting humans and animals (both wild and domestic) such as cattle, dogs, pigs, water buffaloes, rodents (Knopp *et al.*, 2013).

Only three schistosome species are responsible for majority of human disease, with different geographical distribution, morbidity trends, intermediate hosts and clinico-pathological presentations (Squire and Stothard, 2014), namely urogenital schistosomiasis caused by *S. haematobium,* endemic throughout Africa, parts of Arabia, the Near East, Madagascar, Mauritius and recently reported in Corsica, France (Berry *et al.*, 2014; Gautret *et al.*, 2015; Moné *et al.*, 2015); gastrointestinal-hepatosplenic schistosomiasis caused by *S. mansoni* in Africa, Madagascar, Arabia, South America and Caribbean, and *S. japonicum* in China, Philippines, Sulawesi and Thailand. The eggs of each species are distinct and useful in parasitological diagnosis, while the schistosome worm

burden is measured by the number of eggs / ova excreted which can also be correlated to morbidities in the urinary tract or gastrointestinal-hepatosplenic systems (Andrade *et al.*, 2017).

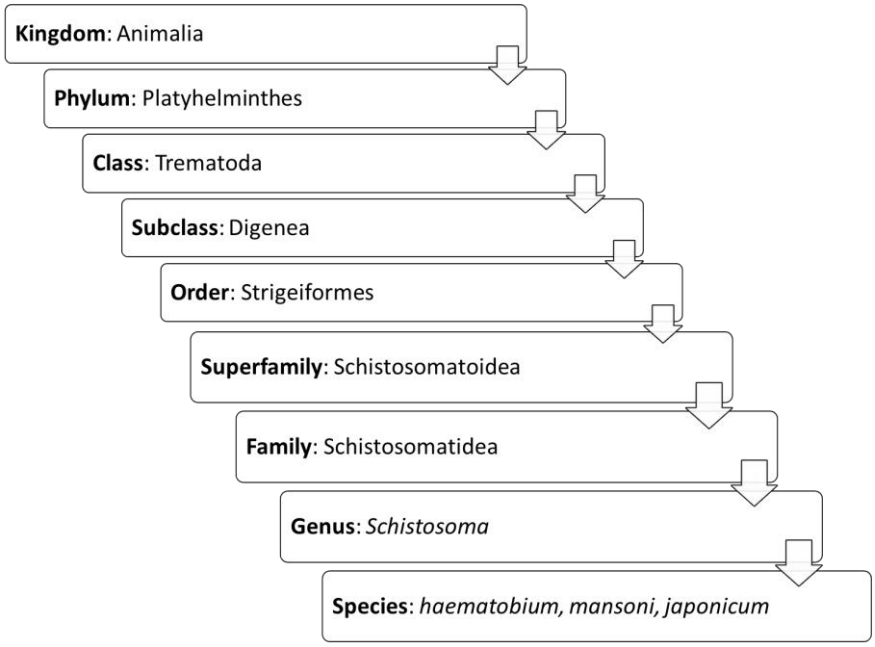


Figure 3: Order of the main schistosome parasites affecting human globally

2.3. Life cycle of schistosomes

The Life cycle of schistosomes (Figure 4) was clearly described by the father of modern helminthology, Robert T. Leiper (1881-1969) between 1915 and 1916 in Egypt (Garnham, 1970; Willmott, 1981; Stothard *et al.*, 2016). Schistosomiasis is distributed along freshwater bodies which create habitats for their particular intermediate snail hosts, namely aquatic sinistral turreted *Bulinus* snails for *S. haematobium* (also *S. intercalatum* and *S. guineensis*); flat ramshorn *Biomphalaria* snails for *S. mansoni* or small amphibious operculate turreted *Oncomelania* snails for *S. japonicum*. These freshwater habitats are areas where both intermediate snails and people come together, mostly those with low-level sanitation, contaminated with urine or faeces harbouring *Schistosoma* ova.

The eggs expelled from the human body through urine or faeces, contain ciliated miracidia which hatch out once in these freshwater habitats and swim in search for suitable specific snail hosts which they penetrate. Thereafter, the miracidia undergoes asexual replication cycle through two generations of sporocysts (mother and daughter sporocysts) resulting in release of minute fork-

tailed cercariae into water 4-6 weeks later (Knopp *et al.*, 2013; Colley *et al.*, 2014). This snail's ability to develop miracidia is usually affected by environmental factors such as high temperature and water chemistry. Once exposed to light, the snails shed hundreds to thousands of infective cercariae into water for several weeks, which survive for 24 – 72 hours unless a suitable host is found.

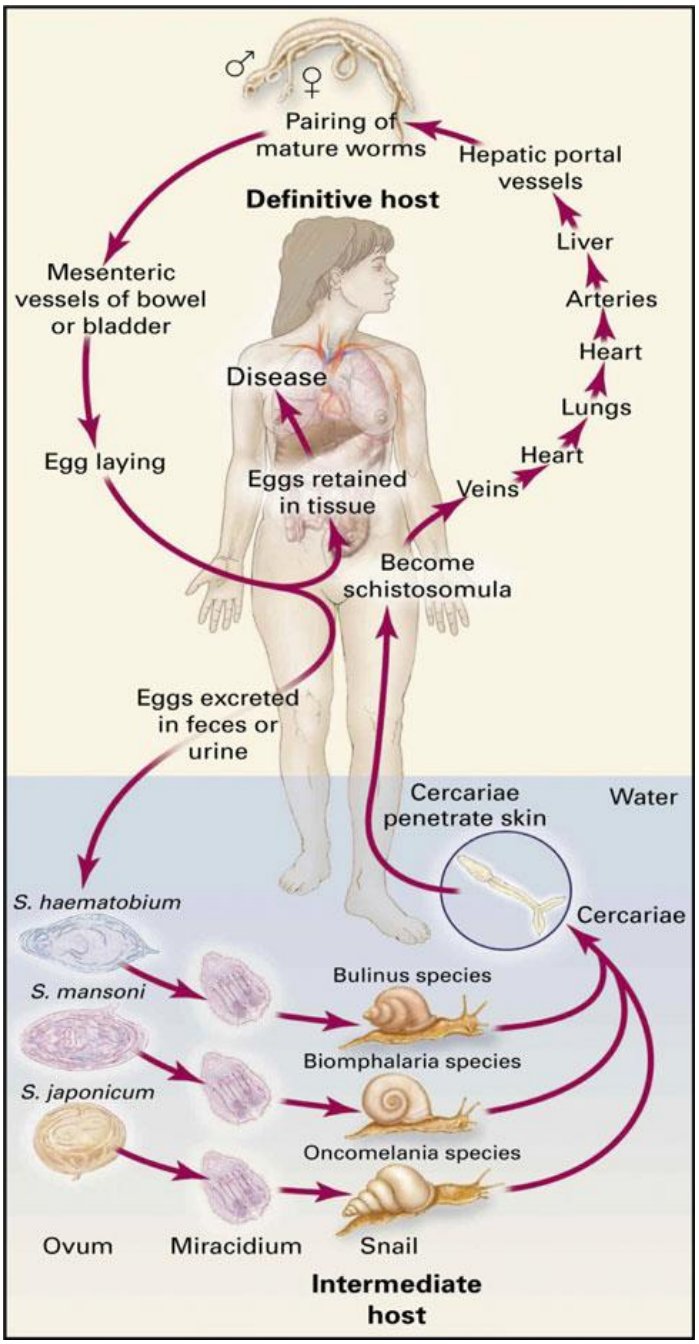


Figure 4: Life cycle of schistosomiasis

Reproduced from (Ross *et al.*, 2012; Ross and Yuesheng, 2017)

Once the cercaria comes into contact with human skin, it penetrates and sheds its tail, becoming a schistosomulum and enters capillary blood or lymph vessels. This penetration may cause itchy maculopapular "swimmer's or fisherman's" skin rash, commonly experienced among visitors and migrants on their first exposure in high endemic areas especially in *S. japonicum* infection than *S. haematobium* and *S. mansoni* (Ross *et al.*, 2002). The schistosomula develop while following circulatory or lymphatic system and through the lungs by passive intravascular migration, causing cough and wheezing, before reaching the liver as juvenile male and female worms, where they reside in the intrahepatic portal vessels. In host circulation, maturing worms survives hostile immune environment due to their ability of regenerating their outer tegument (Colley and Secor, 2014; Ross and Yuesheng, 2017).

The juvenile worms in the liver develops further and mature in 1 – 3 months into adult schistosomes, 1-2 cm long, which are actively motile and pair up in perpetual copulatory position with the female being held in the male *copulo* (gynecorphoric canal). They can live in humans normally for 3 - 10 years but up to 40 years has been reported. The worms feed on erythrocytes, releases energy from glucose metabolism or fatty acid oxidation for egg production (Colley *et al.*, 2014). The schistosomes have no anus as their gut has a blind end, so they regurgitate their waste into blood stream, which is useful for development of blood and urine-based diagnostic assays. Thereafter, the matured paired worms migrate to final habitat in specific venous plexuses, vesical (bladder) for *S. haematobium* and mesenteric for *S. mansoni* and other species, with some possibly migrating to genital vessels where they are responsible for causing genital schistosomiasis.

The adult male and female worms mate to fertilise the eggs which the female lays in the terminal venules of preferred tissues. The eggs are impeded from escaping into circulation by the adult worms obstructing the vessels. The eggs then penetrate the vessel walls and enter tissues of the particular neighbouring organs. The parasite and host adapt to the ova presence which aids rhythmic excretion, with the peak before mid-day (Rollinson, 2009). In addition, movement of the organs propels the eggs towards the lumen of the organ from where they escape into the outside

world through urine or stool. About half of the eggs get trapped in the tissues and die within 2 weeks, causing bulk of pathologies described as chronic schistosomiasis in the next sections (Colley *et al.*, 2014).

Some requirements for transmission of schistosomiasis include contaminated water with viable eggs expelled from human hosts, presence of suitable specific snail hosts in the water, suitable environmental conditions of asexual schistosome development in the snails and human exposure to infested water containing infective cercariae. Therefore, targeted interventions towards these areas are critical in achieving prevention and control of schistosomiasis.

2.4. Epidemiology of schistosomiasis in Africa

At least 17% of world's population is located in Africa, whose population is estimated at 1.3 billion and regarded as the fastest growing continent (UN, 2019). Most of the people live in SSA which experiences diverse weather conditions and has the highest burden of tropical and infectious diseases in the world including HIV and NTDs. Schistosomiasis is endemic in 78 countries globally and the majority of affected people live in poor and rural communities along rivers, dams and lakes in SSA (WHO, 2018b).

At least 779 million people are at risk of the disease in SSA with over 200 million people are infected, making it one of the commonest NTD (Tchuente, 2012; McManus *et al.*, 2018). The global burden of schistosomiasis in 2016 was estimated to be 2.5 million disease-adjusted life years (DALYs), with Africa contributing to 2.2 million DALYs, second largest after malaria among the parasitic and vector-borne diseases (WHO, 2018a). Urogenital schistosomiasis (UGS) affects more people in SSA compared to intestinal schistosomiasis, with 436 million people estimated to be at risk of *S. haematobium* and 112 million are infected, while 393 million are at risk of *S. mansoni* and 54 million are infected. Other species causing intestinal schistosomiasis in Africa include *S. guineensis* and *S. intercalatum* in rainforest areas of Central Africa.

Schistosomiasis mostly affects children (pre-school aged (PSAC) and school aged (SAC)), adolescents, women, fishermen, farmers, irrigation workers and other people when they expose themselves frequently to infective cercariae during their routine domestic chores, recreational or professional activities in infested water. Lack of access to adequate, safe and clean water, poor sanitation and inadequate hygiene make local inhabitants living near infested freshwater bodies, vulnerable to schistosome infection. PSAC and SAC have been observed to harbour the greatest number of worms in endemic areas (Tchuente, 2012; Stothard *et al.*, 2016) and morbidity trends rises to a peak during adolescent age (10-15 years) and falls down steadily to lower levels in adulthood. Heavily infected persons suffer most of the clinical consequences of the infection and are also the major sources of infection for the rest of the community (WHO, 2002).

In highly endemic areas, transmission patterns commonly show that 60% to 80% of SAC are infected while 20% to 40% of adults remain actively infected (Colley and Secor, 2014). The *S. haematobium* infection is commonest and heaviest in children aged between 5 and 15 years who, with highest egg output and frequent exposure to freshwater bodies, are more likely to contaminate the water, being the most important reservoir group of the infection (Montresor *et al.*, 1998; Gryseels *et al.*, 2006). Age-associated decline in infection rates in the adults of endemic population result from antiparasitic immunity rather than reduced contact with infested water (Colley *et al.*, 2014).

2.4.1. Schistosomiasis in Malawi

Malawi is located south of Equator in SSA, bordered by United Republic of Tanzania, People's Republic of Mozambique and Republic of Zambia (Figure 1), with 118,484 square kilometres of land (80%) and water (20%) mostly Lake Malawi, the third largest African lake and renowned tourist destination (NSO and ICF, 2011). Its population of 17.6 million comprise of more women (52%), younger people (51% are aged less than 18 years), majority living in rural areas (84%) and below poverty line (52%) (NSO, 2019).

Schistosomiasis has been reported in Malawi since early 1900s where both *S. haematobium* and *S. mansoni* have been observed to be prevalent and highly focal in distribution along water bodies (Doumenge *et al.*, 1987). *Schistosoma haematobium* has been highly prevalent throughout the country especially shores of Lake Malawi and Shire river, with prevalences of between 32% to 94% in southern and central areas, 75% in northern areas and almost 100% along Lake Malawi since 1980s (Teesdale and Chitsulo, 1985; Makaula *et al.*, 2014). On the other hand, *S. mansoni* predominates Lower Shire river basin, southern highlands, plains of Central and Northern regions, with lower prevalences of mostly less than 40%. Frequent exposures to infested water contributes to reinfections which have been noted along the shores of Lake Malawi with *S. haematobium* infection with rates of 30% to 40% (Madsen *et al.*, 2011).

Bulinus and *Biomphalaria* snail species have been found in Malawi to be responsible for active transmission of schistosomiasis. *Bulinus globosus* and *B. nyassanus* have been observed especially along shores of Lake Malawi causing high transmission and prevalence of *S. haematobium* infections, with subsequent rise in their population recently due to overfishing, thereby reducing molluscivorous fishes which predate on the snails, apart from ecological changes, cyclonic events and migration (Stauffer *et al.*, 1997; Madsen *et al.*, 2011). *Biomphalaria* snails have been observed in swamps, rivers and other water bodies away from Lake Malawi until the recent discovery in the southern part of Lake Malawi in Mangochi District with *S. mansoni* and autochthonous transmission of intestinal schistosomiasis among SAC in the area (Alharbi *et al.*, 2019).

2.5. Clinical and pathological features of schistosomiasis

Schistosomiasis infection has been associated with various clinical and pathological features in infected people. Much of the pathological features are primarily due to egg deposition following their entrapment in various tissues, triggering immune response with the human host (Colley and Secor, 2014). A large proportion of infected people in endemic areas remain asymptomatic while heavily infected persons suffer most of the clinical consequences of the infection (Samuel *et al.*,

2000; WHO, 2002). Clinical features can be categorised as immediate phase, acute phase and chronic phase of schistosomiasis (Ross *et al.*, 2002; Squire and Stothard, 2014).

2.5.1. Immediate phase of schistosomiasis

This occurs soon after penetration of cercariae into the skin, characterised with cutaneous maculopapular rash which can be itchy, also known as *swimmer's itch*. This is mostly associated with avian and animal schistosomiasis but seldom with human schistosomiasis. Usually, the rash is clinical diagnosis, self-limiting and resolves with a few days without treatment.

2.5.2. Acute phase of schistosomiasis

Acute schistosomiasis (also known as Katayama fever) occurs usually at least 2 weeks following contact with contaminated water (Squire and Stothard, 2014). This is rarely seen in residents of endemic area but commonly experienced by visitors during their initial infection with schistosomes. When the worms start laying eggs, soluble antigens leak out of the eggs triggering a Th-1 immune response (Colley and Secor, 2014), antibody production and antigen-antibody (Ag-Ab) complexes formation, resulting in the classical Katayama fever. Other symptoms include urticaria, eosinophilia, fatigue, generalised myalgia, diarrhoea, hepatomegaly, splenomegaly and cachexia.

The eggs migrate from circulation through the tissues to urinary bladder lumen in order escape out of the body. As they move into the bladder, they cause pain (dysuria) and ulcers with blood loss. As they escape, they cause haematuria, dysuria or little blood in stool, which are frequently noted 2 to 3 months after the infection (Samuel *et al.*, 2000). In endemic African countries, *S. haematobium* is the commonest cause of haematuria in children (Figure 5). In both PSAC and SAC, the infections usually result in anaemia, malnutrition, detrimental effects on cognitive development, affecting school attendance and education performance; as well as increased susceptibility to severe bacterial disease caused by *Salmonella* infections (Knopp *et al.*, 2013).

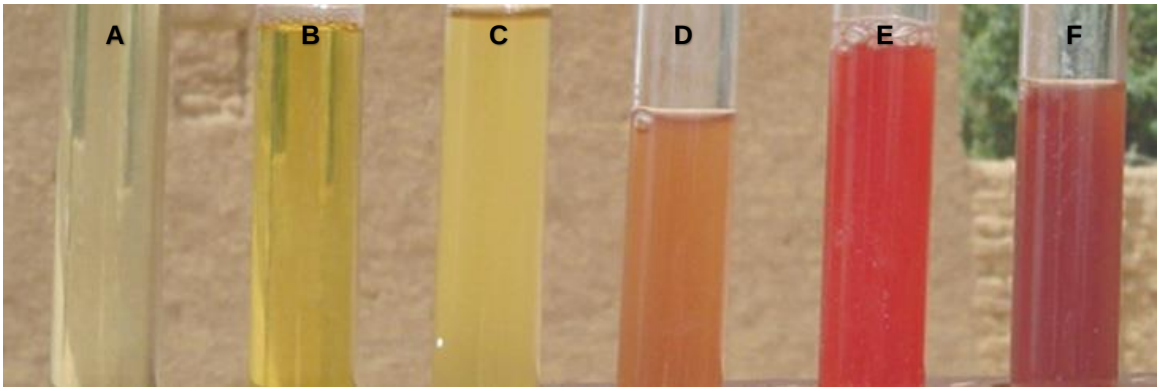


Figure 5: Haematuria with UGS infection caused by *S. haematobium* eggs expelled in urine

A, **B** and **C.** urine without haematuria; while **D**, **E** and **F.** urine with increasing intensity of haematuria.

Image courtesy of J. Russell Stothard, LSTM, August 2019.

Furthermore, schistosome eggs retained in the tissues (Figure 6) trigger intense immune and inflammatory reactions with the host due to the leakage of proteases, soluble egg antigens (SEA) and other potentially toxic moieties once they die in the tissues. This result in granulomata formation, which is characterised by lymphocytes, eosinophils, activated macrophages, foreign body giant cells, plasma cells, Langerhans cells, fibroblasts and multinucleated histiocytes (Ross *et al.*, 2002; Yuesheng and Ross, 2017; McManus *et al.*, 2018). Such immune-mediated response can lead to necrosis and damage to tissues in the liver, gastrointestinal and genitourinary organs among others (Bustinduy and King, 2014; Colley *et al.*, 2014; Squire and Stothard, 2014).

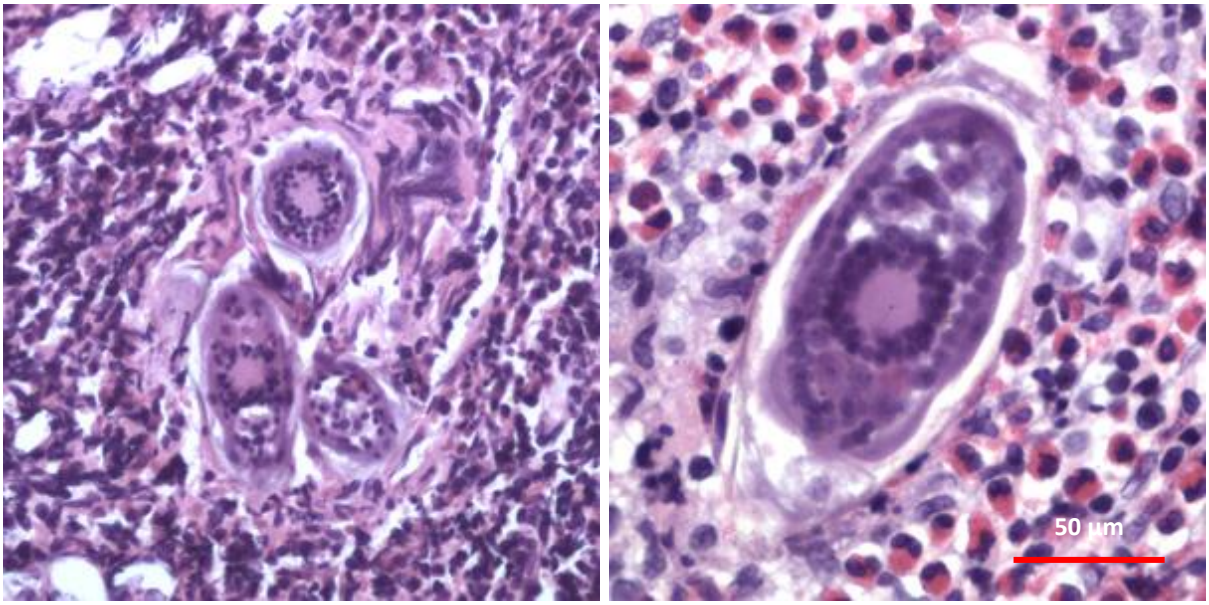


Figure 6: *S. haematobium* eggs trapped in a urinary bladder seen on biopsy, stained with H&E.

Images courtesy of the Michael E. DeBakey V. A. Medical Center, Houston, TX, through https://www.cdc.gov/dpdx/schistosomiasis/index.html.

Some eggs can penetrate some genital organs in both males and females, causing specific pathologies which are described in the subsequent sections.

2.5.3. Chronic phase of schistosomiasis

Chronic schistosomiasis arise from more eggs being trapped in the tissues, stimulating further repeated Th-2 immune reactions from released SEA (Colley and Secor, 2014; Squire and Stothard, 2014; Olveda and Ross, 2017). Continued SEA exposure induces downregulating mechanisms, resulting in granuloma formation, hundred times more than the ova, containing epithelioid and giant cells, anti-IgE antibodies, lymphocytes, eosinophils and other cells. This granulomatous response can cause anatomical and functional obstruction of organs which is reversible after anti-schistosomal chemotherapy (Samuel *et al.*, 2000).

Further granulomatous reactions result in calcification and fibrous tissue development in the affected tissues, whose pathology becomes irreversible even with anti-schistosomal chemotherapy. Some features of chronic schistosomiasis include urinary bladder calcification and carcinoma, obstructive uropathy, renal hydronephrosis and genital schistosomiasis (for *S. haematobium*); colon

pseudopolyposis, liver 'Symmer's pipestem' fibrosis, portal hypertension, hepatosplenomegaly and varices (*S. mansoni, S. japonicum*); and neuroschistosomiasis (ectopy of schistosome eggs into the nervous system (Ross *et al.*, 2012)).

Urinary bladder cancers are the most commonly diagnosed malignancy in many *S. haematobium* endemic areas, commonly in men (Rollinson, 2009). The common cancer pathology associated with chronic *S. haematobium* infection is squamous cell carcinoma. Mechanism of the carcinogens remain still unclear, however *S. haematobium* infection is strongly associated with higher risk for cytological abnormality characterising the malignancy. Early diagnosis and treatment are very important in preventing chronic inflammation and its consequences including fibrosis, malignancy and even death, described above (Samuel and Taylor, 2015).

2.6. Female genital schistosomiasis (FGS)

As described already, women and girls are disproportionately affected by schistosomiasis infection due to their close contact with contaminated water bodies during numerous domestic activities in the community (washing, bathing, water-drawing for their household needs), putting them at risk of schistosome infection.

Female genital schistosomiasis (FGS), defined as infection associated with presence of *Schistosoma* ova in genital organs of women, was first described in 1899 with a papillomatous mass in the submucosa tissue of the vagina of a younger married woman in Egypt, containing numerous ova but none in her urine (Madden, 1899). Since then, schistosomiasis has been observed to be one of the commonest causes of genital lesions in endemic areas, affecting over half of the adult female population(Kjetland *et al.*, 2008).

Up to 75% of women infected with *S. haematobium* in urine living in endemic areas have reported abnormal lesions, nodules and ulcers in genital organs such as cervix, vulva and vagina, which sometimes has been mistaken as sexually-transmitted infections (Samuel and Taylor, 2015),

polyps or malignancy. As much as 23% will have FGS without ova in the urine. There are 9 to 13 million women with FGS globally with the commonest sites in the body being cervix and vagina.

A study conducted in rural Zimbabwe showed that 58% of women with *S. haematobium* ova in urine had FGS, but 41% with FGS had no detectable ova in their urine (Kjetland *et al.*, 2005). Those with FGS had characteristic grainy sandy patches, abnormal blood vessels, neovascularised and friable mucosa on their cervix(Colley *et al.*, 2014). The women also experienced stress incontinence and pollakiuria among other problems. Genital itching, malodorous and abnormally coloured vaginal discharge were significantly associated with *S. haematobium* infection in the women (Kjetland *et al.*, 2008).

Serious complications caused by FGS are ectopic pregnancy, subfertility and infertility, which also contribute to chronic physical and psychosocial consequences to affected women (Rollinson, 2009) like family disputes and divorce. Other consequences associated with FGS include dyspareunia, contact bleeding, menstrual disorders, chronic abdominal pains, chronic cervicitis, vaginal or cervical polyps, spontaneous miscarriages, preterm and low birthweight babies, ascites among more (Talaat *et al.*, 2004; Kjetland, Leutscher and Ndhlovu, 2012; Christinet *et al.*, 2016).

Women with genital schistosomiasis have been observed to have a higher HIV prevalence than others, and also FGS enhance risk of having HIV by 3-fold which highlight the link between FGS and HIV. The sandy patches on the cervix have been suggested as an important risk factor for both acquisition and transmission of HIV (Kjetland *et al.*, 2005; Kjetland *et al.*, 2008; Kjetland, Leutscher and Ndhlovu, 2012; Christinet *et al.*, 2016). In addition, the calcified *S. haematobium* ova in genital tissues increase the density of HIV receptive CD4+ cells, hence lesions around the ova provide entry point for HIV (Jourdan *et al.*, 2011).

Praziquantel treatment among FGS patients result in parasitological cure and stops excretion of new ova in urine and genital tract. Provision of such treatment in to young girls including SAC will prevent FGS development, which represent an innovative preventive AIDS strategy in endemic areas of HIV and schistosomiasis, like SSA (Hotez, Fenwick and Kjetland, 2009). As the cost of

implementing Praziquantel MDA is relatively low, estimated at US$0.32 per person, there's need for women with FGS have access to regular PZQ treatment in endemic areas, in order to avert around 120,000 new HIV cases in the next decade.

2.7. Male genital schistosomiasis (MGS)

Male genital schistosomiasis (MGS) is a chronic manifestation of schistosomiasis, associated with presence of schistosome eggs in genital fluids and tissues and related pathologies in the male genital system (WHO, 2018b). This was first described in 1911 when a lesion seen in spermatic cord of a young Egyptian man was mistaken for a tuberculous lesion (Madden, 1911). MGS is usually underreported and unknown in most endemic areas, despite *S. haematobium* infection causing UGS previously observed to produce characteristic pathologies in reproductive organs (Poggensee and Feldmeier, 2001). Schistosome eggs which pass through or entrapped in tissues of prostate, seminal vesicles, vas deferens, epididymis and testis (Figure 7), trigger immune reactions and granulomata formation resulting in classical symptoms and pathologies, associated with MGS.

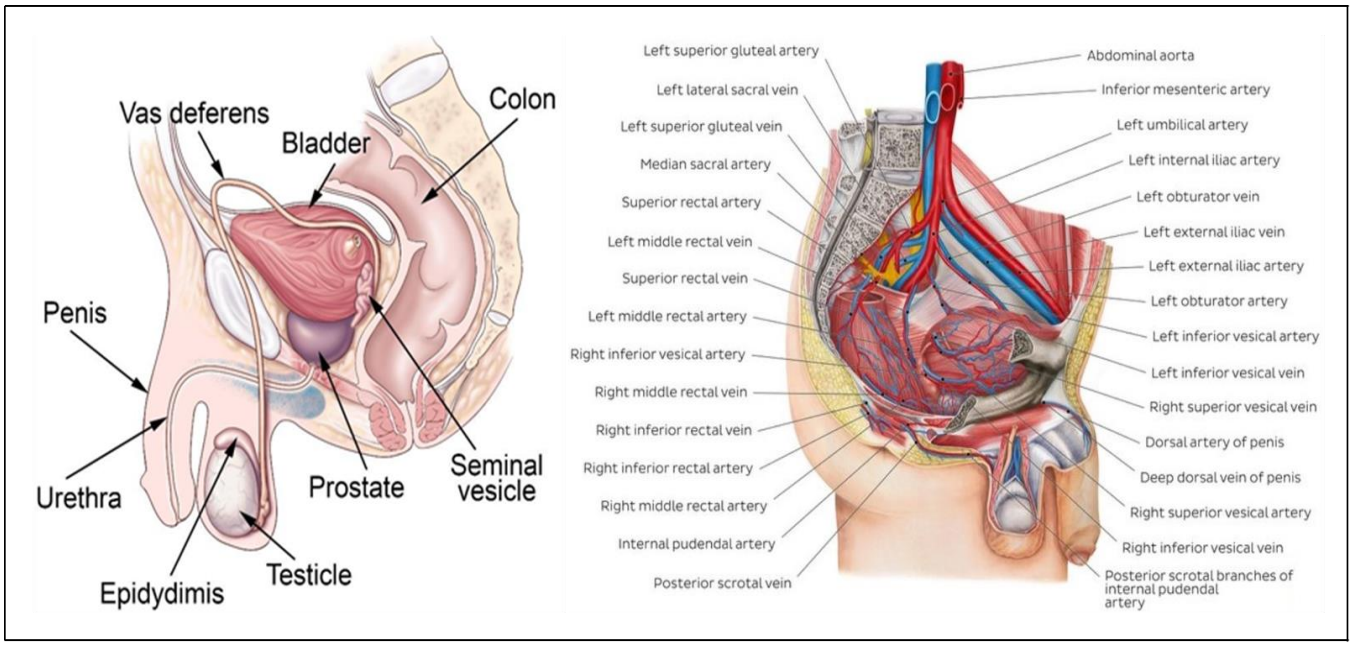


Figure 7: Male genital organs affected by schistosome eggs are passing through or trapped

Image courtesy of https://my.clevelandclinic.org/health/articles/9117-male-reproductive-system and https://www.kenhub.com/en/library/anatomy/the-male-reproductive-system

The symptoms associated with MGS include pelvic pain, especially of the genital organs, coital or ejaculatory pain, haemospermia (presence of blood in the semen), abnormal ejaculates (Figure 8), occasional spermaturia (presence of spermatozoa in urine), infertility and abnormal enlarged organs (Butterworth *et al.*, 2013; Bustinduy and King, 2014; Squire and Stothard, 2014). Although haemospermia is seldom reported in most medical textbooks describing schistosomiasis in tropical countries (Squire and Stothard, 2014), it has been observed in some patients presenting in early stages of MGS (Barlow and Meleney, 1949; Gelfand *et al.*, 1970; Corachan *et al.*, 1994; Feldmeier *et al.*, 1999; Schwartz *et al.*, 2002) and even at a young age (Rambau *et al.*, 2011).

Also post-mortem and histopathological studies have shown that those affected with *S. haematobium* had their seminal vesicles and prostrate frequently affected by the egg-induced lesions similar to urinary bladder, such as granulomatous infiltration, fibrosis and calcifications (Edington *et al.*, 1970; Gelfand *et al.*, 1970; Patil and Elem, 1988). Remarkable genital pathologies have been observed on radiological examinations of those affected with MGS, highlighting the role of ultrasonography and other radiological techniques which can be utilised in endemic areas (Vilana *et al.*, 1997; Al-Saeed *et al.*, 2003; Ramarakoto *et al.*, 2008).

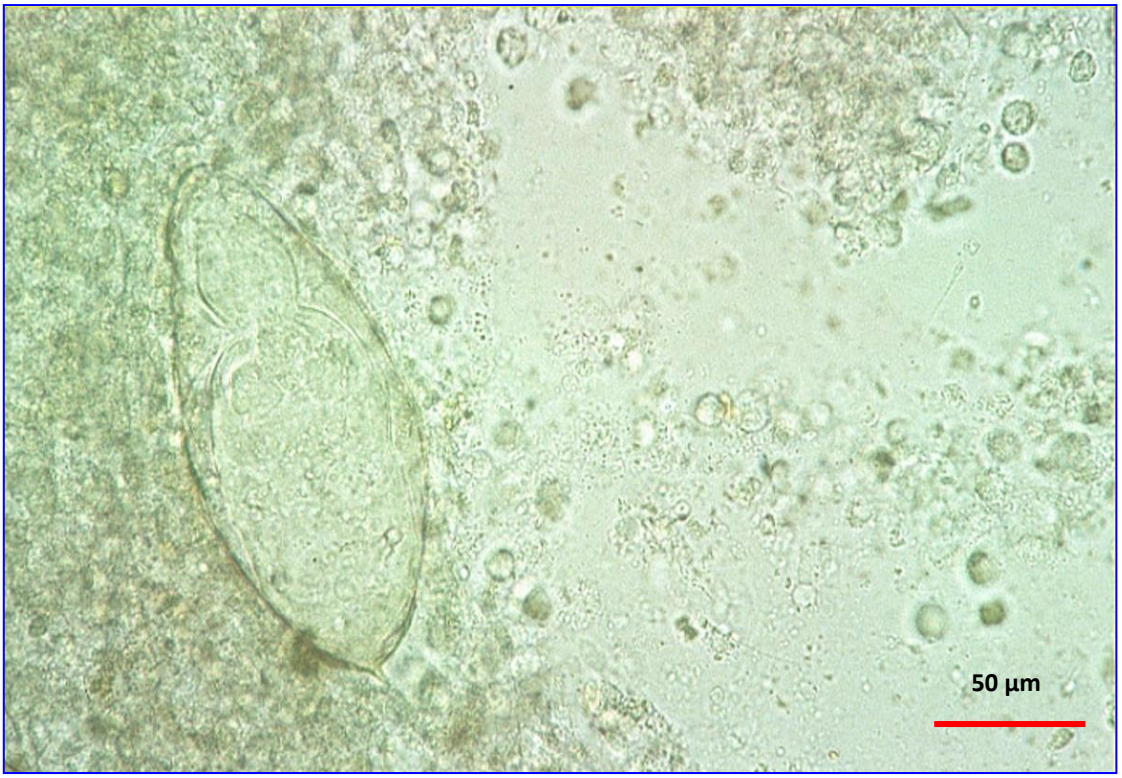


Figure 8: *S. haematobium* egg in semen of a man who swam in Lake Malawi.

Image courtesy of the E. van Lieshout, LUMC, Netherlands (van Delft et al., 2007).

One of the recent research studies in the last decade was conducted by Leutscher *et. al.* among local inhabitants in endemic areas in Madagascar where *S. haematobium* ova were detected in urine and semen samples (Leutscher *et al.*, 2000). Further studies in the same areas observed that increased levels of cytokines and inflammatory markers like eosinophil cationic protein (ECP) in males with schistosome eggs in their semen, suggesting that frequent genital organ infection occur with schistosomiasis (Leutscher *et al.*, 2005). *S. haematobium* infection has been hypothesized to cause increased viral shedding into semen of HIV-infected men as a result of egg-induced inflammation in seminal vesicles and prostate (Leutscher *et al.*, 2005; Leutscher *et al.*, 2008a; Leutscher *et al.*, 2008b; Stecher *et al.*, 2015; Midzi *et al.*, 2017). However, MGS remains underdiagnosed and underreported in local male inhabitants of most endemic areas especially in SSA, with limited research interest on this important infection in the last decade.

2.8. Diagnosis of schistosomiasis

A range of parasitological, immunological and molecular methods have been used for detection of UGS (Stothard *et al.*, 2014). The operational gold standard of diagnosing UGS is direct microscopy of filtered urine (Peters *et al.*, 1976), which has been widely used in high transmission areas to estimate the morbidity upon enumeration of eggs in 10 ml of urine (i.e. ≥50 eggs per 10 m). However, it lacks diagnostic sensitivity in light infections when the number of eggs shed in urine is very few (counting less than 1 egg in 10 ml) and repeated urine samples may need to be inspected. Other less expensive methods that can complement this test include the use of questionnaires in high risk areas for recognition of macrohematuria (presence of red urine), and urine reagent test strips for microhaematuria as a diagnostic indicator and marker of bladder pathology, although these suffer from poor rates of sensitivity (< 75 %) and cannot detect sub-clinical or acute infections (Stothard *et al.*, 2014; Le and Hsieh, 2017).

Antibody-based tests such as enzyme-linked immunosorbent assay (ELISA) for IgG titres against schistosome soluble egg antigen (SEA) present in human serum have been widely used for diagnosis (Le and Hsieh, 2017). Serological methods have much higher sensitivities than filtration and microscopy, especially in travellers originating from non-endemic regions, however they cannot distinguish active from past infections, nor discriminate between species of schistosome. Alternative highly sensitive approaches based on detection of schistosome glycan antigens in blood or urine have been developed to diagnose active *Schistosoma* infections (Utzinger *et al.*, 2015; Le and Hsieh, 2017), which are described below. Nucleic acid amplification tests (NAAT) have increasingly been used as highly sensitive and specific diagnostic tools, utilising several clinical specimens (i.e. stool, urine or tissue biopsy) in diagnosing the infection (Utzinger *et al.*, 2015). Currently NAAT are not widely available, owing to the need for skilled personnel, laboratory equipment and infrastructure which make roll out, especially in endemic areas, difficult. Newer approaches based on loop-mediated isothermal amplification (LAMP), POC magnetic bio-capture probes and microfluidic

devices are being developed for resource poor settings (Minetti *et al.*, 2016; Candido *et al.*, 2018; Poulton and Webster, 2018).

2.8.1. Glycobiology of schistosome antigens and their applications

A number of different schistosome antigens are excreted and secreted into the human circulation, namely cercarial antigens, gut-associated antigens from living juvenile and adult worms and antigens secreted from eggs (van Lieshout, Polderman and Deelder, 2000). Most of the described circulating genus specific antigens in humans are from gut-associated tissues of feeding worms, namely circulating cathodic antigen (CCA) and circulating anodic antigen (CAA). Both CCA and CAA, see Figure 9A, are detectable in the host's serum as well as in urine although relative concentrations can differ and these are thought to have immunomodulating effects within the parasitized host (van Dam *et al.*, 1996; van Dam and Deelder, 1996; van Diepen *et al.*, 2012; Hokke and van Diepen, 2017).

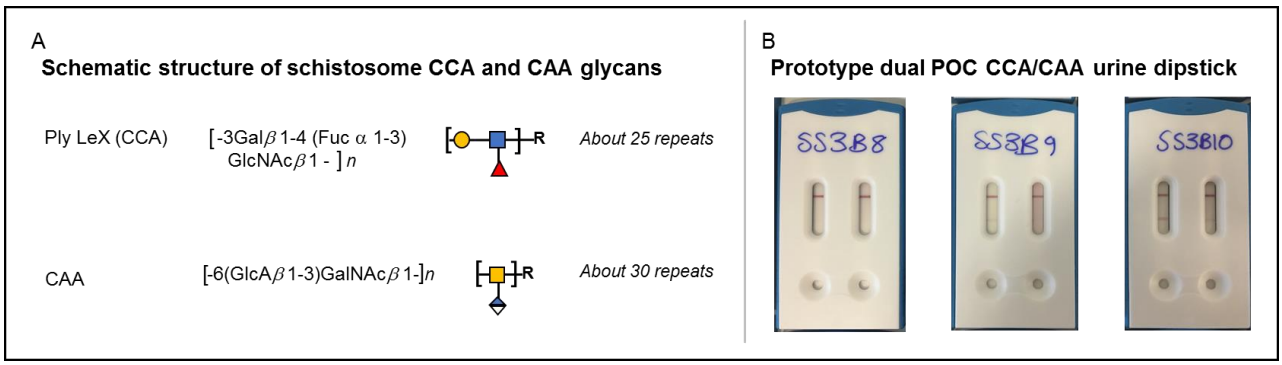


Figure 9: Structures of schistosome CCA and CAA with prototype dual urine dipstick

A. Schematic outline of the chemical and polymeric glycan structures of the two most common schistosome glycoproteins (CCA and CAA) using in rapid urine-antigen detection dipsticks. **B.** An illustration of future developments in POC diagnostics with a prototype dual antigen urine-dipstick detecting each antigen separately (LHS CCA, RHS CAA). Having a dual design could detect and differentiate urogenital and intestinal schistosomiasis co-infection simulatenously, however, this prototype has inadequate sensitivity for detection of urine-CAA and needs reformulation. (*Figure reproduced from (Kayuni et al., 2019b)).*

Glycoproteins containing CCA are produced by the gut epithelium of schistosomes presumably for its protection and are regurgitated into the human bloodstream upon digestion of the blood meal as worms have a blind-end gut. The structure of these positively-charged antigens consists of multiple trisaccharide units (Lewis-X) containing fucose, galactose and N-acetyl-

galactosamine, Figure 9. In addition, CCA epitopes are also present on *Schistosoma* egg secretions. They also evoke high titres of specific IgM/IgG antibodies, which may be responsible for the mild-moderate neutropenia during schistosome infection arising from an inhibitory factor in their sera, causing delay in the maturation of neutrophils in the bone marrow and spleen (van Dam *et al.*, 1996).

Making use of CCA, POC urine-based lateral-flow assays have been developed since the late 90s and have been commercially available since 2002 in the form of reagent dipsticks or cassettes, with carbon- or gold-labelled monoclonal antibodies and interpreted visually (van Dam *et al.*, 2004; Le and Hsieh, 2017). Detectable CCA-levels typically correlate with active schistosome infection, which become undetectable after successful praziquantel treatment. However, upon comparison with intestinal schistosomiasis (caused by *S. mansoni*) these tests perform poorly for UGS see Table 1, hence combining it with urine filtration is needed, and can help with simultaneous detection of co-infected cases (i.e. *S. haematobium* and *S. mansoni*). Since the first use of POC-CCA tests, they have been subject to many evaluations of their performance, with WHO now endorsing these tests as appropriate for estimating prevalence thresholds for intestinal schistosomiasis to guide preventive chemotherapy (Colley, Andros and Campbell, 2017; Bärenbold *et al.*, 2018). Current developments in POC testing include a prototype dual antigen cassette with both CCA and CAA strips included, enabling detection and discrimination of intestinal and urogential schistosomiasis simultaneously *see* Figure 9B. (see https://freebily.eu/about/).

Table 1: Sensitivity and specificity of urine POC-CCA tests to diagnose *S. haematobium* infection, in comparison to urine filtration and microscopy as the gold standard

Source	*N*	Prevalence (%)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Stothard *et al.*, 2009	150	31 - Moderate	9 (2 – 21)	98 (93 – 100)
Ayele *et al.*, 2008	206	48 - Moderate	52 (42 – 62)	64 (54 – 73)
Midzi *et al.*, 2009	265	40 - Moderate	79 (70 – 86)	44 (36 – 52)
Ashton *et al.*, 2011	373	26 - Moderate	37 (26 – 49)	79 (72 – 84)

Obeng *et al*., 2008*	153	Not stated (Case-control study)	41 (Not stated)	91 (Not stated)
El-Ghareeb *et al*., 2016*	600	5	88 (Not stated)	96 (Not stated)
Sanneh *et al*., 2017*	1,954	23	48 (Not stated)	76 (Not stated)
Rubaba *et al*., 2018*	380	40	68 (Not stated)	46 (Not stated)
Range		**5 – 48**	**9 – 88**	**44 – 98**

Data adapted from (Ochodo *et al.*, 2015), where intensities of infection are classed as 'moderate'. Additional sources marked by " * ". Where data is missing this is marked as 'Not stated'. *(Table reproduced from (Kayuni et al., 2019b))*.

The alternative CAA antigens are also gut-associated glycoproteins but are negatively charged. The structure of CAA is made up of carbohydrate chains which consist of multiple disaccharide units containing N-acetyl-galactosamine and glucuronic acid (Figure 9). It binds to the collagen-like stalk of first complement component C1q, probably preventing host complement from attacking the schistosome gut (van Dam *et al.*, 1993). CAA is also present in urine or serum of actively infected people as shown by monoclonal antibody-based antigen detection ELISA's and more recently up-converting phosphor-lateral flow assay (UCP-LF CAA) (Corstjens *et al.*, 2008). This assay has shown to be more sensitive and specific especially in low transmission areas but is unable to differentiate between urogenital and intestinal schistosomiasis (Corstjens *et al.*, 2015; Knopp *et al.*, 2015). The UCP-LF-CAA test therefore has future application in the general monitoring of schistosomiasis as disease control programmes move towards interruption of transmission or endgame scenarios (Corstjens *et al.*, 2017; Stothard *et al.*, 2017).

2.8.2. Focus on male genital schistosomiasis

Substantial progress has been made in developing a gold standard technique for a definite FGS diagnosis, namely colposcopy in gynaecological clinics (WHO, 2015a) often with genital tissue biopsy for histopathology in the hospital laboratory. Conversely, MGS remains largely undefined, an orphan within disease syndromic triage (Kayuni *et al.*, 2019a). At present, semen microscopy is

considered as a standard technique for diagnosing active MGS infection and assessing its severity, since the schistosome eggs are directly visualized. Urine filtration has been used as diagnostic proxy markers in the presence of MGS symptoms; however, there have been reports of seminal schistosome eggs in urine negative patients (Schwartz *et al.*, 2002; van Delft *et al.*, 2007). In addition, genital tissue biopsy and ultrasonography can be applied as diagnostic tools relevant in diagnosing MGS through observation of pathologies associated with the disease in the absence of other genital diseases, which have successfully been studied and reported (Leutscher *et al.*, 2008b).

2.9. Treatment, control and prevention of schistosomiasis

Treatment of all forms of schistosomiasis including MGS is achieved by single dose of a safe and effective anti-helminth drug, PZQ, a pyrazinoisoquinole derivative which remains the drug of choice, with egg-reduction rates of at least 98.7% and cure rates of 63-100%, seen up to 3 months after treatment (Knopp *et al.*, 2013). PZQ is effective on adult stages only and not young developing stages of the schistosomes (Rollinson, 2009; Knopp *et al.*, 2013). It works on the calcium ions channels by binding to actin in the worms' muscles, causing its rapid influx and disrupting homeostasis, which induces intense contractions of muscles and tegumentum, thus exposing the surface membranes to host immune defence mechanisms, Th2, which contributes to the spastic paralysis and killing of the worms (Rollinson, 2009; Knopp *et al.*, 2013). PZQ induces a switch from a predominantly IgA-specific antibody response to a IgG response within 12 weeks, which may be driven by alternations in cytokines levels in response to antigens released by paralysed or dead parasites (Samuel *et al.*, 2000). However, PZQ does not kill juvenile immature worms, prevent re-infection, or reduce transmission of the infection.

Single dose of PZQ is effective in treatment and prevention of schistosomiasis especially in high-risk population living in heavy infested, endemic areas. Significant improvements in language and memory development occur with provision of appropriate treatment to infected children (WHO, 2013b). In addition, impact of PZQ chemotherapy has shown good evidence for reversal of organ

pathology experienced with urogenital schistosomiasis. Furthermore, PZQ has no detrimental effects to humans, as shown from experimental animals who experienced no effects acutely or chronically when given higher doses than routinely given to humans (Olds, 2003). As such WHO recommends PZQ treatment should be given to all children and adults including pregnant and lactating women at any stage. Since polyparasitism is common in endemic areas, co-administration of PZQ and other anti-helminth drugs such as albendazole and ivermectin where necessary has been reported to be safe (WHO, 2002; Mohammed *et al.*, 2008), as a strategy to successfully treat and control prevalent NTDs.

The prevalence, intensity and potential for control of schistosomiasis are largely functions of interactions of social, cultural, behavioural, geographical and economic factors in a given area with local and regional ecological and environmental factors. Furthermore, global control efforts of schistosomiasis has shifted from transmission reduction (snail control) before 1970s to morbidity control (development of diagnostics and effective PZQ chemotherapy) after 1970s (Kloos, 1995; Fenwick, Keiser and Utzinger, 2006). The WHO recommends various interventions for morbidity control (development of diagnostics and effective PZQ chemotherapy) after 1970s (Kloos, 1995). It further advocates a dual strategy (Engels *et al.*, 2002), a strategy for morbidity control in high-burden areas like SSA using preventive chemotherapy with PZQ, and an integrated control strategy in low-endemic areas where elimination is feasible. Most SSA countries are not at an elimination stage yet due to higher transmission potential and prevalence rates (Rollinson, 2013).

There is also need to monitor impact of the control interventions which have been implemented, improve disease surveillance once the morbidity becomes very low (prevalence of less than 10%), improve diagnostic tests and build capacity of personnel involved in schistosomiasis control (Zhou *et al.*, 2011; Solomon *et al.*, 2012). These require strong political will, appropriate legislation and intersectoral collaboration. In addition, with the agenda of schistosomiasis control moving from morbidity to elimination, there are recommendations for intensive implementation of a combination of the successful control strategies, namely health education, preventive

chemotherapy, Intensified case management, provision of safe water supplies, improved sanitation and hygiene and vector (snail) control, which will go a long way to achieve with the available global resources (Rollinson *et al.*, 2013; Colley, 2014).

Despite availability of this safe and effective drug, much still needs to be done to reduce prevalence and intensity of schistosomiasis since the infection continue to persist and long-term sustainable control remains a challenge in many endemic areas of LMICs (Rollinson, 2009; Stothard, Bustinduy and Montresor, 2014). Schistosomiasis control in endemic areas is faced with numerous challenges, namely: mobilisation of required funding for successful interventions; coordination, monitoring and evaluation of control programmes at various levels; scale-up plans; sustainability; limited capacities; lack of country ownership, leadership and political will; poor stakeholder and intersectoral collaborations; operation research; and limited development of improved diagnostic tools Africa (Tchuente, 2012). Success stories in elimination of schistosomiasis in Japan, China and Morocco among others, resulted from setting clear goals for control and elimination, high government commitment, funding mobilisation, leadership and research which are lacking in most endemic countries, especially in sub-Saharan Africa (Tchuente, 2012).

Preventive measures for schistosomiasis include adequate health awareness and education on schistosomiasis to reduce frequency of contact with infested water bodies as well as contamination with schistosome eggs in urine and faeces; improved access and availability of safe water to communities in endemic areas, and treatment of water sources; improved sanitation which includes use of toilets instead of water bodies; preventive chemotherapy through mass drug administration with PZQ in endemic areas (Knopp *et al.*, 2013). Of the 78 endemic countries in the world, 52 countries require preventive chemotherapy with PZQ for at least 220 million children and adults, of which over 90% are in SSA (WHO, 2018c).

Intersectoral snail control measures have been advocated in certain areas which include removal of vegetation around freshwater bodies, biological control using predator fishes, crayfish,

ducks and other snail species. These preventive measures tackle particular stages of the life-cycle of schistosomes, contributing towards elimination of the disease (Colley, 2014), as described below:

a. Preventing specific intermediate snail hosts from getting infected by encouraging infected people from urinating or defecating in water bodies and using toilets. This is achieved through improved health education and awareness, improved sanitation and hygiene and behaviour change, while involving community participation at all levels (Kloos, 1995).
b. Killing or replacing the snail hosts using biological control measures such as competitor snails and snail-eating fishes, chemical control like targeted, biological-friendly molluscicide and environmental modification where removal of vegetation is done to get rid their habitats.
c. Stopping people from getting infected by reducing contact with water bodies harbouring infected snails or cercariae through provision of safe water supplies for personal, household and recreational purposes, adequate health education and awareness on the disease.
d. Killing adult schistosome worms in people laying eggs and causing disease through regular preventive chemotherapy with PZQ to all infected and at-risk populations in endemic areas as well as improved access and availability of diagnosis and treatment at health facilities in endemic areas.

Currently, there's no vaccine available and its progress of development has been slow due to inability to identify appropriate protective antigens eliciting host immune reactions to attack the parasite without cross-reacting with egg antigens which causes severe chronic disease (Rollinson, 2009).

2.10. Human immunodeficiency virus (HIV) infection in Africa

HIV is a lentivirus, belonging to a large, diverse and complex group of retroviruses (Figure 10) which affect the human immune system (Fauci *et al.*, 1996; Coffin, Hughes and Varmus, 1997). The virus slowly destroys the immune CD4 cells, resulting in an advanced and incurable disease, Acquired immunodeficiency syndrome (AIDS) (WHO, 2016b). HIV continues to be a major global public health

issue, with 37.9 million people infected by the virus globally in 2018, 770,000 dying from HIV-related diseases and 1.7 million new infections, especially among the young adults and women (UNAIDS, 2019). Eastern and southern parts of the SSA is the most affected and the epicentre of HIV pandemic, with 20.6 million infected people and over half of the global total of new HIV infections.

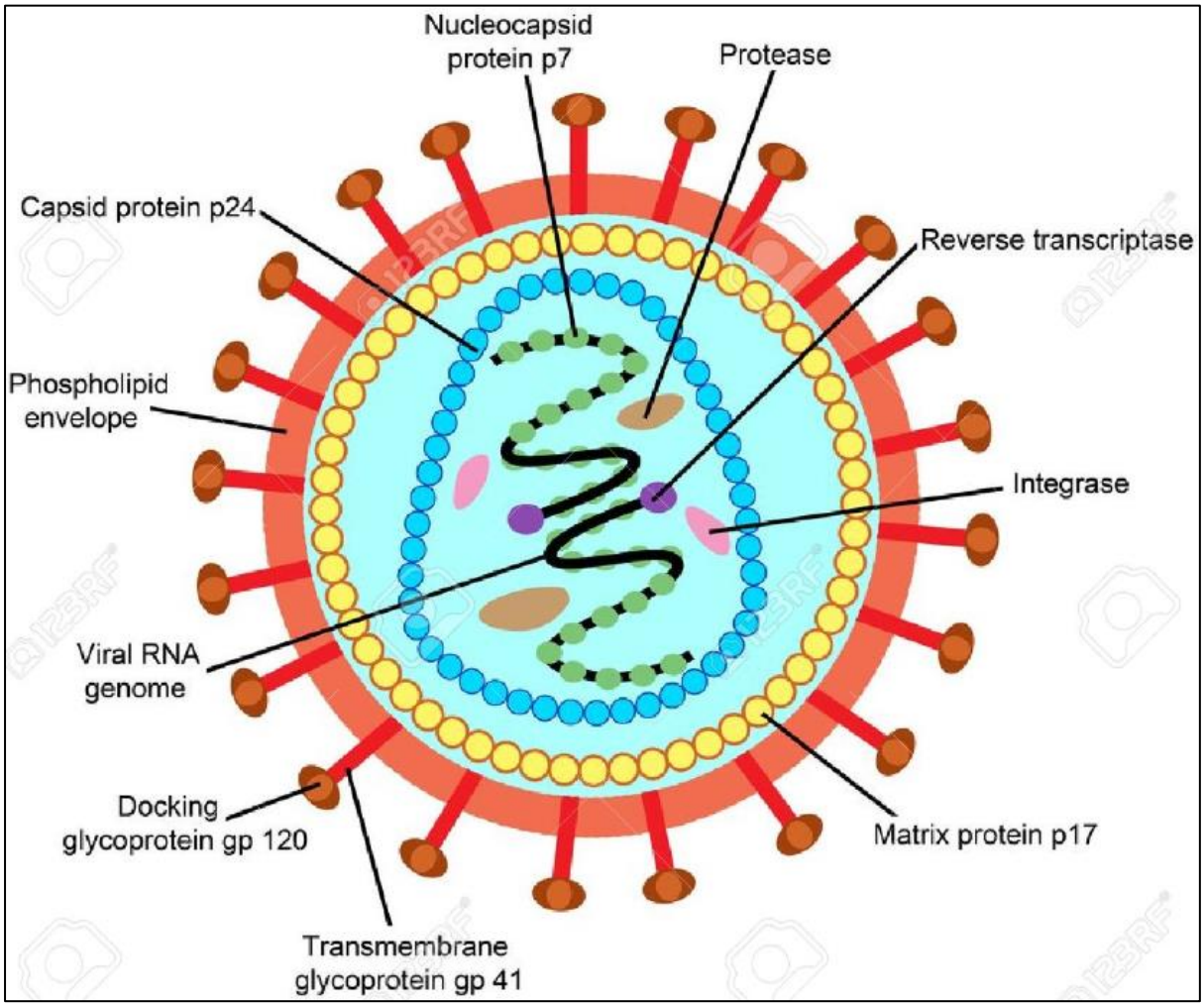


Figure 10: Schematic diagram of the HIV structure with key parts for human cell invasion.

Image courtesy of https://www.123rf.com/phpoto_18649988_structure-of-human-immunodeficiency-virus-hiv-illustration-for-basic-medical-education-for-clinics-a.html

Sexual transmission account for over 85% of all HIV infections despite the lower probability of sexual infection than other routes like blood contact or transfusion (Royce *et al.*, 1997; Cohen, 2004). This raises the idea of possibility of other factors and infections contributing to the epidemic. Evidence has shown that chronic parasitic diseases influence the natural history of HIV infection in a deleterious way (Harms and Feldmeier, 2002). In addition, sex workers and men who have sex with

men (MSM) have more than 20 times increased risk of acquiring HIV infections (UNAIDS, 2019). Unfortunately, only 79% of people globally know their HIV status, with 8.1 million in need of access to HIV testing services.

In order to control HIV infection, several interventions have been developed, implemented and evaluated by WHO, UNAIDS, Ministries of Health in SSA and globally together with other stakeholders. These interventions include provision of accurate HIV testing and counselling services to all people in SSA areas and other areas affected with the disease, treatment to all HIV infected people with standard antiretroviral therapy (ART) in accordance to approved WHO and national guidelines, regular monitoring of treatment and virological suppression, prevention and treatment of opportunistic and severe infections, nutrition support as well as health education and psychosocial support through peers, family and health personnel (Deeks, Lewin and Havlir, 2013; WHO, 2016a;b;2019).

ART are highly active drugs acting at various levels in the HIV life cycle, which have significantly contributed to reduction of HIV transmission to uninfected sexual partners (new infections / incidence), severe diseases related to AIDS (morbidity) and AIDS-related deaths (mortality) of people infected with HIV through viral suppression when regularly taken (Fauci and Folkers, 2012). Although 23.3 million people were accessing ART globally in 2018 (62% of all people living with HIV), 13.8 million (67%) were from the Eastern and southern Africa. Interesting, 92% of all pregnant women with HIV in this African region were on ART to prevent transmission to their child, which is much higher than the global percentage of 82% (UNAIDS, 2019).

The different classes of ART available to people, depending on their site of the action in the virus life cycle (Figure 11), include:

a. Those targeting HIV protein reverse transcriptase (Nucleoside reverse transcriptase inhibitors (NRTIs), Non-nucleoside reverse transcriptase inhibitors (NNRTIs)).
b. Those targeting HIV protein protease (Protease inhibitors (PIs))
c. Those targeting HIV protein integrase (Integrase inhibitors (INSTIs))

d. Those interfering with binding, fusion and entry of HIV into host cell by blocking one of several targets (Early or Fusion inhibitors (FIs))

e. Those that target particular receptor on human helper T-cell, CCR5 (Chemokine receptor antagonists (CCR5 antagonists)).

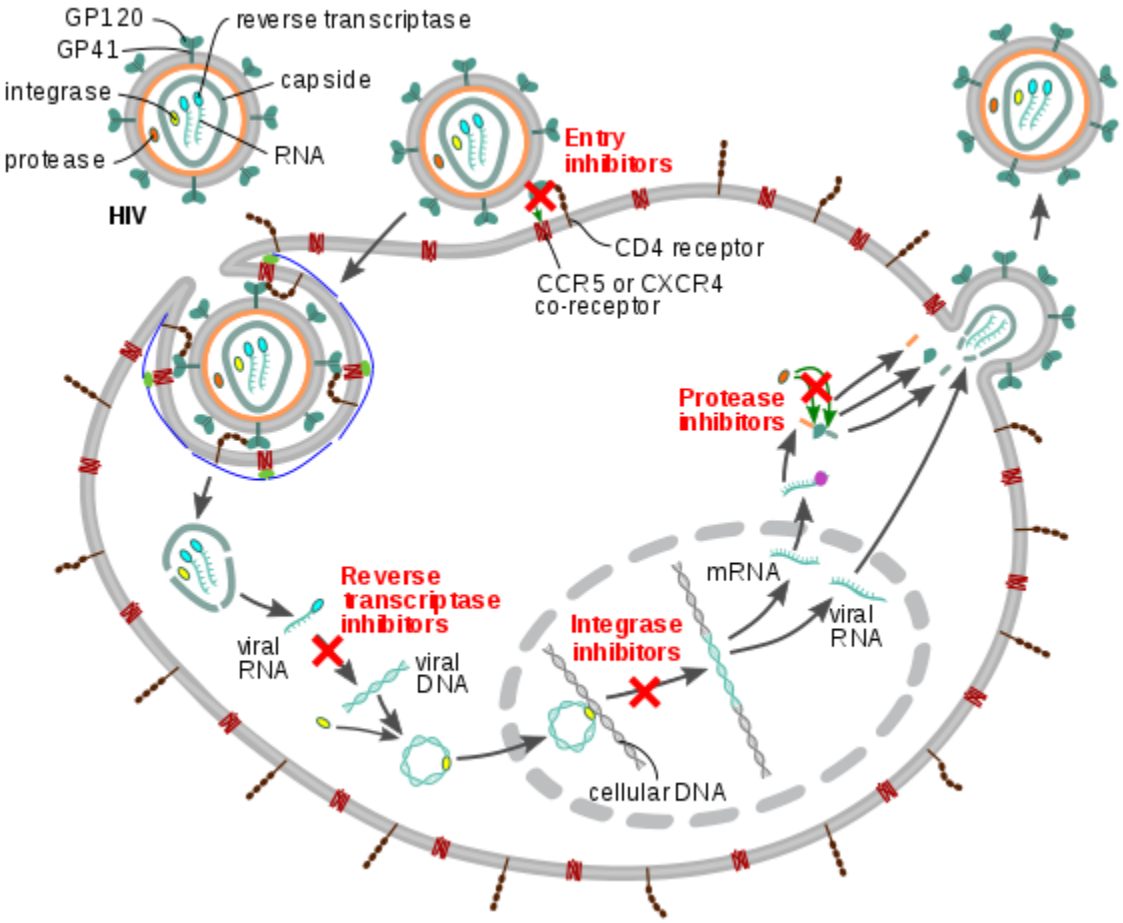


Figure 11: Schematic diagram of the areas in the life cycle of HIV where ART works.

Image courtesy of Parfum BUKANGA Christian, African Institute of Mathematical Sciences, Cameroon, 14th August 2019 (https://www.researchgate.ne/publication/335170083_Community-Based_HIV_Care) *and Thomas Splettstoesser, Nov 2013* (https://www.scistyle.com)

ART is provided in a combination form of 2 or more classes with NRTI/NNRTI as a backbone drug to prevent resistance as much as possible through suppression of the HIV, examples listed in Table 2. Of those on ART in 2018, only 53% achieved virological suppression, posing a significant challenge in achieving the 90-90-90 strategy for HIV control and ending the global AIDS epidemic

(UNAIDS, 2019). The 90–90–90 targets comprise of ensuring that 90% of the people living with HIV know their HIV status, 90% of the people living with HIV who know their HIV status are accessing treatment; and 90% of people with HIV who are receiving treatment have suppressed viral load.

Furthermore, a campaign was launched in 2016, known as **U = U** (Undetectable = Untransmittable), based on that a person with HIV having undetectable VL does not transmit HIV to their partners (PAC, 2016; The Lancet, 2017). This echoes the need to improve access to HIV testing and ART treatment to people especially in the SSA region, in addition to regular VL monitoring to undetectable levels and support to reach and maintain complete viral suppression.

Table 2: Examples of different classes of antiretroviral drugs used in ART combinations

Reverse Transcriptase Inhibitors (RTIs)		**Protease Inhibitors (P.I.)**		**INSTI**	**Entry or Fusion inhibitors**
Nucleosides (NRTIs)	**Non- Nucleosides (NNRTIs)**	**Main**	**Booster**		
Lamivudine	Nevirapine	Atazanavir	Ritonavir	Dolutegravir	Maraviroc
Abacavir	Efavirenz	Darunavir	Cobicistat	Raltegravir	Enfuvirtide
Zidovudine	Etravirine	Lopinavir		Elvitegravir	
Tenofovir disoproxil fumarate	Rilpivirine			Bictegravir	
Tenofovir alafenamide	Doravirine				
Emtricitabine					

As highlighted earlier, the advent of ART has resulted in a number of benefits at both individual and population level. At individual level, ART results in improved health and longer life and at a population level, there is reduction in HIV transmission and acquisition. These benefits accrue from ART's impact on the HIV-1 viral load. An undetectable HIV-1 viral load results in reduced HIV transmission from the index case to the uninfected partner.

This principle however is impacted by other health related issues in both the index case and the uninfected partner. Sexually transmitted illnesses (STIs) have been well known to increase the risk of both HIV transmission (in the index case by raising genital fluid viral load) and HIV-1

acquisition in the uninfected partner (by providing easy access to CD4 carrying cells within the genital tract as a result of inflammation).

2.10.1. HIV in Malawi

Since the first report of AIDS case in 1985, the prevalence of HIV among adults 15 – 64 years in Malawi has been considered to be high, recorded as 10.6% in recent national survey (Ministry of Health, 2017). The HIV prevalence varies from one area to another in the country, with higher rates observed in Mangochi district (11.8%) which bears the south shoreline of Lake Malawi and endemic for schistosomiasis where fishermen are among the high-risk occupational groups in Malawi with higher HIV prevalence, which was 11.5% in 2014 (NSO, 2014; NAC, 2015).

Integrated efforts in HIV prevention and control involving government, communities and various stakeholders have contributed reducing the incidence and mortality, namely robust, abundant health awareness on HIV/AIDS infection, testing and counselling, provision of ART and preventive treatment for opportunistic infections, care and support for people living with HIV and family members.

On ART provision in Malawi, the government through the Ministry of Health develops and disseminate national guidelines for comprehensive HIV treatment, care and support, accepted drugs, coordination, monitoring and supervision of all approved health facilities in the country. These guidelines are adopted from the WHO consolidated guidelines and updated policies for HIV treatment and prevention and ensures harmonisation of ART regimens across the country and relevance with the practice globally.

The ART regimens available in the country comprised of standard and alternate first-line, second-line and third-line regimens of which lamivudine (3TC) is the backbone in all 1st and 2nd line regimens due to its extremely well tolerance and resilient activity even in presence of drug-resistant virus (Ministry of Health, 2016). Since July 2014 following the WHO recommendation for *Test and Treat* that all people living with HIV be provided with ART (WHO, 2016a), the standard 1st line was

also changed to a regimen of fixed dose combination comprising of tenofovir disoproxil fumarate, lamivudine and efavirenz (TNF/3TC/EFV) for adults (Table 3).

Table 3: Standard ART regimens for Malawi from 1st July 2014 to 31st December 2018

Line	Regimen	Used for ART initiation as 'Start regimen'	Adult Formulation	Paediatric Formulation	Alternate regimens
1st	5	Standard for children and adults 35kg+	TDF 300 / 3TC 300 / EFV 600	None	0, 2, 6, 8, 9, NS
1st	2	Standard for children and adults under 35kg	AZT 300 / 3TC 150 / NVP 200	AZT 60 / 3TC 30 / NVP 50	0, 4, 5, 6, 7, 9, NS
1st	0	No	ABC 600 / 3TC 300 + NVP 200	ABC 60 / 3TC 30 + NVP 50	2, 4, 5, 6, 7, 8, NS, ABC/3TC+EFV
1st	4	No	AZT 300 / 3TC 150 + EFV 600	AZT 60 / 3TC 30 + EFV 200	0, 2, 5, 6, 7, 9
1st	6	No	TDF 300 / 3TC 300 + NVP 200	None	0, 2, 5, 8, 9, NS
2nd	7	No	TDF 300 / 3TC 300 + ATV/r 300/100	None	8, 10, 12, NS
2nd	8	No	AZT 300 / 3TC 150 + ATV/r 300/100	None	7, 9, 11, 12
2nd	9	No	ABC 600 / 3TC 300 + LPV/r 200/50	ABC 60 / 3TC 30 + LPV/r 100/25	10, 11, 12
2nd	10	No	TDF 300 / 3TC 300 + LPV/r 200/50	None	7, 8, 11, 12
2nd	11	Preferred start regimen for children under 3 years at sites with extra support	AZT 300 / 3TC 150 + LPV/r 200/50	AZT 60 / 3TC 30 + LPV/r 100/25	7, 8, 10, 12
3rd	12	No	DRV 600 + r 100 + ETV 100 + RAL 400	None	NS

All strengths are in mg. Alternate regimens are provided after possible adverse reaction(s). 3TC = Lamivudine, ABC = Abacavir, ATV = Atazanavir, AZT = Zidovudine, DRV = Darunavir, EFV = Efavirenz, ETV = Etravirine, LPV = Lopinavir, NVP = Nevirapine, r = Ritonavir, RAL = Raltegravir, TDF = Tenofovir. Fixed dose combinations (FDC) shown with a slash (e.g. TDF / 3TC / EFV), combinations made up of separate tablets shown with + (e.g. AZT/3TC + EFV). An "A" is added to the regimen number for adult formulations (e.g. Regimen 2A) and a "P" is added for paediatric formulations (e.g. Regimen 2P).

As of now, since 1st January 2019, the standard 1st line regimen in Malawi has been changed to dolutegravir-based combination (Ministry of Health, 2018), following overwhelming evidence of its superior efficacy over previous TDF / 3TC / EFV regimen (Table 4).

Table 4: Current Standard ART regimens for Malawi since 1st January 2019

Line	Regimen	Used for ART initiation as 'Start regimen'	Adult Formulation	Paediatric Formulation	Alternate regimens
1st	13	New standard for all patients 30kg+	TDF 300 / 3TC 300 / DTG 50	None	5, 7, 8, 10, 11, 14, 15, NS

1st	5	Alternative for patients with (relative) DTG contraindications	TDF 300 / 3TC 300 / EFV 600	None	, 4, 7, 8, 11, 13, 14, 15, 17
1st	4	No	AZT 300 / 3TC 150 + EFV 600	AZT 60 / 3TC 30 + EFV 200	5, 7, 9, 10, 11, 13, 15, 17
1st or 2nd	9	New standard for children under 20kg	ABC 600 / 3TC 300 + LPV/r 200/50	ABC 120 / 3TC 60 + LPV/r 100/25	7, 8, 10, 11, 12, 13, 14, 15
1st or 2nd	14	No	AZT 300 / 3TC 150 + DTG 50	AZT 60 / 3TC 30 + DTG 50	4, 7, 8, 9, 10, 11, 15, 17
1st or 2nd	15	Standard for children 20 – 29.9kg	ABC 600 / 3TC 300 + DTG 50	None	4, 7, 8, 9, 11, 13, 14, 17
1st	16		ABC 600 / 3TC 300 + RAL 400	ABC 120 / 3TC 60 + RAL 25	7, 8, 11, 15, TDF/3TC+RAL
1st	17		ABC 600 / 3TC 300 + EFV 600	ABC 120 / 3TC 60 + EFV 200	4, 5, 7, 8, 9, 11, 13, 14, 15, 16
2nd	7	No	TDF 300 / 3TC 300 + ATV/r 300/100	None	10, 11, 12, 13, 15, NS
2nd	8	No	AZT 300 / 3TC 150 + ATV/r 300/100	None	9, 11, 12, 13, 14, 15, NS
2nd	10	No	TDF 300 / 3TC 300 + LPV/r 200/50	None	7, 8, 9, 12, 13, 14, 15
2nd	11	No	AZT 300 / 3TC 150 + LPV/r 200/50	AZT 60 / 3TC 30 + LPV/r 100/25	8, 9, 12, 13, 14, 15
3rd	12	No	DRV 600 + r 100 + DTG 50 (± NRTIs)	None	NS

All strengths are in mg. Alternate regimens are provided after possible adverse reaction(s). 3TC = Lamivudine, ABC = Abacavir, ATV = Atazanavir, AZT = Zidovudine, DRV = Darunavir, DTG = Dolutegravir, EFV = Efavirenz, ETV = Etravirine, LPV = Lopinavir, NVP = Nevirapine, r = Ritonavir, RAL = Raltegravir, TDF = Tenofovir. Fixed dose combinations (FDC) are shown with a slash (e.g. TDF / 3TC / DTG), combinations made up of separate tablets are shown with + (e.g. AZT/3TC + EFV). An "A" is added to the regimen number for adult formulations (e.g. Regimen 2A) and a "P" is added for paediatric formulations (e.g. Regimen 2P).

2.10.2. Schistosomiasis and HIV co-infections

Observations in SSA indicate substantial geographical overlap of HIV and schistosomiasis in countries with high endemicity (Ndeffo Mbah *et al.*, 2013; Bustinduy *et al.*, 2014). As already stated, schistosomiasis is highly prevalent in fishing communities where opportunities for HIV transmission can be raised (Figure 12). Similar to the high relative risk of HIV infections associated with sexually transmitted diseases causing genital inflammation and elevated viral copies (Cohen *et al.*, 1997; Royce *et al.*, 1997; Dyer *et al.*, 1998; Mabey, 2000; Cohen, 2004), MGS can potentially raise the risk of HIV transmission in dually infected males to their sexual partners, as noted in an observational study among men on ART coinfected with UGS in Zimbabwe (Midzi *et al.*, 2017).

As described earlier, mechanical breach of the genital mucosa caused by the schistosome eggs may render schistosomiasis-infected individuals more prone to contracting HIV during intercourse (Kjetland *et al.*, 2005; Kjetland *et al.*, 2006; Secor, 2006). In addition, local recruitment of CD4+ cells in the genital tissues during granuloma formation around the trapped eggs may also facilitate uptake of HIV viruses and establishment of infection (Leutscher *et al.*, 2005).

Previous studies observed increased levels of inflammatory cells and immunological mediators such as neutrophils, eosinophils, leukocytes, macrophages, CD4+ lymphocytes, eosinophil cationic protein among others in semen harbouring *Schistosoma* eggs, which also necessitate viral attachment and replication, highlighting the possibility of increased risk to HIV acquisition and transmission (Leutscher *et al.*, 2000; Leutscher *et al.*, 2005; Leutscher *et al.*, 2008b).

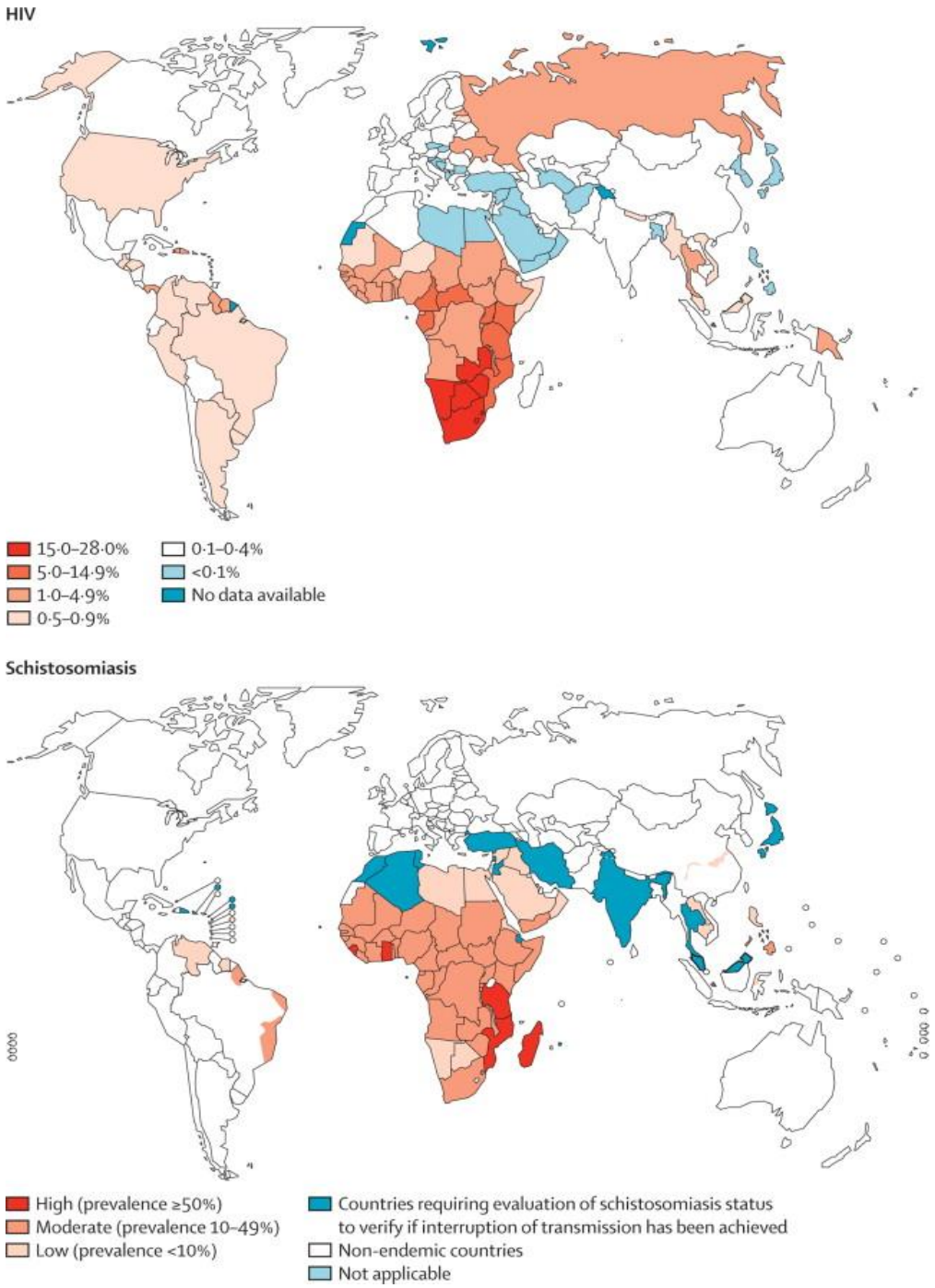


Figure 12: Global distribution of HIV and Schistosomiasis infections in 2015.
Figure reproduced from (Bustinduy et al., 2014)

The current control interventions of schistosomiasis and HIV which are running parallel in dual-epidemic regions, are also leaving out sexually-active men, women and girls who are also at high-risk of both conditions, thereby slowing down efforts of arresting the burden of both diseases (Hotez, Fenwick and Kjetland, 2009; Rollinson *et al.*, 2013; Stothard, Bustinduy and Montresor, 2014; Stecher *et al.*, 2015; Stothard *et al.*, 2016).

2.11. Hypothesis of the MGS cohort studies

Schistosome eggs cause UGS in local men inhabiting endemic areas on the shores of Lake Malawi who further suffer from genital consequences, described as MGS, however the burden remains unknown on the shoreline. Furthermore, MGS could potentially increase the HIV viral shedding in semen of those men with both HIV and MGS infections.

Therefore, the multidisciplinary studies tested the hypotheses that PZQ treatment reduces the prevalence and morbidity of MGS as well as the potential risk of HIV transmission through reduced seminal viral shedding in dually infected fishermen living along south shoreline of Lake Malawi.

2.12. Specific aims and objectives of the cohort studies

The main aims of the cohort research studies were to determine the current prevalence and morbidity of MGS among fishermen in schistosomiasis-endemic areas along shores of Lake Malawi in the southern Malawian district of Mangochi and evaluate the extent of relationship between MGS and HIV-1 VL through potential increased viral shedding in their semen.

Specific objectives are:

1. To assess prevalence of MGS and its associated knowledge, attitudes and practices.
2. To assess morbidity and genital pathology associated with MGS by ultrasonography.
3. To determine efficacy of praziquantel in treating MGS and morbidity resolution.
4. To assess the HIV-1 viral shedding in men co-infected with MGS.

3.1. Summary

This chapter describes the systematic review conducted prior to the longitudinal cohort studies, aimed at providing an update on the epidemiology of MGS in endemic areas, detailing the currently available literature on the epidemiology, clinico-pathological features, co-infections, available diagnostic techniques, treatment, management and control of MGS, as well as the existing gaps for future research prospects. This was published as cited below:

Kayuni, S., Lampiao, F., Makaula, P., Juziwelo, L., Lacourse, E.J., Reinhard-Rupp, J., Leutscher, P.D. and Stothard, J.R., 2018. **A systematic review with epidemiological update of male genital schistosomiasis (MGS): a call for integrated case management across the health system in sub-Saharan Africa.** *Parasite epidemiology and control*, p.e00077, https://doi.org/10.1016/j.parepi.2018.e00077 (Received 13 May 2018, Revised 16 October 2018, Accepted 16 October 2018, Available online 23 November 2018).

My contribution to this manuscript was that I conducted the entire systematic review on all databases and literature on MGS. Thereafter, I wrote the manuscript of the review, detailing the findings of the literature search, and made all the changes suggested by the co-authors and journal referees.

3.2. Abstract

Male genital schistosomiasis (MGS) is a gender specific manifestation of urogenital schistosomiasis (UGS) first described in 1911 by Madden in Egypt. Today, while affecting millions of men and boys worldwide, MGS receives insufficient attention, especially in sub-Saharan Africa (SSA). To provide an epidemiological update and a systematic review of MGS, inspection of both online and hardcopy resources was undertaken. A total of 152 articles were eventually identified with only 32 articles resulting from original research that exclusively focused on MGS.

From these, we discuss pertinent clinico-pathological features, highlight the possible connection between MGS and HIV, and current diagnostic techniques alongside consideration of their use and application in SSA. To appreciate the burden of MGS more fully, in endemic areas there is a clear need for better surveillance and longitudinal population research to investigate the best point-of-care (POC) diagnostic, as well as, explore alternative praziquantel dosing regimens for individual case management in men with or without HIV infection.

Keywords: Male genital schistosomiasis; urogenital schistosomiasis; praziquantel; HIV; control

3.3. Introduction

Schistosomiasis is a snail-borne disease of humans caused by parasitic helminths of the genus *Schistosoma* (Colley *et al.*, 2014). It remains a major neglected tropical disease (NTD) and a significant public health challenge in low and middle-income countries (Chitsulo *et al.*, 2000; Engels *et al.*, 2002; Christinet *et al.*, 2016). There it causes significant morbidity and in certain areas mortality (van der Werf *et al.*, 2003), however, the burden of schistosomiasis is underestimated due to incomplete disease surveillance often by stretched national healthcare systems (King, Dickman and Tisch, 2005; Gryseels *et al.*, 2006) and national control programmes. The latter is more focused on tracking the delivery and treatment coverage of mass treatment campaigns offering donated praziquantel (PZQ) typically to school-aged children (Savioli *et al.*, 2017) rather than monitoring the disease in adults per se.

The consequences and disability caused by gender specific manifestations of urogenital schistosomiasis (UGS) in adults often go unremarked at national and local levels. In contrast to female genital schistosomiasis (FGS), male genital schistosomiasis (MGS), as evidenced by schistosome eggs (usually those of *S. haematobium*) in male genital organs and tracts thereof, remains poorly reported and much understudied. This review was conducted to draw attention to the current evidence on MGS and assess its public health importance.

3.3.1. A brief history of schistosomiasis

Corroborated references to signs and symptoms ascribed to UGS can be traced back to 1,900 BC since haematuria (i.e. frank blood in urine) was described as a common occurrence and linked to 'menstruation' in Egyptian males (Davis and Ansari, 1973). Schistosomiasis is a proven disease of antiquity for *S. haematobium* ova which have been found in kidney tubules of two Egyptian mummies from 1,250 to 1,000 BC (Ruffer, 1910) and more recently *S. japonicum* ova retrieved within Chinese cadavers dated to 206 to 220 AD (Coon, 2005). Schistosomiasis itself was originally described in Egypt by the German pathologist Theodor Bilharz in 1851 who discovered male and

female schistosome worms at autopsy, naming them all *Distomum haematobium*. This led him to ascribe, incorrectly, that UGS and hepato-intestinal disease were linked to this species alone (Rollinson, 2009). Some sixty years later, and again in Egypt, this unfortunate mistake and subsequent confusion was fully resolved by Robert T. Leiper who demonstrated the independent lifecycles of *S. haematobium* and *Schistosoma mansoni* (Leiper, 1916) and their respectively aetiology in urinary and hepato-intestinal disease (Leiper, 1916; Stothard *et al.*, 2016).

Out of the 24 species of schistosomes recognised worldwide, only six cause human diseases, namely *S. haematobium*, *S. mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum* and *S. guineensis* (Rollinson, 2009). The first three species are the most important from a public health perspective. Although there may be exceptions owing to ectopic egg laying sites, *S. haematobium* is exclusively associated with UGS which is widely distributed in Africa and adjacent regions, affecting more people (112 million) than all other species [(WHO) see http://www.who.int/schistosomiasis/epidemiology/table/en/]. *Schistosoma mansoni, S. japonicum* and the other species causes hepato-intestinal schistosomiasis, with *S. mansoni* prevalent in the Caribbean and South America, Africa and Asia, and *S. japonicum* in South East Asia (Colley *et al.*, 2014). Of note, *S. mansoni*, *S. japonicum*, *S. intercalatum* and *S. guineensis* have been reported to cause genital manifestations but even collectively they are minor as compared to *S. haematobium* alone.

3.3.2. Focus on male schistosomiasis

Male genital schistosomiasis is a specific manifestation of schistosomal disease, associated with presence of ova and pathologies thereof in various genital organs and fluids. The first original report of MGS was made by Professor Frank Cole Madden, Professor of Surgery at Kasr-el-Ainy Hospital in Cairo, Egypt. In 1911, he described a 14-year Egyptian boy having enlarged scrotum showing epidydimal schistosomiasis and an English soldier complaining of haemospermia (blood in semen) concurrently with urinary schistosomiasis (Madden, 1911).

Other symptoms of MGS described in literature include pelvic pain appearing spontaneously, during coitus or on ejaculation, ejaculate changes, erection discomfort or dysfunction, infertility (Butterworth *et al.*, 2013; Bustinduy and King, 2014; Squire and Stothard, 2014). Although observations indicate that genital organs are frequently infested with schistosome eggs along with the urinary bladder (*S. haematobium*) or intestines (*S. mansoni*), the current extent of morbidity associated with MGS in endemic areas remains under-researched, since most of the descriptions have been on post-mortem studies and case reports.

On the other hand, FGS has been associated with a three-fold increased risk of human immunodeficiency virus (HIV) infection in women living in endemic areas of SSA (Kjetland *et al.*, 2006; Kjetland, Leutscher and Ndhlovu, 2012). There is a similar plausibility of additional risk of HIV transmission among dually-infected males in schistosomiasis-endemic areas due to observed increase in inflammatory cells and immunological mediators in semen of people with MGS which might increase the viral copies (Leutscher *et al.*, 2005). Hence treatment of MGS with PZQ could support the control of HIV/AIDS in overlapping prevalent areas of both diseases, especially in SSA.

This systematic review on MGS in endemic areas, has the following specific objectives:

1. update the epidemiology of MGS in endemic areas,
2. review the clinicopathological features of MGS including co-infections with other diseases,
3. assess the available diagnostic techniques and treatment of MGS, and
4. determine the existing gaps to develop future research agenda of MGS.

3.4. Methods of literature review

An online literature search was conducted systematically from 9th January 2017 to 30th April 2018 for publications made from 1900 up to 2017, using the main search term 'male genital schistosomiasis' in PUBMED, EBSCOhost (CINAHL Complete, MEDLINE Complete, Global Health, eBook Collection), COCHRANE LIBRARY and WEB OF KNOWLEDGE databases, following the

stipulated guidelines of each database. The main search term was combined with terms for known symptoms of MGS retrieved from the main textbooks on Tropical Medicine (Butterworth *et al.*, 2013; Bustinduy and King, 2014; Squire and Stothard, 2014), which included 'haemospermia', 'haematospermia', 'ejaculate', 'erectile dysfunction', 'infertile', 'sterile', 'painful', 'discomfort', 'spermaturia', 'semen'. In addition, the main term was combined with terms of male genital organs, listed as 'prostate', 'seminal vesicle', 'spermatic cord', 'epididymis', 'vas deferens', 'testis' and 'reproductive organ'.

In the PUBMED database after inputting the main search term, it automatically searched the terms as Medical subject headings (MeSH) and all fields, to produce the total results which were narrowed to those of English language. The search of the main terms in the EBSCOhost database involved all possible forms of the terms, augmented using relevant syntax 'OR', 'AND'; for example, 'male+ OR male* OR man* OR man+' AND 'genital+ OR genital*' AND 'schistosomiasis+ OR schistosomiasis* OR *Schistosoma** OR *Schistosoma*+ OR bilharzia+ OR bilharzia* OR bilharziosis* OR bilharziosis+'. These terms were automatically expanded for equivalent subjects and related words, also narrowed by English language. The search in the COCHRANE LIBRARY followed a similar pattern to the EBSCOhost database. For the WEB OF KNOWLEDGE, the main terms were searched using both field tags 'TOPIC' and 'TITLE' and then combined with Booleans 'OR', and 'AND' appropriately.

The results were compiled together to produce the final list of articles. Additional articles from other sources such as references from the textbooks and known parasitologists were added to the final lists from these four databases. The final articles in French and Portuguese languages were translated into English (Refer to Appendix 1 Supplementary Tables). All the articles were screened in the following five stages:

- **Stage 1:** Lists of articles were checked for possible duplications, which were removed.
- **Stage 2:** Thereafter, the titles of the remaining articles were screened for relevance to MGS and excluded accordingly.

- **Stage 3:** Then, the abstracts of those remaining were read and screened for relevance to MGS. Those articles not related to MGS were removed from the list.
- **Stage 4**: The full text of the remaining articles was retrieved and read through to select those manuscripts on MGS to be included in the review.
- **Stage 5:** The references and citations of the full-text articles included in the review were screened for additional articles not retrieved in the above database searches.

Furthermore, leading articles on FGS and texts from prominent textbooks describing schistosomiasis (Gelfand, 1967; Jordan and Webbe, 1969; Jordan, Webbe and Sturrock, 1993; Kamel and Lumley, 2004; Weiss, 2004) were read to form background knowledge and comparison to MGS where necessary. Alerts were installed on all the databases searched for this review to capture new publications and additional articles relevant to MGS.

3.5. Analysis of assembled literature

The online database search produced a combined total of 3,329 publications using the main search term (Figure 13 & Table 5). Four articles were added from the alerts on the searched databases. After screening through the five stages described earlier, the final articles included in this MGS review are 152 (Supplementary Table 2), of which 32 were original research studies, 96 case reports, 1 article describing voluntary schistosome infection, 2 editorial papers, 3 systematic reviews and 18 literature reviews on schistosomiasis with aspect of male genital pathology. The period of publications was from 1911 to 2018.

Thirty-two original research studies have been published from 1952, with half directly on genital schistosomiasis, while the other half focussed on schistosomiasis with brief descriptions on genital symptoms, pathologies or complications. Sixteen studies reported only on *S. haematobium*, 3 on *S. mansoni*, 9 on mixed *S. haematobium* and *S. mansoni* infections, while 4 had no mention of species (Figure 13). In addition, 26 studies were conducted in Africa [Madagascar-6, Nigeria-6, Egypt-5, Zimbabwe-5, Zambia-2, Ghana-1] and one each in other continents except Australasia. There were

11 necropsy studies, 5 histopathological studies, 6 longitudinal cohort studies, 2 qualitative studies, 1 radiography study and 1 hormonal analysis study. Seven studies involved examination of all genital organs, 2 on seminal vesicles, 1 on prostate only while other studies did not focus on specific genital organ(s).

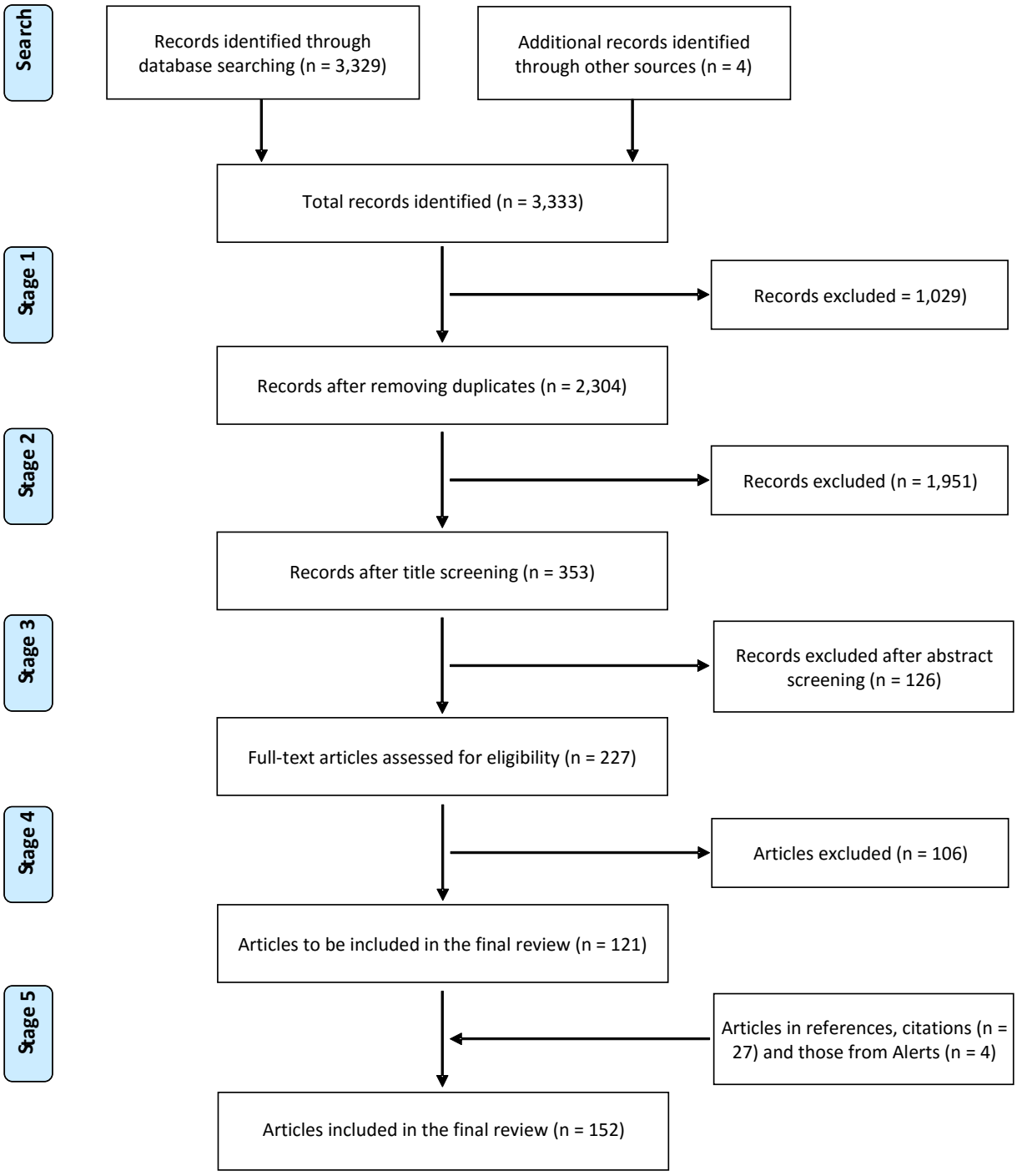


Figure 13: Flow chart showing the results of systematic literature search in the online databases

Table 5: Results of literature search on the online databases conducted from January 2017 to April 2018

Online database	Number of articles from each database		
	'Male genital schistosomiasis'	Combined with 'symptoms'	Combined with 'symptoms' and 'organs'
EBSCOhost	680	837	1,677
PUBMED	181	339	812
WEB OF KNOWLEDGE	140	275	570
COCHRANE LIBRARY	17	96	270
TOTAL	**1,018**	**1,547**	**3,329**

Ninety-six case reports were made between 1911 and 2018, with only five reports published prior to 1952. Fifty-five case reports were from endemic areas mostly in Africa [n=35; 64%] while 40 reports were on travellers or people emigrating from endemic areas to non-endemic countries, especially in Europe [n=30; 75%] (Figure 14). Some travellers diagnosed in non-endemic countries in Europe, Asia and Australia, were infected after swimming or walking in Lake Malawi in SSA, which is endemic mainly for *S. haematobium*. In France, ten of the 12 case reports were of travellers to or emigrants from North, West and Central African countries of Algeria, Cameroun, Central African Republic, Chad, Democratic Republic of Congo (DRC), Egypt, Gabon, Libya, Mali, Mauritania, Niger and Tunisia.

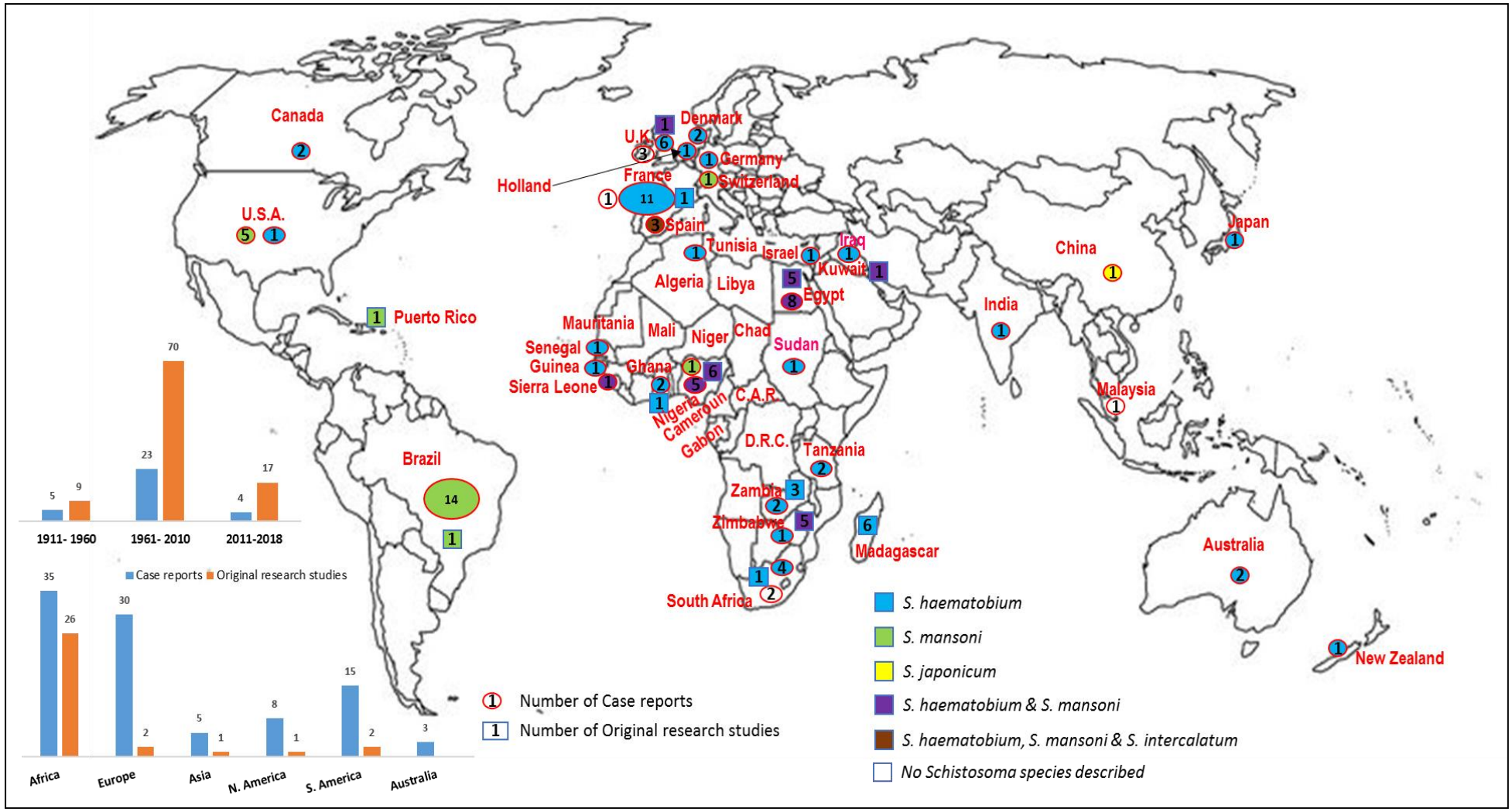


Figure 14: Global map showing distribution of the publications on MGS from 1911 to 2018.

The two charts displaying number of publications in the first and second 50 years and per continent. (The original research studies comprise post-mortem studies conducted in Africa and South America; prospective studies mainly in Africa).

Sixty case reports (63%) were on *S. haematobium*, 22 (23%) on *S. mansoni*, and the rest (5%) on mixed *S. haematobium* and *S. mansoni* (n=2), on *S. haematobium, S. mansoni* and *S. intercalatum* (n=2), on *S. japonicum* (n=1), whereas 9 (10%) had no speciation. The pathological organs described in 74 case reports included scrotum (including testis and vas deferens) [n=39], prostate [n=17], seminal vesicles [n=17], spermatic cord [n=3], epididymis [n=7] and penis [n=1]. The main presenting symptoms or complaints were swelling of scrotum and other genital organs [n=58], pelvic pain [n=23], haemospermia [n=14], hydrocele [n=12], changes in semen / ejaculate [n=11], infertility [n=6] and urethral discharge [n=2].

The 3 systematic reviews were published in 2011 and 2015, discussing the relationship between UGS and HIV (Mbabazi *et al.*, 2011), prostate adenocarcinoma associated with prostatic *S. haematobium* infection (Figueiredo *et al.*, 2015) and MGS treatment as a future HIV prevention tool (Stecher *et al.*, 2015).

3.5.1. Update on the epidemiology of MGS

As described earlier, *S. haematobium* is endemic in Africa where most knowledge originates, and the first recognised description of MGS was made by Madden a century ago (Madden, 1911). However, earlier literature by Chaker, Lortet, Vialleton, Letulle and Madden [1885-1909] described lesions in genital organs like seminal vesicles and prostate which were infiltrated by schistosome ova and granuloma formation (Madden, 1909; Mensah *et al.*, 1972; Guirassay *et al.*, 2008). Other genital organs have been described in the subsequent reports and research studies.

Post-mortem studies were among the earliest research in endemic areas especially in Africa, describing the epidemiology of genital schistosomiasis, four decades after the Madden report, giving the background knowledge to understanding MGS (Mohammed, 1952; Gelfand and Ross, 1953; Edington *et al.*, 1970; Gelfand *et al.*, 1970; Edington, Nwabuebo and Junaid, 1975). Digestive methods were performed using potassium hydroxide (KOH) to harvest the ova from the genital organs with pathologies caused by *S. haematobium* and *S. mansoni.* Seminal vesicles were infected

almost as much as the urinary bladders, ranging from 50% to 80% of vesicles with over 90% of bladders (Mohammed, 1952; Gelfand and Ross, 1953) with approximately 20,000 ova found in the vesicles [Table 6] (Edington *et al.*, 1970; Gelfand *et al.*, 1970; Edington, Nwabuebo and Junaid, 1975). Histopathological examinations were also conducted, in other studies to compare with the digestive methods which showed that more ova were observed with the latter technique (Gelfand *et al.*, 1970).

Table 6: Total number of *Schistosoma* ova in pelvic organs in necropsy studies

Study participants	**Post-mortem studies**	
	Gelfand *et al.* 1970	**Edington *et al.* 1975**
Total number	200	54
Pelvic organs	**Intensity of *Schistosoma* egg distribution**	
	Mean egg count	**Range of egg distribution**
Bladder	105,011	13,260 - 87,100
Seminal vesicles	19,801	4,312 – 12,027
Vas deferens	2,913	-
Prostate	34	169 – 9,828

In endemic areas of *S. haematobium* and *S. mansoni*, the former predominates with more genital pathologies in literature than the latter, similarly to the case reports (Grace and Aidaros, 1952; Alves, Woods and Gelfand, 1955). From these studies, it has been described that MGS affects between 1% to 20% of those in endemic areas at risk and suffering from UGS (Ricosse, Emeric and Courbil, 1980; Fievet *et al.*, 1984). This could be a gross underestimation, because several studies have reported that at least 50% of genital organs are infected by schistosome ova, emphasising that MGS is as common as urinary manifestations of schistosomiasis but with lower intensity of ova

[Table 7] (Edington *et al.*, 1970; Edington, Nwabuebo and Junaid, 1975; Elem and Patil, 1987; Patil and Elem, 1988).

The first identified prospective study on MGS was conducted in Madagascar in 1999-2000, where 19 of 44 participants (43%) had MGS by *S. haematobium* ova in semen (Leutscher *et al.*, 2000). Although the sample size of this study was small, subsequent longitudinal studies in the same country showed similar prevalence of MGS, ranging from 28% in 2005 to 53% in 2009 (Leutscher *et al.*, 2005; Leutscher *et al.*, 2008a; Leutscher *et al.*, 2008b; Leutscher, Host and Reimert, 2009). *Schistosoma* ova were present in semen only in some cases, highlighting fact that the prevalence of MGS is quite significant, similarly to that of UGS in endemic areas, despite not having been studied as extensively.

Table 7: *Schistosoma* ova in male genital organs seen in necropsy studies.

Year	Author(s)	Country	Autopsies	Species	Infected genital organs
1955	Alves *et al.*	Zimbabwe	50	*Sh, Sm*	18% vas deferens; 18% prostate; 4% tunica vaginalis; 2% epididymis
1956	Arban	Brazil	3,233	*Sm*	10/3,233 infected: 20% prostate; 30% testes
1970	Gelfand *et al.*	Zimbabwe	200	*Sh, Sm*	54% seminal vesicles; 39.9% spermatic duct; 20.5% prostate
1975	Edington *et al.*	Nigeria	54	*Sh*	Severe infections: 100% prostate; 100% seminal vesicles; 57% testes; 57% epididymis
1987	Elem & Patil	Zambia	50	*Sh*	62% bladder; 58% seminal vesicles; 50% prostate
1988	Patil & Elem	Zambia	100	*Sh*	62% bladder; 58% seminal vesicles; 50% prostate

Sh - S. haematobium; Sm - S. mansoni

3.5.2. Clinico-pathological features of MGS including co-infections

From our search, genital organs with schistosomal pathologies have been recorded in case reports from Africa, namely prostate, seminal vesicles, vas deferens, testis and scrotum which were

more associated with *S. haematobium* than *S. mansoni* (Cerqua, 1930; Mohammed, 1930; Makar, 1937; Gelfand and Davis, 1940). An early report from South America associated with *S. mansoni*, presented of enlarged scrotum, thickened seminal vesicles and hydrocele (Armbrust, 1951). Subsequent reports indicate that a higher burden of MGS is in *S. haematobium* - endemic areas of Africa than other schistosome - endemic areas in the world.

Although most of the MGS pathologies have been reported on *S. haematobium* in inhabitants and travellers to endemic areas, similar reports have been made on *S. mansoni, S. intercalatum* and *S. japonicum* (Corachan *et al.*, 1994; Vilana *et al.*, 1997; Yu *et al.*, 2013). Infestation of genital organs result in several early symptoms of MGS. One major symptom observed in early stages is haemospermia resulting from egg penetration and release into seminal vesicle lumen, causing ulceration of mucosal lining, and pain during coitus and ejaculation (Madden, 1911; Makar, 1937; Mohammed, 1952; Kato-Hayashi *et al.*, 2013; Lang, Minion and Wong, 2017). Haemospermia can occur as the only symptom or first symptom preceding haematuria, occurring within three months of exposure to infection (Becquet, 1966; Pedro Rde, Barros Rde and Amato Neto, 1973; Corachan *et al.*, 1994; Schwartz *et al.*, 2002).

Of interest, this symptom has been described more frequently among travellers than inhabitants of endemic areas, in 8 of the 12 case reports found in the search. This could be due to failure to recognise the symptoms, societal acceptance of condition as male menstruation and maturing from boyhood to adulthood, not knowing or making an association with MGS, being mistaken with sexually transmitted infections (STIs) or infidelity (Ukwandu and Nmorsi, 2004; Yirenya-Tawiah, Ackumey and Bosompem, 2016). In relation to haemospermia, other reported symptoms include alteration in semen quality and appearance with discolouration (McKenna, Schousboe and Paltridge, 1997; Torresi *et al.*, 1997; Hawary *et al.*, 2012), subjective change (Davies and Hamdy, 1998), lumpy semen (Lewis, al-Adnani and Murphy, 1996; Lang, Minion and Wong, 2017), rice grains with increased volume (Pedro Rde, Barros Rde and Amato Neto, 1973) and

reduced viscosity or volume (Perignon, Pelicot and Consigny, 2007; van Delft *et al.*, 2007; Knapper, Morrell and Lomax, 2012).

The symptoms associated with mucosal thickening and enlargement of organs such as seminal vesicles cause irritation of the sympathetic nervous system leading to sexual hyperexcitability, night dreams and frequent painful erections (Mohammed, 1952). However, these symptoms have not been reported in the last four decades, raising the question of their reliability in the earlier studies or non-reporting in the recent studies, possibly due to the sensitive descriptive nature. The enlargement of genital organs has also been mistaken for other diseases such as tuberculosis or malignancy resulting in extraneous surgical interventions where PZQ treatment provided earlier might have prevented the surgery (Madden, 1911; Chippaux, Cornet and Datchary, 1957; Eltayeb, 1969; Kazzaz and Salmo, 1974; Fievet *et al.*, 1984; Githae, 1992; Ferreira *et al.*, 2015). Untreated, the organs chronically become nodular, firmer, smaller and non-functional.

More recently reported symptoms of MGS include spermaturia (sperm in urine) as a result of fibrosis and abnormal cystic dilatation of seminal vesicles (Etribi, Girgis and Saleh, 1967), hydrocele formation (Gelfand and Davis, 1940; Armbrust, 1951; Van Beukering and Vervoorn, 1956; Pawel *et al.*, 2008; Ramarakoto *et al.*, 2008; Rambau *et al.*, 2011), epididymitis (Alves, Assis and Rezende, 2004), funiculitis (Durand *et al.*, 2004), orchitis (Mikhail *et al.*, 1988; Ihekwaba, 1992; Al-Qahtani and Droupy, 2010), prostatitis (Cerqua, 1930; Alexis and Domingo, 1986; Patil and Elem, 1988; Cohen, Edgar and Cooper, 1995; Fender, Hamdy and Neal, 1996; Basilio-de-Oliveira *et al.*, 2002; Al-Saeed *et al.*, 2003; Lambertucci, Voieta and Barbosa, 2006; Bacelar *et al.*, 2007; Sharma *et al.*, 2015), infertility from oligospermia, azoospermia either obstructive from blockage of vas deferens, spermatic cord, epididymis, tunica vaginalis or non – obstructive through infarction (Kini *et al.*, 2009; Abdel-Naser *et al.*, 2018a; Abdel-Naser *et al.*, 2018b), fibrotic lesions (Edington, Nwabuebo and Junaid, 1975) or functional lymphotic infiltration in testis (Adisa *et al.*, 2012). While egg load in the bladder tissue has been found to correlate with pathological severity, few ova in seminal

vesicles, prostate and other genital organs have been associated with severe extensive pathological changes (Edington *et al.*, 1970; Edington, Nwabuebo and Junaid, 1975).

On malignancies of genital organs, our search showed that MGS has been reported among travellers and those emigrating from endemic areas, apart from testicular or scrotal schistosomiasis simulating neoplasia (Alexis and Domingo, 1986; Cohen, Edgar and Cooper, 1995; Ma and Srigley, 1995; Basilio-de-Oliveira *et al.*, 2002; Bacelar *et al.*, 2007; Lopes, de Almeida and Jacobino, 2007; Figueiredo *et al.*, 2015). Prostatic adenocarcinoma has been observed to occur together with *Schistosoma* ova, resulting in epithelial granulomata, marked fibrosis and organ enlargement, which have been described in reports of tissue histopathology and cancer spread to other genital organs affected by MGS. Despite an accepted link between chronic UGS and squamous cell carcinoma of the bladder (Honeycutt *et al.*, 2014), the mechanism of association between prostatic cancer and schistosomiasis remains unknown.

Our search produced recent systematic reviews addressing extensively the interactions of both MGS and HIV and were included in this review (Mbabazi *et al.*, 2011; Stecher *et al.*, 2015). As one of the leading causes of morbidity and mortality in the world, HIV has its epicentre in the SSA region (UNAIDS, 2016) where coincidentally schistosomiasis is endemic (Figure 15). Female genital schistosomiasis (FGS) has been observed in 33-75% of women having UGS living in endemic areas in SSA (Kjetland, Leutscher and Ndhlovu, 2012). In addition, FGS has been associated with a 3-fold increased risk of HIV infection with characteristic sandy-grainy patches present in egg-infected genital organs, abnormal blood vessel formation and increased levels of inflammatory cells expressing CD4+ receptors triggered by *Schistosoma* granuloma (Kjetland *et al.*, 2006; Christinet *et al.*, 2016). More knowledge is needed on the risk of MGS on HIV infection.

Various hypotheses have been proposed regarding the impact of MGS on HIV transmission. As described in Leutscher *et al* (2005) study and the systematic reviews by Mbabazi *et al* (2011) and Stecher *et al* (2015), men with MGS have elevated levels of eosinophils and lymphocytes among other inflammatory cells expressing CD4+ receptors together with cytokines IL-4, 6, 10, TNF-α. These

recruit more HIV-infected cells into semen, upregulating viral replication and increasing viral concentration (Leutscher *et al.*, 2005; Mbabazi *et al.*, 2011; Stecher *et al.*, 2015). With the chronic inflammation and cell recruitment to the male genital tract, these may increase HIV viral load in semen, similar to that seen with STIs (Mabey, 2000).

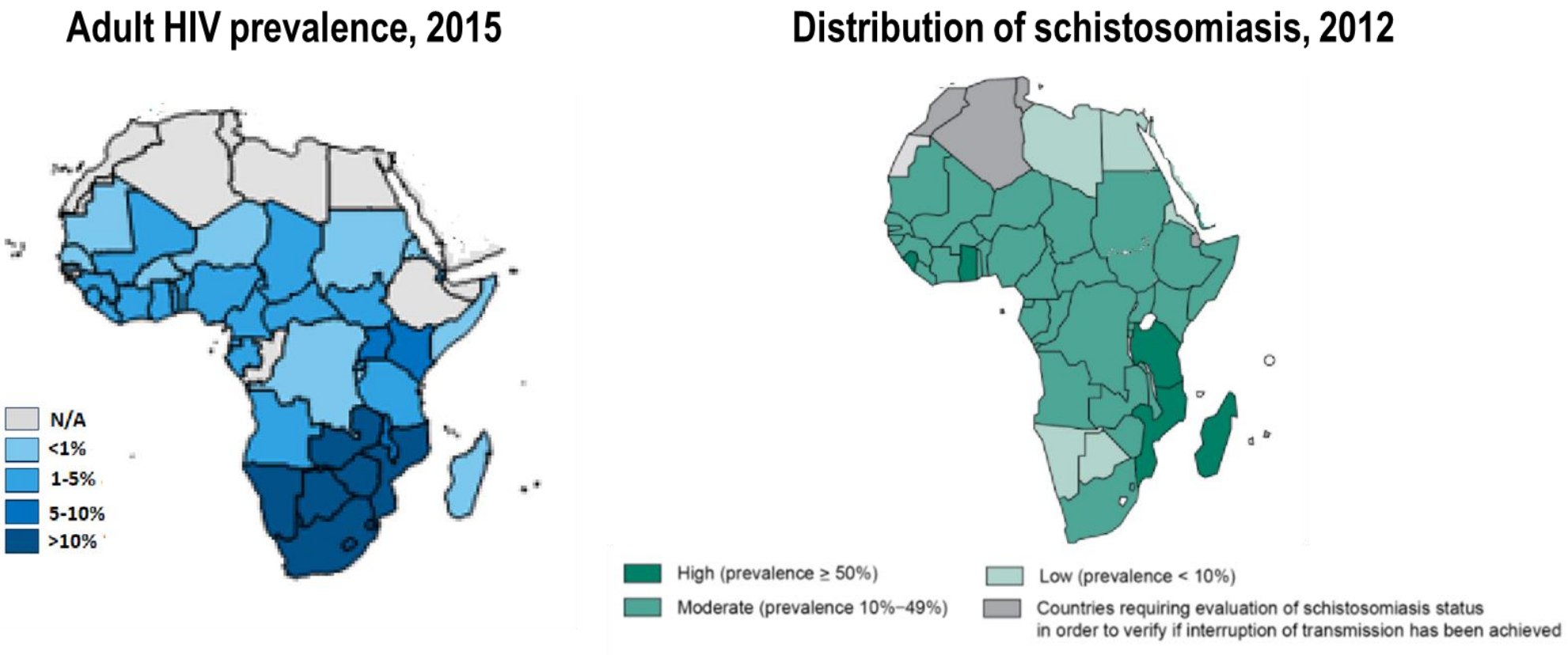


Figure 15: Map of Africa showing the correlation of the prevalence of HIV and schistosomiasis. Produced from (WHO, 2014; Kaiser-Family-Foundation, 2016)

A recent observational pilot study in endemic SSA area demonstrated a reduction of viral load shedding in semen of HIV positive men coinfected with UGS 10 weeks after PZQ treatment (Midzi *et al.*, 2017). Further case-cohort or randomized studies are needed to be conducted in endemic areas to explore these critical findings further.

3.5.3. Techniques for detection of MGS

Urine microscopy remains the definitive way for identifying schistosome ova (mainly of *S. haematobium*) to diagnose UGS. While it is the gold standard (Le and Hsieh, 2017) and also considered as a useful proxy for diagnosing MGS, our findings indicate challenges in its reliability due to presence of ova in semen or histological specimens in the absence of ova in urine (or stool), as well as other schistosome species that may on occasion cause MGS (Corachan *et al.*, 1994; Torresi *et al.*, 1997; Leutscher *et al.*, 2000; Schwartz *et al.*, 2002; Lang, Minion and Wong, 2017). As such, there is a direct need to conduct microscopy on semen and biopsy material from suspicious genital lesions/tissues to diagnose MGS.

Also, semen should be analysed repeatedly with periods of abstinence to cater for the daily diurnal variations in excretion of ova from the genital organs and increase likelihood of maximum egg yield, similar to the recommended consecutive urine analyses (Leutscher *et al.*, 2008b; Le and Hsieh, 2017). However, perceptions, beliefs and sensitivity of people in endemic communities with regards to semen samples can affect the collection or submission and analyses, therefore there will be need for more health education and counselling of those required to submit and the community to ensure acceptability and success in collection of the sample in the community (Price *et al.*, 2005; Midzi *et al.*, 2017).

Leutscher and colleagues report on use of eosinophil cationic protein (ECP), circulating anodic antigen (CAA) and soluble egg antigen (SEA) as blood-based markers of MGS which showed positive correlation to urine egg count, with ECP significantly correlating with urine count and declining after PZQ treatment, highlighting its importance in diagnosis (Leutscher *et al.*, 2000;

Leutscher *et al.*, 2008b). However, other helminth, bacterial or viral infections and inflammatory conditions elevate ECP hence affecting the reliability in co-morbidities which are common in endemic areas. PCR and DNA-based tests on the other hand have shown to be highly sensitive and specific diagnostic tools, which can be used in urine, semen, and many other specimens (Le and Hsieh, 2017). These tests are still expensive for field use in endemic areas of Sub-Saharan Africa, hence the need to develop easier, accessible, low-cost tests.

Our findings showed that ultrasonography is useful in diagnosing lesions caused by MGS and monitoring morbidity of the pathologies (Richter, 2000; Al-Saeed *et al.*, 2003; Ramarakoto *et al.*, 2008). The pathological lesions seen in genital organs have been described as echogenic lesions and calcifications, with the former improving with treatment (Richter, 2000; Ramarakoto *et al.*, 2008). Availability of portable sonography machines could be more cost-effective in endemic areas since other radiological techniques such as computerised tomography (CT) and magnetic resonance imaging (MRI) are very expensive, not feasible and almost non-existent in these regions. However, there has been limited radiological research on MGS in endemic areas, hence there is a need for more field studies to study the resolution of pathologies after treatment.

3.5.4. Current treatment options for MGS

Praziquantel has remained the mainstay treatment for most forms of schistosomiasis, including MGS (WHO, 2013a). It is effective with population cure rates of over 90% and targets adult worms thereby reducing egg excretion and averting morbidity, however, praziquantel does not successfully kill juvenile worms (Rollinson, 2009). Most identified case reports and studies used the recommended traditional dosage of 40mg/kg in treating MGS with some failure cases requiring further repeated doses or higher dose of 60mg/kg (Schwartz *et al.*, 2002; Alonso *et al.*, 2006; Perignon, Pelicot and Consigny, 2007). It has been suggested that higher doses are more efficacious in MGS treatment than the traditional dose (Lang, Minion and Wong, 2017).

Use of PZQ in most African programs are based on morbidity control through mass drug administration versus specific case management. The former is an attempt to keep prevalence and intensity down to an acceptable level, below 10% in the endemic population and obtain the greatest cost-benefit outcome at a populational level. There are well-known gaps in this approach, for example, school-aged children are targeted with donated stocks of PZQ ring-fenced (restricted) for this use in school-based programs. As an indirect consequence, many adolescents and adults rarely receive adequate treatment and PZQ is not always available in peripheral health clinics which further affects management of schistosomiasis in an individual case management setting (Christinet *et al.*, 2016; McManus *et al.*, 2018).

3.5.5. Existing gaps for further research of MGS

This review conducted a systematic search to elucidate the burden of MGS in endemic areas, a century after the first recognised report in 1911. Despite the detailed epidemiology of schistosomiasis in the world highlighting the enormous impact of UGS, much remains unknown of the burden of genital manifestations of schistosomiasis either FGS or MGS, specifically. More description and research studies of UGS especially in endemic areas have concentrated on urinary system and associated pathologies, however, with the growing interest in cervical cancer screening there are opportunities to integrate surveillance of FGS (Christinet *et al.*, 2016). On the other hand, for men, no such screening programmes exist and therefore the prevalence and morbidity of MGS in endemic areas will remain under-reported.

In addition, the limited description of MGS is compounded by difficulties in diagnostic techniques and approaches, these include deficits in standardised protocols for analysis of semen. Indeed, future methods which involve molecular assays will be challenging to carry out in primary health facilities in SSA. Future research studies to explore the deployment of low-cost techniques and methods are urgently required. These would be particularly important regarding treatment and management of MGS, as currently there is a clear gap in our understanding of the optimal dose of

PZQ to treat MGS, whether single, repeated or higher dosages would be effect a parasitological cure (Schwartz *et al*., 2002; Alonso *et al.*, 2006; Lang, Minion and Wong, 2017), notwithstanding tracking the dynamics of lesions in the genital tract. This highlights the need for further prospective longitudinal studies in endemic areas and more clinical research exploring an agenda of how best to integrate preventive treatment and management of MGS alongside ongoing interventions for HIV in SSA.

3.6. Discussion

This review has revealed that genital organs are infested with schistosome ova in the early stages of the infection, similar to other forms of the disease. These organs are infected with substantial numbers of ova as much as urinary bladder or intestines, further indicating the higher levels of MGS in endemic areas. Clinical manifestations associated with MGS in this review have been described previously by Barlow after self-infection with cercariae (Barlow and Meleney, 1949) and are regarded as major symptoms and diagnostic for MGS.

As symptoms like haemospermia can also present in other diseases such as hypertension, prostatitis or STIs (Feldmeier *et al.*, 1999), raising the need to exclude other conditions before concluding the diagnosis of MGS. Underreporting and misconceptions of these symptoms which may have negative perception in the community, contribute to misdiagnosis and underestimation of MGS in these endemic areas (Ukwandu and Nmorsi, 2004; Yirenya-Tawiah, Ackumey and Bosompem, 2016). Furthermore, co-existence of MGS and prostatic metaplasia and malignancies require further research to understand the link and develop diagnostic and therapeutic interventions.

Although urine microscopy has been considered as a proxy for diagnosing MGS, our findings observed challenges of ova found only in semen without any in urine or stool, hence the need to consider semen microscopy as a definitive way of diagnosing MGS. Accessible, low-cost molecular tests should be developed to address this diagnostic challenge. Similarly, radiological techniques like

field-based ultrasonography should be rolled out into endemic areas to monitor the morbidity and resolution of MGS pathologies.

MGS appears to be prevalent in areas endemic for UGS, which coincidentally are high prevalent areas for HIV. Some people in these areas in SSA have higher HIV prevalence and also at higher risk for schistosomiasis due to their lifestyles and daily activities, as reported about fishermen in Malawi (NSO, 2014; NAC, 2015). With some evidence of MGS potentially upregulating viral replication, increasing the concentration of HIV particles in the semen and exponentiating the infectiousness of dually infected males, treatment of MGS could be an importance tool in helping to avert new HIV infections in SSA (Stecher *et al.*, 2015). Interestingly, one of the current effective intervention of HIV prevention, male circumcision, was considered by ancient Egyptians around 2300 BC as an intervention to prevent schistosomal infection among men bathing in infested waters, though later disputed (Allen, 1909; Madden, 1919; Jordan, 2000; Weiss, 2004).

Although this review followed general principles of a systematic review, it was not registered with PROSPERO and did not include 2 reviewers for the literature screening as recommended by the PRISMA guidelines. The recent systematic review on UGS and HIV, Zirimenya study (https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=116648), was registered with PROSPERO in 2018, however there are no obvious published outputs from it.

3.7. Conclusion

MGS is an under-appreciated manifestation of UGS and has been reported worldwide but its current distribution is most tightly linked with areas endemic for *S. haematobium*. In SSA, MGS likely blights the lives of millions of men who currently do not have adequate access to point-of-care diagnostics or access to optimal praziquantel treatment regimes. We propose that MGS should be considered specifically in a new light of individual case management approaches as being used for other NTDs.

3.8. Supplementary Tables

Table 1: Additional articles on MGS received from Parasitologists and Alerts from databases

Articles added to those retrieved from online search before the screening stages			
No.	**Authors**	**Year**	**Key facts**
1.	Barlow and Meleney	1949	Voluntary schistosomal infection with haemospermia in early stages
2.	Feldmeier *et al.*	1999	Male genital schistosomiasis and haemospermia
3.	Rollinson	2009	Review of history, life cycle and clinic-pathologies of schistosomiasis
4.	Le and Hsieh	2017	Review of current diagnostics, challenges and future advances
Articles added from the Alerts on the online searched databases after the screening stages			
1.	Midzi *et al.*	2017	Decreased in seminal HIV-1 viral load after PZQ treatment of UGS coinfection in HIV-positive men
2.	Aber-Naser *et al.*	2018	Testicular schistosomiasis in an obstructive azoospermic man
3.	McManus *et al.*	2018	Review of the epidemiology, pathophysiology, diagnosis, management and control of schistosomiasis, some facts on genital manifestations
4.	Aber-Naser *et al.*	2018	Review of schistosomiasis as an important cause of male infertility in endemic areas

Table 2: All articles included in the review

Original research studies (* those focussed specifically on MGS; ǂ those from references)					
No.	**Authors**	**Year**	**Country**	**Organs / Specimens**	**Key findings**
1.	Mohammed*	1952	Egypt	Post-mortems: Seminal vesicles Prostate	80% seminal vesicles and 19% prostates infected with bladder; dense fibrosis and calcified eggs
2.	Grace and Aidaros*ǂ	1952	Egypt	Autopsies: Seminal vesicles	95% seminal vesicles involved in infected autopsies, bilateral, 94%

					with *S. haematobium* calcified eggs
3.	Gelfand and Ross‡	1953	Zimbabwe	Post-mortems: all organs, males and females	*S. haematobium* more common than *S. mansoni* n prostate despite rectum close proximity
4.	Alves *et al.**	1955	Zimbabwe	Autopsies: male genital organs	76% of autopsies showed bilharzial eggs; prostate, vas deferens, seminal vesicles involved most.
5.	ArbÁN	1956	Puerto Rico	Autopsies and surgical removed specimens	10 genitourinary lesions due to *S. mansoni*; kidneys, bladder, testes, prostate and seminal vesicles
6.	King	1965	South Africa	Urine microscopy Radiological exam	More epididymo-orchitis in no urine eggs than with; prostatitis in 0.11% with eggs, 0.16% without
7.	Etribi *et al.**	1967	Egypt	Histopathological analyses of thick vas deferens	62.7% of sub fertile men had Bilharziasis (eggs in semen, urine, calcifications), obstructive testicles
8.	Gelfand *et al.**	1970	Zimbabwe (S. Rhodesia)	Autopsies: prostate, seminal vesicles, vas deferens	Seminal vesicles, spermatic cord, prostate infected more with *S. haematobium* than *S. mansoni* eggs
9.	Edington *et al.*	1970	Nigeria	Autopsies: all organs	Mean egg load more in males; eggs in seminal vesicles, prostate, vas deferens, epididymis; calcified.

10.	Edington *et al.**	1975	Nigeria	Autopsies; male and female genital organs, appendix, brain	Seminal vesicles, prostate, testes, epididymis were most infected; atrophy, fibrosis with calcified eggs
11.	Aboul *et al.**	1977	Egypt	Seminal vesicles	Seminal vesiculitis due to severe bilharzial infiltration showed no obstructive infertility.
12.	Cheever *et al.* ‡	1978	Egypt	Autopsies: pathology of extrahepatic organs	Active lesions in seminal vesicles, prostate and ejaculatory ducts; eggs, fibrosis, muscle hypertrophy
13.	Ricosse *et al.*	1980	France	Pathological specimens	Testes: hard, irregular, hydrocele Epididymis: nodular mass, inflamed
14.	Bello and Idiong	1982	Nigeria	-	42.2% of the urethral discharge students had *S. haematobium* eggs
15.	Gwavava *et al.* ‡	1984	Zimbabwe	Surgical biopsies from all organs	Penile ulcer, swollen testis / epididymis, hydrocele haematuria
16.	Elem and Patil*	1987	Zambia	Autopsies: bladder, seminal vesicles, prostate; urine, semen	11 haemospermia men - no eggs; 100 semen – no eggs but 2 in urine; 58% prostate, 50% seminal vesicles
17.	Patil and Elem*	1988	Zambia	Post-mortems: bladder, seminal vesicles, prostate	Digestion vs histology exams: s. vesicles – 50% vs 25%, prostate- 50% vs 9%, bladder – 62% vs 20%

18.	Ogunbanjo *et al.*	1989	Nigeria	-	2/54 seminal fluids from infertile men had *S. haematobium* eggs
19.	Skelly *et al.**	1994	Brazil	-	No significant differences in testosterone levels between infected and non-infected males
20.	Leutscher *et al.**	2000	Madagascar	-	57% with urine eggs, 43% in semen ECP in urine and semen correlated with number of eggs excreted
21.	Al-Saeed *et al.*	2003	Kuwait	Seminal vesicles	Solid masses of seminal vesicles observed on prostate transrectal sonography; chronic infection
22.	Ukwandu and Nmorsi	2004	Nigeria	-	66% with eggs in urine; 4.3% in semen; 22% had sexual pain.
23.	Leutscher *et al.**	2005	Madagascar	-	67% in urine, 28% semen; cytokines, leucocytes, lymphocytes increased; declined with PZQ
24.	Mohammed *et al.*	2007	Nigeria	Endoscopic or surgical biopsies (GIT, GUT)	10% male genital organs affected: prostate, testis, epididymis
25.	Leutscher *et al.**	2008	Madagascar	-	62% eggs in urine, 42% in semen; ECP, SEA, CAA correlated with egg count, with CAA performing well
26.	Leutscher *et al.*	2008	Madagascar	-	55% eggs in urine; 17% had STI; urethral discharge,

					haematuria, dysuria, painful ejaculate.
27.	Ramarakoto *et al.**	2008	Madagascar	Urogenital organs with *S. haematobium*	Hyperechogenic and calcified lesions in seminal vesicles and prostate; enlarged
28.	Leutscher *et al.**	2009	Madagascar	-	53% egg in semen, lower volume, leukocytospermia; apoptosis correlate with ECP, lower with PZQ
29.	Coltart *et al.*	2015	UK	-	6/10 patients with haematospermia had eggs in urine
30.	Yirenya-Tawiah *et al.*	2016	Ghana	-	94.4% males knew UGS, sexual dysfunction, urethral discharge, haemospermia, itchy scrotum; 12.3% UGS help HIV acquisition
31.	Lima *et al.*	2017	Brazil	Histopathological diagnosis for ectopic forms of *S. mansoni*	5 cases of male genital *S. mansoni*: testis (3, 2 had orchiectomy for prostatic adenocarcinoma; 1 with chronic orchitis); penis (1) with squamous cell carcinoma; epididymis (1) with epididymitis.
32.	Midzi *et al.*	2017	Zimbabwe	Urine & stool for schistosomiasis diagnosis; blood for HIV-1 diagnosis & RNA viral load monitoring; semen for RNA viral load monitoring	18 HIV-positive men (6 ART-naïve & 12 ART-experienced) with *S. haematobium* in urine recruited and treated at baseline; followed up for 10 weeks. Mean HIV viral

					load reduction on follow-up after PZQ & no *S. haematobium* in urine

Case reports (#Letters to the Journal Editors, ‡ those from references)					
No.	**Authors**	**Year**	**Country**	**Organs / Specimens**	**Key findings**
1.	Madden‡	1911	Egypt	Right scrotum Spermatic cord Epididymis Seminal vesicles	Hard, painless swelling; epididymis replaced by nodules full of eggs. Haemospermia, haematuria, pain, thick seminal vesicle, eggs in urine
2.	Cerqua‡	1930	Egypt	Prostate	Frequency, scalding erection; painless elastic swelling with eggs
3.	Mohammed‡	1930	Egypt	Seminal vesicle Vasa deferentia	*S. haematobium* in urine; both organs fused with calcified eggs
4.	Makar‡	1937	Egypt	Prostate Seminal vesicles Urethra	Haemospermia; enlarged prostate, seminal vesicles, calcifications, *S. haematobium* eggs in urine, semen
5.	Gelfand and Davis‡	1940	Zimbabwe	Left testis Epididymis	Enlarged, knobby testis; hydrocele, obliterated epididymis; also, eggs
6.	Armbrust‡	1951	Brazil	Scrotum	Enlarged scrotum, hydrocele with dark fluid, *S. mansoni* in testes
7.	Van Beukering and Vervoorn	1956	Ghana (Gold Coast)	Scrotum	Infertility; thick funiculi; bilateral hydrocele with eggs; azoospermia

8.	Chippaux *et al.*	1957	France	Seminal vesicles	Haematuria, mass near seminal vesicle, with *S. haematobium* eggs
9.	Piganiol *et al.*	1957	Senegal	Seminal vesicles	2-3 times increase in volume and hypertrophy of seminal vesicles
10.	Joshi‡	1962	Ghana	Scrotum	Bilateral painless swelling; mass with testis, epididymis and cord fused, infarcted with calcified eggs
11.	Cayret *et al.*	1963	France	Seminal vesicles	7 cases presenting with haematuria, pelvic pain, enlarged vesicles, orchitis
12.	Klerk	1964	South Africa	Seminal vesicles	Dysuria, groin pain, firm seminal vesicles felt; calcified, thickened
13.	Houston	1964	Zimbabwe (S. Rhodesia)	Right testis	Enlarged abnormal; with bladder; having bilharzial pseudo-tubercles
14.	Chaves and Figueiredo	1965	Brazil	Scrotum	Pruritic eruptions, ulcer and oedema; nodules, papules with eggs
15.	Becquet	1966	France	-	Haemospermia, viable *S. haematobium* eggs in seminal fluid
16.	Joshi	1967	Sierra Leone	Right scrotum	Painless hard swelling; calcified eggs and fibrosis
17.	Eltayeb	1969	Sudan	Right testis Epididymis	Painless swelling, non-tender irregular with hard nodules and hydrocele; many eggs in epididymis
18.	Chatelain *et al.*	1969	France	Epididymis	Haematuria, dysuria, urethral shrinkage; fistulae lesion left

					epididymis, large seminal vesicle
19.	Rosenberg *et al.*	1971	France	Left testicle	Painless testicular mass with dilated calcified urethra, urine eggs
20.	Mensah *et al.*	1972	Senegal	Prostate Epididymis	Perineal pain, haematuria, enlarged prostate, hydrocele, discharge
21.	Richaud *et al.*	1972	France	Right scrotum	Nodule of cord lower part with eggs and sclero-inflammation
22.	Monnet *et al.*	1972	France	Left testicle	Swelling of left testicle in a 12-year-old boy from Tunisia
23.	Pedro Rde *et al.*	1973	Brazil	-	Haemospermia, watery red-brown semen with rice grains, many eggs
24.	Kazzaz and Salmo‡	1974	Iraq	Epididymis	Enlarged nodular swelling, multiple granuloma, calcified eggs, fibrosis
25.	Nwokolo	1974	Zambia	-	Purulent urethral discharge
26.	Steinberger *et al.*	1975	U.S.A.	Left scrotum	Left groin pain and swelling; necrosis and granuloma with eggs
27.	Mampilly and Sunkwa-Mills ‡	1976	Zambia	Scrotum	Left inguino-scrotal swelling; thickened epididymis and spermatic cord
28.	Badejo *et al.* ‡	1978	Nigeria	Penis	Ulceration of penis with eggs in dermis; almost autoamputation
29.	Elbadawi *et al.* ‡	1978	U.S.A.	Left testicle	Firm enlarged testis and prostate; tubular hyaline with calcified eggs
30.	Adeyemi-Doro *et al.*	1979	Nigeria	Perineum	Numerous, discrete, firm, painless papular eruptions;

					biopsy with viable and calcified *S. haematobium* eggs
31.	Lembeli and Venkataramaiah[#]	1981	Tanzania	-	*S. haematobium* eggs in semen
32.	Peyer and Graber	1982	Switzerland	Testis	Testicular pain, haematuria; budding lesions with eggs and inflammation
33.	Fievet *et al.* [‡]	1984	France	Right testicle	nodule inferior pole; hypoechoic, epididymal nodule with pus, eggs
34.	Alexis and Domingo	1986	U.S.A.	Prostate	Urinary obstruction; *S. mansoni* eggs with adenocarcinoma
35.	Bambirra *et al.*	1986	Brazil	Right testicle	Enlarged firm testis, nodule, granulomas, viable and dead eggs
36.	Bac *et al.*	1987	South Africa	Left scrotum	Haematuria, hydrocele, firm irregular enlarged testis, nodules with many viable and calcified eggs
37.	Hamida *et al.*	1987	Tunisia	Seminal vesicles	Back pain, dysuria, haematuria, hypogastric mass; no urine eggs; cyst in right vesicle and eggs in muscularis
38.	Mikhail *et al.*	1988	Egypt	Right scrotum Seminal vesicles Spermatic cord	Swelling, heaviness, sensation loss, hydrocele; *S. haematobium* and *S. mansoni* eggs in testis
39.	Elem *et al.*	1989	Zambia	Right testis Epididymis	Painless enlarged testis; nodules on fibrocellular mass with eggs
40.	Fataar *et al.*	1990	New Zealand	Seminal vesicles	Seminal vesicle calcifications and granuloma seen on CT scan
41.	Wedel and Jess	1991	Denmark	Right testis	Haematuria, granulomas with eosinophils and necrosis plus eggs

42.	Ihekwaba	1992	Nigeria	Left testes	Enlarged left testes; hydrocele; granuloma with calcified eggs
43.	Githae	1992	South Africa	Left testicle	Left hydrocele, hard, irregular non-tender testis, granuloma formation
44.	Bornman *et al.*	1992	South Africa	-	Primary infertility; *S. haematobium* eggs in semen, low sperm motility
45.	Fall *et al.*	1992	Senegal	Left testicle	Pelvic pain, haematuria; painless, firm, multinodular testis; orchidectomy with *S. haematobium* eggs found.
46.	Godec *et al.* ‡	1992	U.S.A.	Prostate	Lower back pain, diffuse indurated prostate; bone metastases and prostate adenocarcinoma with *S. mansoni* eggs
47.	Obel and Black	1994	Denmark	-	Left inguinal pain; thin clotting semen with numerous *S. haematobium* eggs, none found in urine
48.	Corachan *et al.*	1994	Spain	Prostate Seminal vesicle	Haematospermia, perineal and coital discomfort, calcifications in prostate and seminal vesicle
49.	Cohen *et al.*	1995	South Africa	Prostate	Elevated PSA, adenocarcinoma, with *S. haematobium* viable and calcified eggs; also, adult worms
50.	Ma and Srigley	1995	Canada	Prostate Seminal vesicles	Both enlarged; adenocarcinoma of both with calcified *S. haematobium* eggs; no granuloma or fibrosis
51.	Fender *et al.*	1996	U.K.	Prostate Seminal vesicle	Fever, urgency, frequency, loin pain, haematuria,

					haemospermia, boggy prostate and seminal vesicle
52.	Ingram *et al.*	1996	U.K.	Testicles Epididymis	Testicular pain, haemospermia, *S. haematobium* eggs in semen
53.	Lewis *et al.*	1996	U.K.	Testicles	Testicular pain; 2mm white lumps in semen, *S. haematobium* eggs
54.	Torresi *et al.*	1997	Australia	-	Thin, brown discoloured semen, with many *S. haematobium* eggs
55.	Vilana *et al.*	1997	Spain	Prostate Seminal vesicle	Haemospermia, perineal discomfort, calcifications, enlargement on scan
56	McKenna *et al.*	1997	U.K.	-	Subjective ejaculate change, low volume and viscosity (watery)
57.	Davies and Hamdy[#]	1998	U.K.	-	Subjective change in ejaculate
58.	Soans and Abel	1999	Australia	Right testis	2cm mass, hypoechoic lesions; necrotizing granuloma with eggs
59.	Basilio-de-Oliveira *et al.*	2002	Brazil	Prostate	Small nodule; adenocarcinoma with scattered *S. mansoni* eggs
60.	Schwartz *et al.*	2002	Israel	-	Haematospermia, haematuria, pain after ejaculation, eggs in semen
61.	Durand *et al.*	2004	France	Spermatic cord	Pain at coitus, haematuria, many *S. haematobium* eggs in semen
62.	Alves *et al.*	2004	Brazil	Left epididymis	Chronic pain, hardening; chronic granulomatous process with eggs

63.	Mortati Neto *et al.*	2004	Brazil	Right testis	2cm solid nodule; granulomatous lesion with schistosome egg.
64.	Faucher *et al.*	2004	France	Seminal vesicles Ejaculatory duct	Haematuria, dysuria, ejaculatory pain; hyperechoic vesicles and ducts, bladder biopsies with eggs
65.	Alonso *et al.* ‡	2006	Spain	Testis	Dysuria, discomfort; pain on coitus; brownish, watery semen; eggs in semen
66.	Lambertucci *et al.*	2006	Brazil	Prostate	Elevated PSA, characteristic granuloma around *S. mansoni* eggs
67.	Dauda and Rafindadi	2006	Nigeria	Left testicle	Painless swelling, *S. haematobium* eggs viable and dead, granulomata
68.	Perignon *et al.*	2007	France	-	Yellow coloured ejaculate, reduced in viscosity; semen *S. haematobium* eggs
69.	Lopes *et al.*	2007	Brazil	Seminal vesicle	Elevated PSA and adenocarcinoma of the prostatic; unviable eggs in s. vesicle
70.	van Delft *et al.*	2007	Netherlands	-	Cough, wheezing, scrotal pain, watery semen, haematospermia; eggs in semen
71.	Lopes *et al.*	2007	Brazil	Right testis	2cm nodule, hypoechoic; *S. mansoni* eggs with granuloma formation
72.	Bacelar *et al.*	2007	Brazil	Prostate	Prostatic adenocarcinoma; isolated lesions, viable eggs and granulomas

73.	Pawel *et al.*	2008	U.S.A.	Right scrotum	Pain, swelling; hydrocele; intense egg infiltrate, eosinophils; granulomas
74.	Athanazio and Athanazio[#]	2008	Brazil	Testicles	Massive calcified egg load in right testis; left testis only sparse calcified eggs
75.	Guirassay *et al.* [‡]	2008	Guinea	Prostate	Pollakiuria, dysuria, terminal haematuria; enlarged prostate, fibrous with eggs
76.	Kini *et al.*	2009	U.K.	Testicles	Primary infertility; azoospermia, reduced size, normal testes morphology
77.	Lambertucci and Lippi	2010	Brazil	-	Few matured eggs in semen after vasectomy; none in stool, rectal biopsy
78.	Stevens *et al.*	2010	U.K.	Rectum	Haemospermia; rectal bleeding, chronic inflammation with eggs
79.	Al-Qahtani and Droupy	2010	France	Right testis	Primary infertility; azoospermia, white cells; small mass with eggs, granuloma
80.	Rambau *et al.*	2011	Tanzania	Left scrotum	Pain, testicular mass; hydrocele, atrophic granulomas with schistosome eggs
81.	Periyasamy *et al.*	2011	Malaysia	Left testicle	Genital discomfort; firm nodular swelling; eggs surrounded by granulomatous tissue
82.	Hassan *et al.*	2011	Egypt	Right testis	Painless large tense swelling; pus drained out; many eggs with granuloma reactions
83.	Hawary *et al.*	2012	U.K.	-	Yellow watery semen, particles; beaded seminal vesicles; eggs in urine, semen

84.	Knapper *et al.*	2012	U.K.	-	Orange-coloured watery semen; no eggs in urine or semen; ELISA positive
85.	Adisa *et al.*	2012	Nigeria	Left testicle	Erectile dysfunction, oligospermia; eggs of *S. mansoni* and lymphocytes, left testis
86.	Kato-Hayashi *et al.*	2013	Japan	-	Haematuria, dysuria, haematospermia; eggs in urine and semen
87.	Yu *et al.*	2013	China	Prostate	Frequent micturition; firm, enlarged prostate; with hyperplasia and eggs
88.	Ehsani and Osunkoya	2013	U.S.A.	Prostate	Haemospermia, elevated PSA levels; prostate needle biopsy showed *S. haematobium* eggs, no cancer
89.	Ze Ondo *et al.* ‡	2014	Senegal	Testicles	2 cases in 5 years of testicular nodule; orchidectomy done, histological analysis showed *S. haematobium* eggs
90.	Sharma *et al.* ‡	2015	India	Prostate	Unable to urinate, enlarged prostate with inflammatory infiltrate with eggs
91.	Wobser *et al.*	2015	Germany	Right scrotum	Indurated subcutaneous nodule; granulomas with central necrotic areas
92.	Ekenze *et al.*	2015	Nigeria	Left testicle	Irregular hard mass; non-caseating granulomas on viable and calcified eggs
93.	Ferreira *et al*	2015	Brazil	Right testicle	Difficulties in urinating, hardened nodule, hypoechoic; chronic granuloma reaction

94.	Alves *et al.*	2017	Brazil	Right testicle	Orchiepididymitis; extensive loss of testicular structure and *Schistosoma* egg-induced granulomas, plus Zika virus
95.	Lang *et al.*	2017	Canada	Seminal vesicle	Painful ejaculation, haematospermia; cystic dilated left s. vesicle; ova in semen
96.	Aber-Naser *et al.*	2018	Egypt	Genital organs especially both testis	Persistent azoospermia with low semen volume, absent fructose & seminal vesicles, intact spermatogenesis; *S. haematobium* & *S. mansoni* eggs in right testicular biopsy, none in the left

Editorial articles			
No.	**Authors**	**Year**	**Key facts**
1.	Feldmeier *et al.*	1999	Male genital schistosomiasis and haemospermia
2.	Murdoch	2003	Haematospermia associated with male genital schistosomiasis among travellers

Systematic reviews			
No.	**Authors**	**Year**	**Topics**
1.	Mbabazi *et al.*	2011	Examining the relationship between urogenital schistosomiasis and HIV Infection. MGS and FGS implications in endemic areas.
2.	Figueiredo *et al.*	2015	Prostate adenocarcinoma associated with prostatic infection due to *S. haematobium*. Causal or incidental finding
3.	Stecher *et al.*	2015	Considering treatment of male genital schistosomiasis as a tool for future HIV prevention. More clinical studies to be conducted

Literature reviews (‡ those from references)				
No.	**Authors**	**Year**	**Organs / Specimens**	**Key facts**

1.	Madden‡	1909	Penis	Bilharziosis of glans, prepuce, body, erectile and subcutaneous tissues
2.	Al-Ghorab‡	1968	Prostate Seminal vesicles	Radiological manifestations of Genito-urinary bilharziasis
3.	Nozais *et al.*	1983	All genital organs	Bilharzia of seminal vesicles, prostate, testicles, epididymis
4.	Richter	2000	-	Ultrasonographic changes on schistosomal pathologies after therapy and exposure
5.	Scrimengeour and Daar	2000	-	Schistosomiasis review with relevance to surgeons in Australasia, includes epididymitis, orchitis, prostatitis
6.	Bichler *et al.* ‡	2001	-	Critical review of diagnostics and treatment of schistosomiasis, with mention of MGS
7.	Corachan‡	2002	-	Manifestations of schistosomiasis acquired during international travel including MGS
8.	Ghoneim	2002	-	Bilharzial manifestations of seminal vesiculitis and prostatitis
9.	Richens	2004	All genital organs	Genital manifestations of schistosomiasis which include bleeding and egg deposition in semen, calcifications of prostate and vesicles
10.	de Cassio Saito *et al.*	2004	Scrotum	Ultrasound of scrotum with schistosomiasis show enlarged testes with hypoechoic solid masses (granuloma) of increased vascularity
11.	Vennervald and Dunne	2004	-	An update on the morbidity of schistosomiasis with description of genital manifestations
12.	Coon	2005	-	Detailed history and life cycle of schistosomiasis including aspects of MGS
13.	Maranya *et al.*	2007	Prostate Seminal vesicles	Bilharzial seminal vesiculitis and prostatitis associated with haemospermia, painful ejaculation, low back pain and calcifications
14.	Rollinson	2009	-	Overview of the history, life cycle and clinic-pathological manifestations of schistosomiasis including MGS and its health implications

15.	Shebel *et al.*	2012	Prostate Seminal vesicles	Pathological radiographic findings in Genitourinary schistosomiasis
16.	Le and Hsieh	2017	-	Review of current diagnostics and their challenges, with future advances in progress
17.	Aber-Naser *et al.*	2018	Genital system, especially testis	Review of schistosomiasis as an important, but rarely reported cause of male infertility in endemic areas
18.	McManus *et al.*	2018	-	Review of the current epidemiology, pathophysiology, diagnosis, management and control of schistosomiasis

Chapter 4: Description of the cohort study

4.1. Summary

This chapter provides the detailed description of our cohort for the longitudinal study assessing the current prevalence of MGS among local fishermen on the south shoreline of Lake Malawi in Mangochi District in Malawi. Further details of the methods of diagnosing UGS and MGS, used in this research are highlighted, which have been published as cited below:

Kayuni S.A., Corstjens P.L., LaCourse E.J., Bartlett K.E., Fawcett J., Shaw A., Makaula P., Lampiao F., Juziwelo L., de Dood C.J., Hoekstra P.T., Verweij J.J., Leutscher P.D.C., van Dam G.J., van Lieshout L. and Stothard J.R. (2009). **How can schistosome circulating antigen assays be best applied for diagnosing male genital schistosomiasis (MGS): an appraisal using exemplar MGS cases from a longitudinal cohort study among fishermen on the south shoreline of Lake Malawi.** *Parasitology*, Volume 146, Issue 14, pages 1785-95, https://doi.org/10.1017/S0031182019000969. (Received: 20 February 2019, revised: 30 June 2019, accepted: 3 July 2019, published online: 27 August 2019).

My contribution to this manuscript was that I conducted the literature review on MGS and the research fieldwork along south shoreline of Lake Malawi. Thereafter, I wrote the manuscript and made all the changes suggested by the co-authors and journal referees.

4.2. Introduction to the longitudinal MGS cohort study

As a way of understanding the burden of MGS in local inhabitants of schistosomiasis-endemic areas in SSA, the focus was placed on local fishermen along the south shoreline of Lake Malawi in Mangochi District in Malawi.

Malawi is one of the South Eastern African countries where both *S. haematobium* and *S. mansoni* are prevalent and highly focal around most water bodies (Teesdale and Chitsulo, 1985; Makaula *et al.*, 2014). The shoreline of Lake Malawi, the third largest lake in Africa, is endemic for urogenital schistosomiasis (UGS), with a high prevalence of egg-patent urine *S. haematobium* infections (Madsen *et al.*, 2011; Stauffer, Madsen and Rollinson, 2014). More recently, with the discovery of *Biomphalaria pfeifferi* there is also emergence of autochthonous transmission of intestinal schistosomiasis by *S. mansoni* (Alharbi *et al.*, 2019). More broadly, due to frequent contact with cercariae-infested waters, children, women, farmers and fishermen are at greater risk of UGS as well as growing threat of intestinal schistosomiasis.

The prevalence of HIV in Malawi is considered high (10.6%), especially in this lakeshore region (11.8%), despite the control efforts contributing to reducing the incidence and mortality (NAC, 2015; Ministry of Health, 2017). Despite the wide awareness for the significant burden of UGS in the area, MGS typically remains undiagnosed and underreported among men. With no information about the burden of MGS on the south shoreline of Lake Malawi in Mangochi District, the research study was set out to determine the current prevalence and morbidity of MGS among local fishermen on the shoreline and the potential risk of raised HIV transmission through viral load shedding in semen.

4.3. Study methodology

4.3.1. Study area and population

The research study was conducted among fishermen living in fishing communities (villages) identified and selected along the south shoreline of Lake Malawi in Mangochi district from October

2017 to December 2018 (Figure 16). Mangochi is the largest district in the southern region of Malawi, covering 6,729 km^2 of land with at least 1.1 million people and population density of 171 people per km^2 (NSO, 2019). The district has a tropical continental climate with a longer dry season of cold weather from May to August and hot weather from September to November, and a relatively shorter wet season from December to April (NSO and ICF, 2011).

The Ministry of Health through the District Health Office (D.H.O.) is responsible for provision and management of all health services delivery in the district. The services are provided through government health centres, faith-based and private facilities which are monitored by the D.H.O. Each health centre has a catchment area comprised of villages / communities that it serves. Fishing communities in the study area where most fishermen live and carry out their routine fishing related activities, were selected from the information provided by the D.H.O. of Mangochi District, together with latest prevalence rates of schistosomiasis and HIV.

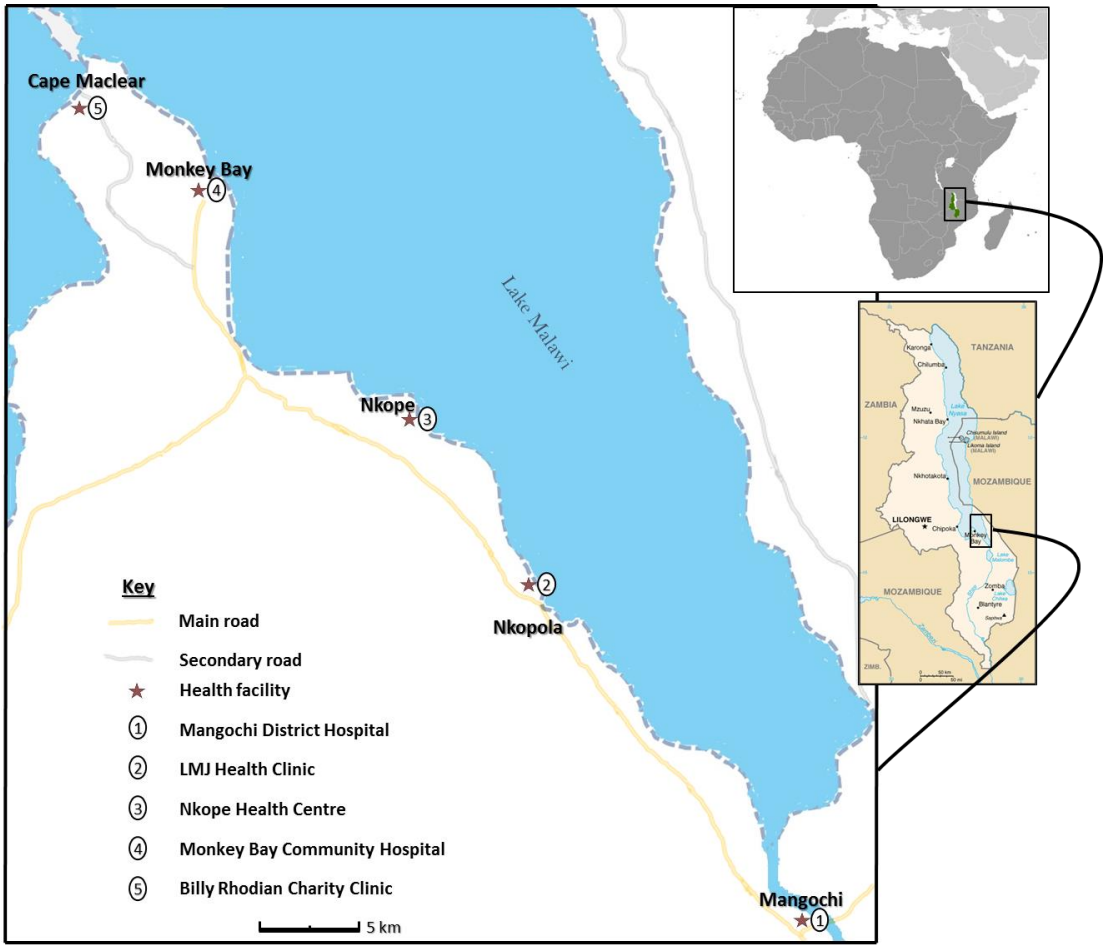


Figure 16: Schematic map of Study area showing health facilities along Lake Malawi

(The study map was produced by Dr Sekeleghe Kayuni (4th August 2019), while the maps of Africa and Malawi were reproduced from the maps at the Central Intelligence Agency (CIA) website, public domain: https://www.cia.gov/library/publications/the-world-factbook/attachments/locator-maps/MI-locator-map.gif and https://www.cia.gov/library/publications/the-world-factbook/attachments/maps/MI-map.gif)

4.3.2. Study sampling

This was a longitudinal cohort study, comprising of baseline surveys of MGS among fishermen and follow-up studies after praziquantel (PZQ) treatment, conducted in villages and nearby health centres. All fishermen aged at least 18 years willing to provide written informed consent were eligible and invited to participate in the study. Using the estimated 20% prevalence of *S. haematobium* UGS in adults from previous studies and assuming 10% having MGS, plus the formula, ($\mathbf{Z}^2$ x **p** x [1 - **p**]) ÷ $\mathbf{e}^2$, where **Z** is the value from normal distribution curve (at a desired confidence interval of 95% = 1.96), **p** is the expected prevalence (0.2), and **e** is the acceptable level

of precision (0.05), a minimum sample size of 275 fishermen (adjusted for assumed 10% loss to follow-up), was planned to be recruited for the study to measure the current prevalence of MGS and subsequent follow-up studies (Kirkwood and Sterne, 2006; CDC, 2014).

In order to assess impact of MGS on the risk of HIV transmission, a pilot HIV study was conducted alongside this cohort study, recruiting a minimum sample size of 20 HIV positive participants co-infected with MGS (as study cases) and 20 HIV positive participants without MGS (study controls). This was due to no similar previous studies conducted in endemic areas.

Ethical clearance for the study was granted by both the Liverpool School of Tropical Medicine Research Ethics Committee (LSTM REC, Approval number: 17-018, Appendices 1 and 2) and the National Health Sciences Research Committee of Malawi (NHSRC, Approval number: 1805, Appendices 3 - 5).

Figure 17: Sensitisation meeting with some fishermen during the MGS cohort study. *Photo credit: Dr Sekeleghe Kayuni, Malawi, June 2018.*

4.3.3. Data collection and analysis

Data were collected by the Principal Investigator (PI) and study team members, comprising of Health Surveillance Assistants (HSAs) from the health centre(s) in study area, Laboratory and

Radiology technicians, who were trained prior to the onset of the study. The D.H.O. and personnel at the health centre(s) in the study area were briefed followed by the community leaders. The HSAs were orientated during the training on the research objectives, sensitization of community leaders, mobilization of potential participants and the process of questionnaire interviews., Thereafter, the HSAs visited villages in the study areas, briefed the community leaders on the study and afterwards approached the potential study participants for recruitment into the study (Figure 17). Adequate research information was given to the potential study participants together with information leaflets (Appendices 6 and 7) describing the study objectives and methods. The following were the data collection methods and analyses used in the study:

4.3.3.1. Individual questionnaires

After briefing about the study and obtaining written informed consent (Appendices 8 and 9), fishermen were recruited in their communities (Appendices 10 and 11) and interviewed with individual questionnaires (Figure 18), collecting information on demographic, health, hygiene, sanitation and socio-economic characteristics (Appendices 12 - 15). This information assessed their knowledge, perceptions, attitudes and practices on MGS and HIV.

The questionnaires were developed from standardised questions administered elsewhere in a similar study (Ukwandu and Nmorsi, 2004). The questionnaires were piloted on the first 10 participants to assess the reliability of the questions. After the questionnaire interviews, the participants were invited to the nearby health facility to submit urine, semen, blood and for ultrasonography examination.

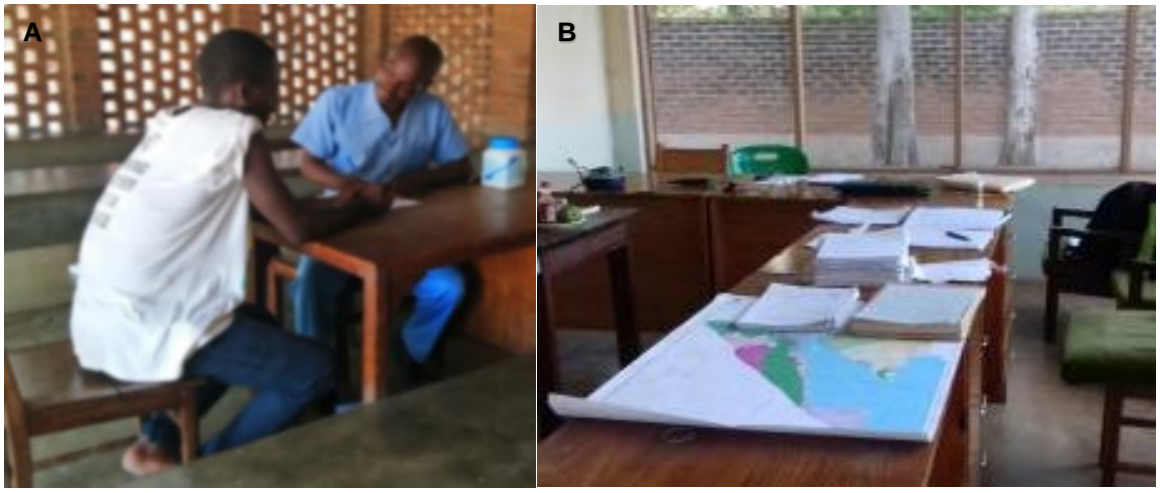


Figure 18: Individual questionnaires interviews with participants during the MGS study

A. Interviewing a fishermen during the study. **B.** Screening and reviewing the questionnaires.

Photo credit: Dr Sekeleghe Kayuni, Malawi. June 2018.

4.3.3.2. Parasitological analyses

At the health facility, they were provided with a clean sample container to submit urine, between 10am and 2pm for filtration to examine for schistosome eggs (confirming UGS). Semen was submitted in a clear, transparent, self-sealing plastic bag, *see* Figure 21, after abstaining from coitus for two days to examine for MGS, defined in the study as the presence of schistosome eggs in semen.

4.3.3.3. Urine analysis including filtration

Urine was analysed (Appendix 16) immediately for macrohematuria by visual inspection using a urine colour card (scores 0, 1, 2 or 3), and then for microhaematuria, leukocytes and proteinuria using reagent strips (Siemens multistix 10G) and scores were recorded in the following categories: negative, trace, +, ++ and +++ (Figure 19). Point-of-care circulating cathodic antigen (POC-CCA) test was conducted on the urine to assess for possible intestinal infection by *S. mansoni*, following manufacturer's instructions (Rapid Medical Diagnostics, South Africa; batch no. 171103130) and as described previously (van Dam *et al.*, 2004) (Figure 20). Urine was measured and

recorded accordingly, before conducting filtration following approved standard guidelines (WHO, 1991; Cheesbrough, 2009).

Figure 19: Urine analyses for macrohaematuria and microhaematuria.

A and **B.** Analysing urine using Siemens multistix 10 SG reagent strips (microhaematuria); **C** and **D.** using visual colour card (scores: **0** = normal; **1** – **3** = macrohaematuria (mild, moderate, severe)) and recording on datasheets.

The entire volume of urine was filtered through a disinfected filter containing a clean polycarbonate membrane with 20 µm pores to trap all *S. haematobium* eggs in the sample. The membrane was removed, placed on a standard glass slide and examined under the microscope. A drop of Lugol's iodine was added to visualise the eggs distinctly. The number of eggs was calculated by first, dividing the total eggs observed by the total volume filtered and then multiplying by 10, to express the resultant egg count per 10 ml of urine. Highest infection intensity for UGS was defined as egg count of ≥ 50 eggs per 10 ml urine as widely described (Cheesbrough, 2009).

Figure 20: Analysing urine using POC-CCA test and filtration during the cohort study

A and **B.** Urine testing using POC-CCA test cassettes. **C.** Intepretation of POC-CCA test results. **D.** Conducting urine filtration using filter holder containing polycarbonate filter membrane. **E.** *S. haematobium* eggs observed on a filter membrane placed on a microscope slide after adding Lugol's iodine, or **F.** without addition of iodine.

4.3.3.4. Seminal microscopic analysis

After submission, the bag with semen was placed under room temperature on a clean bench surface to allow the semen to liquefy (Appendix 17). Thereafter, the semen was pushed gently to one corner of the clear plastic bag. Then the bag was heat-sealed to evenly concentrate the semen for easy visualization during microscopy (Figure 21). Direct examination of the semen bag was conducted under a microscope using x40 and x100 magnification to check for schistosome eggs and the presence of leukocytes (WHO, 2010), thereafter the results were recorded as per ml of ejaculate.

Afterwards, the semen was measured and centrifuged at 5, 000 rpm for 5 minutes to collect the seminal plasma. The sediment was re-dissolved in 0.5 ml normal saline for wet mount inspection using 2-3 drops and placed on a slide with a coverslip for microscopy, followed by recording of the results. Thereafter, 0.5 ml of ethanol was added to the remaining sediment for preservation and stored together with the seminal plasma at -80°c in preparation for shipment to the United Kingdom for real-time polymerase chain (PCR) analysis of *Schistosoma* DNA and HIV viral load for those

participants on ART. After semen submission, the participants were interviewed on any problems or challenges experienced during submission of semen sample using the plastic bag (Appendices 18 and 19).

Figure 21: Description of the seminal analysis for schistosome ova.

A pictorial methodology of visualisation of schistosome ova in semen with a clean, non-sterile transparent plastic bag**. A.** Semen is concentrated to one corner; **B.** the bag is heat sealed to trap the liquid; **C.** the bag is placed on microscope stage and inspected at x40 or x100 magnification; **D.** an egg of *S. haematobium* with miracidium inside. *(Figure reproduced from (Kayuni et al., 2019b).*

4.3.3.5. UCP-LF CAA seminal analysis

A trichloroacetic acid (TCA) extraction was performed on the seminal plasma following standard methods used for serum with an equal volume of 4% w/v TCA (Corstjens *et al.*, 2008) (Figure 22). Small volume extraction (50 µl seminal plasma with 50 µL TCA) in microfuge tubes resulted in a clear supernatant after centrifugation (5 min, 13,000 rpm). The up-converting phosphor-lateral flow (UCP-LF) CAA analysis was performed according to standard methods with 20 µl of the clear supernatant, with a cut-off threshold of 10 pg/ml. High volume extraction (0.5 ml seminal plasma with 0.5 ml TCA and a cut-off threshold of 1 pg/ml) required extended centrifugation time (30 min) before a clear supernatant was obtained; the resulting pellet was not rigid. Amicon 10 kDa centrifugal filtration devices (Merck Millipore) were used to concentrate 0.5 ml of the clear supernatant targeting concentration to 20 µl following standard methods used for serum undergoing centrifugation for 30 min at 13,000 rpm (Corstjens *et al.*, 2014).

Figure 22: Preparing urine and semen samples for UCP-LF CAA analyses.

A. Extracting of sample supernatants in preparation for centrifugation. **B.** Making oral presentation on the MGS study fieldwork in Malawi to the team at Leids Universitair Medish Centrum (LUMC), indicated on **C**. *Photo credit: Ptysje Hoekstra, Leids Universitair Medish Centrum (LUMC), Leiden, Netherlands. Sept 2018.*

4.3.3.6. *Schistosoma* DNA real-time PCR analysis

The ethanol preserved semen sediment was defrosted and centrifuged for 1 minute at 10,000 rpm. The ethanol layer was removed, and the pellet washed twice with 1 ml of phosphate buffered saline (PBS) (Figure 23). The pellet was suspended in 0.4 ml of PBS containing 2% polyvinylpolypyrrolidone (PVPP) (Sigma, Steinheim, Germany). The suspension was heated for 10 min at 95°c and stored frozen overnight at -20°C. DNA was extracted using the QIA symphony DSP virus / pathogen midi kit and pathogen complex 400 protocol of the QIA symphony Sample Processing (SP) system (Qiagen, Hilden, Germany). In each sample, a fixed amount of Phocine Herpes Virus 1 (PhHV-1) was added within the isolation lysis buffer, to serve as an internal control for the isolation procedure and to monitor inhibition of the real-time PCR. *Schistosoma* genus-specific real-

time PCR was performed using primers and probes as described previously (Obeng *et al.*, 2008; Kenguele *et al.*, 2014).

Figure 23: Preparing semen pellets for real-time PCR using automated QIA symphony.

Photo credit: Dr Sekeleghe Kayuni, LSTM, U.K. September 2018.

4.3.3.7. Blood collected for further analysis

Participants were requested for their consent to have their venous blood collected following the standard aseptic and infection prevention guidelines using approved personal protective equipment into 4ml EDTA-coated tubes (Cheesbrough, 2005). All safety precautions were observed to minimize the risks associated with blood collection. Thereafter, the blood was centrifuged at 5, 000 rpm for 5 minutes to harvest plasma.

After the preparation, the blood plasma together with seminal plasma were shipped under dry ice at - 80°c to LSTM in United Kingdom for comparative HIV viral load analyses and *Schistosoma* soluble egg antigen (SEA) enzyme-linked immunosorbent assay (ELISA).

4.3.3.8. *Schistosoma* serology SEA ELISA

Schistosoma serology SEA ELISA microwell kits (SCHISTO-96 IVD IgG/M, New Life Diagnostics LLC, Lot. 1729) manufactured by IVD Research Inc. (Carlsbad, CA 92010 USA. www.ivdresearch.com) were used to run ELISA on the participants' plasma to detect schistosome antibodies. The kits were

placed on bench at room temperature for half an hour after removal from 2-8˚C cold room before opening and use. The plasma was defrosted and then spun at 13,000 rpm for seven minutes.

Using the microwells, 100 µl of prediluted negative control was first added into designated well, followed by 100 µl of prediluted positive control from the kit, and finishing 100 µl of diluted (1:40 and 1:80) plasma from participants to the remaining wells. The negative and positive controls, designed by the manufacturer for easy validation and quality control of the kit, were also added to the last two wells.

The microwells was then incubated at room temperature for 10 minutes before shaking out the contents and washing three times with diluted wash buffer. Two drops of enzyme conjugate was added to each well and incubated at room temperature got another 10 minutes, before shaking out contents and washing three times. Two drops of chromogen (substrate) was added to each well and incuabted for 5 minutes, beofre two drops of Stop solution were added to each well.

Thereafter the well were read visually and then using Fluostar Omega BMA-LABTECH spectrophotometer with filters at 450 nm (Figure 24). Absorbance reading equal or greater than 0.2 OD units were recorded as positive while those less than 0.2 were negative. Further quality control of this ELISA was conducted on 33 participants' plasma, using DRG IgG test kits (Lot. G062, IQA reference: 2462/19) at an accreditated clinical laboratory at LSTM whose results are described in chapters 6 and 8.

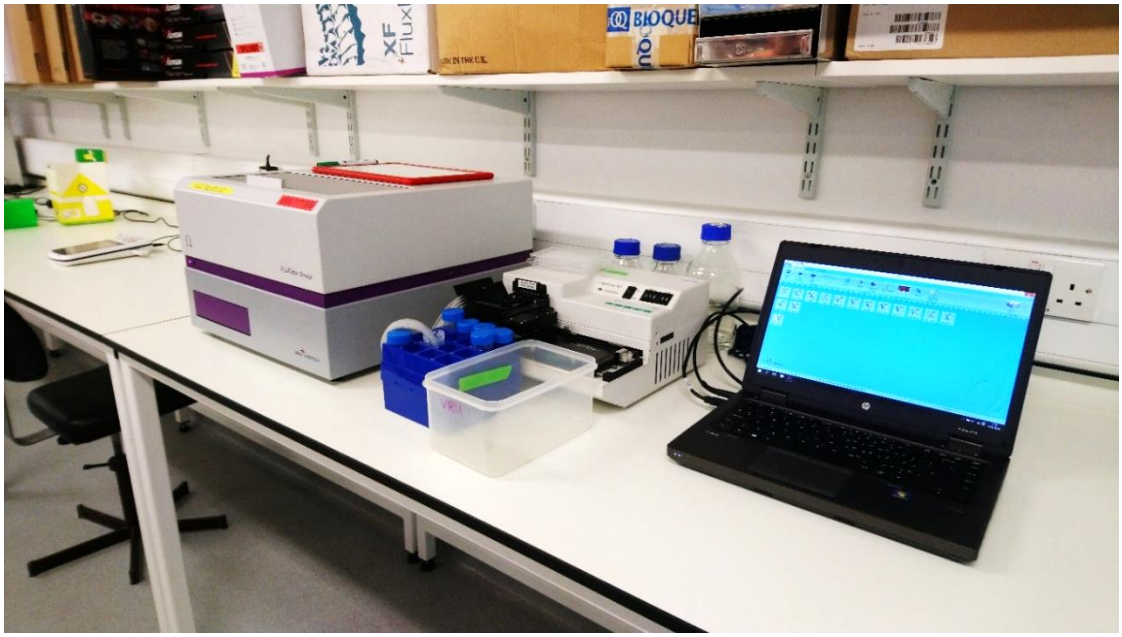


Figure 24: FluoSTAR Omega Spectrophotometer used for reading ELISA kit microwells

Photo credit: Dr Sekeleghe Kayuni, LSTM, U.K. September 2019.

4.3.3.9. Point-of-care Prostate specific antigen (POC-PSA)

A rapid test for the semi-quantitative detection of Prostate specific antigen (PSA) in whole blood, serum or plasma (ALL TEST PSA Rapid Test Cassette, MedNet GmbH, Ref. TPS-402, Lot.: PSA18110014), manufactured by Hangzhou All Test Biotech Company Limited. (Hangzhou - 310018, P.R. China, www.alltests.com.cn) was conducted on some participants with abnormal ultrasonograophy results on their prostates for possible malignancy. The frozen plasma sample was used in this test, after completely thawing, mixing well.

A drop of plasma (approximately 40 µl) was transferred using the dropper provided with the kit, to the specimen well, labelled S on the cassette, and then a drop of the buffer also provided was added to the specimen well (Figure 25). Thereafter a timer was started to run for 5 minutes before reading the results. The results area was expcted to display coloured lines, namely control (C), reference (R) and test (T) lines, for reading the test results. Positive results were interpreted with presence of all the 3 coloured lines while negative results only showed the control and reference lines, without the test line (Figure 26).

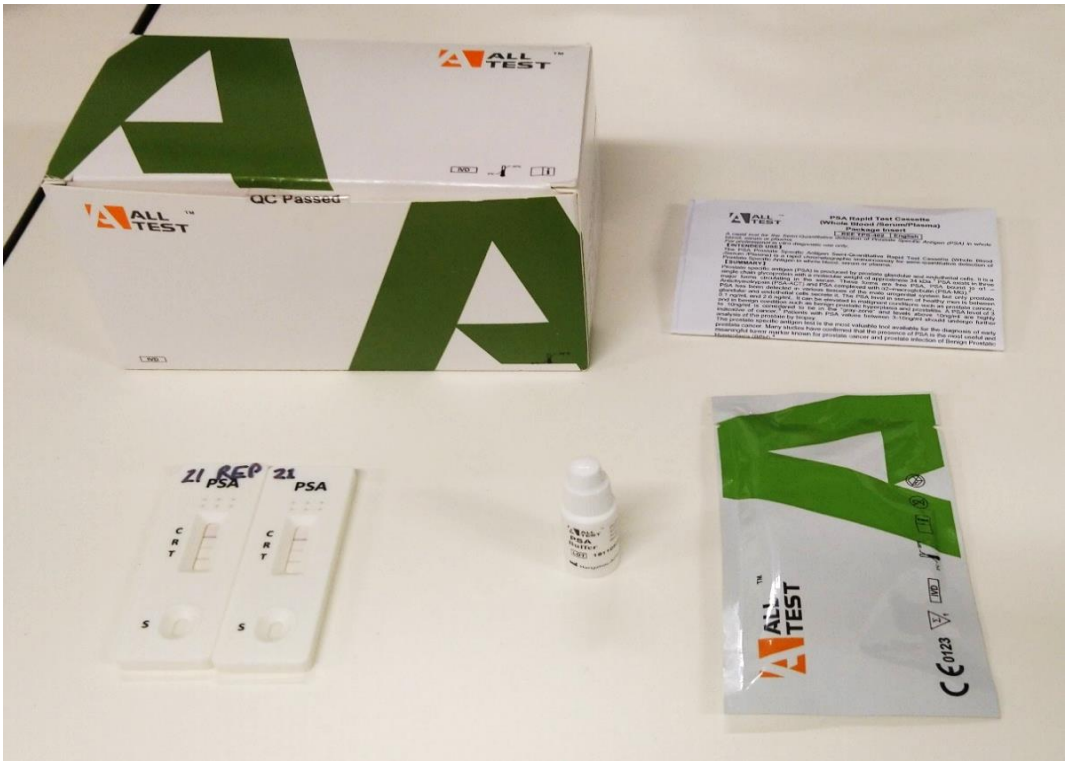


Figure 25: ALL TEST PSA Rapid Test showing the test kit, buffer and used cassettes.

Photo credit: Dr Sekeleghe Kayuni, LSTM, U.K. July 2019.

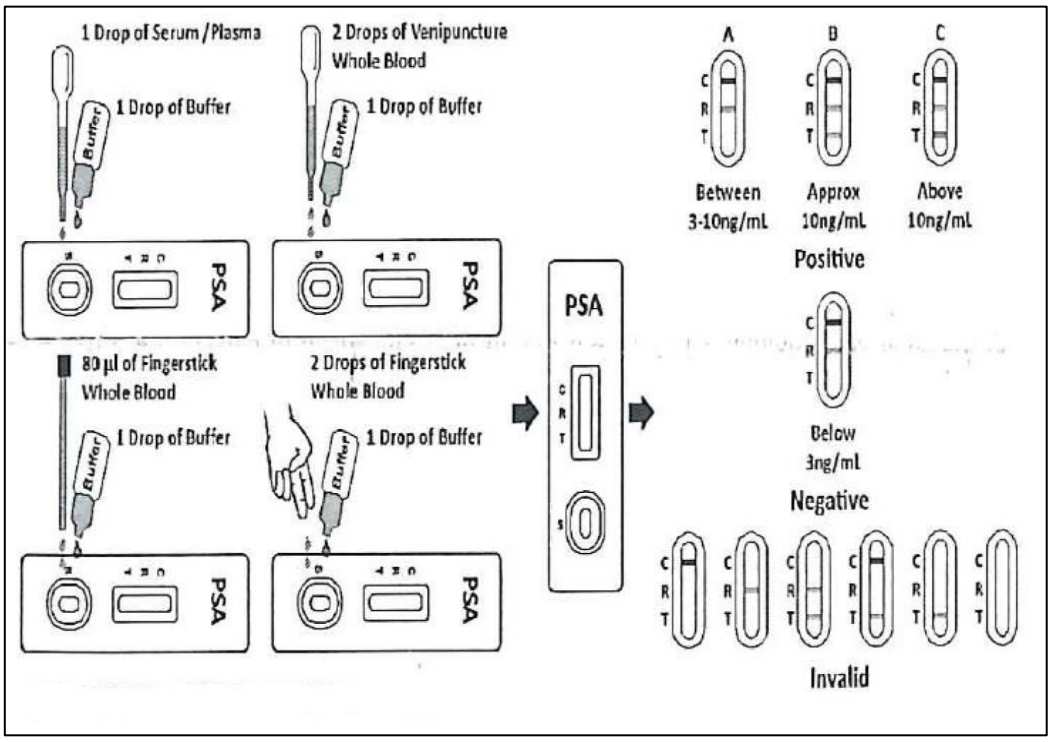


Figure 26: Outline of the procedure for ALL TEST PSA rapid test, in accordance to the manufacturer's instructions showing the expected results.

4.3.3.10. HIV-1 RNA detection

HIV-1 RNA detection on the collected blood and semen plasma samples were conducted using a commercially available Cepheid Xpert® assay for qualitative and quantitative plasma detection of HIV-1 (Cepheid, 2019). The Xpert system is a semi-automated quantitative real-time polymerase chain reaction (RT-PCR) amplification test system. The system is designed to combine automated and integrated sample preparation, nucleic acid extraction, amplification and detection of HIV-1 target sequence using real-time reverse transcription PCR within 90 minutes of test initiation. The system is a fully closed system that includes an internal control and utilises a single disposable cartridge (Figure 27) for each run minimising the risk of contamination and biological hazard (WHO, 2017). The assay quantifies HIV-1 Group M, N and O with a range of 40 to 10^7 copies/ml.

Figure 27: Cepheid Xpert cartridge

Image courtesy of https://www.cepheid.com/en/.

Firstly, validation experiments were carried out using semen samples from two HIV-negative and schistosome-negative men from the study area. These semen samples were spiked with the third WHO reference standard for HIV-1 RNA (NIBSC 10/152) reconstituted to 1 millilitre with a final concentration of 10,000,000 viral copies per millilitre. In the first set of experiment, the detection

threshold of the Xpert HIV QUAL assay was confirmed in the absence of semen inhibition by performing the experiments using a 10-fold dilution factor.

In these experiments, semen sample was spiked with the HIV standard to different concentration ranging from 10^5 to 10^2 copies/ml (10^5, 10^4 ,10^3 and 10^2) including an unspiked plasma sample from a HIV-1 negative patient for quality control. In the second experiments, the HIV-1 RNA concentration was kept fixed at 500 IU/ml (equivalent to 291 copies/ml) whereas the proportion of semen sample to diluent was varied to different proportions each totalling 1 ml. In this experiment, semen (200, 300, 400, 500 and 900 µl) was diluted to an input totalling 1 ml prior to testing. Samples were then tested with the Cepheid Xpert HIV QUAL assay, which has a reported lower limit of detection of 278 copies/ml (equivalent to 478 IU/ml).

The second set of experiments was performed in duplicate, with or without a prior spin step following dilution. The spin step involved wash step and low-speed centrifugation at 500 rpm to separate possible sediments without precipitation of the virus. Analysis of data from these experiments subsequently determined the approach for testing the patients' semen samples. Upon successful completion of the validation, quantification of the HIV-1 VL was conducted on participants in the study.

Plasma were separated in the field study area from collected whole blood samples in 4ml plain vacutainer tubes by centrifugation at 5,000 rpm for 5 minutes. The semen collected was put in sterile 2ml microtubes following liquefaction and then centrifugation at 5,000 rpm for 5 minutes to harvest seminal plasma. The seminal pellets were mixed with equal volumes of saline and ethanol to preserve the samples for schistosome real-time PCR, described above. The plasma samples were shipped frozen and monitored at -80^{o}c to Liverpool School of Tropical Medicine (LSTM) and Institute of Infection and Global Health in University of Liverpool (IGH) in United Kingdom.

Samples were prepared and diluted in the IGH extraction laboratory, packed in Saf-T-Pak and taken for HIV-1 RNA viral load testing to the virology department of the Royal Liverpool and Broadgreen University NHS Hospital where the Xpert infinity platform was located. For plasma, one

millilitre of sample input was transferred into the cartridge and processed on the platform. For seminal plasma testing, samples were diluted based on sample availability ranging from 2.5-fold dilution (400 µl of semen sample to 600 µl of diluent) to 10-fold dilution (100 µl of semen sample to 900 µl of diluent) using molecular grade water as diluent and loaded into the cartridge for testing on the Infinity test platform.

4.3.3.11. Ultrasonography examination of genital organs

a. Preparations for the procedure

Safety precautions including use of appropriate protective wear and gloves were ensured during the ultrasonography examinations to prevent exposure to hazards (Appendix 20). Participants were briefed on the transabdominal and scrotal ultrasonography procedures to be conducted on them using a portable Chison Q5 ultrasound scanner (Figure 28) with 3.5MHz probe (Mount International United Services Ltd, Gloucester, United Kingdom). The scanner was put on the right side of the participants and set on the appropriate urology mode for the procedure. Participants were asked to present with a full bladder, before the procedure to increase the visualisation and validity of the images. Whenever possible, room lightning was turned off to maximise screen visibility.

b. Outline of the ultrasonographic procedure

The participant's study number was registered in the ultrasound machine and report form prior to commencing the procedure. The participant was positioned supine on the examination couch and a reasonable amount of ultrasound gel was put on the probe. The scanning procedure investigated the appearance, size and abnormalities of the following key pelvic and genital organs, namely urinary bladder, seminal vesicles and scrotum (tests, epididymis). Visualization conditions were recorded first of each section on report and then absence/presence of pathological findings were documented.

Figure 28: The portable Chison Q5 ultrasound scanner in an examination room

Photo credit: Dr Sekeleghe Kayuni, June 2018.

i. ***Urinary bladder***

Probe position: The probe was placed transverse (TS) above the pubic symphysis with probe orientation projecting the right side of the patient on the left side of the screen. Transverse sweeps through the bladder were performed to assess the shape (distension) and wall thickness of the bladder, as well as the distal ureters where possible. Care was taken to adjust depth and gain settings appropriately for anterior and then posterior bladder wall/ureters to avoid artefacts and reduced visibility of bladder wall. Longitudinal (LS) sweeps were also be performed in similar way.

Description of results: Normal finding was the bladder being fully distended and having a regular, rectangular shape; the bladder wall was of regular thickness and not thicker than 5 mm. Normal distal ureters not being visible. Pathological findings of schistosomiasis-related urinary pathologies included a rounded or irregular shape of the bladder, wall thickening with diffuse or focal thickening of > 5 mm (mild: 6 - 10 mm; severe: ≥11 mm), bladder wall calcifications and masses

or pseudopolyps protruding in the bladder lumen; the distal ureters were considered pathological when dilated.

Storage of images and clips: Bladder wall thickness were measured in mm and stored as a separate still image. After performing several sweeps through the bladder, the best representative sweep was stored under the label “bladder”. In case of any pathologic findings additional still images with relevant measurements were stored. Once the bladder wall thickness was abnormal, the kidneys were scanned for evidence of hydronephrosis.

ii. ***Prostate***

Probe position: The prostate was visualised with the probe directed deep into the pelvis, after scanning the bladder. Care was also taken to adjust depth and gain settings appropriately to clearly and fully visualise the prostate.

Description of results: Normal finding was the prostate being normal when volume is 30 mm^3 or less with smooth outline. Pathological findings of schistosomiasis-related pathologies included nodules or masses above 1 cm, and calcifications of the prostate.

Storage of images and clips: The prostate was measured in mm^3 or ml and stored as a separate still image. After performing several sweeps through the prostate, the best representative sweep was stored under the label “prostate”. In case of any pathologic findings, additional still images with relevant measurements were stored.

iii. Seminal vesicles

The seminal vesicles were visualised adequately with several sweeps, just after scanning the prostate. Description of results: Normal finding was the seminal vesicles being symmetrical, measuring 15 mm or less in antero-posterior (AP) plane with smooth outline. Pathological findings for schistosomiasis-related pathologies included enlarged and or asymmetrical vesicles with nodular, echogenic appearance.

Storage of images and clips: if the vesicles measured larger than 15 mm in AP plane, their measurement were stored as a separate still image. Following performing several sweeps through

the vesicles, the best representative sweep was stored under the label “SV”. In case of any pathologic findings, additional still images with relevant measurements were stored.

iv. Scrotum

Probe position: The probe was placed transverse on the scrotum with probe orientation projecting the right side of the patient on the left side of the screen. Axial sweeps on the scrotum were performed to assess both testes. Care was taken to adjust depth and gain settings appropriately.

Testis: if found abnormal, a description on absence or presence of nodules, masses, atrophy or calcifications; Epididymis whether normal or enlarged; and other abnormalities like hydrocele.

c. **Disinfection at the completion of the procedure**

At the end of the procedure of each participant, the probe was cleaned with tissue paper to remove the gel, and with methylated spirit. Similarly, the gel on the procedure was wiped off with tissue paper and disposed together with used gloves, disposable protective wear and other waste in accordance with the approved standard infection prevention protocols for the Ministry of Health in Malawi. All participants were notified of pathological findings that day, and further appropriate investigations and management were organised in accordance with standard clinical practice. Thereafter, praziquantel treatment at 40 mg/kg as a single dose was offered along with an invitation to follow-up studies after 1-, 3-, 6- and 12-months.

4.3.4. Statistical analyses

All the information collected during the study was screened and quality-controlled before entry into Microsoft Excel and SSPS computer packages (Appendices 21 and 22). No double data entry was conducted. Screening for errors using descriptive analyses and cleaning were conducted, before commencing statistical analyses to present the results of the study. All video clips and digital images were coded for data protection and were then stored onto the device before transferring to a password-protected external hard drive for further analyses. A sample of 15% of the scan images

were randomly selected and re-read by specialist radiologist for quality control, who conducted training on ultrasonography of genital organs related to schistosomiasis.

All video clips and digital images collected from ultrasonography were stored onto the device before transferring to the external hard drive for further analyses. The data collected from clips, images and report forms comprising from symmetry, thickness, echogenicity, calcifications, nodules, polyps, masses and hydroceles, were screened to clear all errors before entry into IBM SSPS programmes in accordance to evidence-based recommendations and guidelines on specific ultrasonography (Vilana *et al.*, 1997; WHO, 2000; Martino *et al.*, 2014). Summary statistics were calculated to explore the data and thereafter correlations and significant tests were conducted to describe and interpret the results further, mainly using nonparametric tests.

4.3.5. Ethical considerations

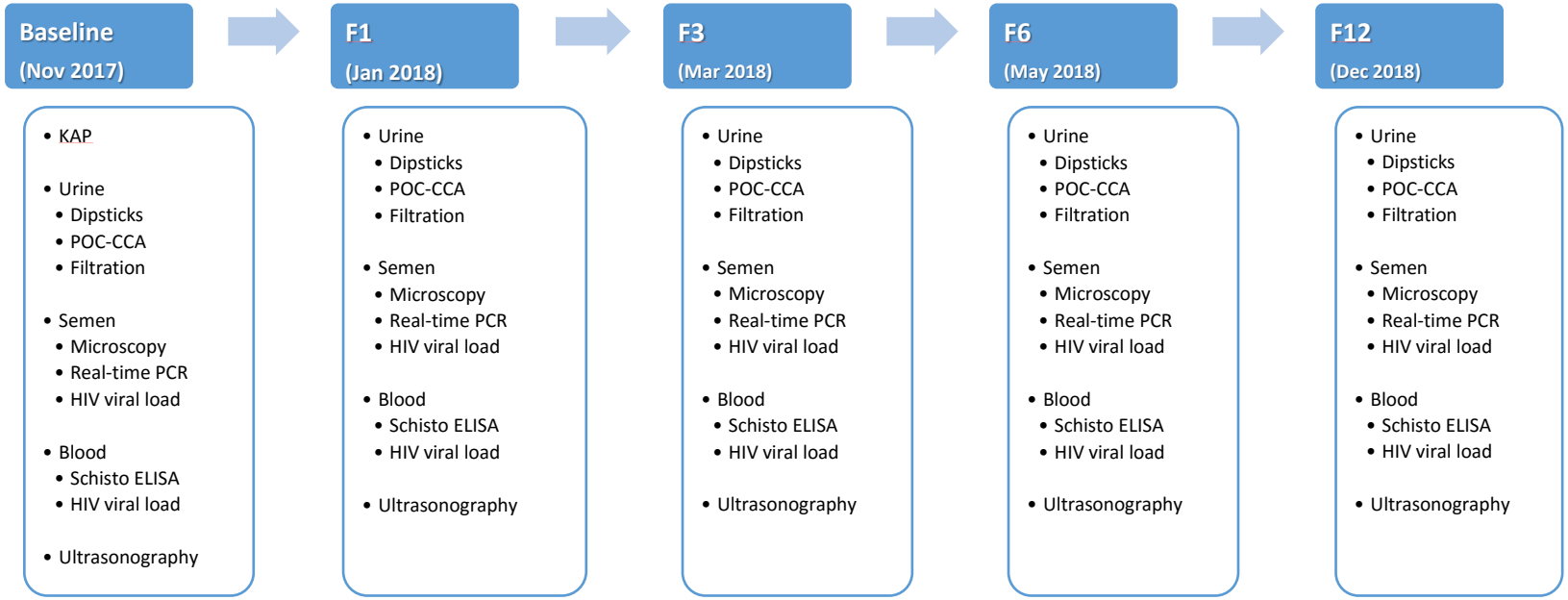
Utmost privacy and confidentiality were maintained in the study and where necessary, the information was anonymised to protect the identity of the participant. Participants were informed of their right to opt-out at any stage of the study if they wish to do so. No disruption was caused to their normal daily activities or seeking other services at any health facility. Since this was a test-to-treat study, participants were offered praziquantel treatment at the end of the visit before inviting them to the next follow-up study.

4.4. Description of the study cohort

A total of 384 fishermen were approached on the shorelines and fishing communities along of Lake Malawi and briefed of the MGS study, of which 376 accepted and were recruited into the study (Figure 29). These participants included 56 who were HIV infected and taking ART for at least 6 months prior to the study. All were interviewed with questionnaires and thereafter invited to the nearby health facilities for parasitological and ultrasonography examinations.

The participants came from 39 villages located in two Traditional Authorities (T/A) of Mponda and Nankumba, along the shoreline within the study area. Further description of the cohort and subsequent results have been outlined and discussed in the subsequent chapters.

The cohort was not randomly selected as any eligible fishermen found in the study area were approached and recruited into the study which introduces possible recruitment bias and may affect the generalisability to the whole population of Malawian fishermen. In addition, the absence of HIV testing and reliance on participants' self-declared results from past testing pose a challenge on interpreting the participants' HIV status since people's recall of their test results can be limited and may not give full true picture of the HIV infection status in the cohort.



KAP, all urine and semen microscopy results at Baseline are presented in Chapter 5; Semen real-time PCR and blood Schisto ELISA at all time-points and all urine results at F1, F3, F6 and F12 are in Chapter 6; Ultrasonography at all time-points are in Chapter 7; and Semen and Blood HIV viral load results are in Chapter 8.

Figure 29: Outline of the research data collection during the MGS study along Lake Malawi shoreline

Chapter 5: Baseline prevalence of MGS and associated knowledge, attitudes and practices

5.1. Summary

This chapter described the results of the longitudinal cohort study on male genital schistosomiasis (MGS) conducted at baseline, from the individual questionnaires administered to local fishermen aged 18+ years (study participants) along the south shoreline of Lake Malawi, regarding their knowledge, attitudes and practices related to schistosomiasis especially MGS and HIV infection.

In addition, results of the field parasitological tests on the urine and semen samples submitted from participants are described in this chapter. Prevalence of urogenital schistosomiasis (UGS) in the cohort was 17.1% among those participants who submitted urine samples (n = 210, median = 0.9 eggs / 10 ml, range = 0.1 – 186.0) and for MGS was 10.4% of those who gave semen samples (n = 114, median = 5.9 eggs / ml ejaculate, range: 0.4 – 30.0).

Furthermore, more participants with MGS reported during questionnaire interviews to have experienced dysuria, frequency, haematuria and blood in stool, compared to those without MGS. In contrast, only one of 12 MGS-positive participants reported having experienced classical symptoms of MGS, namely pain in their genital organs, compared to 22 MGS-negative participants who had haemospermia, pain in their genital organs, during coitus and on ejaculation. This elaborates that classical symptoms are not always present in those men excreting schistosome eggs in semen.

5.2. Introduction

Although schistosomiasis is a prevalent parasitic, neglected tropical disease (NTD) affecting over 200 million people globally, especially in SSA (McManus *et al.*, 2018; WHO, 2018b), its chronic genital consequences have been overlooked. Male genital schistosomiasis (MGS) is a specific gender manifestation of schistosomiasis especially urogenital schistosomiasis (UGS), associated with snail-borne schistosome eggs and related pathologies in genitalia of men inhabiting or visiting endemic areas in SSA (Feldmeier *et al.*, 1999; Leutscher *et al.*, 2000). Despite the first recognised MGS patient report described by Madden in 1911 (Madden, 1911), the epidemiology, diagnostic testing and case management of MGS are not well described owing to limited research and diminishing focus over several decades.

Schistosome eggs evoke immunological responses causing granuloma formation and pathological consequences in genital organs (Poggensee and Feldmeier, 2001; Leutscher *et al.*, 2008b). Men suffering from MGS in endemic areas experience pelvic, coital or ejaculatory pain, abnormal ejaculates, haemospermia, abnormal swelling of genital organs and infertility, which is generally underreported (Butterworth *et al.*, 2013; Bustinduy and King, 2014). Schistosome eggs and pathologies have also been observed in seminal fluids and tissues of seminal vesicles, spermatic cord, vas deferens and prostrate during parasitological, histopathological and radiological tests (Al-Ghorab, 1968; Gelfand *et al.*, 1970; Fataar *et al.*, 1990; Vilana *et al.*, 1997).

Furthermore, observations from female genital schistosomiasis (FGS) studies has shown increased risk of HIV infection in women having schistosomiasis due to characteristic genital mucosal breach, increased abnormal vasculature, inflammatory cells and mediators which could facilitate Human immunodeficiency virus (HIV) acquisition and replication (Kjetland *et al.*, 2006; Downs *et al.*, 2017b). This has also been observed in males with MGS, highlighting the plausible risk for acquisition, intensive shedding and increased transmission of HIV (Leutscher *et al.*, 2005; Leutscher *et al.*, 2008b), which can be reduced after PZQ treatment, the mainstay antihelminth for schistosomiasis (Midzi *et al.*, 2017).

Malawi is one of the SSA countries where both *S. haematobium* and *S. mansoni* are prevalent and highly focal around most water bodies, especially Lake Malawi (Teesdale and Chitsulo, 1985; Makaula *et al.*, 2014). In addition, HIV prevalence among adult population (15 – 49 years) is considered high at 10.6% in SSA region, despite the control efforts including provision of antiretroviral treatment (ART) (NAC, 2015; Ministry of Health, 2017; UNAIDS, 2019). Fishermen are one of the high-risk occupational groups in Malawi with higher HIV prevalence, also with a plausible risk of increased HIV transmission to their sexual partners, if dually infected MGS.

With no information about the burden of MGS on southern shoreline of Lake Malawi, this longitudinal cohort study was set out to assess prevalence of MGS among local fishermen dwelling along the southern shoreline of Lake Malawi in Mangochi district and their knowledge, attitudes and practices related to MGS.

5.3. Specific objectives of the study

The specific objectives of the study were:

a. to determine the prevalence of MGS among local fishermen at the baseline of the longitudinal cohort study.
b. to assess the knowledge, attitudes and practices of the fishermen related to MGS and HIV infection.

5.4. Methodology

5.4.1. Study area, population and sampling

The research study was conducted among fishermen living in fishing communities (villages) identified and selected along southern shoreline of Lake Malawi in Mangochi district, the largest district in southern region of Malawi, from October 2017 to December 2018. Most of fishermen in the area live in specific fishing villages, closer to the lake to carry out their routine activities.

Fishermen aged ≥ 18 years willing to provide written informed consent were eligible to participate in the study. They were asked about results of their recent HIV test, with the last 12 months and reported accordingly. A minimum sample size of 275 fishermen was planned to be recruited for the study to measure the current prevalence of MGS and subsequent follow-up studies (Kirkwood and Sterne, 2006; CDC, 2014), as described in Chapter 4.

5.4.2. Study data collection and analysis

5.4.2.1 Individual questionnaires

As described in Chapter 4, briefing about the study were conducted before obtaining written informed consent from the fishermen and recruiting them into the study. Individual questionnaires developed from previous similar study (Ukwandu and Nmorsi, 2004) and piloted in the study area, were administered to the participants, collecting information on demographic, health, hygiene, sanitation and socio-economic characteristics.

5.4.2.2 Parasitological analyses

After the questionnaire interviews, the participants were invited to the nearby health facility where they submitted urine, semen and underwent ultrasonography examination. They were provided with a clean 120 ml sample container to submit midmorning urine for visual inspection for macrohaematuria, reagent multistix dipstick for microhaematuria, point-of-care circulating cathodic

antigen (POC-CCA) test for possible intestinal *S. mansoni* schistosomiasis and finally for filtration to detect and confirm UGS (WHO, 1991; Cheesbrough, 2009).

Semen was submitted into a transparent, sealable plastic bag to examine for MGS in the field, defined in the study as presence of schistosome eggs in semen. Participants were advised to abstain from coitus for at least two days before submitting semen sample. After the field tests, the seminal plasma and pellet were harvested after centrifugation and preserved in preparation for shipment to the United Kingdom for real-time polymerase chain reaction of *Schistosoma* genus DNA and HIV viral load accordingly. Detailed description of the methodology has been described in Chapter 4.

5.4.3. Statistical analyses

All the data collected during the study was screened and quality-controlled before entry into Microsoft Excel and SSPS computer packages. Screening for errors and cleaning were done, before conducting descriptive statistics to describe the data, producing frequencies, proportions, medians and ranges of the variables of interest, defining the prevalences of UGS and MGS. Thereafter, the data was explored to further assess the association of different variables related to MGS as well as explore any differences existing between the groups of participants in the study. Non-parametric statistics were used to analyse the data due to its lack of normal distribution, resulting from low numbers of participants especially those with MGS.

5.4.4. Ethical considerations

Ethical clearance to conduct the study was provided by the National Health Sciences Research Committee (NHSRC) of Malawi and Liverpool School of Tropical Medicine (LSTM) Research Ethics Committee (LSTM REC), as outlined earlier. Utmost privacy and confidentiality were maintained in the study and where necessary, the information was anonymised to protect the identity of the participant. Since this was a test-to-treat study, participants were offered PZQ treatment at the end of each visit before inviting them to the next follow-up study.

5.5. Results

After the briefing and sensitisation about the study, 384 fishermen expressed interest in the study, with 376 recruited and interviewed with questionnaires (Figure 30). Fifty-six participants were HIV positive and taking anti-retroviral therapy (ART) for at least 6 months. The participants came from 39 villages located in two Traditional Authorities (T/A) of Mponda and Nankumba, along the shoreline within study area.

5.5.1 Demographic information of the study participants

The median age of the participants was 30.0 years with a range of 18.0 to 70.0 years (Interquartile range [IQR]: 13) and their duration of stay in the fishing village ranged from 1 month to 70 years (median: 20.0 years, IQR: 24.3, range: 0.1 – 70.0 years; Table 8). There was a strong, positive correlation between the age and duration of stay (Spearman's coefficient *rho* = 0.44, n = 318, $p < 0.001$). The median weight of the participants was 58.3 kg (IQR: 8.7, range: 43.0 – 85.0 kg).

For the HIV-positive participants, the median age was 39.5 years (IQR: 17.0, range: 21.0 – 65.0 years), the duration of stay was 24.0 years (IQR: 24, range: 1.0 – 59.0 years) and weight of 56.4 kg (IQR: 8.8, range: 43.0 – 74.0 kg). There was a significant difference in the median age between HIV-positive participants and those who were negative (independent samples Mann-Whitney test $U = 10{,}917.5$, $z = 5.80$, $p < 0.001$).

Regarding their education status, 11.4% never went to school, 48.4% did not finish primary school while only 7.7% finished secondary school (Table 9). Of the total participants, 65.7% were married and 67.3% had children regardless of their marital status (range: 1 – 16 children). There was a positive correlation between participant's age and number of his children (*rho* = 0.70, n = 260, $p < 0.001$), but negative correlation with education status (*rho* = -0.24, n = 370, $p < 0.001$). Apart from fishing, 20% of participants were involved in other activities including farming, business, studies and household duties.

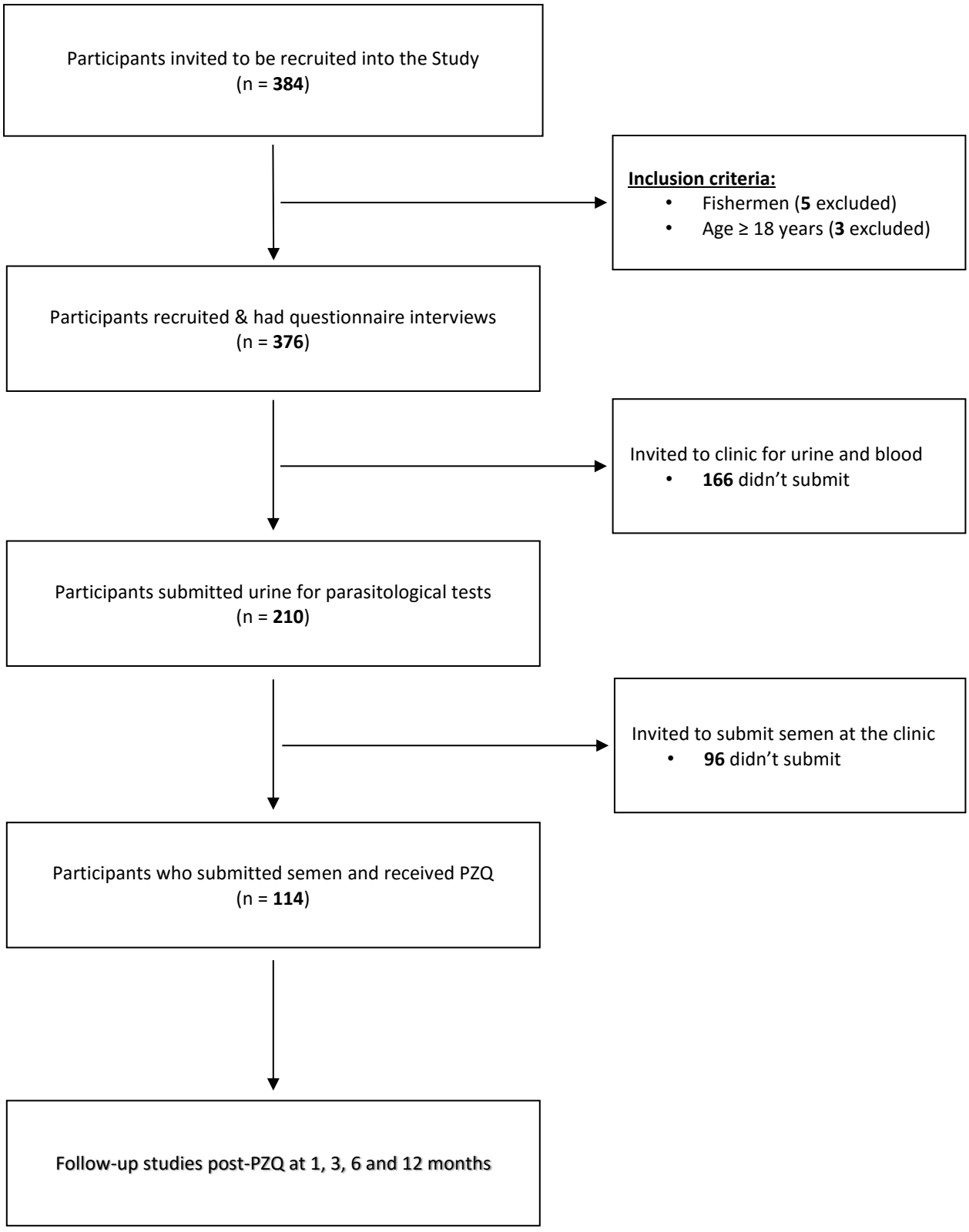


Figure 30: Consort diagram showing the outline of the study

Table 8: Demographic information of the study participants at baseline

Variable		*N*	Median	Range	Interquartile Range (IQR)
All participants	**Age (years)**	376	30.0	18 – 70	13.0
	Duration in the area (years)		20.0	0.1 – 70.0	24.3
	Weight (kg)		58.3	43 – 85	8.7
HIV-negative participants	**Age (years)**	320	28.0	18 - 70	13.0
	Duration in the area (years)		19.0	1 – 70	21.5
	Weight (kg)		59.0	44 - 85	8.1
HIV-positive participants	**Age (years)**	56	39.5	21 – 65	17.0
	Duration in the area (years)		24.0	1 – 59	24.0
	Weight (kg)		56.4	43 – 74	8.8
Samples submitted	**Urine**	210	30.0	18 – 70	15.0
	Semen	114	29.0	18 – 67	15.0

Table 9: Additional demographic information of the study participants at baseline

Variable		*N*	Percent (%)
Level of education	Never went to school	43	11.4
	Didn't complete Primary school	182	48.4
	Completed Primary school	41	10.9
	Didn't complete Secondary school	70	18.6
	Completed Secondary school	29	7.7
	Didn't complete Tertiary school	4	1.1
	Completed Tertiary school	1	0.3
Marital status	Single	83	22.1
	Married	247	65.7
	Co-habiting / engaged	2	0.5
	Divorced	21	5.6
	Other (widowed)	2	0.5
Children	No	64	17.0
	Yes	253	67.3
Other occupation	Farming	2	0.5
	Business	2	0.5
	Household work	69	18.4
	Student	4	1.1
	Unemployed	1	0.3

5.5.2 Prevalence of UGS and MGS in the study cohort

Out of the total recruited participants, only 210 submitted urine after questionnaires (55.9%) and 114 submitted semen (30.3%). Forty-three participants on ART submitted urine while 26

submitted semen samples. The median age of participants who submitted urine was 30.0 years (IQR: 15, range: 18.0 – 70.0) and for semen was 29.0 years (IQR: 15, range: 18.0 – 67.0; Table 8 and Figure 31). Urine examination for macrohaematuria using colour-score card revealed that most of the urine was clear in appearance (97.1%) while few samples were cloudy (2.9%).

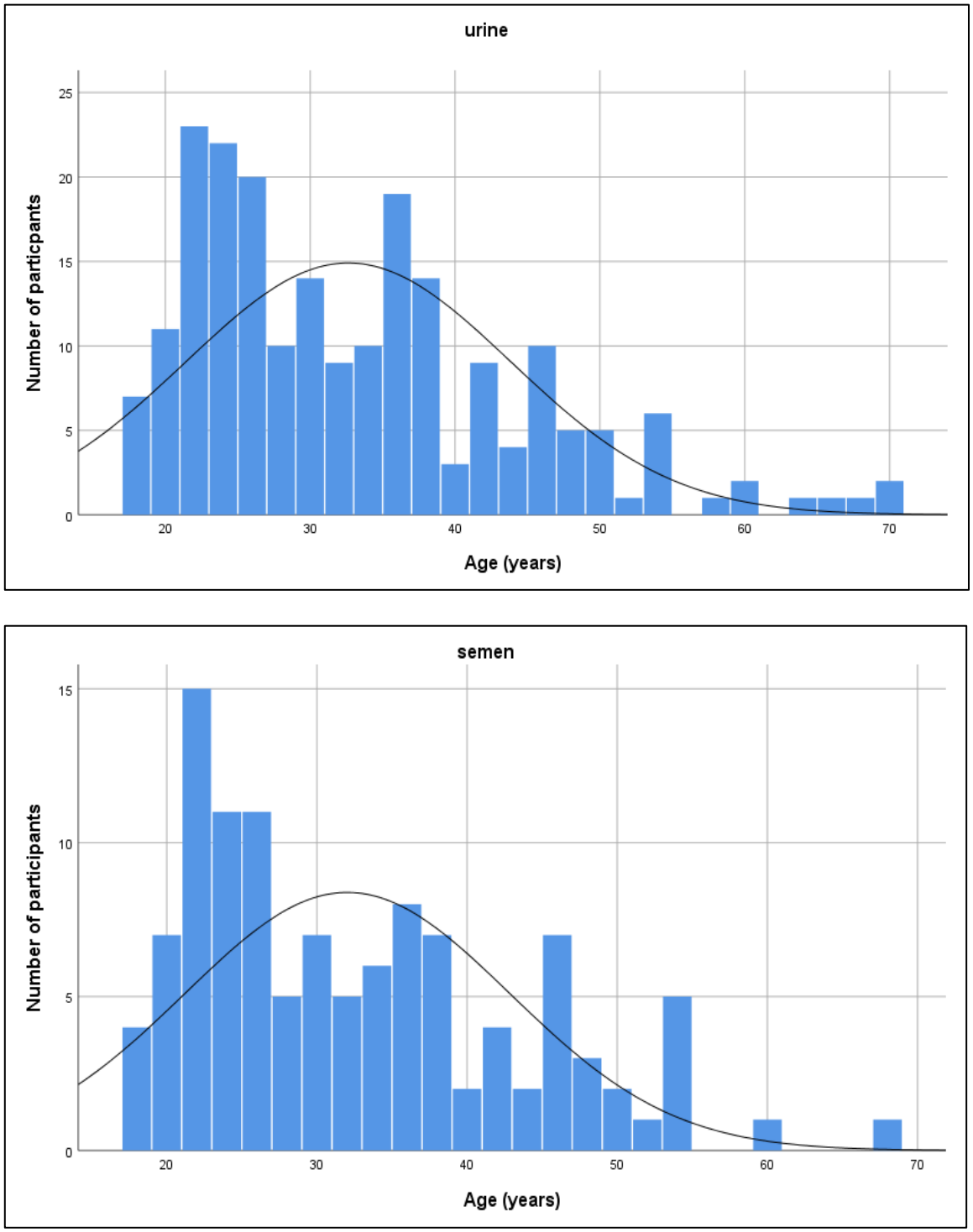


Figure 31: Histograms showing age distribution of participants who submitted samples

Further examination of the urine using reagent Siemens multistix strips showed most of the urine was negative for leukocytes (82.4%), blood (72.9%) and protein (63.8%; Table 10). None of the urine was positive for glucose, suggestive of no glycosuria associated by other diseases.

Table 10: Proportion of 210 participants who submitted urine according to results of reagent strip

Reagent strip score	Leucocytes	Blood	Protein
Negative	173 (82.4%)	153 (72.9%)	134 (63.8%)
Trace	14 (6.7%)	28 (13.3%)	29 (13.8%)
+	11 (5.2%)	10 (4.8%)	34 (16.2%)
++	11 (5.2%)	8 (3.8%)	9 (4.3%)
+++	1 (0.5%)	11 (5.2%)	3 (1.4%)
++++	0 (0.0%)	0 (0.0%)	1 (0.5%)

After urine filtration, 36 participants (17.1%) had *S. haematobium* eggs in urine (UGS), their median egg count was 0.9 eggs per 10 ml (IQR: 5.4, range: 0.1 - 186.0 eggs; Table 11). Only three participants had the highest infection intensity, defined as 50+ eggs per 10 ml of urine (92, 137.8 and 186 eggs). The urine submitted by participants ranged from 10 ml to 240 ml.

Eight (22.2%) of those 36 participants with UGS were on ART, representing 14.3% of HIV-positive participants who submitted urine in the study cohort. There was no significant difference in the urine egg count according to the participants' HIV infection status (median: 1.0 egg, n = 21 [Negative]; median: 0.1 egg, n = 8 [Positive]; Mann-Whitney test $U = 85.0$, $z = 0.05$, $p = 0.96$). In addition, the age of participants did not correlate with UGS infection (*rho* = -0.16, n = 36, $p = 0.35$).

Eight participants of those who submitted urine (3.8%, n = 210) had a positive POC-CCA test, suggestive of possible *S. mansoni* intestinal schistosomiasis infection. There was a positive correlation between reagent strip scores for leukocytes, blood and proteins with urine egg count

(*rho* = 0.23, *p* = 0.001; *rho* = 0.36, *p* < 0.001; and *rho* = 0.25, *p* = 0.001 respectively), while there was no correlation between urine colour card with egg count (*rho* = 0.01, n = 210, *p* = 0.89).

For those who submitted semen, 12 (10.4%) had *S. haematobium* eggs in semen (MGS), with median egg count of 2.9 per ml of ejaculate (IQR: 6.3, range: 0.4 - 30.0 eggs) and seminal volume ranged from 0.1 to 4.5 mL (mean: 1.4 ml). None of the semen had blood in it or of abnormal colour. The semen bag method identified 8 participants (66.7%) whose mean egg count was 1.7, while the centrifugation method identified 9 participants (75%) with mean of 5.3 eggs, and only 5 participants (41.7%) were observed to have MGS by both methods simultaneously (Figure 32). There was no statistical difference in the egg count between the methods. Eight participants (66.7%) with MGS had no eggs in urine.

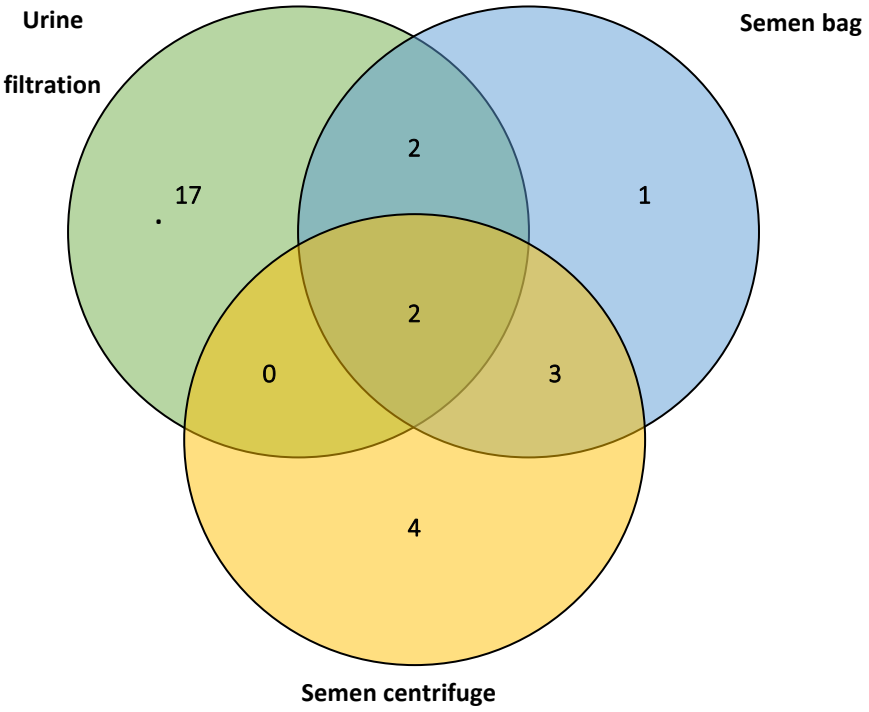


Figure 32: Venn diagram showing positive results of the urine filtration, semen microscopy using bag and centrifugation methods on those participants who submitted semen at baseline (n = 114)

The median age of those with MGS was 46.0 years (IQR: 23.0, range: 18 – 54) while those who were MGS negative, it was 29.0 years (IQR: 14, range: 18 – 67), with statistically significant differences (Mann-Whitney test $U = 833.0$, $z = 2.04$, $p = 0.04$). The ages of participants with MGS correlated significantly with their egg count (*rho* = 0.19, n = 114, $p = 0.001$). Furthermore, there was no correlation between semen egg count with urine card score, POC-CCA test results or urine egg count.

Twenty of those participants (17.5%) who submitted semen had leukocytes in their semen, with 13.2% (n = 15) had less than 50 leukocytes, 1.8% (n = 2) had 51 - 100 leukocytes and 2.6% over 100 leukocytes. None of the participants with leukocytes had schistosome eggs in their semen while only 5 participants with *S. haematobium* eggs in urine (UGS), with no correlation and statistical difference (urine: *rho* = 0.035, n = 98, $p = 0.73$, Mann-Whitney test $U = 801.5$, $z = 0.27$, $p = 0.79$; semen: *rho* = 0.09, n = 98, $p = 0.37$, Mann-Whitney test $U = 581.5$, $z = 1.01$, $p = 0.31$).

Table 11: Parasitological analyses on urine and semen of the study participants at baseline

Variable		*n/N*	Median	Range	Interquartile Range (IQR)
All participants	**Eggs in urine (per 10 ml)**	36/210	0.9	0.1 – 186.0	5.4
	Eggs in semen (per ml)	12/114	2.9	0.4 – 30.0	4.3
	Eggs in semen bag (per ml)	12/114	0.8	0.0 - 9.3	2.5
	Eggs by centrifugation (per ml)	12/114	2.9	0.0 - 30.0	6.25
HIV-negative participants	**Eggs in urine (per 10 ml)**	28/166	1.0	0.1 – 137.8	5.1
	Eggs in semen (per ml)	7/88	3.0	0.8 – 9.3	4.0
	Eggs in semen bag (per ml)	7/88	2.5	0.5 – 9.3	3.3
	Eggs by centrifugation (per ml)	7/88	4.7	0.8 – 6.7	4.3
HIV-positive participants	**Eggs in urine (per 10 ml)**	8/44	1.4	0.1 – 186.0	28.9
	Eggs in semen (per ml)	5/26	2.7	0.4 – 30.0	16.3
	Eggs in semen bag (per ml)	5/26	1.2	0.4 – 2.0	-
	Eggs by centrifugation (per ml)	5/26	5.0	2.7 – 30.0	-

Table 12: Proportion of all 376 participants who reported experiencing symptoms of schistosomiasis including MGS

Symptoms		*n*	Percent (%)
General	Fever	110	30.9
	Weakness	85	22.6
	Abdominal pain	130	34.5
Schistosomiasis	Dysuria	104	27.6
	Urinary frequency	94	24.9
	Urine colour change	177	47.1
	Haematuria	74	19.6
	Blood in stool	27	7.3
MGS	Haemospermia	4	1.0
	Pain on sex	18	4.8
	Pain on ejaculation	12	3.2
	Pain in genital organs	22	5.9

5.5.3 Symptoms and diseases reported by study participants and their spouses

Regarding symptoms related to schistosomiasis, participants reported in their questionnaire interviews among others, change in urine colour (47.1%), dysuria (27.6%), frequency (24.9%), haematuria (19.6%) and blood in stool (7.3%) (Table 12). Specifically, for MGS, fewer participants reported to have experienced pain in their genital organs (7.6%), during coitus (4.8%) and on ejaculation (3.2%), haemospermia (1%), with none of those with MGS reporting any of these classical symptoms. On the diseases reported, 28.6% had schistosomiasis, malaria (32.1%), diarrhoea (31.7%), worm infestation (7.2%) and sexually transmitted infections (STI, 6.4%) (Tables 13 and 14). On their genital symptoms, two participants thought they were related to STIs (11.2%) and majority were not

sure of the cause (66.7%). The participants said that their spouses thought they had STIs (15.4%) and schistosomiasis (7.7%) among others.

Table 13: Proportion of all 376 participants who reported the diseases and treatment received in the preceding months before the study

Variable		*n*	Percent (%)
Disease	Malaria	120	32.1
	Diarrhoea	119	31.7
	Dysentery	24	6.4
	Worm infestation	27	7.2
	Skin disease	49	13.1
	Sexually transmitted infections (STI)	24	6.4
	Schistosomiasis	107	28.6
Treatment in last 12 months	Antimalarials	134	39.0
	Albendazole	77	22.4
	Praziquantel (PZQ)	123	34.8
Easily accessible to PZQ	No	232	61.7
	Yes	129	34.3

Table 14: Comparison of 114 participants who reported experiencing symptoms of schistosomiasis according to the MGS infection status

Symptoms		MGS-positive *(N = 12)*		MGS-negative (*N = 102*)	
		n	Percent (%)	*n*	Percent (%)
General	Fever	2	16.7	34	33.3
	Weakness	3	25.0	20	19.6
	Abdominal pain	3	25.0	38	37.3
Schistosomiasis	Dysuria	4	33.3	27	26.5
	Urinary frequency	3	25.0	27	26.5
	Urine colour change	6	50.0	41	40.2
	Haematuria	13	11.4	13	12.7
	Blood in stool	1	8.3	5	4.9

MGS	**Haemospermia**	0	0.0	1	1.0
	Pain on sex	0	0.0	8	7.8
	Pain on ejaculation	0	0.0	5	4.9
	Pain in genital organs	1	8.3	8	7.8

With regards to symptoms reported by the participants' spouses to them, 5.9% had a miscarriage, primary infertility (1.9%) and secondary infertility (2.7%), which have been previously described to be consequences of female genital schistosomiasis (FGS) (Table 15). Furthermore, 2.7% and 0.8% reported to experiencing pain during coitus and bleeding afterwards respectively which are also thought to be associated with FGS.

Regarding the spouses' symptoms, majority of participants thought they were due to either normal body functioning (22.4%) or contraceptives from health facility (20.4%), while some thought it was unknown disease (14.3%), pregnancy (8.2%), schistosomiasis (2.0%) and other conditions, with one surprisingly saying, *"I think her blood is bad, doesn't relate well with mine"*. The spouses themselves had similar thoughts of normal body functioning (19.4%), contraceptives (19.4%), pregnancy (8.1%), unknown diseases (6.5%) among others, with one saying, *"I think this is as a result of witchcraft"*. Most of participants expressed their need for assistance with their symptoms and diseases (39.1%), diagnostic testing (13.0%) and treatment (8.7%) of schistosomiasis, and further information on the common diseases in the area (30.4%), namely schistosomiasis, malaria, HIV/AIDS, contraceptives use. Of particular interest, one participant commented that, *"do not use modern contraceptives from health facility, rather use traditional methods from the village"*.

Table 15: Proportion of all 376 participants who reported the symptoms and conditions experienced by their spouses in the preceding months before the study

Variable		***n***	**Percent (%)**
Symptoms	Abdominal pains	68	18.1
	Pain on sex	10	2.7
	Bleeding after sex	3	0.8

	Menstrual pains	36	9.6
	Menstrual change	36	9.6
Conditions	Miscarriage	22	5.9
	No children in marriage	10	2.7
	Infertility	7	1.9

Praziquantel was accessed by 34.8% of participants in the last 12 months, 22.4% received albendazole, and 61.7% reported that they had difficulties to access treatment for schistosomiasis (PZQ), however no significant correlation with MGS in this study.

5.5.4 Water contact, fishing and sanitation information of the study participants

The participants admitted to swimming or walking (92.5%), bathing and washing their bodies and clothes (94.1%) in the lake, at least four times in a week. The main reasons for using the lake were because of its convenience to their daily routine since they spend vast time in the lake (44.9%), and its clear, fresh and free water (41.9%) which encourages them to be clean (6.4%), while a small proportion reported that the borehole water was salty (1.0%) or located at a far distance (3.7%).

With regards to their fishing, the median duration of fishing was 7.0 years (IQR: 9.3, range: 0.2 - 60 years) and participants spending an average of 4 days per week on fishing in the lake (IQR: 5, range: 1 – 7 days; Table 16). There was a strong, positive correlation between participant's age and number of years he has been fishing (*rho* = 0.51, n = 298, $p < 0.001$) as well as frequency of fishing in a week (*rho* = 0.14, n = 352, $p = 0.008$), highlighting the potential increased risk of exposure to schistosomiasis.

Table 16: Proportion of participants who reported on their water contact and fishing history in the Lake in the preceding months before the study

Variable	*N*	Median	Range	Interquartile Range (IQR)
Swim / walk in the Lake (times per week)	371	6	1 – 7	4

Bath / wash in the Lake (times per week)	373	4	1 – 7	5
Protective wear in the Lake (times per week)	372	5	1 – 7	4
Fishing in the Lake (number of years)	298	7.0	0.2 – 60	9.3
Frequency of fishing (days per week)	352	3	1 – 7	5
Fish migration to other areas (times per year)	354	3	1 – 20	2

Close to two-thirds (66.2%) admitted to fish in other areas, more than 3 times in a year (IQR: 2, range: 1 - 20), mainly following larger fish catches at a particular time (91.6%), as well as increased availability of fishing boats in specific area (3.8%), following friends migrating to other areas (peer pressure, 3.4%) and better fish prices (1.3%).

Since the lake closes to fishing from December to January every year, in accordance to Malawi government regulations for fish breeding season, most of the participants do other activities including farming (50.4%), casual work, other skilled works (hair-cutting, tailoring, carpentry, building, welding: 7.7%), while some do nothing (22.2%). The duration of fishing was negatively correlated with education status of the participants, which was statistically significant (*rho* = - 0.16, n = 295, $p = 0.005$).

Only 12.7% use protective wear during their water contact. The reasons for not using protective wear include lack of special wear (17.3%), inconveniencing as they have difficulties work with them (19.7%), fear of drowning due to heavy weight once soaked (26.5%), while some find them not necessary (7.1%) and 10% stated that there are rules against use of protective wear or any heavier clothing when fishing in the lake.

Half of study participants used their home toilet for urination (56.9%), followed by those using the lake (32.3%), both lake and home toilet (9.7%) and only 3 use the bush (0.8%). Similarly,

more participants used their home toilet (60.8%) for defecation, some still use the lake (30.8%), both places (9.7%) and the same number used the bush. Thus, indicating the participants do increase the risk of being infected during frequent water contact and transmitting the disease due to their poor sanitary behaviour.

Most have home toilet (92.4%), which they used on average 6.3 days in a week (95% C.I.: 6.2 – 6.5 days), contradicting to their earlier response. For those participants without, use toilet of their parents (50%), neighbour (30%) or drinking bar (10%).

5.5.5 Multivariate analysis of reported symptoms, diseases, water contact and tests results

Further statistical analyses were conducted to explore the relationship between the MGS infection status and reported symptoms, diseases, water contact and fishing history. There was a significant correlation between MGS infection and the frequency of fishing in a week (*rho* = - 0.25, n = 100, *p* = 0.01) and fishing in other areas (*rho* = 0.23, n = 73, *p* = 0.05), while only the duration of stay in the study site was slightly significant correlated with UGS infection (*rho* = 0.16, n = 178, *p* = 0.03).

There was no correlation between infection status and number of days involved in swimming, walk, work, bath or wash in the lake per week, use of protective wear in the lake or use of home toilet. However, there was statistical difference in MGS infection status with regards to their use of the lake for bathing or washing (*p* = 0.01), explanation for their use of the lake (*p* = 0.008) and their village of stay (*p* = 0.001).

Furthermore, there was a significant statistical difference in the fishing duration in a week according their MGS infection using the Mann-Whitney *U* test (median: 3 days, n = 93; median: 1 day, n = 7; *U* = 142, *z* = -2.54, *p* = 0.01, *r* = 0.03; respectively), suggesting that those with less number of fishing days were likely to have MGS. There was no difference in the frequency of water contact during swimming, bathing, washing, number of years fishing and frequency of fishing in other areas. All the variables were not statistically difference with their UGS infection status.

There was no statistical difference in the infection status with the response to the symptoms and diseases experienced by the participants, during and in the months preceding the study. On the participants' response to PZQ access for schistosomiasis treatment, there was no significant difference in MGS and UGS infection status. Similarly, on the easy access to PZQ treatment for schistosomiasis was not significantly different between those participants with and without MGS, similarly to those who either or not received treatment in the last 12 months preceding the study.

One-way between groups analysis of variance (ANOVA) was conducted to explore the impact of participants' education status on the MGS and UGS infection, which were divided into 4 groups according to their responses: never went to school, primary education, secondary education and college education. With no violation of homogeneity of variances, there was no statistically significant difference in UGS and MGS infection among the education status groups of the participants: F (2, 204) = 0.20, p = 0.82 (UGS), and F (2, 108) = 0.07, p = 0.92 (MGS). Of the MGS participants in the study, 9 (75%) attended primary education while the remaining 3 (25%) attended secondary education.

5.5.6 Multivariate analyses of the different diagnostic tests for schistosomiasis

On the relationship between the tests conducted in the study, there was no statistical difference between the urine colour-card scores and the urine egg counts using Kruskal-Wallis tests (n = 210, p = 0.89), while there was significant difference in the reagent strip scores and urine schistosome egg counts (n = 210, p = 0.012 (leukocytes), p < 0.001 (blood, protein)). The reagent strip scores correlated with the semen egg count (*rho* = 0.23, p = 0.001 [leukocytes]; *rho* = 0.36, p < 0.001 [blood]; and *rho* = 0.25, p = 0.001 [protein]), while the colour card scores were not correlated with semen egg counts.

Comparing the urine tests with semen egg results, there was statistical difference between the reagent strip scores for protein and semen egg counts (n = 114, p = 0.01), with no difference in reagent strip scores for leukocytes, blood and colour card scores. When compared, the semen bag

and centrifugation methods had a Kappa measure of agreement value was 0.56, with statistical significance ($p < 0.001$). Using semen centrifugation method as a reference test, the sensitivity of semen bag was 55.6% while specificity was 97.1%, illustrate the capability of bag method in MGS testing.

5.6. Discussion

Looking at the literature, this is the first original research study investigating MGS among local inhabitants along the shoreline of Lake Malawi in a schistosomiasis-endemic area. Previous reports have been case descriptions of travellers or non-dwellers visiting the lake for recreation or business (Schwartz *et al.*, 2002; van Delft *et al.*, 2007; Kayuni *et al.*, 2019a).

Despite its first description over a century ago by Madden (Madden, 1911), MGS remains unknown, unrecognised, misdiagnosed and underreported among men in endemic areas like as Lake Malawi shoreline, thereby suffering from its consequences ranging from persistent genital, coital and ejaculatory pain, abnormal ejaculates, haemospermia, swollen organs and infertility. Coupled with the long known poor health-seeking behaviour of men, certainly MGS impacts the male reproductive health negatively, making it an ignored aspect of an NTD and a public health concern in such endemic areas.

5.6.1. On detection of MGS among local fishermen along Lake Malawi

Our study population comprised young and middle-aged fishermen who had spent most of their life on the lake shoreline in the fishing communities, as observed from their median age of 30.0 years. This is similar to the national trend of the country population, pegged at 17.6 million in 2018 with over 230,000 men aged ≥ 18 years in the district and over 70,000 in the two T/A of the study area. In such endemic areas, people get infected as earlier in life as in infancy (Poole *et al.*, 2014) and as they grow, repeated exposure through contact with infested lake water, results in re-infections and progressive development of chronic manifestations of schistosomiasis, including MGS (Madsen *et al.*, 2011; Colley *et al.*, 2014). Despite some case reports on MGS being in young children (Rambau *et al.*, 2011; Ekenze *et al.*, 2015), most reports on MGS has been in adults beyond adolescence, similar to our observation for the mean age of those with MGS being higher and significantly different to those without disease (Kayuni *et al.*, 2019a).

The prevalence of MGS observed in our study was similar to the assumed prevalence (10.4%), but lower from previous studies in other endemic countries (Leutscher *et al.*, 2000; Leutscher *et al.*, 2008b). As observed in this study, majority of MGS participants (66.7%) had no eggs in urine (UGS) which also explains the non-correlation of both tests. Urine filtration has been used as a proxy to diagnosing MGS, due to challenges encountered in semen submission with individual perceptions around the sample (Price *et al.*, 2005). However, filtration is known to have low sensitivity and specificity especially when the prevalence starts declining (Le and Hsieh, 2017), especially with the mass drug administration (MDA) campaigns with PZQ, which the national control programme in Malawi conducts annually. Interestingly, in the study, the scores of the urine reagent multistix strips correlated significantly with MGS, hence the need to develop more accessible, affordable, point-of-care sensitive and specific diagnostic tests for MGS (Stecher *et al.*, 2015).

The novel semen bag method described and used in the study showed reasonable sensitivity and specificity when compared to the standard semen examination technique (centrifugation method) which has been routinely used (WHO, 2010). This could serve as the first-line examination tool for semen in diagnosing MGS in endemic areas, owing to the readily availability of the tool, compared to most sample collection tools like non-spermicidal condoms or disposable containers.

In addition, the use of POC-CCA urine tests could assist in determination of intestinal schistosomiasis (van Dam *et al.*, 2004; Colley, Andros and Campbell, 2017; Le and Hsieh, 2017), which happen to be an emerging infections having autochthonous transmission on the shoreline (Alharbi *et al.*, 2019). Further investigation of positive men on POC-CCA can elucidate this infection and explain the absence of *S. mansoni* eggs in semen unlike previous reports from other areas (Pedro Rde, Barros Rde and Amato Neto, 1973; Lambertucci, Voieta and Barbosa, 2006; Lambertucci and Lippi, 2010).

5.6.2. Clinical symptomatology, attitudes and practices related to MGS

With regards to the symptoms, fewer participants reported having the classical symptoms of UGS and specifically MGS, certainly none of the symptoms had a significant relationship with MGS infection status. This was similar to their perceptions of the symptoms not related to schistosomiasis but rather other diseases including STI, similarly observed in other studies (Ukwandu and Nmorsi, 2004; Yirenya-Tawiah, Ackumey and Bosompem, 2016). In previous reports, such classical symptoms were like haemospermia, abnormal ejaculates were observed in naïve individuals visiting endemic areas and displaying early stages of schistosomiasis preceding diagnosis of MGS (Ross *et al.*, 2002; van Delft *et al.*, 2007; Knapper, Morrell and Lomax, 2012).

On their water contact during bathing, washing or swimming in the lake, there was no correlation observed with MGS infection status which was surprising. However, their frequency of fishing in the lake per week was noted to correlate with the infection, supporting the known fact that repeated exposure to infested water increases the risk of schistosome infection, its intensity and afterward development of MGS, coupled with their low usage of protective wear (Gryseels *et al.*, 2006; Colley *et al.*, 2014). The mean frequency of fishing was significantly different between the infection statuses which suggest more days of fishing contribute to more exposure which subsequently result in MGS infection.

Furthermore, practices of some participants in using the lake for urination and defecation instead of toilets contribute to continuation of the life cycle because the intermediate snail hosts will be infected with miracidia from their hatched eggs. This does not appear surprising observing that the level of education had no impact on the infection status, since all the MGS participants attended primary and secondary education, although education is supposed to contribute towards modification and transformation of behaviour with regards to schistosomiasis (Kloos, 1995). There is need for comprehensive health education through diverse available channels like beach committee meetings which involves all fishermen, community radio programs, health meetings among others in order to engage the men on schistosomiasis and MGS in particular. Certainly, there is need to

develop local tailor-made water, sanitation and health (WASH) interventions together with the men to address issues of poor hygiene and sanitation observed in the study.

5.6.3. Limitations of the MGS research study

The drop-out of participants in submitting samples especially semen limit the generalisation of the study results to male population in the country and endemic region. This could be explained by negative perceptions and myths associated with semen in rural communities, however previous study examining semen in the district did not encounter such challenges (Kipandula and Lampiao, 2015). In addition, the rumours of blood suckers (vampires) visiting and terrorising the local communities negatively affected the trust and confidence local men had on the study team, thus additional sensitisation and discussions were conducted with local traditional and opinion leaders, health workers and police officers. Also, some participants could be reluctant to submit samples at the health centres, due to poor health-seeking behaviour. However, previous studies describing MGS had similar or even lower number of participants submitting semen, hence our results contribute to the current knowledge of MGS in local inhabitants of a schistosomiasis endemic area.

5.7. Conclusion

In conclusion, male genital schistosomiasis remains a prevalent, unrecognised manifestation of schistosomiasis, especially UGS, commonly in men with frequent exposure to infested waters in endemic areas like Lake Malawi shoreline. This study illustrate the need for more education on schistosomiasis, specifically MGS among men, to timely seek medical assistance to access PZQ which has excellent egg-reduction and cure rates of up to 100% (Knopp *et al.*, 2013).

Chapter 6: Molecular diagnosis of MGS and progression after treatment

6.1. Summary

This chapter described the diagnostic outcomes for male genital schistosomiasis (MGS) using molecular real-time polymerase chain reaction (PCR) on semen samples. At baseline, real-time PCR detected a higher MGS prevalence of 26.6% among the study participants (n = 64, Ct-vale range: 18.9 – 37.4), compared to 10.4% prevalence using semen microscopy. In total, 25 of those participants who submitted semen at baseline (21.9%, n = 114) were detected with MGS using either semen microscopy or real-time PCR.

Furthermore, the results of parasitological and molecular tests conducted at 1-, 3-, 6- and 12-months follow-up time-points are being described in this chapter, indicating varying prevalences despite receiving standard single-dose PZQ treatment.

Application of more sensitive and specific diagnostic tests on semen such as real-time PCR technique improves diagnosis of MGS in men living in schistosomiasis-endemic areas. Despite PZQ treatment being able to acutely clear schistosome eggs in semen, re-exposure and re-infection significantly contributes to recurrence of MGS, hence the need for research study on the impact of repeated doses, and higher dosing on burden of MGS.

6.2. Introduction

Male genital schistosomiasis (MGS) is a severe chronic consequence of urogenital schistosomiasis (UGS), which was first reported in 1911 (Madden, 1911), and is generally unrecognised in most endemic areas (Leutscher *et al.*, 2000; Bustinduy and King, 2014; Squire and Stothard, 2014). Currently, semen microscopy to visualise schistosome eggs is considered as a standard technique for diagnosing active MGS infection, with urine filtration being a diagnostic proxy marker in the presence of MGS symptoms (Kayuni *et al.*, 2019b).

However, there have been reports of seminal schistosome eggs in negative urine filtrations, raising serious diagnostic challenges in MGS, as opposed to substantial progress in defining female genital schistosomiasis (FGS) diagnosis gold-standard (WHO, 2015a). Similarly, sensitivity and specificity of semen microscopy is affected by the diurnal variation of schistosome egg excretions observed in the ejaculate (Ramarakoto *et al.*, 2008). In addition, pathologies in genital tissues have been observed during histopathological examinations and ultrasonography, in absence of other genital diseases, which can also be applied as additional diagnostic tools for MGS (Leutscher *et al.*, 2008b). Molecular techniques like PCR can been applied to MGS, which could improve the diagnosis, treatment and monitor the progress of the disease.

In addition, schistosomiasis has been associated with increased risk of Human immunodeficiency virus (HIV) infection due to the possible alterations of genital mucosa, chronic immunomodulation of the host susceptibility to the virus, thereby facilitating increased risk in HIV acquisition in women and transmission in co-infected men (Leutscher *et al.*, 2005; Kjetland *et al.*, 2006; Stecher *et al.*, 2015). Praziquantel (PZQ) treatment for schistosomiasis could be one of the preventive strategies in controlling the possible transmission of HIV among dually infected men in addition to Antiretroviral therapy (ART) (Ndeffo Mbah *et al.*, 2013; Midzi *et al.*, 2017). Malawi being one of the SSA countries endemic to schistosomiasis along most water bodies (Teesdale and Chitsulo, 1985; Makaula *et al.*, 2014) as well as HIV infection (NSO, 2014), which put most high-risk

populations like fishermen at vulnerable position to MGS and transmitting HIV to their sexual partners.

As described in Chapter 5, our longitudinal cohort study among fishermen along the south shoreline of Lake Malawi in Mangochi district observed a prevalence of 17.1% for UGS and 10.4% for MGS on semen microscopy at baseline. In order to describe further diagnosis of MGS among high-risk men in an endemic area, this research study was set out to assess the application of seminal real-time PCR to determine the prevalence of MGS among local fishermen living along the southern shoreline of Lake Malawi in Mangochi district and monitor the progress of infection after PZQ treatment.

6.3. Objectives of the Study

The specific objectives of the study were:

a. to determine the performance of seminal real-time PCR in diagnosing MGS.
b. to describe the efficacy of PZQ in treating MGS by clearing seminal schistosome eggs and negative real-time PCR.

6.4. Methods

6.4.1. Study area, population and sampling

As described in Chapter 4, this research study recruited fishermen living in fishing villages along the south shoreline of Lake Malawi in Mangochi district (NSO and ICF, 2017) from October 2017 to December 2018. This longitudinal cohort study comprised of baseline and follow-up studies after PZQ treatment in fishing villages and nearby health centres, with a minimum sample size of 275 recruited fishermen aged ≥ 18 years, eligible to participate in the study (Kirkwood and Sterne, 2006; CDC, 2014).

6.4.2. Data collection

The methods of this study data collection as described earlier in Chapter 4, comprised of individual questionnaires administered to recruited fishermen on the shoreline and communities and parasitological analyses of the collected urine and semen samples and transabdominal ultrasonography in the nearby health facilities located in the study area. (WHO, 1991; Ukwandu and Nmorsi, 2004; van Dam *et al.*, 2004; Cheesbrough, 2009). Blood was also collected where plasma was harvested and shipped together with preserved semen at -80°C for further analyses including SEA ELISA using SCHISTO-96 IVD IgG/M microwell kits (New Life Diagnostics LLC, Lot. 1729, IVD Research Inc., USA) and DRG IgG kits (Lot. G062, IQA ref.: 2462/19), point-of-care Prostate Specific Antigen (PSA) using ALL TEST PSA rapid test cassettes (Ref. TPS-402, Lot.: PSA18110014, Hangzhou All Test Biotech Company Ltd, P.R. China) and comparative HIV virological load analyses in the UK.

Semen was collected in a clear, transparent, self-sealing plastic bag for direct microscopy after liquefication and later centrifugation (WHO, 2010), and the results were recorded as per ml of ejaculate. Afterwards, 0.5 ml of ethanol was added to the semen sediment for preservation before shipment to the United Kingdom and Netherlands for real-time polymerase chain reaction (real-time

PCR) of *Schistosoma* genus DNA. Due to the little volume of some semen samples, not all participants who submitted semen underwent real-time PCR.

6.4.3. Real-time PCR for *Schistosoma* DNA

Ethanol was removed from the preserved semen sediments after centrifugation, as described in Chapter 4. The pellet was washed twice with phosphate buffered saline (PBS) before suspending in PBS containing 2% polyvinylpolypyrrolidone (PVPP) (Sigma, Steinheim, Germany). The suspension was then heated and frozen overnight, before DNA extraction using the QIA symphony Sample Processing (SP) system (Qiagen, Hilden, Germany). Phocine Herpes Virus 1 (PhHV-1) was added as an internal control and monitor for inhibition of the real-time PCR, which was performed using primers and probes described previously (Obeng *et al.*, 2008; Kenguele *et al.*, 2014).

6.4.4. Statistical analyses

The data collected from diagnostic, serological and molecular tests during the study was screened and quality-controlled before entry into Microsoft Excel and SSPS programmes. Summary statistics were calculated to explore the data and thereafter correlations and significant tests were conducted to describe and interpret the results further, mainly using nonparametric tests.

6.4.5. Ethical considerations

Ethical clearance to conduct the study was provided by the National Health Sciences Research Committee (NHSRC) of Malawi and Liverpool School of Tropical Medicine (LSTM) Research Ethics Committee (LSTM REC). Utmost privacy and confidentiality were maintained in the study and where necessary, the information was anonymised to protect the identity of the participant. Since this was a test-to-treat study, participants were offered PZQ treatment at the end of the visit before inviting them to the next follow-up study.

6.5. Results

6.5.1. Study population

A total of 376 fishermen were recruited into the study, from 39 villages located in two Traditional Authorities (T/A) of Mponda and Nankumba along the shoreline and had questionnaire interviews. Fifty-six participants had HIV infection and were on anti-retroviral therapy (ART). Out of the total recruited participants, only 210 submitted urine (55.9%) and 114 submitted semen (30.3%). The median age of participants who submitted urine was 30.0 years (Interquartile range [IQR]: 15, range: 18.0 – 70.0) and for semen was 29.0 years (IQR: 15, range: 18.0 – 67.0) (Table 17).

Table 17: Age distribution of the study participants who submitted samples for diagnostic tests

Sample submitted	N	Median	Range	Interquartile Range (IQR)
Urine	**210**	30.0	18 – 70	15.0
Semen	**114**	29.0	18 – 67	15.0

6.5.2. Urine filtration and semen microscopy of the study cohort

Examination of the urine using reagent Siemens multistix® 10 G strips showed most of the urine was observed to be negative for leukocytes (82.4%), blood (72.9%), protein (63.8%) or glucose (Table 18).

Table 18: Proportion of 210 participants who submitted urine according to results of reagent strip

Reagent strip score	Leucocytes	Blood	Protein	Glucose
Negative	173 (82.4%)	153 (72.9%)	134 (63.8%)	210 (100.0%)
Trace	14 (6.7%)	28 (13.3%)	29 (13.8%)	0 (0.0%)
+	11 (5.2%)	10 (4.8%)	34 (16.2%)	0 (0.0%)
++	11 (5.2%)	8 (3.8%)	9 (4.3%)	0 (0.0%)
+++	1 (0.5%)	11 (5.2%)	3 (1.4%)	0 (0.0%)

++++	0 (0.0%)	0 (0.0%)	1 (0.5%)	0 (0.0%)

Urine filtration showed that 36 participants (17.1%) had *S. haematobium* eggs in urine (UGS) (median egg count: 0.9 per 10 ml; range: 0.1-186.0; IQR: 5.4; volume range: 10-240 ml (Table 11). Eight (3.8%) were positive for POC-CCA, possibly intestinal *S. mansoni* schistosomiasis. Twelve participants (10.4%, n=114) had *S. haematobium* eggs in semen (MGS) (median: 2.9 eggs per ml of ejaculate; range: 0.4-30.0; volume range: 0.1-4.5 ml. Eight participants (66.7%) with MGS had no schistosome eggs in urine.

6.5.3. *Schistosoma* serology SEA ELISA

A total of 106 blood plasma samples from all time points were retrospectively analysed with SCHISTO-96 IVD IgG/M kits. Using absorbance cut-off value of 0.20 OD units, 74 participants were positive on ELISA, giving a cohort *Schistosoma* seroprevalence of 69.8%. Further ELISA results are described alongside with real-time PCR results at the specific time-points. Comparing the IVD ELISA results with that of an accreditated clinical laboratory (DRG IgG ELISA) conducting the external quality control, 54.5% of 33 semen samples were positive using IVD ELISA while 48.5% were positive on the DRG ELISA. Using the DRG ELISA as a reference test, the sensitivity of the IVD ELISA was observed to be 81.3% and specificity was 70.6%, witha significant Kappa measurement of agreement (value = 0.516, p = 0.003) and correlation (rho = 0.52, p = 0.002).

6.5.4. Real-time PCR for *Schistosoma* DNA

The real-time PCR was conducted on semen samples collected from all the time-points, as well as on urine samples collected at 6- and 12-months' time-points (Figure 33). Threshold cycle (Ct-value) of less than 45 was considered positive result for schistosomiasis.

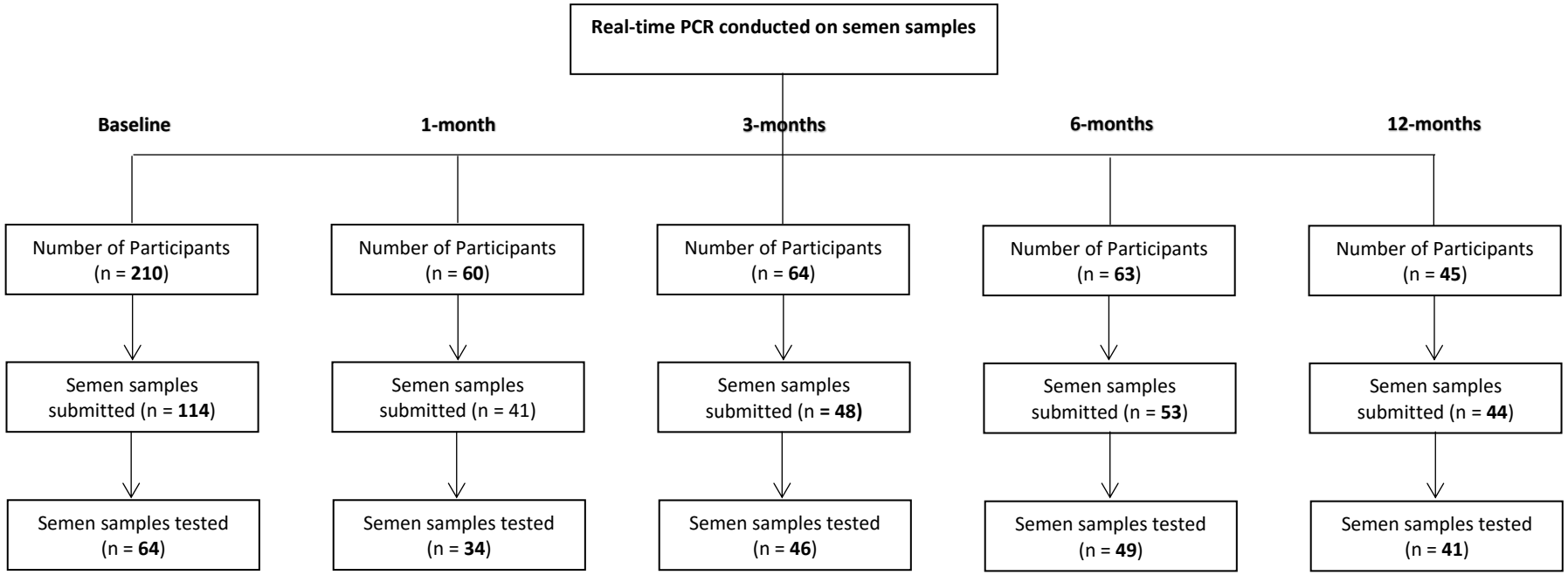


Figure 33: Diagram showing the number of study participants at each time-point, the semen samples submitted and tested using real-time PCR

6.5.4.1 Baseline time-point

Real-time PCR was conducted on only 64 semen samples submitted at baseline, due to limited volume of the samples from some participants. The median age of the participants was 32.5 years (IQR: 22.0, range: 18.0 – 67.0 years), duration of stay was 22.0 years (IQR: 23.8, range: 0.5 – 54.0 years) while their bodyweight was 57.4 kg (IQR: 7.5, range: 43.0 - 75.4 kg). Seventeen participants (26.6%) were positive for real-time PCR, with median Ct-value of 26.5 (IQR: 8.5, range: 18.9 – 37.4). Of those MGS participants, eight had no eggs in semen or urine, six had eggs in semen only, while 27 had eggs in urine only (Figure 34). For the cohort, 25 participants were detected with MGS using semen microscopy and real-time PCR, raising the prevalence of MGS from 10.4% to 21.9% (n = 114).

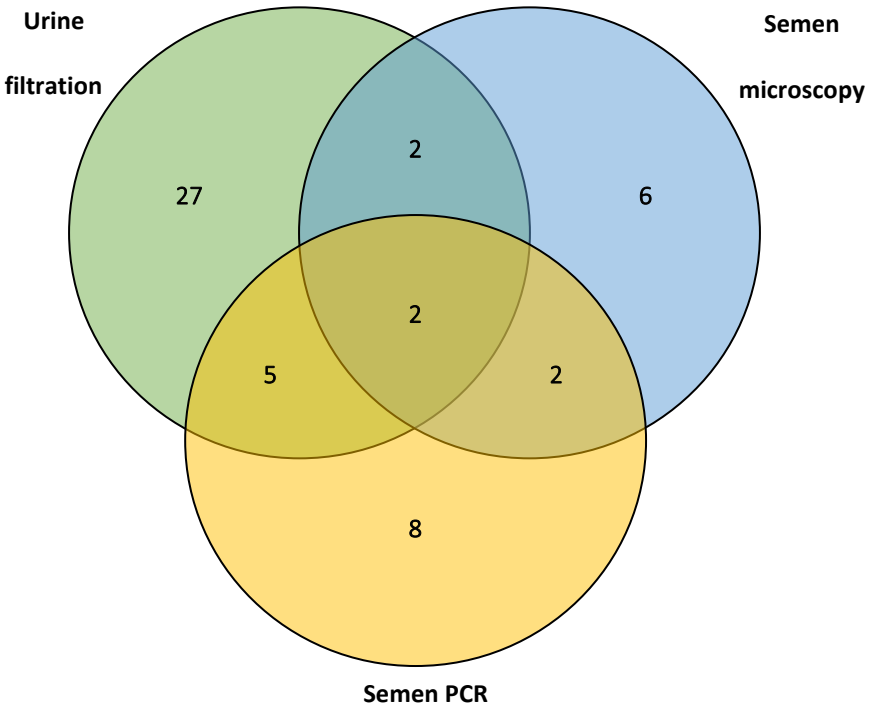


Figure 34: Venn diagram showing positive results of the different diagnostic tests at baseline of the study (n = 52)

Comparing results of all diagnostic tests with age, the proportion of positive semen microscopy and real-time PCR increased with age, compared to ELISA and urine filtration (Figure 35).

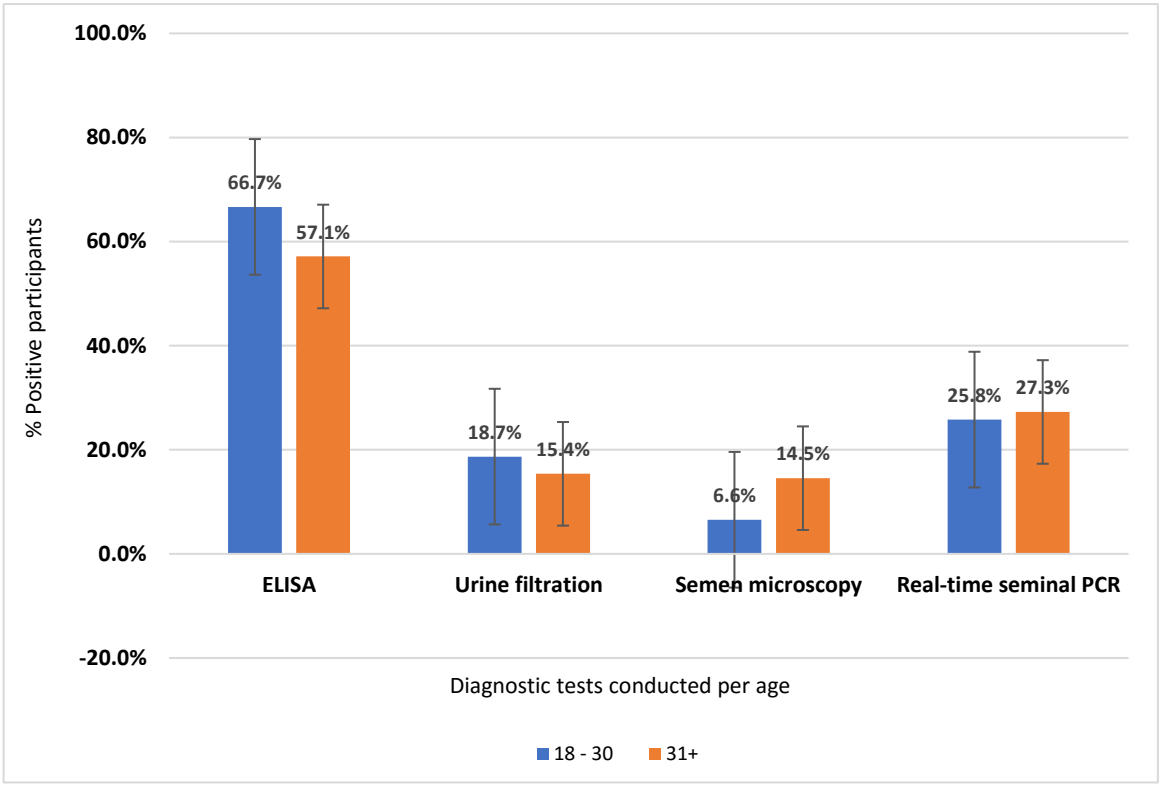


Figure 35: Baseline clustered bar graph of proportion of positive participants on urine filtration, semen microscopy, ELISA and real-time PCR per age group.

(There is no statistically significance difference between the age groups)

6.5.4.2 1-month time-point

Of all the 114 participants who submitted semen at baseline and invited for follow-up, only sixty participants returned and were examined at 1-month follow-up time-point. Real-time PCR conducted was conducted on 34 out of 41 semen samples submitted at this time-point. The median age of the participants was 34.5 years (IQR: 18.0, range: 19.0 – 49.0 years). While none of the 41 participants who submitted semen had schistosome eggs, nine were positive for real-time PCR, showing MGS prevalence of 26.5%, with median Ct-value of 28.7 (IQR: 9.1, range: 23.7 – 37.0). Of the positive real-time PCR participants, only three had eggs in urine only.

Six participants (10.5%) had *S. haematobium* eggs in urine (median: 12.8, IQR: 22.9, range: 0.1 - 29.6) while nine (69.2%) were SEA ELISA seropositive (median: 0.31, IQR: 0.22, range: 0.24 – 0.65) with all except one had no urinary schistosome eggs and negative semen real-time PCR. None

of the participants were positive for POC-CCA test. Looking at all diagnostic tests with age at this time-point, only the proportion of positive real-time PCR increased with age (Figure 36).

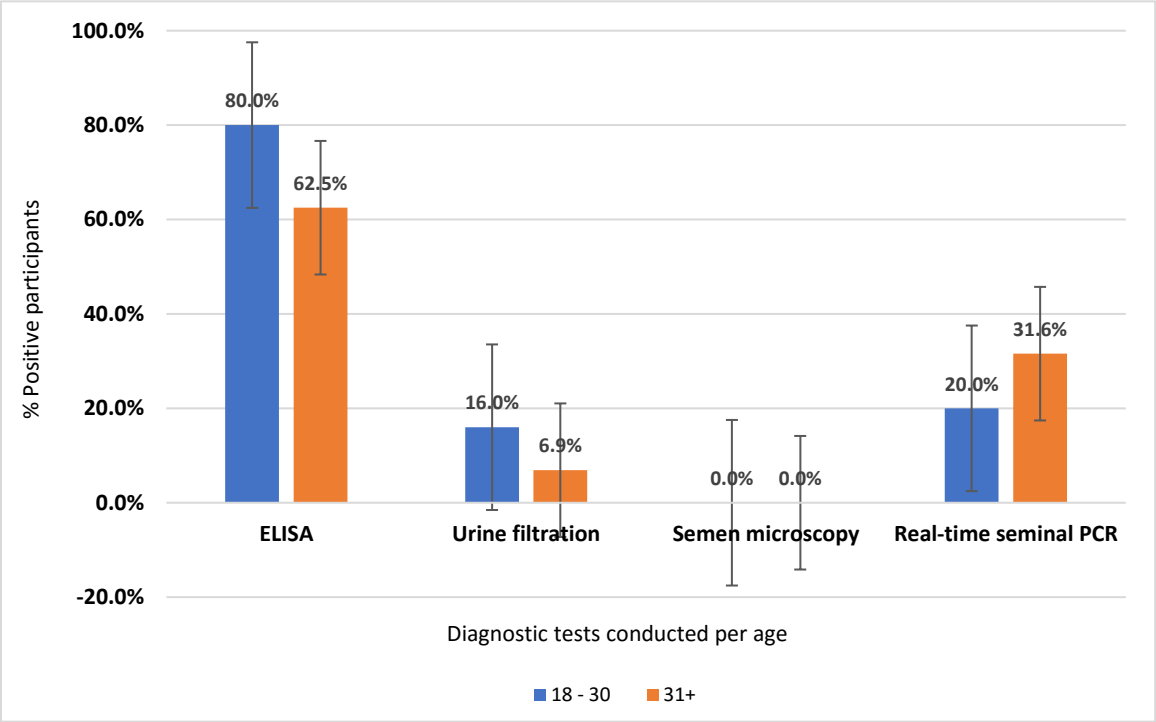


Figure 36: 1-month follow-up clustered bar graph of proportion of positive participants on urine filtration, semen microscopy, ELISA and real-time PCR per age group.

(There is no statistically significance difference between the age groups)

6.5.4.3 3-months' time-point

The real-time PCR was conducted on 46 semen samples submitted from 48 participants, and their median age was 33.0 years (IQR: 21.0, range: 19.0 – 67.0 years). Sixty-four participants were followed at this timepoint. Thirteen participants (28.3%) were positive for real-time PCR, with median Ct-value of 28.3 (IQR: 8.5, range: 22.5 – 36.9). Seven of the positive real-time PCR participants had no schistosome eggs in semen or urine, one had eggs in urine only, two in semen only while four had eggs in both urine and semen (Figure 37). In total, 14 participants were detected with MGS using semen microscopy and real-time PCR, showing the MGS prevalence of 29.2% (n = 48) at this time-point.

Seven participants (14.6%) had *S. haematobium* eggs in urine (median: 9.0, IQR: 19.2, range: 0.3 - 69.0), 5 (10.4%) in semen (median: 2.5, IQR: 7.3, range: 0.8 – 10.0), while 8 (88.9%) were ELISA seropositive (median: 0.3, IQR: 0.42, range: 0.20 – 1.05).

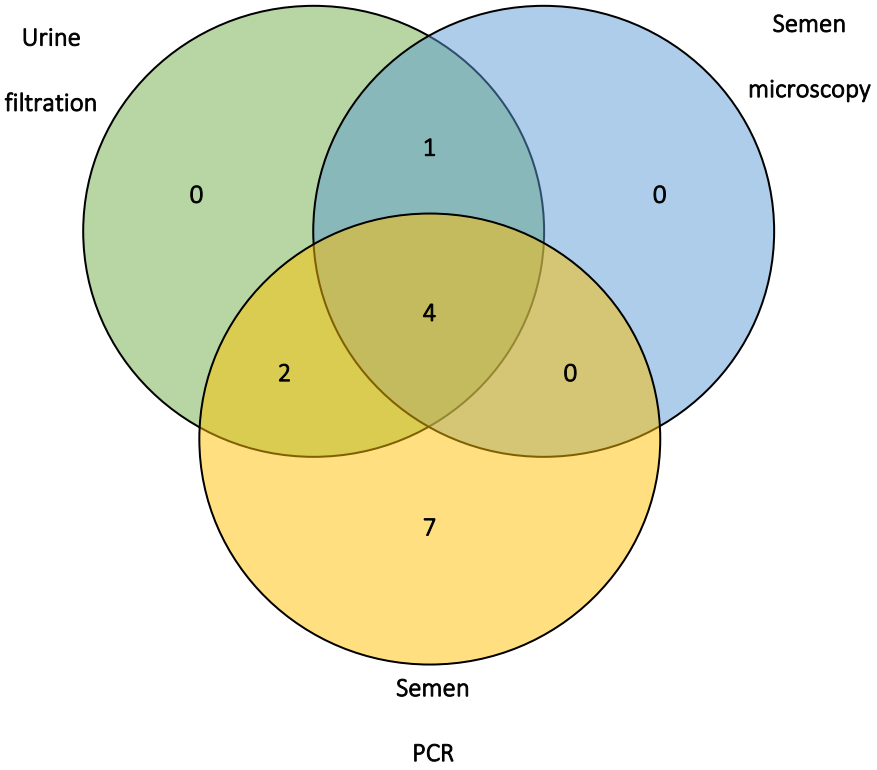


Figure 37: Venn diagram showing positive results of the different diagnostic tests at 3-months follow-up time-point of the study (n = 14)

Comparing the diagnostic tests with age, only proportion of positive participants on urine filtration, semen microscopy and real-time PCR decreased with age, as opposed to ELISA (Figure 38).

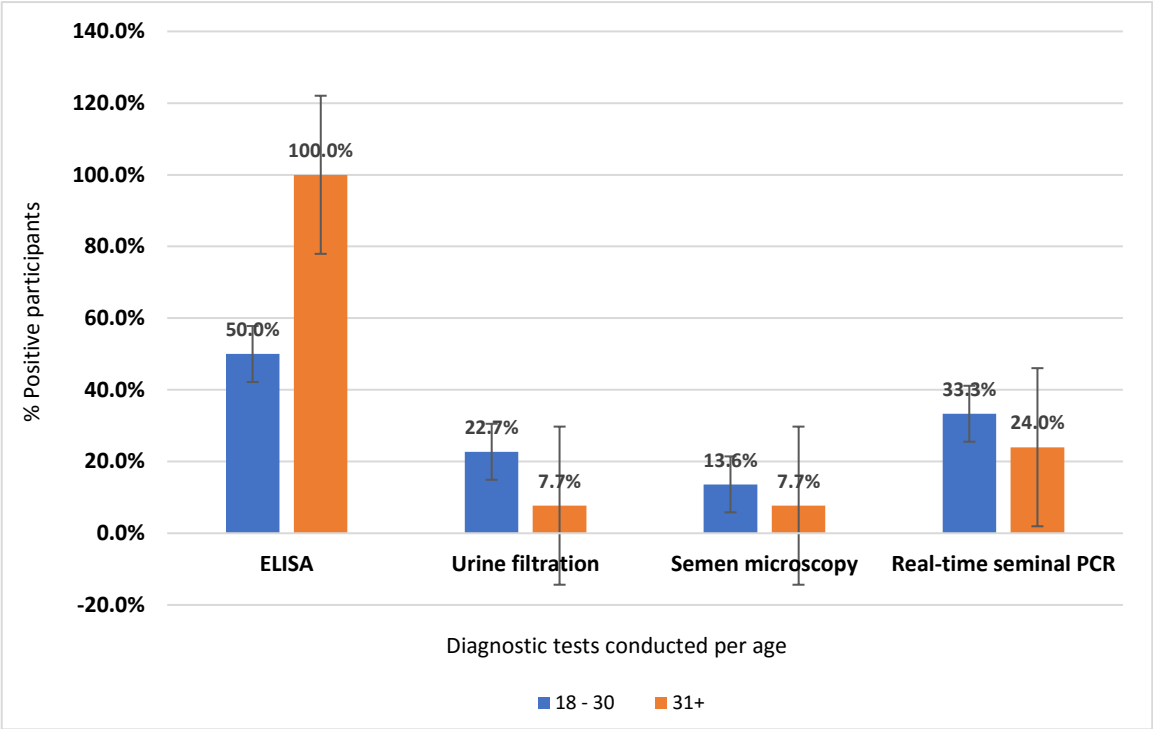


Figure 38: 3-months follow-up clustered bar graph of proportion of positive participants on urine filtration, semen microscopy, ELISA and real-time PCR per age group.

(There is no statistically significance difference between the age groups)

6.5.4.4 6-months' time-point

Semen samples submitted from 49 of the 53 participants at 6-months follow-up timepoint underwent real-time PCR analyses, and the participants' median age was 31.0 years (IQR: 18.0, range: 18.0 – 54.0 years). A total of 63 participants took part in the study at this time-point. Eight participants were positive for real-time PCR (16.3%), with median Ct-value of 31.3 (IQR: 5.0, range: 23.4 – 36.1), and had no schistosome eggs in urine or semen. Ten participants were detected with MGS using semen microscopy and real-time PCR, showing MGS prevalence of 18.9% (n = 53).

Two participants (3.3%) had *S. haematobium* eggs in urine (mean: 1.95, SD: 2.62), 2 (3.8%) in semen (mean: 0.85, SD: 0.78), while 22 (71.0%) were ELISA seropositive (median: 0.37, IQR: 0.46, range: 0.20 – 1.51). Looking at the diagnostics tests used at this time-point, the proportion of positive participants of all the tests increased with age, as opposed to previous time-points, presented earlier (Figure 39).

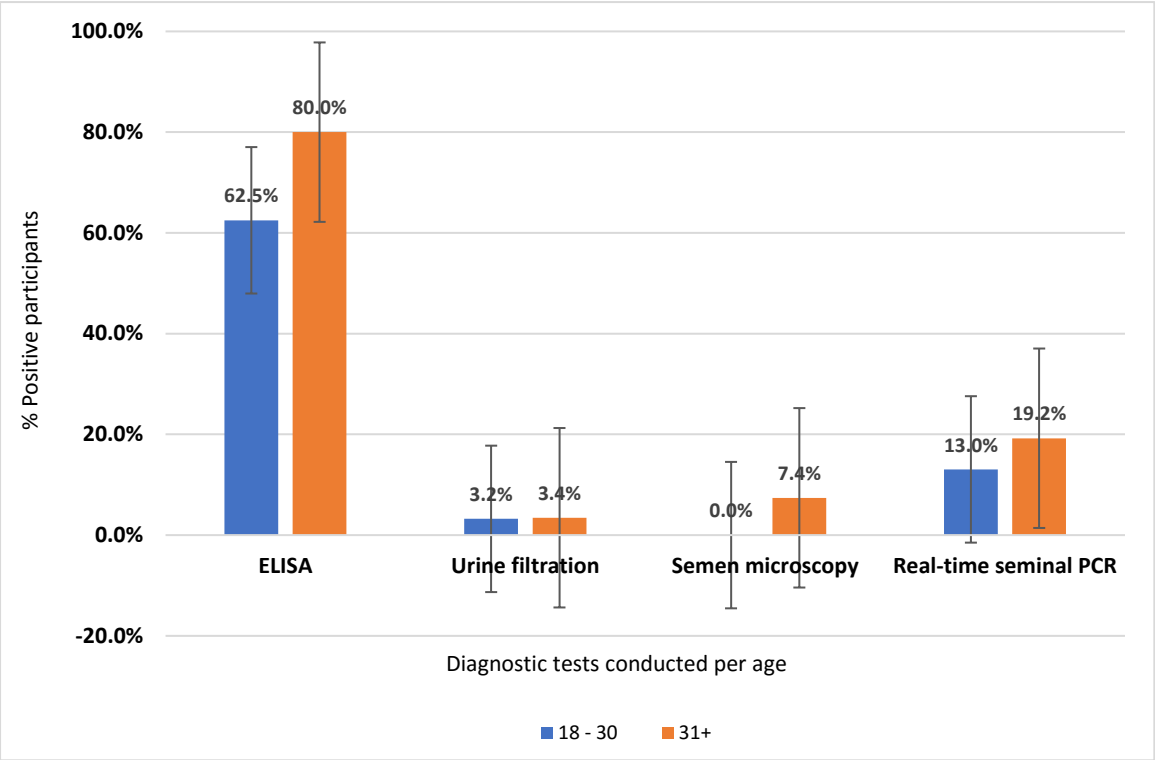


Figure 39: 6-months follow-up clustered bar graph of proportion of positive participants on urine filtration, semen microscopy, ELISA and real-time PCR per age group.

(There is no statistically significance difference between the age groups)

6.5.4.5 12-months' time-point

The real-time PCR was conducted on 41 out of 44 semen samples submitted at 12-month follow-up timepoint, and the median age was 35.0 years (IQR: 21.0, range: 18.0 – 67.0 years). Forty-five participants were examined at this time-point. Twelve participants were positive for real-time PCR, showing MGS prevalence of 29.3%, with median Ct-value of 28.4 (IQR: 14.6, range: 17.6 – 36.6). Of the positive real-time PCR participants, 8 had no schistosome eggs, 2 had eggs in urine only, 1 in stool and another one in semen. In addition, real-time PCR was also conducted on 48 urine and 3 stool samples at this time point, with only 3 (6.3%) urine samples being positive (mean Ct-value: 31.0, SD: 5.43, range: 25.1 – 35.8).

Four participants (8.3%) had *S. haematobium* eggs in urine (median: 3.05, IQR: 2.8, range: 0.8 – 4.1), one (2.3%) in semen (mean: 0.85, SD: 0.78), one (33.3%) in stool (mean: 0.85, SD: 0.78) and 23 (69.7%) were ELISA seropositive (median: 0.41, IQR: 0.20, range: 0.20 – 2.18). Comparing the

results of all diagnostic tests with age, a similar trend to that of 6-months' follow-up was observed with all the proportions of positive participants decreasing with age (Figure 40).

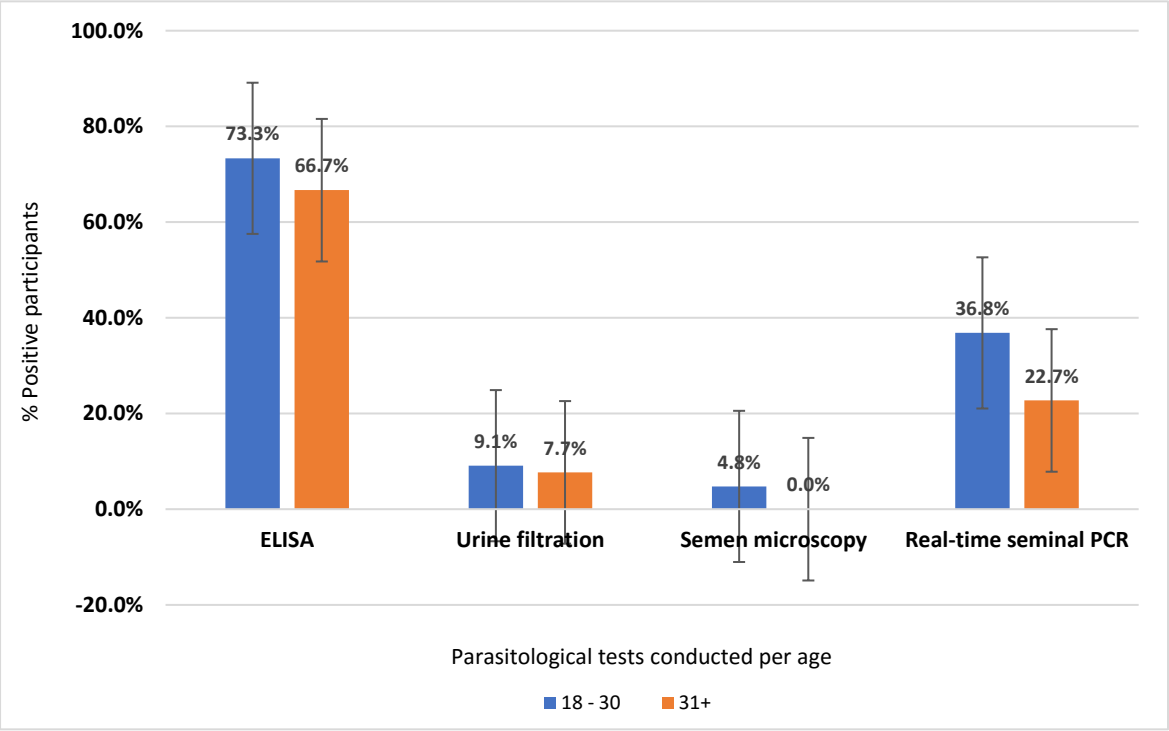


Figure 40: 12-months follow-up clustered bar graph of proportion of positive participants on urine filtration, semen microscopy, ELISA and real-time PCR per age group.

(There is no statistically significance difference between the age groups)

6.5.5. Comparison of the different diagnostic tests

Using semen real-time PCR as reference test, the sensitivities of the other diagnostic tests ranged from 14.3% (semen microscopy) to 76.2% (ELISA) while the specificities ranged from 30.5% (ELISA) to 93.2% (urine filtration) (Table 19). Only urine filtration correlated significantly with real-time PCR, with higher Kappa measure of agreement value and stronger statistical significance than other tests. The sensitivity increased while specificity decreased when any positive results from parasitological and antigen tests were compared with real-time PCR results, demonstrating correlated and statistically significant differences. When results of the diagnostic tests are combined to describe the prevalence of MGS, it shows that prevalence goes up from 10.4% (using semen microscopy only) to 30%, as shown in Figures 41 and 42, using results from the Study baseline.

Table 19: Outcomes of the diagnostic tests compared to semen real-time PCR as reference test

Test	Total (*N*)	Sensitivity (%)	Specificity (%)	Kappa		Spearman Correlation		Chi-squared test	
				value	*p-value*	Coefficient (rho)	*p-value*	value	*p-value*
Urine filtration	**210**	**33.3**	**93.2**	**0.31**	**0.002**	**0.34**	**0.002**	**9.21**	**0.002**
Semen microscopy	114	14.3	91.5	0.07	0.45	0.09	0.45	0.58	0.45
POC-CCA (antigen)	210	52.4	67.9	0.18	0.10	0.19	0.11	2.64	0.10
ELISA (antibody)	106	76.2	30.5	0.04	0.56	0.07	0.57	0.34	0.56
Positive by any parasitological test	210	33.3	84.7	0.20	0.08	0.20	0.08	3.16	0.08
Positive by any parasitological or antigen test	**210**	**71.4**	**59.3**	**0.24**	**0.02**	**0.27**	**0.02**	**5.86**	**0.02**
Positive by any of the four tests	210	95.2	15.3	0.06	0.21	0.14	0.22	1.56	0.21

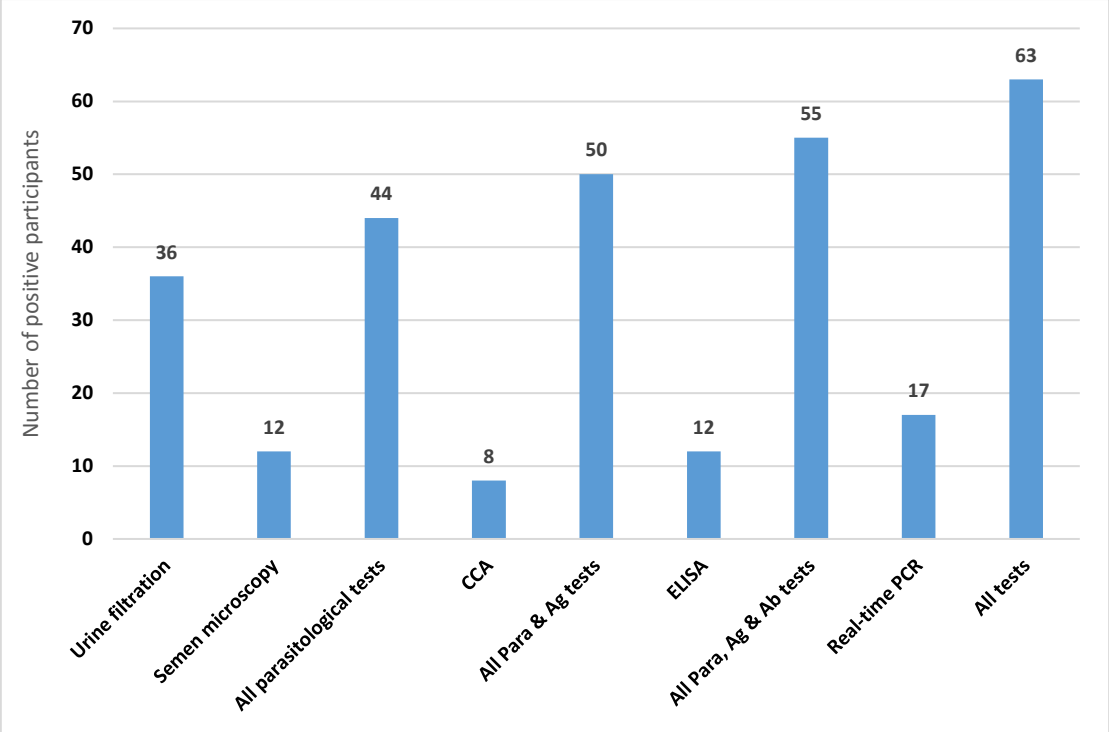


Figure 41: Bar graph of number of positive participants on diagnostic tests at the study baseline

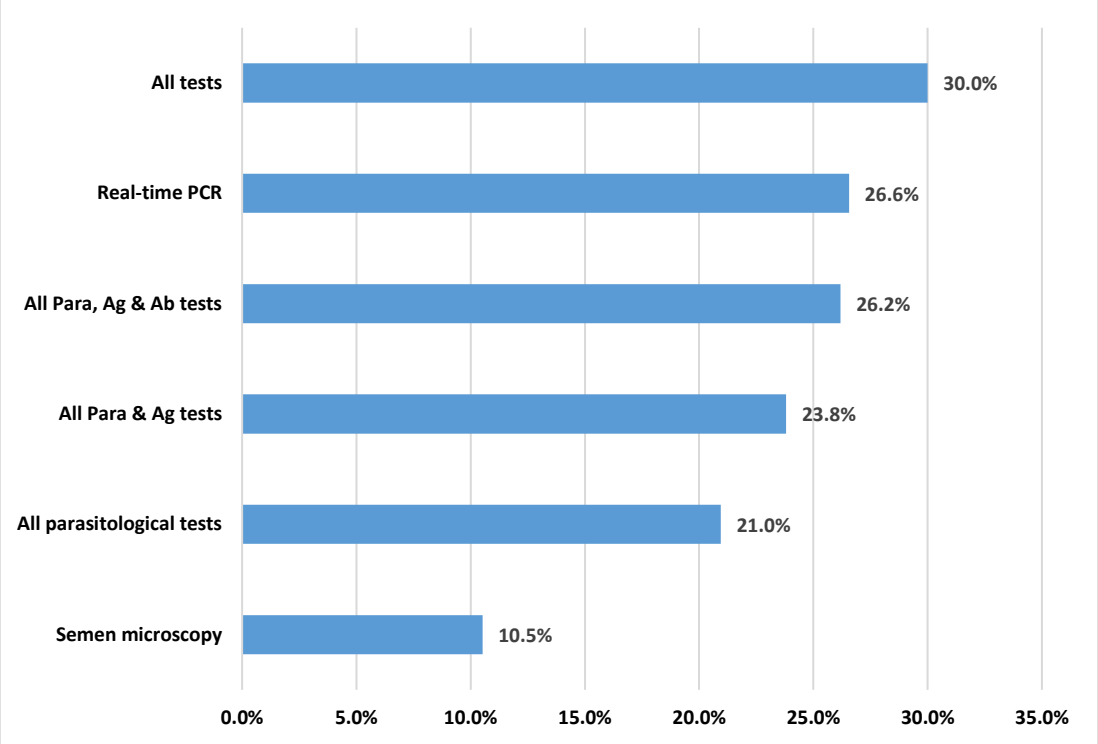


Figure 42: Bar graph of proportion of positive participants on diagnostic tests at the study baseline

6.6. Discussion

Since its first description over a century ago by Madden (Madden, 1911), MGS remains unknown, unrecognised, misdiagnosed and underreported among men in endemic areas such as shores of Lake Malawi. Coupled with the long known poor health-seeking behaviour of men, MGS certainly impacts the male reproductive health negatively, making it an ignored aspect of an NTD and a public health concern in such endemic areas. This longitudinal cohort study among local fishermen along the south shoreline of Lake Malawi was the first original research to investigate the prevalence of MGS in a schistosomiasis-endemic area, following several previous case descriptive reports of travellers or non-dwellers visiting the lake for recreation or business (Schwartz *et al.*, 2002; van Delft *et al.*, 2007; Kayuni *et al.*, 2019a).

6.6.1. Diagnostics for UGS with special focus on MGS among local inhabitants

The diagnosis of UGS includes inexpensive, urine filtration which suffer low sensitivity and specificity and can be lacking in most endemic areas due to the limited resources and inadequate laboratory capacity, overburdened with other important prevalent diseases like malaria, diarrhoea, pneumonia, HIV. Although semen microscopy is considered as a standard diagnostic method for MGS, perceptions and challenges encountered in semen submission and analysis (Price *et al.*, 2005) have resulted in urine filtration being used as a proxy to diagnosing MGS. Both methods suffer from the day-to-day diurnal variations in excretion of eggs into urine and semen, affecting their sensitivity and specificity which requires repeated sample submission and examination on consecutive days in order to improve detection of infected people (Le and Hsieh, 2017).

This MGS study, comprising of fishermen aged between 18 and 70 years old, who spent most of their life on lake shoreline, observed a 17.1% prevalence for UGS and 10.4% for MGS using semen microscopy at baseline (Kayuni *et al.*, 2019b). Also, most MGS participants in the study at baseline (66.7%) did not have schistosome eggs in urine, highlighting the need for more sensitivity and specificity diagnostic tests such as molecular techniques.

6.6.2. Novel findings with real-time PCR diagnostics for MGS

Development of PCR has revolutionised the field of medicine, improving diagnosis of prevalent diseases especially NTDs. Detection of parasitic DNA in specimens from people exposed to infective parasites in endemic areas highly indicate presence of the infection, which demonstrates the high sensitivity and specificity of this diagnostic technique and contribute to control, treatment and prevention. Real-time PCR of schistosomiasis has also demonstrated better diagnostic performance in areas of low transmissions and non-endemic populations (Le and Hsieh, 2017).

This technique has been applied to various active or preserved samples including urine, stool and vaginal secretions to detect *Schistosoma* DNA in comparison traditional standard tests and producing significant results in schistosomiasis diagnosis (Obeng *et al.*, 2008; Aryeetey *et al.*, 2013; Kenguele *et al.*, 2014). However, this molecular technique is quite expensive and not readily available in endemic areas, just like in Malawi. It is worthy to note that our study is the first original research in an endemic area to apply semen real-time PCR to diagnose MGS, detecting 26.6% of participants at baseline, with median Ct-value of 26.5 and increasing the overall prevalence of MGS by 2-fold.

Conducting other diagnostic tests in addition to urine filtration, such as like urine reagent multistix strips (which correlated with MGS in our cohort prevalence study), POC-CCA and ELISA can help in improving the diagnosis of schistosomiasis, identifying other infected people with other *Schistosoma* species (van Dam *et al.*, 2004; Colley, Andros and Campbell, 2017; Le and Hsieh, 2017) and subsequently MGS. Our study demonstrated a significant Kappa agreement of measurement between any positive parasitological or antigen test with semen real-time PCR as a reference test (p = 0.015), correlation (p = 0.015) and statistical difference (p = 0.015). Also, urine filtration showed low sensitivity of 33.3%, high specificity of 93.4%, a significant Kappa agreement of measurement (p = 0.002), correlation (p = 0.002) and statistical difference (p = 0.002). In all, the number of people with schistosomiasis was seen to increase with multiple diagnostic methods, showing a higher prevalence of schistosomiasis than individual method.

At the baseline of the study, eight of the 17 participants who were positive on semen real-time PCR had no eggs in urine or semen which highlights the need for multiple diagnostic platform as well as testing and use of more sensitive tests to detect more cases of MGS. Also, real-time PCR did not pick up all MGS cases as observed in participants with semen eggs but negative on PCR, which could explain an old infection with dead eggs migrating in the genital tissues and then released into ejaculatory ducts and seminal fluid.

Furthermore, looking at the trend of the tests with age, the proportion of MGS participants diagnosed by semen microscopy and real-time PCR increased with age, compared to urine filtration and SEA ELISA. This could be due to MGS developing later in life as a result of repeated exposure to infested water bodies, having re-infections (Madsen *et al.*, 2011; Colley *et al.*, 2014) and presenting as a chronic consequences of schistosomiasis, especially UGS (Kayuni *et al.*, 2019a).

However, there have been reports of MGS at younger age (Rambau *et al.*, 2011; Ekenze *et al.*, 2015), showing since schistosomiasis starts as early as infancy (Poole *et al.*, 2014), MGS can develop even before adolescence, as the children have frequent exposure to infested waters, experience high re-infection rates despite at times being provided PZQ treatment which has good cure rates and reverses early pathologies (Knopp *et al.*, 2013). Of note, children especially in schools are targeted either with individual treatments or through annual mass drug administration (MDA) campaigns by national control programmes including Malawi, which leaves adults out. As part of this study, PZQ was provided during the study and assessed the clearance of MGS among the participants.

6.6.3. Efficacy of standard single-dose PZQ treatment on progression of MGS

Following the MGS participants after PZQ treatment, the study showed that schistosome eggs were cleared in semen 1-month later, despite 26.5% of 34 participants tested with real-time PCR being positive. This indicates presence of *Schistosoma* DNA in infected participants who were not excreting eggs in semen and limiting semen microscopy in MGS at this time-point, supporting

the low sensitivity of microscopy in diagnosing schistosomiasis. Also, comparison of the trend of diagnostics tests with age was similar as a baseline. At 3-months' time-point, schistosome eggs were detected in 10.4% of participants' semen with 28.3% being positive for real-time PCR, increasing the MGS prevalence to 29.2%.

Similarly, at 6- and 12-months' time-points, the prevalence of MGS was 16.7% and 29.3% respectively, demonstrating the possible treatment failure and re-infection of the study participants due their repeated exposure to the infested lake waters where they continued to conduct the income-generating fishing activities. This highlights the need for comprehensive control strategies for schistosomiasis like adequate health education on modify and transform behaviour schistosomiasis (Kloos, 1995), engaging men in water, sanitation and health (WASH) interventions, intermediate snail- host control in addition improving access to treatment in local health facilities and to inclusion and participation in PZQ MDA campaigns (WHO, 2013b), to reduce the prevalence and complications of MGS.

6.6.4. Limitations and recommendations from the MGS cohort study

The low number of participants in submitting semen samples limit the generalisation of the study results to male population in the country and endemic region. This could be explained by negative perceptions and myths associated with semen in rural communities, however previous study examining semen in the district did not encounter such challenges (Kipandula and Lampiao, 2015). In addition, the rumours of blood suckers (vampires) visiting and terrorising the local communities negatively affected the trust and confidence local men had on the study team, thus additional sensitisation and discussions were conducted with local traditional and opinion leaders, health workers and police officers.

Also, some participants could be reluctant to submit samples at the health centres, due to poor health-seeking behaviour. However, previous studies describing MGS had similar or even lower

number of participants submitting semen, hence our results contribute to the current knowledge of MGS in local inhabitants of a schistosomiasis endemic area.

With the study findings, development of more accessible, affordable, point-of-care sensitive and specific diagnostic tests for MGS (Stecher *et al.*, 2015) is strongly recommended since this molecular test remains unavailable and not feasible in endemic areas where currently can immediately benefit from a combination of tests to diagnose MGS among at-risk men. Also, timely provision of individual PZQ treatment and consideration to increase the frequency of PZQ preventive treatments given through MDA campaigns from annually to bi-annually, to prevent re-infections and monitoring of the disease regression.

Furthermore, with the possibility of co-morbidities among local inhabitants of these endemic areas arising from other prevalent NTDs and infectious diseases including HIV, further diagnostics such as HIV viral load analyses are critical in integrated, holistic management of those affected men with dual or multiple infections, as described in chapter 8.

6.7. Conclusion

In conclusion, applying more sensitive and specific semen real-time PCR technique improves diagnosis of MGS, a prevalent, unrecognised gender manifestation of schistosomiasis in men in endemic areas like Lake Malawi shoreline, whose prevalence rose by 2-fold. It was also observed that PZQ treatment was able to acutely clear schistosome eggs in semen, although possible re-exposure and re-infection increased the prevalence again at longer follow-up time-points.

Chapter 7: Morbidity of MGS described by ultrasonography

7.1. Summary

This chapter described the genital abnormalities observed on transabdominal and scrotal ultrasonography conducted on study participants, describing morbidity associated with male genital schistosomiasis (MGS), in reference to the different parasitological and molecular tests.

This study observed that nine (6.9%) of those 130 participants (out of 376 participants) had abnormalities in prostate, seminal vesicles, testis and testis at baseline. Four of these participants were negative on all parasitological and molecular schistosome tests. Over the follow-up time points, some of the abnormalities resolved, other persistent while new abnormalities appeared. In total, 146 participants were scanned during the entire cohort study, and abnormalities were observed in 16 participants at various time-points.

Some of the results from the ultrasonography have been published as case reports in two journals, as cited below:

Kayuni S.A., Corstjens P.L., LaCourse E.J., Bartlett K.E., Fawcett J., Shaw A., Makaula P., Lampiao F., Juziwelo L., de Dood C.J., Hoekstra P.T., Verweij J.J., Leutscher P.D.C., van Dam G.J., van Lieshout L. and Stothard J.R. (2009). **How can schistosome circulating antigen assays be best applied for diagnosing male genital schistosomiasis (MGS): an appraisal using exemplar MGS cases from a longitudinal cohort study among fishermen on the south shoreline of Lake Malawi.** *Parasitology*, Volume 146, Issue 14, pages 1785-95, https://doi.org/10.1017/S0031182019000969.

Kayuni, S. A., LaCourse, E. J., Makaula, P., Lampiao, F., Juziwelo, L., Fawcett, J., Shaw, A., Alharbi, M., Verweij, J. J. and Stothard, J. R. (2019). **Case Report: Highlighting Male Genital Schistosomiasis (MGS) in Fishermen from the Southwestern Shoreline of Lake Malawi, Mangochi District.** *The American Journal of Tropical Medicine and Hygiene*, 101(6), pages 1331 – 1335, doi: https://doi.org/10.4269/ajtmh.19-0562.

My contribution to these manuscripts were that I conducted the literature review on MGS, research fieldwork along south shoreline of Lake Malawi, wrote first drafts of the manuscript and made all the changes suggested by the co-authors and journal referees.

7.2. Introduction

Schistosomiasis is prevalent in SSA where more than 90% of infected people live, causing considerable morbidity and some deaths (McManus *et al.*, 2018; WHO, 2018b). The pathological lesions of schistosomiasis give characteristic manifestations which can be detected by non-invasive radiological techniques such as ultrasonography which are becoming widely available and successfully utilised in endemic areas (WHO, 2011).

These techniques are important, safe, effective and valuable diagnostic methods in tropical diseases, which are useful specially the control of NTDs (WHO, 2000; Ramarakoto *et al.*, 2008). Ultrasonography is acceptable method which has undergone improvements producing affordable, portable instruments. Such equipment can be easily moved from one health facility to another in endemic areas, thereby supporting the semen microscopy and other available point-of-care diagnostics tests for schistosomiasis.

Transrectal ultrasonography (TRUS), computed tomography (CT) and magnetic resonance imaging (MRI) have been noted to be very important and useful in schistosomiasis (Shebel *et al.*, 2012). These methods detect hypoechogenic and hyperechogenic lesions concomitant with granulomas and calcifications, determine the sizes of enlarged organs, as well as demonstrating calcified schistosome ova in the tissues (Fender, Hamdy and Neal, 1996; Vilana *et al.*, 1997; Al-Saeed *et al.*, 2003; de Cassio Saito *et al.*, 2004).

Such pathologies have been observed to be correlated with clinical features specific to MGS and subsequently they are noted to resolve more especially in earlier stages compared to irreversible, late presentation. With the abundant evidence of ultrasound validity of renal tract and liver, Niamey ultrasonography guidelines have been developed by radiologists which clearly defined the pathological lesions associated with urogenital schistosomiasis (UGS) as well as intestinal schistosomiasis (WHO, 2000). Although ultrasonography can be used as a surveillance method in MGS, currently there's limited evidence in literature from few case reports of scrotal/lower abdominal studies in travellers describing MGS and impact of PZQ in an endemic setting, which

necessitate for further prospective studies to validate the use of ultrasonography in MGS and understand the evolution and resolution of the associated genital pathologies.

As part of our novel longitudinal cohort study on MGS among fishermen in south-western shoreline of Lake Malawi, known to harbour both urogenital and intestinal schistosome species (Makaula *et al.*, 2014; Alharbi *et al.*, 2019), a ultrasonographical study was conducted to determine the morbidity associated with MGS.

7.3. Objectives of the research

The specific objectives of the study were:

a. to document the morbidity associated with MGS using ultrasonography making specific reference to genital organ abnormalities.

b. to determine by ultrasonography if, after PZQ treatment(s), there was morbidity resolution of encountered cases through time.

7.4. Methods

7.4.1. Study area, population and sampling

The research aspect of this investigation by ultrasonography was embedded within the larger longitudinal cohort study (see Chapter 4) as conducted among fishermen living in fishing villages identified and selected along the south shoreline of Lake Malawi in Mangochi District (NSO and ICF, 2017), from October 2017 to December 2018. The study comprised of baseline and follow-up studies after PZQ treatment in fishing villages and nearby health centres.

7.4.2. Data collection

The study methods utilised for the data collection comprising individual questionnaires, parasitological analyses of urine and semen samples collected in health facilities, and blood collection. Further serological and molecular analyses for diagnosing schistosomiasis were conducted in the United Kingdom. In addition, all participants were invited to undergo transabdominal pelvic and scrotal ultrasonography examination (Figure 43) to assess morbidity features of MGS in the prostate, seminal vesicles, testes and epididymis. More description of the ultrasonography procedure has been outlined in Chapter 4.

Figure 43: Conducting transabdominal ultrasonography on a participant at a health facility.

Photo credit: Dr Sekeleghe Kayuni, June 2018.

7.4.3. Statistical analyses

The data collected from video clips, digital images and report forms comprising from symmetry, thickness, echogenicity, calcifications, nodules, polyps, masses and hydroceles, were entered into IBM SSPS programmes in accordance to evidence-based recommendations and guidelines on specific ultrasonography after quality control by a specialist radiologist (Vilana *et al.*, 1997; WHO, 2000; Martino *et al.*, 2014). Summary statistics were calculated to explore the data and thereafter correlations and significant tests were conducted to describe and interpret the results further, mainly using nonparametric tests.

7.4.4. Ethical considerations

Ethical clearance to conduct the study was provided by the National Health Sciences Research Committee (NHSRC) of Malawi and Liverpool School of Tropical Medicine (LSTM) Research Ethics Committee (LSTM REC). Utmost privacy and confidentiality were maintained in the study and

where necessary, the information was anonymised to protect the identity of the participant. Since this was a test-and-treat study, participants were notified of the ultrasonography results including pathological findings at the end of the procedure and where necessary, further appropriate investigations and management were organised in accordance with standard clinical practice. Treatment with PZQ at 40 mg/kg as a single dose was offered before inviting them to the next follow-up studies at 1-month, 3-, 6- and 12-months' time-points. Details of observed treatment were recorded when subsequent follow-ups were performed.

7.5. Results

Out of 376 fishermen recruited into the study, only 130 participants returned to the health facility and for the ultrasonography examinations at baseline of the study.

7.5.1. Demographic information and diagnostic results

The median age of the 130 scanned participants was 32.0 years with a range of 19.0 to 70.0 years (Interquartile range [IQR]: 18) and their duration of stay in the fishing village ranged from 2 months to 70 years (median: 22.0; IQR: 24.5; Table 20). The median weight of the participants was 59.0 kg (IQR: 9.0, range: 43.0 – 75.4 kg).

All participants except one submitted urine and 81 submitted semen (62.3%). Examination of urine visually for macrohaematuria using colour-score card revealed that most of the urine was clear in appearance (97.7%) while few samples were cloudy (2.3%). Further examination of the urine using reagent Siemens strips showed that more participants were negative for leukocytes (80.6%), blood (67.4%) and protein (65.1%; Table 21). None of the urine was positive for glucose (no glycosuria), suggestive of no possibility of diabetes mellitus, metabolic and renal diseases.

After urine filtration, 27 participants (20.9%) had *S. haematobium eggs* in urine (UGS), their mean egg count was 19.1 eggs per 10 ml and ranging from 0.1 to 186.0 eggs (median: 1.0, IQR: 5.8). Six participants (4.9%) had a positive POC-CCA test, suggestive of possible intestinal *S. mansoni* infection. For the 81 participants who submitted semen, 10 (12.3%) had *S. haematobium* eggs in

semen (MGS), mean egg count was 3.9 per ml of ejaculate (median: 2.9 eggs), ranging from 0.4 to 9.3 eggs and volume of semen ranged from 0.1 to 4.5 mL (mean: 1.6 ml). The real-time PCR conducted on 57 semen samples revealed that 16 participants (28.1%) were positive. Four participants were positive on both semen microscopy and real-time PCR.

Table 20: Demographical information, diagnostic analyses on urine and semen with ultrasonography results of the 130 study participants

Variable	*N*	Median	Range	Interquartile Range (IQR)
Age	130	32.0	19.0 – 70.0	18.0
Duration of stay in village (years)	120	22.0	0.2 – 70.0	24.5
Weight (kgs)	115	59.0	43.0 – 75.4	9.0
Eggs in urine (filtration, 10 ml) *	129	1.0	0.1 – 186.0	5.8
Eggs in semen (ml)*	80	2.9	0.4 – 9.3	4.6
Seminal real-time PCR (Ct-value)	57	26.4	18.9 – 36.6	10.5
Prostate size (ml)	122	13.4	5.4 – 61.3	5.4

Table 21: Proportion of 130 participants who submitted urine according to results of reagent strip

Reagent strip score	Leucocytes	Blood	Protein
Negative	104 (80.6%)	87 (67.4%)	84 (65.1%)
Trace	7 (5.4%)	18 (14.0%)	21 (16.3%)
+	6 (4.7%)	8 (6.2%)	16 (12.4%)
++	11 (8.5%)	7 (5.4%)	8 (6.2%)
+++	1 (0.8%)	9 (7.0%)	0 (0.0%)

7.5.2. Baseline results of the ultrasonography exanimations

Of the participants who had ultrasonography, 10 (7.7%) had one or more abnormalities in genitourinary (GU) organs, with 9 (6.9%) having abnormalities in prostate, seminal vesicles and/or scrotum (testis and epididymis) (Table 22).

Table 22: Proportion of abnormal findings in particular organs at baseline

Organ	Total scans	Number of Abnormal scans	Percentage (%)
Urinary Bladder	106	2	1.9%
Kidneys*	4	1	25.0%
Prostate	126	3	2.4%
Seminal vesicles	117	1	0.9%
Testis[#]	129	1	0.8%
Epididymis[‡]	129	1	0.8%
Scrotum[α]	129	6	4.7%

**Kidneys scanned for those who had severe bladder abnormalities; [#]Left testis; [‡]Right epididymis; [α]Hydroceles were observed in scrotums of five participants*

Three participants (3.1%) were observed to have abnormalities in at least two GU organs, and two had MGS, confirmed by parasitological and/or molecular tests, as shown in Table 23 below.

Table 23: Abnormalities observed in participants with at least 2 GU organs affected at Baseline

Participant	Age (years)	Eggs in urine (per 10 ml)	Eggs in semen (per ml)	Real-time PCR (Ct-value)	Abnormalities observed
1	19	0	0	N/D[#]	Irregular bladder wall and severe polypoid thickness, with bilateral hydronephrosis
2	22	0	0	25.4	Irregular bladder wall with severe flattened thickness, irregular prostate with hyperechoic nodule (Figure 44)
3	69	0	N/A[‡]	N/A[‡]	Severely enlarged prostate (volume = 61.3 ml) and right epididymis, with bilateral hydrocele

[#] Test not done, inadequate sample; [‡] Sample not submitted, test not done

Eighteen of all the scanned participants had UGS only, confirmed by *S. haematobium eggs* observed in urine, 15 had MGS only (six had semen eggs only while 9 were positive for real-time PCR) and only two participants were positive for all three diagnostic tests (Figure 45). Only 4 participants of the 10 with abnormalities had MGS by semen microscopy and real-time PCR, two participants had schistosome eggs in urine and semen and positive real-time PCR, while none was positive for POC-CCA. In all, 18 (13.8%) of the scanned participants could be considered to have MGS at baseline, by being positive on semen microscopy, real-time PCR or pathological abnormality on ultrasonography in genital organs.

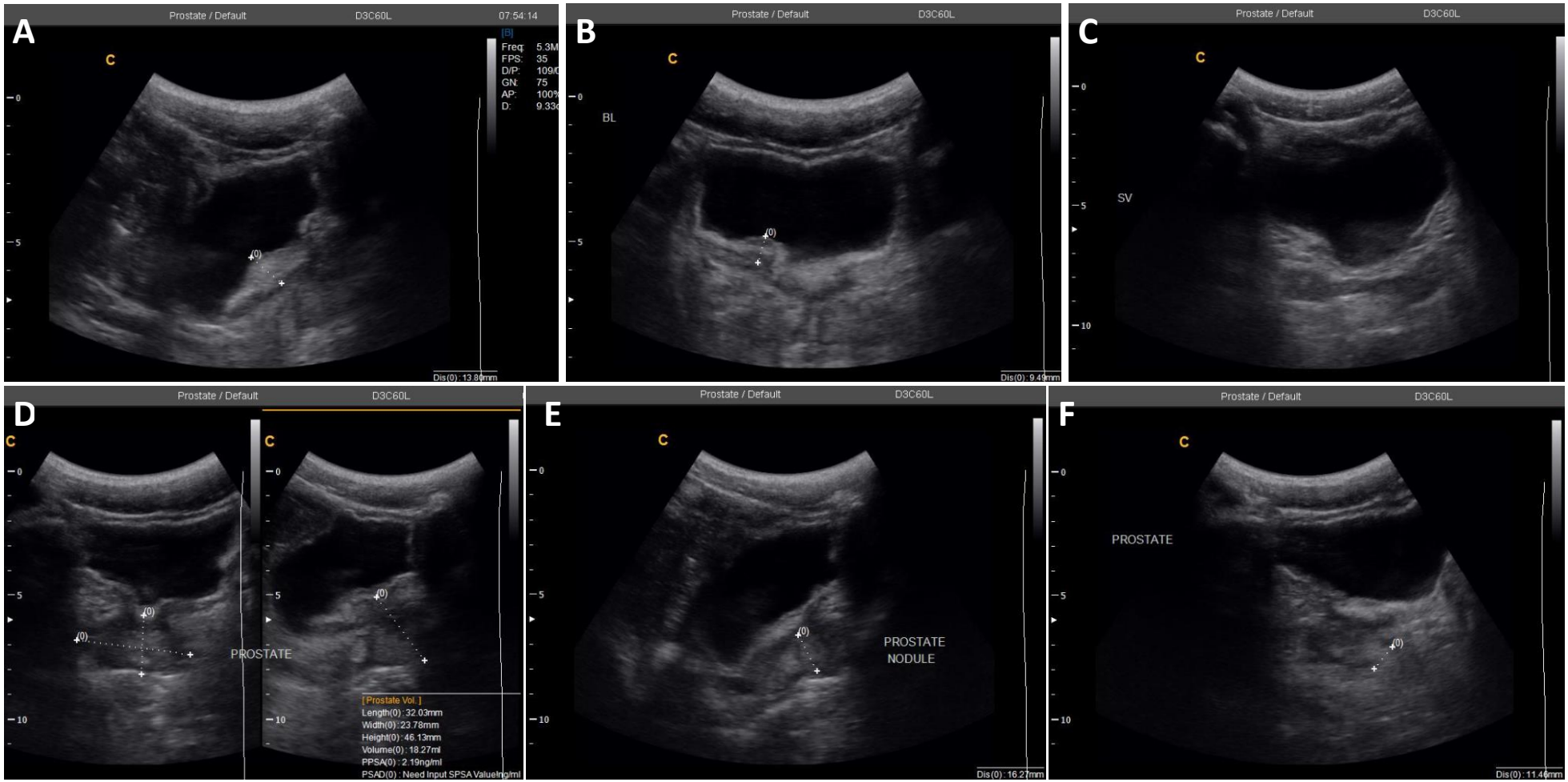


Figure 44: Ultrasonographic images of an MGS positive study participant with abnormalities in the bladder and prostate at Baseline

A and **B.** Irregular urinary bladder wall and severe flat thickness, measuring up to 13.6 mm. **C.** Normal symmetrical seminal vesicles. **D.** Prostate with abnormal irregular outline, but normal volume size of 18.3 ml. **E** and **F.** Hyperechoic nodule in the prostate, measuring 11.4 mm by 16.2 mm

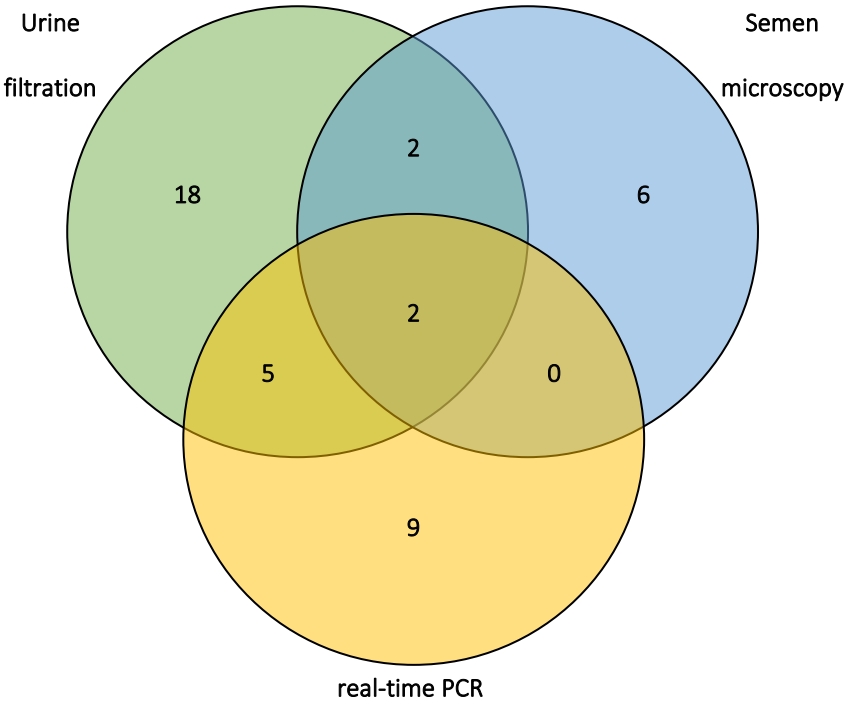


Figure 45: Venn diagram showing positive diagnostic tests on all scanned participants at baseline

7.5.2.1. Urinary bladder and Kidneys

Two participants had irregular outline of their urinary bladder with severe wall thickness of at least 11 mm (20% of total abnormal scans (AS); 1.9% of scanned bladders (SC)). One participant (number 1, Table 23) was negative for urine filtration and semen microscopy, whose ultrasonography of the kidneys revealed bilateral hydronephrosis. The other participant (number 2, Table 23) had MGS, by real-time PCR.

7.5.2.2. Prostate

Three participants (2.3%) had abnormal prostate appearance, two aged 51 and 69 years old, had enlarged prostates with volumes of 39.1 ml and 61.3 ml, while one, aged 22 years, had irregular prostate outline and hyperechoic nodule, (Figure 44), among other severe bladder abnormalities and positive real-time PCR. None had schistosome eggs in semen and point-of-care prostate specific antigen (POC-PSA) described in chapter 4, was negative (Figure 46).

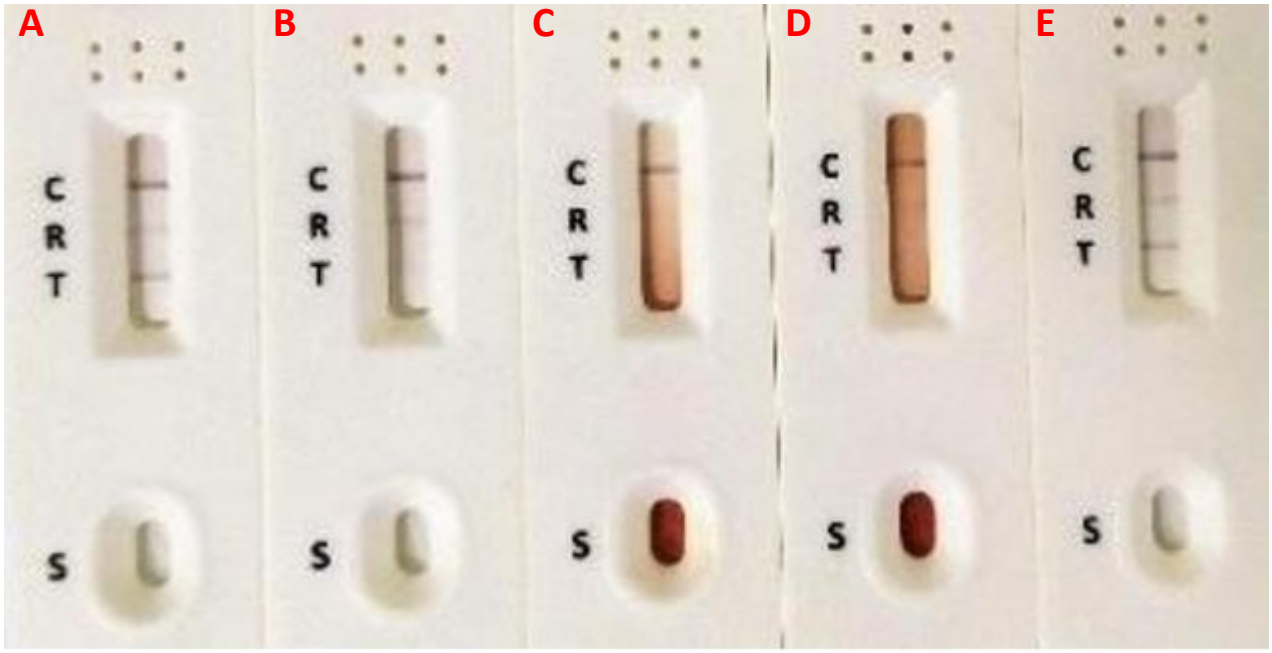


Figure 46: Images of the POC-PSA test conducted on participants with Prostate abnormalities

A and **E.** Strong positive POC-PSA with no urine or semen eggs and no abnormalities. **B.** Negative POC-PSA with semen real-time PCR (Ct-value: 25.4), irregular prostate outline and hyperechoic nodule. **C.** Negative POC-PSA with no urine eggs, but had grossly irregular, enlarged prostate (61.3 ml), abnormal epididymis and bilateral hydrocele. **D.** Negative POC-PSA with no urine or semen eggs, negative real-time PCR but had irregular, enlarged prostate (39.1 ml).

7.5.2.3.Seminal vesicles

Although three participants (8.5%) had enlarged seminal vesicles, measuring at least 15 mm, only one had asymmetrical, hyperechoic vesicles, aged 24 years with *S. haematobium* eggs in urine and semen, as well as positive real-time PCR. The measurements for the scanned participants' right vesicles ranged from 4.8 mm to 18.5 mm and the left vesicles from 3.7 mm to 17.5 mm.

7.5.2.4.Scrotum

Abnormalities observed on scrotal ultrasonography were particularly in testis, epididymis and coverings. One participant (0.8%) had left testicular nodule, with *S. haematobium* eggs detected in urine and semen (Figure 47). Only one participant was observed to have an abnormal enlarged right epididymis, among other abnormalities described above and had no had *S. haematobium* eggs in urine (participant 3, Table 23).

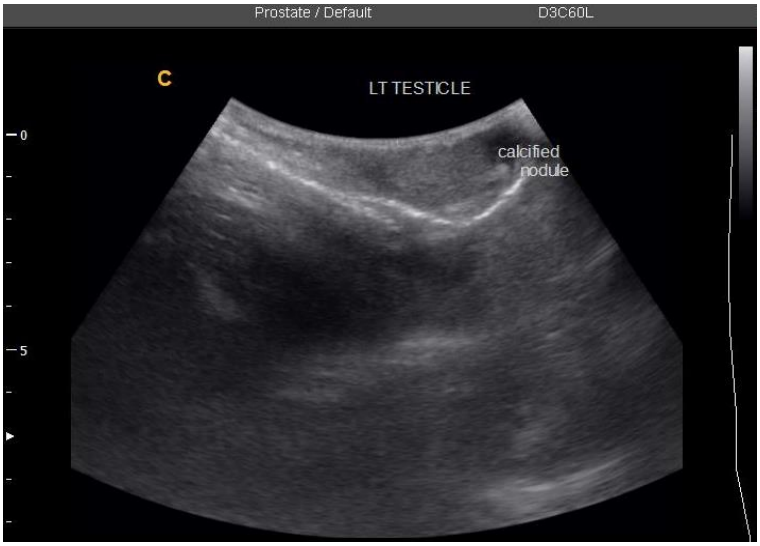


Figure 47: Ultrasonographic image of study participant with left testicular nodule at baseline.

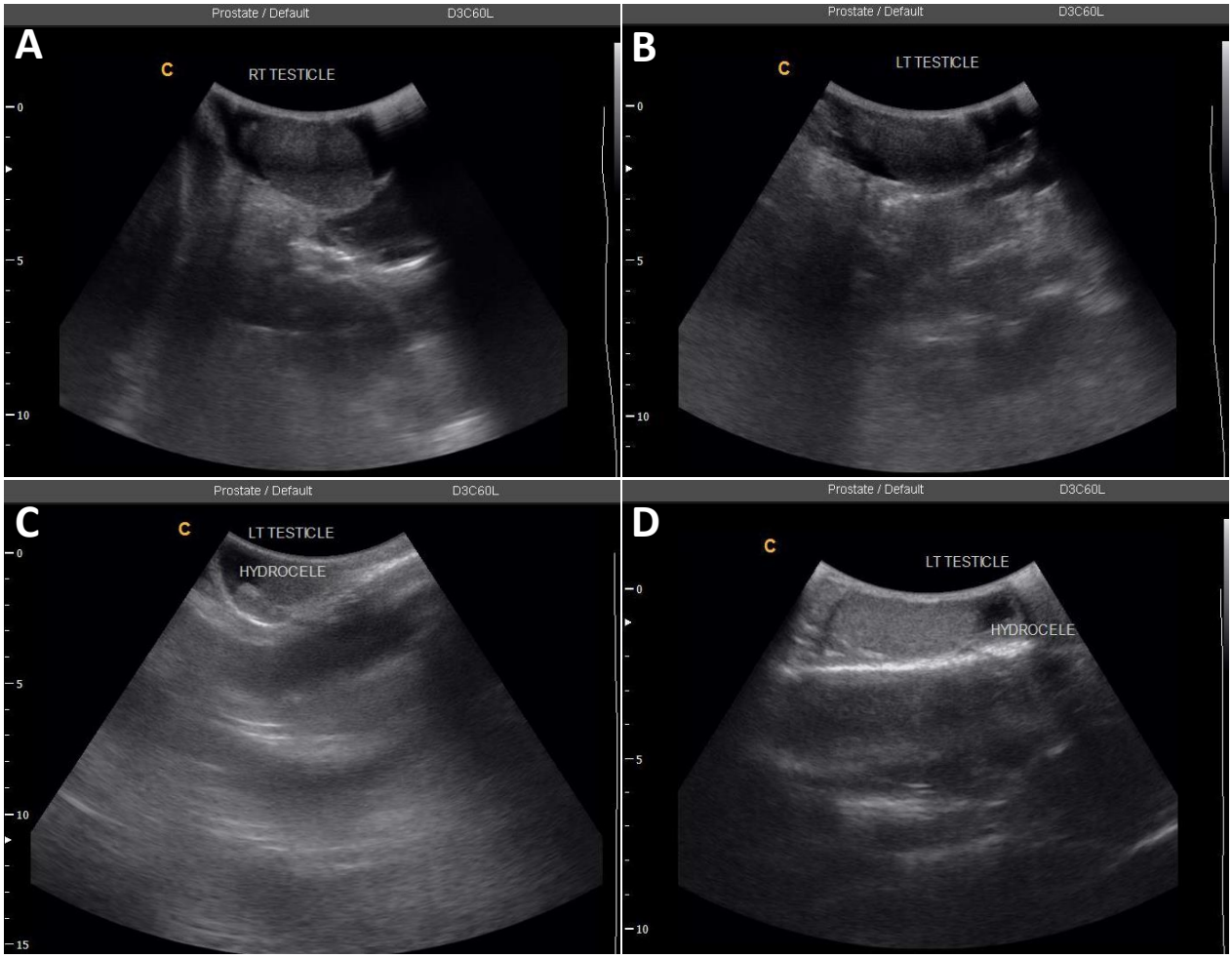


Figure 48: Ultrasonographic images of participants with hydroceles at baseline in Table 23.
A and **B** Bilateral hydroceles of participant C showing larger amount of scrotal fluid, compared to moderate left hydroceles, **C** of participant A and **D** of participant B.

Six participants (4.7%) were observed to have hydroceles of which three had bilateral hydroceles (Figure 48). Only one participant with left hydrocele was positive for all the diagnostic tests (participant B, Table 24).

Table 24: Study participants with Hydroceles on Transscrotal ultrasonography at Baseline

Participant	Age (years)	Location of Hydrocele	Eggs in urine (per 10 ml)	Eggs in semen (per ml)	Real-time PCR (Ct-value)
A	29	Left	0.0	N/A[ǂ]	N/A[ǂ]
B	30	Left	37.3	2.0	25.7
C	49	Bilateral	0.1	0.0	45.0*
D	49	Bilateral	1.0	0.8	N/D[#]
E	54	Bilateral	0.0	0.0	45.0*
F	69	Bilateral	0.0	N/A[ǂ]	N/A[ǂ]

** Ct-value of 45.0 is Negative; [ǂ] Sample not submitted; [#] Test not done, inadequate sample*

In context with all the abnormalities described above, there was no correlation between age, duration of stay, diagnostic tests' results and abnormalities.

7.5.3. Follow-up ultrasonography exanimations

At the end of the ultrasonography examinations at baseline, PZQ treatment was provided to the participants on exit of the study after submitting semen sample for resolution of the abnormalities. The participants were invited to follow-up ultrasonography examinations at 1-, 3-, 6- and 12 months' time-points.

7.5.3.1. 1-month follow-up

Only 29 participants were scanned out of the 60 participants who returned at 1-month follow-up, with 4 participants scanned for the first time and no abnormalities observed. Two of the repeat participants had *S. haematobium* eggs in urine, three were positive on semen real-time PCR

while none had *S. haematobium* eggs in semen. One of the repeat participants had abnormalities at this time point, who had no abnormalities at baseline.

Three participants had hydroceles, with two being bilateral and were also present at baseline, namely participant II in Table 25 (participant C in Table 24) and participant III (participant D in Table 24), showing persistent abnormalities despite PZQ treatment (Table 26). Two of the participants had MGS, by semen real-time PCR.

Table 25: Study participants with hydroceles on ultrasonography at 1-month follow-up

Participant	Age (years)	Eggs in urine (per 10 ml)	Eggs in semen (per ml)	Real-time PCR (Ct-value)	Location of Hydrocele
I	44	23.1	0.0	23.7	Left
II	49	0.0	0.0	25.0	Bilateral
III	49	0.0	0.0	45.0*	Bilateral

** Ct-value of 45.0 is Negative*

Table 26: Observations on ultrasonography of 2 participants at baseline and 1-month follow-up

Age (years)	Baseline		1-month Follow-up	
	Test results	Abnormalities observed	Test results	Abnormalities observed
49	Eggs in urine, none in semen; negative PCR	Bilateral hydrocele	Eggs in urine, semen; positive real-time PCR	Bilateral hydroceles
49	Eggs in urine, semen; no real-time PCR done	Left testicular nodule, mild bilateral hydroceles	No eggs in urine or semen; negative PCR	Bilateral hydroceles

7.5.3.2. 3-months follow-up

Sixty-four participants were followed up at 3-months' time-point of which 32 had ultrasonography examinations, and 4 were scanned for the first time. On diagnostic examinations, 5 had *S. haematobium* eggs in urine (17.2%), 4 in semen (13.8%), 4 had trace POC-CCA test while 5 had positive semen real-time PCR. Pathological abnormalities were observed in three participants (28.1%). One participant had abnormalities for the first time in the study, while 2 participants remained with abnormalities from the previous 1-month time point (Table 27).

Table 27: Abnormalities observed at 3 time points in 2 scanned participants

Participant	Age (years)	Baseline	1-month follow-up	3-month follow-up
G	44	No abnormality	Left hydrocele	Left hydroceles
H[β]	49	Bilateral hydroceles	Bilateral hydroceles	Bilateral hydroceles

[β] *This is participant C in Table 24 and II in Table 25*

Of the 3 participants with abnormalities at this time point, two had left hydroceles while one had bilateral hydroceles. One of the participants with left hydroceles was positive on the all diagnostic tests (participant G in Table 27, same as K in Table 28) and previously had the same abnormality at preceding time point, while the other one presented with abnormality for first time in the study (participant J in Table 28).

Table 28: Study participants with hydroceles on ultrasonography at 3-months follow up time point

Participant	Age (years)	Eggs in urine (per 10 ml)	Eggs in semen (per ml)	Real-time PCR (Ct-value)	Location of hydrocele
J	33	0.0	0.0	45.0*	Left
K	44	15.2	7.5	26.7	Left
L	49	0	0	45.0*	Bilateral

** Ct-value of 45.0 is Negative*

7.5.3.3. 6-months follow-up

Sixty-three participants were followed up at 6-months' time-point of which 38 had ultrasonography examinations, and 4 were scanned for the first time. On diagnostic examinations, 2 had *S. haematobium* eggs in urine (5.3%), 1 in semen (2.6%), 4 (10.5%) positive and 2 (5.3%) trace POC-CCA test results, while 3 had positive semen real-time PCR (7.6%).

Pathological abnormalities were observed in 4 participants (13.2%), with 3 reported for the first time while one had the abnormalities reported at all previous time points (bilateral hydroceles) (Table 29). Two participants had left hydroceles, while two participants had no abnormalities at the time point which were observed previously.

Table 29: Abnormalities observed in more GU organs of participants at 6-months follow-up

Participant	Age (years)	Eggs in urine (per 10 ml)	Eggs in semen (per ml)	Real-time PCR (Ct-value)	Abnormalities observed
M	25	0.0	0.0	N/D#	Left hydroceles
N	33	0	0	N/D#	Enlarged, asymmetrical seminal vesicles (Figure 49)
O	39	0.0	0.0	45.0*	Left hydrocele
P	49	0.0	0.0	45.0*	Bilateral hydroceles

** Ct-value of 45.0 is Negative; # Test not done, inadequate sample*

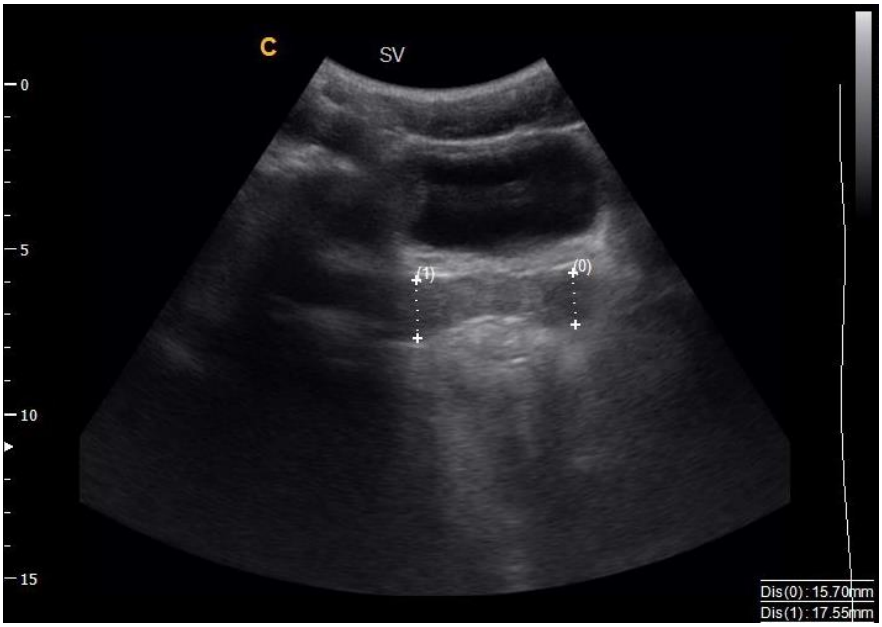


Figure 49: Ultrasonographic image of enlarged, asymmetrical seminal vesicles in participant N, Table 28 at 6-months follow-up time point.

7.5.3.4. 12-months follow-up

Forty-five participants were reviewed at 12-months' time-point of which 17 had ultrasonography examinations, and 4 were scanned for the first time. One participant had abnormal bladder wall thickness and left kidney mass. On diagnostic examinations, the participants were negative for POC-CCA, urine filtration and semen microscopy, with 2 participants being positive on semen real-time PCR (11.8%).

Table 30 illustrates the progress of abnormalities detected at baseline, over the course of the study and Table 31 shows those with no abnormality at baseline but detected during the follow-up. In total, 146 participants were scanned in the study and abnormalities were noted in 16 participants at various time points. Table 32 shows the proportion of participants with abnormalities in GU organs on ultrasonography in accordance with their MGS status at all time-points.

Table 30: Ten participants with abnormalities in GU organs on ultrasonography at baseline and progression over the time points in the study

Age (years)	Baseline	1-month	3-months	6-months	12-months
19	Bladder thickening, severe hydronephrosis	Lost to follow-up			
22	Bladder thickening, prostate nodule	*Didn't show up*	No abnormality	Inadequate bladder filling, not reported for repeat scan	Lost to follow-up
24	Enlarged asymmetrical seminal vesicles	Inadequate bladder filling, not reported for repeat scan	Lost to follow-up		
29	Left hydrocele	Lost to follow-up			
30	Left hydrocele	*Didn't show up*	*Didn't show up*	*Didn't show up*	No abnormality
49	Bilateral hydroceles	Bilateral hydroceles	Bilateral hydroceles	Bilateral hydroceles	Lost to follow-up
49	Testicular nodule, mild bilateral hydroceles	Bilateral hydroceles	Lost to follow-up		
51	Enlarged prostate	*Didn't show up*	*Didn't show up*	No abnormality	Lost to follow-up
54	Bilateral hydroceles	Lost to follow-up			
69	Enlarged prostate, bilateral hydroceles	Lost to follow-up			

Table 31: Six participants with abnormalities in GU organs on ultrasonography during follow-up time-points but none at baseline in the study

Age (years)	Baseline	1-month	3-months	6-months	12-months
25	No abnormality	No abnormality	*Didn't show up*	Left hydrocele	Lost to follow-up
33	No abnormality	*Didn't show up*	Left hydrocele	*Didn't show up*	No abnormality
33	No abnormality	*Didn't show up*	*Didn't show up*	Enlarged, asymmetrical seminal vesicles	Lost to follow-up
39	No abnormality	*Didn't show up*	*Didn't show up*	Left hydrocele	Lost to follow-up
44	No abnormality	Left hydrocele	Left hydrocele	No abnormality	Lost to follow-up
53	No abnormality	*Didn't show up*	*Didn't show up*	*Didn't show up*	Bladder thickening, left kidney mass

Table 32: Proportion of participants in the study with abnormalities in GU organs on ultrasonography in accordance to their MGS status at all time-points

		MGS									
		Baseline (n = 130)		1-month (n = 29)		3-months (n = 32)		6-months (n = 38)		12-months (n = 17)	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
USS	Positive	4	5	2	1	1	2	0	4	0	0
	Negative	14	107	1	25	3	26	1	33	2	15

7.6. Discussion

To our knowledge, this is the first prospective ultrasonographic study of MGS in Malawi and south-eastern Africa on the fishermen cohort to determine its morbidity, through observations of pathological abnormalities in genital organs, as well as look at changes through time in men after standard dose-regimen of PZQ treatment.

7.6.1 Detection of genital consequences of schistosomiasis on ultrasonography

Genital manifestations like MGS are among the complications of schistosomiasis which remains unknown among local inhabitants frequently exposed in infective cercariae harbouring their freshwater bodies as well as health professionals working in the areas which result in undiagnosed, under- or mis-treating and underreporting of the disease, contributing further to morbidity among affected men. In some instances, people suffer from social prejudice and discrimination arising from the consequences of the genital complications, such as infertility, abnormal organ swelling, reduced sexual prowess, coital pain, genital bleeding among other with women severely and disproportionately (Yirenya-Tawiah, Ackumey and Bosompem, 2016; Kukula *et al.*, 2019).

In order to improve awareness and knowledge of MGS, other diagnostic methods can be added to help in detection of the disease. Radiological techniques have been observed to improve diagnosis of schistosomiasis at various stages, mostly with chronic complications. Ultrasonography is considered as an acceptable, safe and less-invasive tool in diagnosis, management and monitoring control of NTDs, including schistosomiasis. Recent advances in this technology has resulted in development of portable, high quality scanning devices which can be easily mobile to limited-resource endemic areas and utilised in the available infrastructure, in detecting the genital pathological abnormalities affecting rural population among other conditions, as observed in our study.

7.6.2 Morbidity of MGS in Malawian fishermen

Our MGS cohort study among local fishermen along the south shoreline of Lake Malawi observed a 17.1% baseline prevalence of UGS, 10.4% for MGS using semen microscopy and 26.6% by semen real-time PCR. Among those 130 participants who were scanned at baseline, the prevalence of UGS was 20.9%, 4.9% for possible intestinal *S. mansoni* schistosomiasis, 12.3% for MGS using semen microscopy and 28.1% by semen real-time PCR.

Previous studies have described abnormalities observed in genital organs like prostate, seminal vesicles, ejaculatory ducts, vas deferens, epididymis, tests, scrotal sac among other structures, which include organ enlargement, shrinkage, dilatations, thickening, echogenic lesions, calcification, hydroceles among others, which can mimic other diseases. In schistosomiasis-endemic areas, detection of such pathological abnormalities together with classical genital symptoms like genital, coital or ejaculatory pain, haemospermia, abnormal ejaculates, reduced libido or suspected infertility, should suggests a diagnosis of MGS (Kayuni *et al.*, 2019c). Since, similar clinical presentation and findings can be associated with other diseases prevalent in these endemic areas, affecting the genital organs such as sexually transmitted infections (STIs), tuberculosis (TB), malignant hypertension or cancer, proper clinical assessment and extensive diagnostic examinations are very important to be conducted to ensure appropriate diagnosis, treatment, care and management of affected men.

The findings of this study showed that 10 participants had pathological abnormalities in their GU organs at baseline, of which nine participants (6.9%) having them in prostate, seminal vesicles and scrotum. These abnormalities suggest consequences of previous or current schistosomal infection acquired from their frequent exposure during fishing and other routine activities in the lake, which is known to harbour schistosomes. The abnormalities observed ranged from ‘mild’ ones present in majority of participants to severe ones, with three participants (3.1%) had abnormalities in multiple organs. Seminal vesicles had the most abnormalities on ultrasonography, with majority of participants having asymmetrical normal-sized vesicles and only three participants showing severely

enlarged vesicles. This is consistent with evidence from previous studies and literature which describes that seminal vesicles are among the frequent affected genital organs with schistosomiasis (Alves, Woods and Gelfand, 1955; Gelfand *et al.*, 1970; Kayuni *et al.*, 2019a).

Further results show that urinary bladder had abnormal wall thickening, in some cases severe polypoid structures and associated bilateral hydronephrosis which required referral for further medical management at district hospital, unfortunately, participant was lost to follow-up. Such observations have been widely described in literature which originate from matured paired schistosome worms migrating from hepatic veins to reside in vesical plexus and continually deposit massive number of eggs, which get trapped in the bladder wall, causing granulomatous reactions, fibrosis, calcifications and architecture destruction, compromising bladder functioning (Bustinduy and King, 2014; Colley *et al.*, 2014). As the infection progresses and bladder malfunctions, urine backflow compromises the ureters (hydroureter) which later affects the kidneys, resulting in hydronephrosis. Early diagnosis and management are critical in preventing such chronic and fatal consequences of schistosomiasis.

Interestingly, schistosome worms have been thought to reside in venous plexus around genital organs like prostate, seminal vesicle and testes, with eggs being trapped in the tissues due to its tough architecture in comparison to the urinary bladder. This can result in echogenic lesions, calcifications, organ enlargement, atrophy, hydroceles among the pathological abnormalities in these genital organs which can be detected on ultrasonography (Richter, 2000; Al-Saeed *et al.*, 2003; Richens, 2004). These abnormalities were observed in this study in the prostate, epididymis and testis, with some participants being positive on the urine and semen diagnostic tests. Prostate abnormalities were observed in 3 participants, of which two had grossly enlarged prostates and one had hyperechoic prostatic nodule and positive semen real-time PCR. These can be attributed to MGS after exclusion of other possible diseases like STIs, TB, benign prostatic hyperplasia or prostate malignancy. In addition, the lack of statistically significant differences among those with abnormalities in accordance to age, duration of stay and diagnostic tests' results demonstrate that

the lesions can present at any age since they could have developed from a young age, as reported previously (Rambau *et al.*, 2011; Ekenze *et al.*, 2015).

7.6.3 Long-term monitoring for MGS in line with regression of pathologies

Monitoring of disease morbidity especially MGS is critical in controlling the disease and prevention of severe irreversible pathological abnormalities which later could contribute to mortality. As a mainstay treatment, PZQ has shown to be effective in treating both forms of schistosomiasis, registering cure rates of over 90% in most endemic areas (Knopp *et al.*, 2013; WHO, 2013a). Currently, it is utilised by most national control programmes in endemic areas as one of the key control interventions through the MDA campaigns, which commonly targeted school-aged children. PZQ has also been used in treating MGS, clearing the schistosome eggs in semen and resolving some pathological abnormalities, while adjusting the standard dose of treatment in some cases to ensure complete cure (Kayuni *et al.*, 2019a).

From the study follow-up after PZQ treatment to the participants, most participants were observed to have resolved their pathological abnormalities at 1-month time-point, except two participants who had persistent abnormalities. This could illustrate the knowledge that early mild lesions will be resolved by standard PZQ treatment as it kills those adult-laying worms, hence reducing further damage to the organs. Chronic, long-standing abnormalities like hydroceles require further assessments, medical and surgical interventions to resolve these, as it was done in the study where such participants were referred to the bigger district hospital.

During further follow-ups at the 3-, 6- and 12-months' time-points, some participants developed new abnormalities, which highlighted the repeated exposure to the infested lake water. Other participants showed resolution of their abnormalities over time, supporting the need for repeated PZQ treatment to completely resolve the severe lesions which are reversible, while providing additional control interventions like adequate awareness and health education, provision of adequate, portable, safe and clean water, encouraging construction and utilisation of household

and community sanitation facilities, as well environmental control to reduce intermediate snail host population. Only two participants were observed to have abnormalities at the last 12-months' time-point which shows the efficacy of praziquantel in resolving MGS pathologies.

7.6.4 Study limitations and ultrasonographic diagnostic challenges in MGS

The low number of participants undergoing the ultrasonography examinations limit the generalisation of the study results to male population in endemic region. This could be explained by lack of experience with the method as most participants reported this was their first time to undergo such examinations. Negative perceptions with regards to new techniques in rural communities and the longer time spent during the examination especially among those presenting with inadequate bladder filling could have deterred more study participants from taking part. Also, some participants could be reluctant to take part at health centres, due to poor health-seeking behaviour. More sensitisation and discussions which were done can help to address such and other concerns, thereby stimulate more participants to such important studies.

The low sensitivity of transabdominal ultrasonography compared to TRUS, CT or MRI could result in missing some lesions in the genital organs, resulting in poorly described burden of genital diseases like MGS. However, cost implications associated with these sensitive techniques, their unavailability and inaccessibility as well as low acceptability among local participants could further jeopardise the implementations of such examinations in rural endemic areas.

In addition, advanced radiological expertise and extensive training on genital ultrasonography are required in order to detect pathological lesions arising from MGS, which could be easily mistaken for those from other prevalent genital diseases such as STIs, TB or cancer. Also, further ultrasonography studies with larger cohort as well as inclusion of more advanced portable devices such as TRUS are necessary in endemic areas to describe more on morbidity of MGS.

7.7. Conclusion

In conclusion, pathological abnormalities caused by MGS can be detected using portable transabdominal and scrotal ultrasonography, as demonstrated in our study, which could be used in describing the morbidity of MGS. This method could aid in improving diagnosing MGS among affected population, due to advances made in developing portable scanning devices which can be easily accessible in endemic areas especially SSA. Our study also showed good resolution of abnormalities at follow-up time-points, with only one participant having pathological abnormalities on ultrasonography at 12-months' time-point, highlighting the role of this technique in monitoring progress of intervention in schistosomiasis control interventions.

Chapter 8: Seminal HIV-1 RNA detection among HIV-positive in men with MGS

8.1. Summary

This chapter presents the results of HIV-1 viral load analyses of the paired blood and semen plasma samples. Blood and semen samples were collected from all participants at entry into the study for analysis of plasma and semen HIV-1 viral load. Participants that were found to have schistosomiasis were treated with praziquantel (PZQ) and followed up. All participants had been on ART for six months or more at entry into the study.

Fifteen participants with male genital schistosomiasis (MGS, cases) had their HIV blood and semen plasma viral loads compared with 16 participants without MGS (controls) at baseline, 1-, 3-, 6- and 12-months after treatment with PZQ among the cases. At baseline, more cases (12/15, 80.0%) had detectable HIV-1 virus in plasma samples compared with the controls (5/16, 31.3%). Among the cases, there were three participants (20%) with detectable and quantifiable VL in semen plasma samples compared to two (12.5%) among the controls

During follow-up at all time-points, HIV viral load declined among the cases. This may be attributable to the PZQ among the cases. We have demonstrated in this pilot study that men with MGS have higher viral loads in both blood and semen plasma compared with men without. We have also demonstrated that PZQ treatment in men with MGS results in reduction of HIV-1 viral load in both blood and semen plasma.

To our knowledge, this is the first study in Malawi that has looked at seminal HIV-1 shedding in men with schistosomiasis. Although our data is limited by the small number of study participants, high loss to follow-up and not controlling for adherence to ART and other confounders that may increase HIV-1 viral load in both semen and blood including sexually transmitted illnesses, there is a possible significant role of MGS among men in fuelling this transmission, which suggest for further prospective larger trials.

8.2. Introduction

The SSA region remains at the epicentre of the HIV epidemic (Caldwell and Caldwell, 1996; Kopelman and van Niekerk, 2002; UNAIDS, 2019), as illustrated in Figure 12 in Chapter 2.

The advent and effective use of highly active combined antiretroviral therapy (ART) in HIV infected people has shown greater ability to suppress viral load (VL) in blood plasma below detection threshold and reducing VL in genital secretions, thereby contributing significantly to decreased HIV transmission (Marcelin *et al.*, 2008; Sheth *et al.*, 2009). Despite the high rate of viral suppression and reduced HIV transmission, occasional HIV-1 transmission has occurred in those people on ART, with some men continuing to shed HIV-1 RNA in seminal secretion (Halfon *et al.*, 2010; Cohen *et al.*, 2011).

Schistosomiasis, which contributes significantly to the disease burden in SSA (Butterworth *et al.*, 2013; McManus *et al.*, 2018; WHO, 2018b), overlaps geographically with the HIV epidemic (Mbabazi *et al.*, 2011; Ndeffo Mbah *et al.*, 2013). Studies from East Africa among fisher folks have demonstrated increased prevalence of HIV among their communities (Allison and Seeley, 2004; Kissling *et al.*, 2005; Seeley, Tumwekwase and Grosskurth, 2009), where urinary schistosomiasis is prevalent. Male genital schistosomiasis (MGS) remains a little known, underreported manifestation of schistosomiasis in endemic SSA areas (Leutscher *et al.*, 2000; Kayuni *et al.*, 2019a).

A recent study in SSA demonstrated a reduction of seminal viral load shedding of newly diagnosed HIV-infected men coinfected with urogenital schistosomiasis (UGS) 10 weeks after praziquantel (PZQ) treatment (Midzi *et al.*, 2017), highlighting a possible impact of schistosomiasis treatment on HIV control (Stecher *et al.*, 2015).

MGS and HIV-1 co-infection interactions within men in respect to the impact on VL is not fully understood in SSA, especially those on regular ART using the tenofovir disoproxil fumarate - lamivudine - efavirenz regimen (TNF/3TC/EFV). In order to evaluate the extent of relationship between MGS and HIV-1 VL in blood and semen, a pilot study was conducted among men with HIV

infection along Lake Malawi in Mangochi District, to assess the viral shedding in semen of those co-infected with MGS.

8.3. Methods

8.3.1. Study area, population and sampling

This study was conducted in the ART clinics of the health facilities located in the study area, (NSO and ICF, 2017), as described in Chapter 4. Men aged 18+ years who were HIV infected and taking ART were eligible to participate in the study, with those testing positive for schistosomiasis enrolled as study cases, and those negative regarded as study controls. A minimum sample size of 20 study cases and 20 study controls was planned for the pilot study.

8.3.2. Data collection and analyses

Data collection methods comprised of individual questionnaires, parasitological schistosome tests on urine and semen samples, transabdominal ultrasonography examination, as described in Chapter 4, blood for comparative HIV-1 RNA detection and molecular real-time PCR to detect schistosome DNA in semen and serological ELISA tests. Blood and semen plasma were analysed for HIV VL using Cepheid Xpert HIV-1 assay (Cepheid, 2019) as described in Chapter 4.

8.3.3. Statistical analyses

After screening, and quality-control and data entry, descriptive statistics were calculated to explore the data and produce frequencies, medians and ranges of each study groups, including correlations and nonparametric tests.

8.3.4. Ethical considerations

Ethical clearance to conduct the study was provided by the National Health Sciences Research Committee (NHSRC) of Malawi and Liverpool School of Tropical Medicine (LSTM) Research

Ethics Committee (LSTM REC). Since this was a test-to-treat study, participants were offered PZQ treatment at the end of the visit before inviting them to the next follow-up study.

8.4. Results

8.4.1. Study population

Seventy-four participants were invited to take part and were recruited into the study and 31 participants submitted paired semen and blood samples for VL analyses, whose results are presented in this chapter (Figure 50).

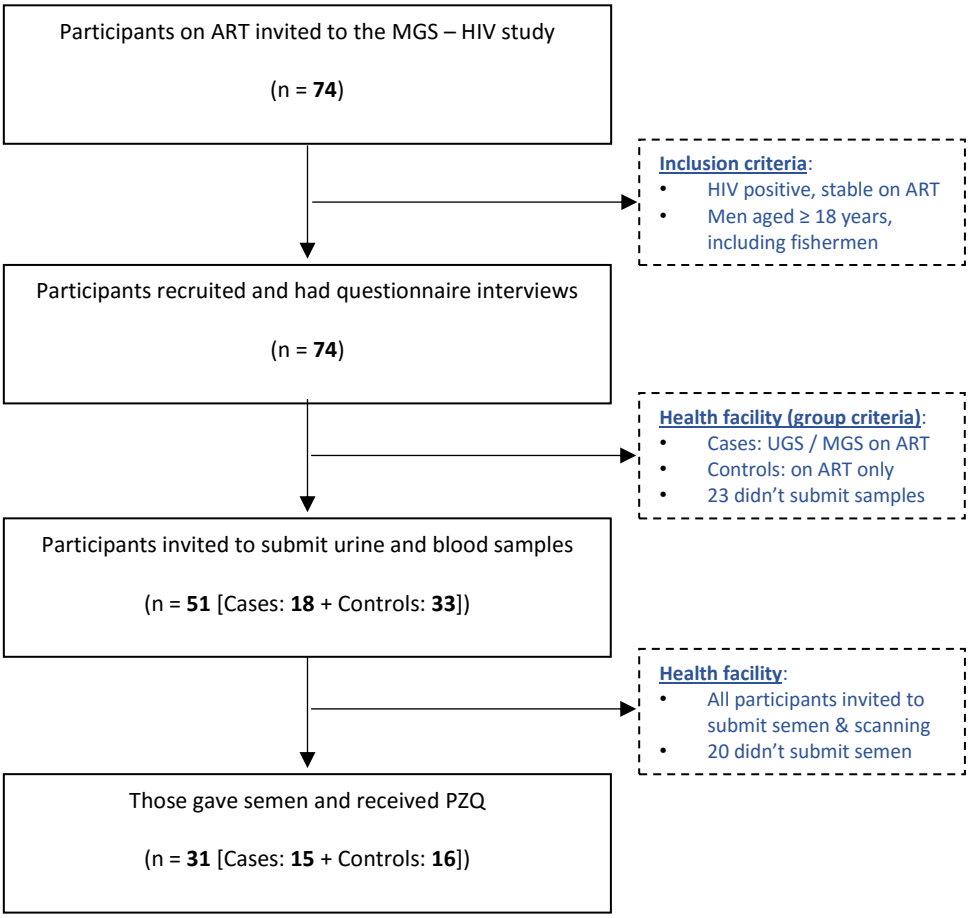


Figure 50: Diagram of the participants who took part in the study

Fifteen participants had MGS on basis of schistosome eggs in semen and / or positive semen real-time PCR and categorised as cases while 16 were negative for schistosomiasis and categorised as controls.

8.4.2. Demographic information of the study participants

The median age of study cases was 42.0 years (interquartile range (IQR): 11.8), similar for study controls (median: 42.0 years, IQR: 17.3), with no statistical difference (Independent Mann-Whitney test $U = 120.0$, $p = 0.78$) (Table 33). The median time after HIV diagnosis was 7.5 years (cases) and 7.8 years (controls) while duration on ART was 4.8 years (cases) and 6.1 years (controls) (Table 34), with no significant statistical difference.

Table 33: Description of the study participants

Characteristic	Cases	Controls	*p-value*
Total number of participants, *n*	15	16	
Age, median years (IQR, range)	42.0 (11.8, 43.0)	42.0 (17.3, 40.0)	0.78
Time since HIV diagnosis, median years (IQR, range)	7.5 (11.0, 14.4)[a]	7.8 (6.3, 11.3)[b]	0.50
ART duration, median years (IQR)	4.8 (10.3, 12.2)[c]	6.1 (7.1, 12.3)[d]	0.43
Time on ART[e]			
< 1 year (%)	4 (28.6)	1 (7.1)	-
> 1 year (%)	10 (71.4)	13 (92.9)	-
Current ART regimen[f]			
TDF/3TC/EFV	14	14	-
TDF/3TC+NVP	1	0	-

[a]Data available from 13 cases; [b]Data available from 12 controls; [c]Data available from 14 cases; [d]Data available from 14 controls; [e]Data available from 14 cases and 14 controls; [f]Data from commonly used first-line ART regimen.

Table 34: Description of the study participants

Characteristic		Cases	Controls
Age	**N**	15	16
	Median	42.0	42.0
	Range	25.0 – 65.0	22.0 – 65.0
	Interquartile Range (IQR)	11.8	17.3
Time since HIV diagnosis	**N**	13	12
	Median	7.8	7.5
	Range	1.5 – 12.8	0.2 – 14.4

	Interquartile Range (IQR)	6.3	11.0
Time on ART	**N**	14	14
	Median	4.8	6.1
	Range	0.1 – 12.3	0.1 – 12.3
	Interquartile range (IQR)	10.3	7.1

8.4.3. Schistosomiasis diagnosis in the study cases

Of the 15 study cases, ten were positive for semen microscopy and / or real-time PCR at baseline, two for first-time at 1-month and three at 3-months' time-point (Table 35).

Table 35: Parasitological, serological and molecular results of the study cases

Study Case ID	Baseline					1-month			3-months			6-months			12-months		
	UF	SEM	IVD	DRG	PCR	UF	SEM	PCR	UF	SEM	PCR	UF	SEM	PCR	UF	SEM	PCR
A	-	-	+	-	+										-	-	-
B	-	-	+	-	-	-	-	+									
C	-	+	+	+	-												
D	-	+	-	-	-	-	-	-	-	-	-				-	-	-
E	+	-	+	+	+	-	-	-	-	-	+	-	-	+	-	-	-
G	-	+	-	-	-	-	-		-	-	-	-	+	-	-	-	-
H	-	-	-	-	-	-	-	-	-	-	+				-	-	-
I	+		+	+		+	-	+	+	+	+	-	-	+	-	-	-
J	-	-	-	-	+	-	-	-				+	-	-			
K	-	-	+	+	+												
L	+	+	+	+	-							-	-	-	-	-	+
M	+	-	+	-					+	-	+	+	+	-			
N	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-
O	+	+	+	+	+										-	-	-
P	-	-	-	+	+												

UF = urine filtration, SEM = semen microscopy, IVD and DRG = serological ELISA tests, PCR = real-time PCR, "-" = negative result, "+" = positive result. * positive real-time PCR on urine, not semen

8.4.4. Patterns of HIV-1 viral detection in blood and seminal plasma of the study participants

8.4.4.1. Study cases

Seven cases (7/15 participants) had discrepant detectable HIV-1 VL in their paired samples at baseline of the study (Table 36). Two participants (2/7) had detectable but unquantifiable VL in

semen. Three cases had discrepant detectable and quantifiable VL in plasma, with a median VL of 3.83 $\log_{10}$ copies /ml (IQR: 4.65$\log_{10}$ copies/ml).

Table 36: Results of the HIV viral load analyses in the study cases

Study case ID	Baseline		1-month		3-months		6-months		12-months	
	BLOOD	SEMEN	BLOOD	SEMEN	BLOOD	SEMEN	BLOOD	SEMEN	BLOOD	SEMEN
A	UD	UD^{α}							D	UD
B	UD	UD	UD	UD						
C	UD	D^{β}								
D	UD	D^{α}	UD	UD	UD	UD			UD	UD
E	UD	UD	UD	D^{α}	UD	**2.09**	UD	D^{α}	UD	UD
G	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
H	D	UD	UD	UD	D	UD				
I	UD		UD	UD	D	UD	UD	UD	UD	UD
J	**2.32**	UD	**1.98**	UD			D	UD		
K	UD	UD								
L	**3.32**	UD					**2.91**	UD	**2.44**	UD
M	UD	UD^{α}			D	UD				
N	D	UD	D	UD	D	UD	UD	UD		
O	UD	UD^{α}							D	UD
P	**4.75**	UD								

D = detectable but not quantifiable in blood (VL: 1.34 – 1.59 $\log_{10}$ copies/ml), UD = undetectable in blood (VL < 1.34 $\log_{10}$ copies/ml); D^{α} = detectable but not quantifiable in semen (VL: 1.74 – 2.04 $\log_{10}$ copies/ml), D^{β} = detectable but not quantifiable in semen (VL: 2.34 – 2.60 $\log_{10}$ copies/ml), UD = undetectable in semen (VL < 1.74 $\log_{10}$ copies/ml), UD^{α} = undetectable in semen (VL < 2.04 $\log_{10}$ copies/ml), UD^{β} = undetectable in semen (VL < 2.34 $\log_{10}$ copies/ml)

In observing subsequent time-points after PZQ treatment, VL of two cases (J and L) showed a downward trend to undetectable level, while one (P) was lost to follow-up. One case (E) had detectable but unquantifiable VL at 1-month, quantifiable VL of 2.09 $\log_{10}$ copies / ml at 3-months, reduced back to unquantifiable level at 6-months and undetectable VL at 12-months' time-point.

In all, 12 cases (80.0%) had detectable VL in either blood or semen plasma at some time-point during the study. At baseline, out of 10 cases with positive schistosome tests, five had discrepant detectable VL Out of all study cases, five had detectable and quantifiable HIV VL (Table 37) which were discrepant between blood and semen plasma samples during the longitudinal cohort study, demonstrating shedding of HIV viruses in blood among four participants with schistosomiasis and one in semen, as illustrated in Figure 51.

Table 37: Comparative results of the schistosome parasitological, serological and molecular tests with detectable HIV viral load in 5 study cases

Study case ID	BASELINE							1-MONTH					3-MONTHS					6-MONTHS					12-MONTHS				
	Schistosome					HIV VL		Schistosome			HIV VL		Schistosome			HIV VL		Schistosome			HIV VL		Schistosome			HIV VL	
	UF	SEM	IVD	DRG	PCR	BLD	SM	UF	SEM	PCR	BLD	SM	UF	SEM	PCR	BLD	SM	UF	SEM	PCR	BLD	SM	UF	SEM	PCR	BLD	SM
E	2.1	0.0	0.32	0.61	36.1	UD	UD	0.0	0.0	NEG	UD	D^{α}	0.0	0.0	22.5	UD	2.09	0.0	0.0	23.4	UD	D^{α}	0.0	0.0	NEG	UD	UD
F	0.0	0.0	0.10	0.12	NEG	4.06	UD	0.0	0.0	NEG	4.16	UD	0.0	0.0	NEG	4.15	UD						0.0	0.0	25.1	4.46	UD
J	0.0	0.0	0.09	0.17	36.6	2.32	UD	0.0	0.0	NEG	1.98	UD						0.6	0.0	NEG	D	UD					
L	0.1	0.4	0.24	0.74	NEG	3.32	UD											0.0	0.0	NEG	2.91	UD	0.0	0.0	35.0	2.44	UD
P	0.0	0.0	0.24	0.46	21.3	4.75	UD																				

Schistosome = schistosome parasitological, serological and molecular tests (▢ = positive result), HIV VL = HIV viral load analyses (in $\log_{10}$ copies/ml, ▢ = number of detected viral copies, ▢ = detectable but unquantifiable viral copies); UF = urine filtration (eggs / 10ml urine), SEM = seminal microscopy (eggs /ml ejaculate), IVD and DRG = serological ELISA tests, PCR = seminal real-time polymerase chain reaction (PCR) analyses (Ct-value, NEG = negative), BLD = blood plasma, SM = seminal plasma. D = detectable but not quantifiable in blood (VL: 1.39 – 1.59 $\log_{10}$ copies/ml), UD = undetectable in blood (VL < 1.39 $\log_{10}$ copies/ml); D^{α} = detectable but not quantifiable in semen (VL: 1.74 – 2.04 $\log_{10}$ copies/ml), D^{β} = detectable but not quantifiable in semen (VL: 2.34 – 2.60 $\log_{10}$ copies/ml), UD = undetectable in semen (VL < 1.74 $\log_{10}$ copies/ml), UD^{α} = undetectable in semen (VL < 2.04 $\log_{10}$ copies/ml), UD^{β} = undetectable in semen (VL < 2.34 $\log_{10}$ copies/ml).

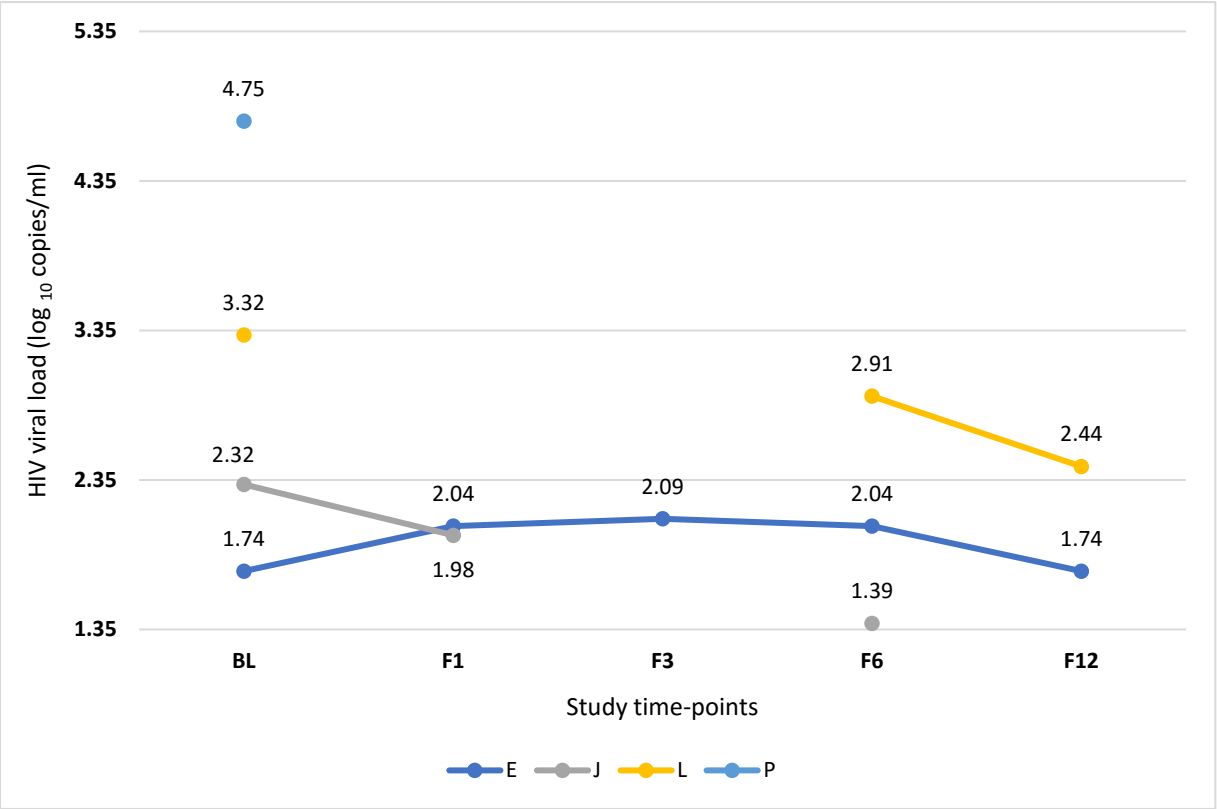


Figure 51: Progression of HIV-1 viral levels of detectable cases at all the study time-points

HIV VL from blood plasma from all cases except semen plasma for case E; blood plasma VL of 1.39 $\log_{10}$ copies/ml or less is classified as undetectable; semen plasma VL of 1.74 $\log_{10}$ copies/ml or less is classified as undetectable, VL of 2.04 $\log_{10}$ copies/ml is detectable but not quantifiable.

8.4.4.2. Study controls

Of the 16 participants who were already on ART and recruited as study controls at baseline, three (I, VI and XV) had detectable HIV VL, with two (I and XV) having discrepant detectable VL in blood plasma, whilst one (VI) had concordant VL in blood and semen plasma (Table 38). Two other control participants had detectable and quantifiable VL, one (II) at 1-month in blood plasma only, and the other (V) at 3-months in both blood and semen plasma.

Table 38: Results of the HIV viral load analyses in the study controls

Study control ID	Baseline				1-month		3-months		6-months		12-months	
	IVD	DRG	BLOOD	SEMEN	BLOOD	SEMEN	BLOOD	SEMEN	BLOOD	SEMEN	BLOOD	SEMEN
I	+	+	D	UD							D	UD
II	-	-	UD	UD	**1.81**	UD					D	UD
III	+	-	UD	UD$^{\beta}$			UD	UD$^{\beta}$				
IV	+	+	UD	UD$^{\beta}$								
V	-	-	UD	UD$^{\alpha}$			**4.03**	**2.72**	UD	UD	D	UD
VI	+	+	**4.43**	**3.68**								

VII	-	**+**	UD	UD	UD	UD	UD	UD	UD	UD		
VIII	**+**	**+**	UD	UD								
IX	**+**	**+**	UD	UD								
X	-	-	UD	UD					UD	UD		
XI	-	-	UD	UD								
XII	-	-	UD	UD								
XIII	**+**	**+**	UD	UD			UD	UD				
XIV	-	-	UD	UD								
XV	-	-	**1.61**	UD								
XVI	-	**+**	UD	UD$^{\beta}$			UD	UD$^{\alpha}$			UD	UD$^{\alpha}$

IVD and DRG = serological ELISA tests; D = detectable but not quantifiable in blood (VL: 1.34 – 1.59 log $_{10}$ copies/ml), UD = undetectable in blood (VL < 1.34 log $_{10}$ copies/ml); UD = undetectable in semen (VL < 55 copies/ml), UD$^{\alpha}$ = undetectable in semen (VL < 2.04 log $_{10}$ copies/ml), UD$^{\beta}$ = undetectable in semen (VL < 2.34 log $_{10}$ copies/ml)

8.4.5. Comparison of detectable HIV-1 viral levels between study cases and controls

Twelve of 15 cases (80.0%) had detectable HIV VL compared to 5 of 16 controls (31.3%) in the study. Three cases (J, L and P) had detectable and quantifiable VL in blood plasma at baseline, ranging from 2.32 to 4.75 log $_{10}$ copies/ml (mean VL: 3.32 log $_{10}$ copies/ml), which was higher compared to that of two control participants, 1.61 and 4.43 log $_{10}$ copies/ml (mean VL: 3.02 log $_{10}$ copies/ml).

On follow-up after PZQ treatment, the VL showed a downward trend as the schistosome infection was clearing. The blood plasma VL of one case participant (J) present at 1-month time-point reduced by 0.34 log $_{10}$ copies/ml, observed at 1.98 log $_{10}$ copies/ml, where all the schistosome tests were negative, and at 6-months' time-point, had detectable but unquantifiable VL despite having UGS (schistosome eggs in urine). Similarly, blood plasma VL of the other MGS participant (L) followed up at 6- and 12-months' time-points, declined to 2.91 and 2.4 log $_{10}$ copies/ml respectively.

8.5. Discussion

This is the first epidemiological pilot study to measure the HIV-1 viral levels in blood and semen plasma of men on ART with MGS in endemic areas of SSA. It reveals a potential impact of MGS on HIV management and monitoring of viral suppression, from ART, and as a pilot study, suggests larger surveys are warranted to elucidate fully this relationship.

It is recognised that the risk of HIV-1 sexual transmission among serodiscordant couples is zero when the infected partner is on effective ART regime and has a fully suppressed VL (Cohen *et al.*, 2011; Rodger *et al.*, 2016; Rodger *et al.*, 2019).

However, those on ART with suppressed VL in blood can have intermittent shedding and detectable HIV RNA in semen and genital tract fluids, even in absence of STIs and other inflammatory infections (Kalichman, Di Berto and Eaton, 2008; Lambert-Niclot *et al.*, 2012), which raises significant concerns at a public health level. In this study, where more case participants with schistosomiasis, specifically MGS, had higher detectable and quantifiable VL at baseline compared with the control participants, it is suggestive of increased risk of HIV transmission. This is in line with evidence that schistosome egg excretion has been associated with increased HIV-1 VL in plasma (Karanja *et al.*, 1997; Mwanakasale *et al.*, 2003).

This pilot study has detected a decline in VL on follow-up time-points after standard single-dose PZQ treatment, where case participants also had reduced schistosome infections. Other prospective studies in SSA have reported similar results (Kallestrup *et al.*, 2005a; Erikstrup *et al.*, 2008), coinciding with suggestions by a Cochrane review that treatment of helminth infections in HIV co-infected people may contribute to decline of their HIV-1 RNA VL (Means *et al.*, 2016). A recent observation study on Zimbabwean men reported high VL in HIV-1 positives with UGS, which decreased 10 weeks after PZQ treatment (Midzi *et al.*, 2017).

In terms of longitudinal dynamics, further follow-up of the participants revealed rebound detectable VL among some case participants, despite continued long-term ART and PZQ treatment. This could be due to re-infection with schistosomes, incomplete cure of previous schistosome

infection, low ART adherence, presence of other infections like malaria, TB, other helminths or STIs, which were not detected in the study, as well as poorer ART performance (possible failure), other host or viral genetic factors.

Although some case participants with MGS in our study were observed to have small amounts of VL reported as detectable but unquantifiable VL, it has been recognised not to correlate with HIV transmission risk due to negligible risk achieved on fully suppressive ART consistently for at least 6 months (BHIVA, 2018). However, discrepant detectable VL suggest the different suppressive nature of ART in various body compartments especially TNF regimen which have been reported before (Ghosn *et al.*, 2004; Lowe *et al.*, 2006). Currently, the HIV treatment in Malawi has changed in 2019 to dolutegravir regimen.

Though this pilot study has shown the potential for a relationship between MGS, PZQ treatment and HIV VL, the study was limited in its statistical power by low number of participants submitting fewer samples during time-points, high numbers lost to follow up and meagre field resources, affecting the comparability of our results to previous studies and generalisation to the male population in the country and SSA region. This reaffirms the call for more prospective studies on co-infection of MGS and HIV with larger sample size of this high-risk male population in endemic areas.

8.6. Conclusion

In conclusion, detectable VL was noted in some HIV-positive men who had MGS and displayed a downtrend after PZQ treatment on subsequent time-points while others had rebound detectable VL and schistosome re-infection. Therefore, PZQ treatment in endemic areas as multiple or repeated doses, bi-annual MDA is essential with synergising of schistosomiasis control strategies in the HIV program for cost-effective impact of both diseases among co-infected men, following more robust, large prospective studies.

Chapter 9: General discussion

9.1. Summary

This chapter discusses the main findings of the longitudinal cohort study on MGS, in line with the research hypothesis and objectives. Special focus is being put on the recommendations for diagnosis and management of MGS which reflects on a proposed algorithm, and role of other point-of-care examination tools such as ultrasonography in determining the morbidity of MGS. This is critical to be incorporated in the activities for the national schistosomiasis control programme in Malawi and other endemic countries in SSA and globally.

The interrelationship of HIV and MGS infections is also highlighted and possible role of both PZQ and ART together with regular viral load monitoring, adherence and treatment of other opportunistic infections, in controlling and managing both infections. Integration of control programmes in Malawi and other affected areas is very important in successful control of both diseases.

Finally, suggestions for future work are presented to produce more evidence and improve knowledge among key health professionals in endemic areas.

9.2. Prevalence of MGS

Schistosomiasis remains a prevalent parasitic disease affecting over 200 million people in tropical countries, especially SSA, where 85% inhabit, contributing to significant morbidity and considerable mortality in the world (WHO, 2018b). This neglected tropical disease (NTD) poses a significant public health challenge in most endemic rural and peri-urban areas, with its burden in 2016 estimated at 2 543 364 disease-adjusted life years (DALYs) (WHO, 2018c), most arising for urogenital *Schistosoma haematobium* infections.

The World Health Organisation (WHO) recommends preventive chemotherapy (PC) through periodic mass drug administration (MDA) of anthelmintic praziquantel (PZQ) as an appropriate measure for morbidity control, targeting at least 75% of school-aged children (SAC) in all schistosomiasis-endemic countries by 2020. In addition to PC, other recommended strategies include strengthening health systems, improving water and sanitation, hygiene education and snail control (WHO, 2012). Apart from SAC, other people considered for PC include adults at-risk such as fishermen, farmers and women in contact with infested waters, pre-school aged children (Pre-SAC) as well as entire communities in high-risk areas (WHO, 2006). As of 2017, 98.7 million people received PC globally, with the African region where 90.4% of those requiring PC live, treating 75 million SAC and 12.4 million adults. Renewed commitments in accomplishing schistosomiasis control have been highlighted by various key stakeholders in NTDs.

Despite the tremendous progress in delivery of PZQ treatments in endemic areas, the burden of schistosomiasis remains high with most people suffering the chronic consequences of the disease. These chronic consequences usually develop insidiously during childhood and present in later years of life as illustrated in Figure 52 (Bustinduy *et al.*, 2014). In *S. haematobium* infection causing urogenital disease of which its complications have been described in literature, genital consequences have been understudied, and its epidemiology has been ignored in the recent decades (Stecher *et al.*, 2015).

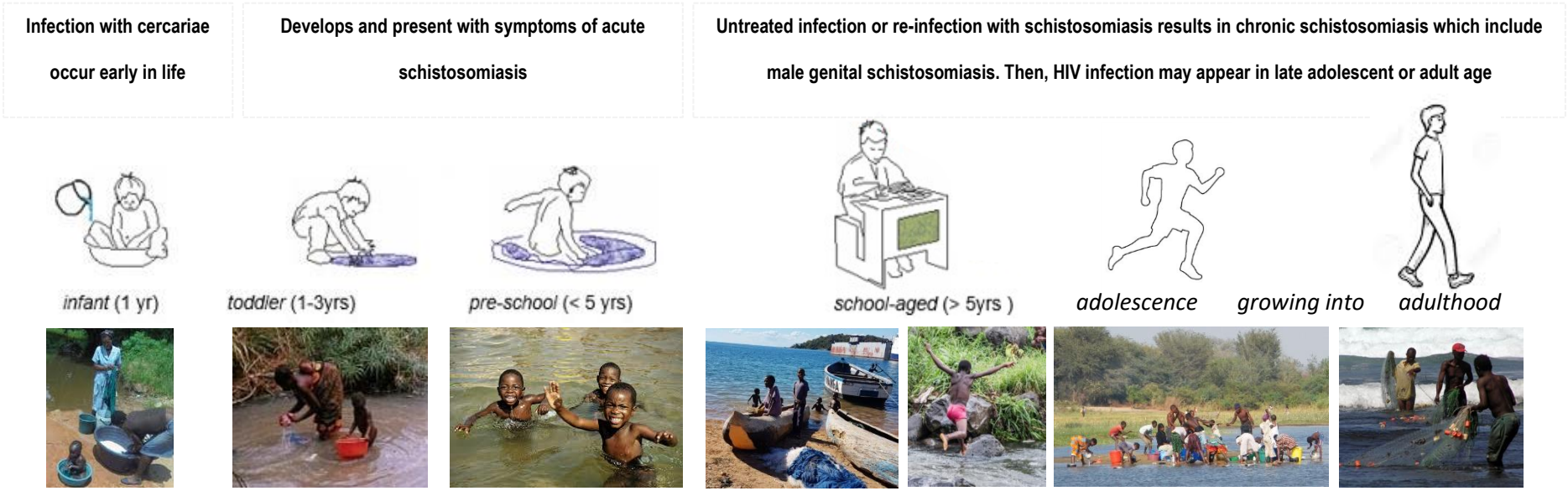


Figure 52: Stages of schistosomiasis disease from infection with schistosome cercariae to chronic consequences and HIV interplay

Photo credits: Dr Sekeleghe Kayuni, LSTM, June 2018.

Among such chronic genital consequences, male genital schistosomiasis remains the most unknown, unrecognised and undertreated manifestation of chronic urogenital schistosomiasis (UGS), unlike female genital schistosomiasis (FGS) which has received increased attention (Kjetland, Leutscher and Ndhlovu, 2012; Christinet *et al.*, 2016). This arises from undefined standard method for diagnosing MGS which result in under- and misdiagnosis as sexually transmitted infections (STIs) which are also prevalent in most schistosomiasis-endemic areas especially among young sexually active men.

On the other hand, there have been advances in describing the definite standard tool and diagnostic algorithm for FGS (WHO, 2015a). By contrast, wet mount semen microscopy is the method commonly used for diagnosing MGS, however due to the logistical challenges with semen submission and handling, urine microscopy has been used as proxy method instead, which poses challenges with regards to its diagnostic sensitivity and specificity.

This longitudinal cohort study on MGS which was conducted among local fishermen from a schistosomiasis-endemic area, along the south shoreline of Lake Malawi in Mangochi District, observed that 36 study participants had urine-patency *S. haematobium* eggs, giving a baseline prevalence of 17.1% for UGS. Also, twelve participants had *S. haematobium* eggs in semen, thus 10.4% prevalence for MGS. This highlights the existence of MGS in local male inhabitants of an endemic area in Malawi which has never been reported previously, at similar level as that of UGS.

Furthermore, it was noted that a substantial proportion of participants (66.7%) had schistosome eggs in semen only but none in urine, which illustrate the possible locations of the schistosome worms in blood vessels surrounding genital organs like prostate, seminal vesicles, testes and scrotum. This also demonstrates that using urine filtration as a proxy technique in diagnosing of MGS is challenged by limited sensitivity and specificity as this will not detect all infected people. As such, there is need for more research to aid in developing better diagnostic tools (Kayuni *et al.*, 2019b).

In addition, the classical symptoms of MGS in literature were reported by less than 5% of participants and none of them had schistosome eggs in semen. This shows that local people in endemic areas with MGS do not commonly present with symptoms, which would likely result in underdiagnosis or mis-diagnosed as STIs (Ukwandu and Nmorsi, 2004; Yirenya-Tawiah, Ackumey and Bosompem, 2016). However, some of those with symptoms had schistosome eggs in urine, which highlights the underlying infection and unspecific symptomatology of MGS.

Although *S. mansoni* eggs which cause intestinal schistosomiasis were not detected in semen of our study cohort as shown in previous studies and literature (Adisa *et al.*, 2012; Lima *et al.*, 2017; Abdel-Naser *et al.*, 2018b), 3.8% of the study participants submitting urine had a positive POC-CCA test, showing possible intestinal schistosomiasis infection. Although stool Kato-Katz analysis done at subsequent follow-up time-points did not find any *S. mansoni* eggs, *Biomphalaria pfeifferi* snails were found on the shoreline in the study area, together with autochthonous transmission of intestinal schistosomiasis (Alharbi *et al.*, 2019). This elucidates the possibility that *S. mansoni* infection may also contribute to MGS in men, located in the vicinity of the transmission spots. It is also worthy to note that *S. mansoni*-associated MGS usually occurs as co-infection with urogenital *S. haematobium* (Kayuni *et al.,* 2019a).

This study presented novel results from longitudinal analyses of seminal samples using molecular real-time polymerase chain reaction (PCR) in a local at-risk male population in an endemic area of SSA. The prevalence of MGS with the highly sensitive diagnostic technique increased by more than 2.5 times from 10.4% by semen microscopy to 26.5% using real-time PCR, showing that microscopy suffer from lower sensitivity and specificity as it misses out more infected men in the area.

Interesting, some participants who had schistosome eggs in semen were noted to be negative on real-time PCR people, alluding to possibility of excretion of dead eggs or egg shells into urine and semen which could not be leaking schistosome DNA to be detected by PCR, thereby picking up active MGS infection, rather than chronic, controlled infection by host immunity, which is

established in adulthood. Although, this discrepancy could also arise from other components in the semen acting as inhibitors, thereby affecting the performance and depicting as a limiting factor for this molecular technique in diagnosis of MGS, it was observed that our real-time PCR did not suffer any inhibition during the analyses.

Therefore, molecular techniques have a critical role to play in diagnosis of MGS where available, emphasizing the need for development of more accessible and affordable sensitive tools, such as point-of-care dipstick tests, utilising the relevant background scientific knowledge. At present, no single method can diagnose all possible cases of MGS, hence a combination of disease symptomatology, clinical findings of examination and current readily available methods is needed as proposed in a structured algorithm which will help in improving the diagnosis of MGS, as illustrated in Figure 53.

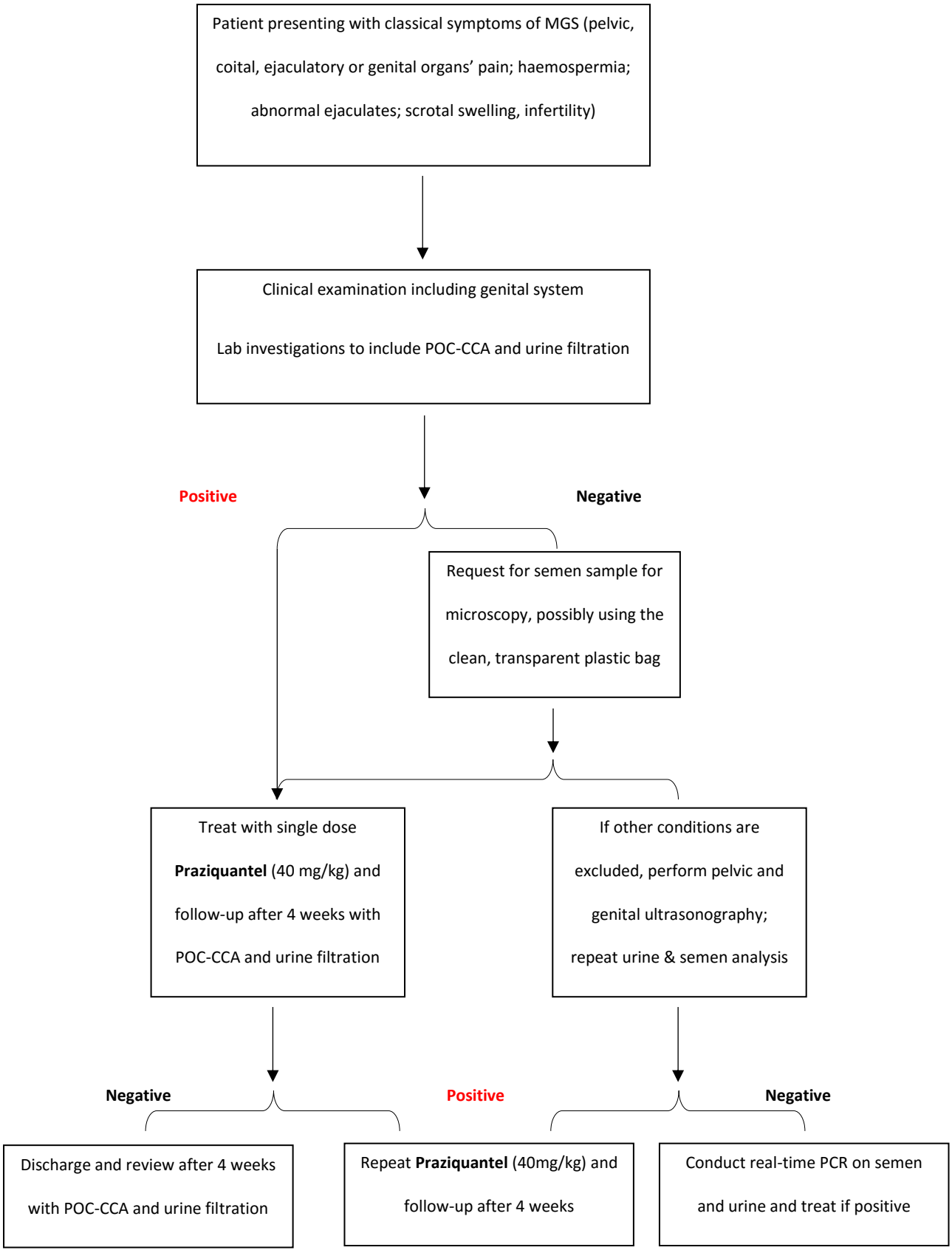


Figure 53: Suggested algorithm for investigating and managing a suspected MGS patient

9.3. Morbidity and treatment of MGS

Chronic schistosomiasis such as MGS is associated with pathological lesions in various genital organs in infected men, arising from the inflammatory reactions, granulomata, fibrosis and calcifications. This is an on-going process which will arise from younger age progressing into later years of life, if no PZQ treatment is provided. These lesions have been reported to be detectable using radiological techniques such as ultrasonography (US), computerised tomography (CT) or magnetic resonance imaging (MRI) (Richter *et al.*, 1996; Richens, 2004; Ramarakoto *et al.*, 2008; Shebel *et al.*, 2012).

Specialised US such as transrectal US (TRUS) have been shown to be essential in definitively describe genital lesions associated with diseases affecting genital organs including tuberculosis, prostatitis, benign prostatic hyperplasia, malignancy, MGS among others. However, in rural endemic areas, portable transabdominal USS are seen to be useful in eliciting pathological lesions arising in genital organs from schistosomiasis.

This cohort study utilised a portable Chison Q5 scanner with acceptable convex 3.5 MHz probe to perform transabdominal and scrotal ultrasonography on the consenting participants. At baseline, nine participants (6.9%) were observed to have abnormalities in prostate, seminal vesicles, epididymis and testes. The observed abnormalities which could suggest MGS include grossly enlarged prostates (3 with one having nodule), enlarged hyperechoic seminal vesicles (3), testicular nodule (1) while four had hydrocele which could arise from other infections. Excluding the four with hydroceles, four of these 5 participants had no schistosome eggs in urine or semen and were negative for semen real-time PCR. Three of the four who had hydrocele were also negative for MGS. Two of the 9 participants only reported experiencing pain of their genital organs in the month preceding the onset of the study, among the possible classical symptoms associated with MGS including haemospermia, coital or ejaculatory pain.

These baseline findings illustrate that transabdominal and scrotal ultrasonography plays an important role in identifying genital lesions arising from MGS, as more diagnostic tests-negative

participants were observed to have such lesions. Radiological examination provided at first healthcare facility in the rural endemic area such as portable US scanner can contribute to improving detection of MGS. Furthermore, other possible diseases were excluded during the study from individual questionnaire interviews in lack of constitutional symptoms of diseases like tuberculosis, diagnostic tests such as prostatic specific antigen which were negative as well as long-term follow during the 12-months cohort study.

Interestingly, follow-up of study participants revealed new lesions in previously normal participants, resolution of previous lesions as well as persistent lesions, which have also been described in published manuscript in Appendix 23 (Kayuni *et al.*, 2019c). This was associated with clearance of infection due to PZQ which was provided at each time-point, especially at 1-month follow-up where none of the participants had schistosome eggs detected in semen but resurfaced at the subsequent time-points. Also, those chronic pathological lesions such as hyperechoic vesicles or hydroceles can be irreversible and not respond to PZQ treatment, let alone standard single dose, which explains the persistence in some participants. Such participants require additional medical management procedures such as surgical drainage of hydroceles to correct the pathology.

Re-infections arising from re-exposure to same infested water bodies could contribute to new lesions developing despite previous treatment. This echoes the need for comprehensive control strategies including water, sanitation and health interventions which advocate for access to adequate, safe and clean water to deter people from re-exposing themselves to cercariae-infested waters. Another intervention is more involvement of men especially those at high-risk for schistosomiasis infection like fishermen in PC during MDA with PZQ. Malawi does conduct regular annual MDA campaigns which also targets adults in high risk areas such as along shoreline of Lake Malawi.

However, from this study 34.8% of participants accessed PZQ treatment in the last 12 months at baseline, with 61.7% reporting experiencing difficulties in accessing the treatment. There is need to elicit more information to understand factors contributing to this low access of PZQ in

men, and devise interventions to include men in this PC with MDA program. In addition, increasing the frequency of MDA especially in high-risk areas to all populations could be a way forward in arresting this infection and control its morbidity while introducing other strategies like snail control.

Although transabdominal ultrasonography is a non-invasive diagnostic tool in schistosomiasis, it is neither sufficiently sensitive nor specific in detecting morbidity lesions of MGS as observed in this longitudinal cohort study as compared to transrectal ultrasonography, CT scanning and MRI. From this study, it remains unproven as a method for securing a clinical diagnosis for assessing prostate and seminal vesicles, affecting sensitivity, despite being only feasible method where available in this setting.

Furthermore, the WHO developed practical guide to standardise use of ultrasonography for assessment of schistosomiasis-related morbidity, known as the 'Ultrasound in Schistosomiasis Niamey protocol' over two decades ago (WHO, 2000). However, this protocol in addition to recent manual of diagnostic ultrasound (WHO, 2011) does not provide detailed guidelines and descriptions of morbidity associated with genital schistosomiasis especially MGS. With regards to our novel findings on MGS lesions in genital organs, which resolve with PZQ treatment, updated standard guidelines on genital schistosomiasis is being advocated to aid in diagnosis of parasitological negative but at-risk people in rural and peri-urban schistosomiasis-endemic areas.

9.4. HIV and MGS co-infection

Co-infections of HIV and schistosomiasis have been seldomly researched in endemic areas despite the two sharing geographical overlap in their epidemiology (Ndeffo Mbah *et al.*, 2013). The risk of HIV infections has been observed to increase in women suffering from genital schistosomiasis by 2-3 fold , arising from mucosal structural breach which necessitate easier viral entry, inflammations triggering increased CD4+ receptor cells to the genital regions thereby increasing viral uptake in addition abnormal vasculature formation which increase blood flow and cells (Kjetland *et al.*, 2006; Downs *et al.*, 2011; Jourdan *et al.*, 2011). On the other, schistosomiasis in men has been associated with some increased risk of HIV transmission through elevated inflammatory cells and cytokines which could increase viral load (VL) in semen (Leutscher *et al.*, 2005; Leutscher *et al.*, 2008b; Downs *et al.*, 2017a). In addition, men with both infections have been noted to have lower CD4+ cells levels as well as higher viral load which improve after PZQ treatment (Kallestrup *et al.*, 2005a; Midzi *et al.*, 2017).

Although, this evidence illustrates the critical role of schistosomiasis treatment on HIV disease progression and control, other studies have shown no beneficial or adverse effects of PZQ treatment on HIV infection, resulting in raising further reduction of CD4+ cells and increasing VL (Lawn *et al.*, 2000). This has energised the need for more studies to understand the interactions between coinfections of HIV and MGS and impact of regular PZQ treatment and PC in at-risk men of endemic areas.

Our longitudinal study recruited participants co-infected with HIV infections and active schistosomiasis especially MGS as cases and only HIV infections as controls in a pilot design due to the lack of previous studies and limited resources. At baseline, more study cases were observed to have discrepant detectable and quantifiable HIV VL in blood plasma which was higher than control participants. This suggest that the study participants had a larger number of viral copies in circulation which could means higher possibility of transmitting HIV to their sexual partners. Although these study cases had no detectable VL in semen, it is widely recognised that detectable

blood plasma VL is associated with increased HIV infectiousness and transmission. In this study, the cases with detectable VL were noted to have MGS as well, which allude to the point that despite the effective action of combined highly-active ART in suppressing HIV, schistosomiasis could have had resilient systemic immunomodulatory effect, compromising their effect.

Furthermore, after providing PZQ treatment, follow-up showed some clearing of MGS infection as well as downward trend in the VL of those participants which supported the existing evidence of the important role played by this antihelminth in helping supress the virus, in addition to other factors like ART adherence. Antihelminth treatment have been advocated to co-infected with HIV and helminth in order to aid in managing the HIV disease, as such regular PZQ treatment through PC should be given at least twice a year to men and women on ART in schistosomiasis-endemic areas. This should be provided alongside intermittent treatment where symptoms of schistosomiasis or MGS present or diagnosis of infection is made, in order to reduce the possibility of detectable viruses.

The effects of active and past schistosome infections on HIV infection dynamics with regards to viral suppression in era of ART remain unknown. In this study, using results of two serological diagnostic tests, there was no direct association with detectable VL in the study cases although two of the 3 study controls with detectable blood plasma VL were seropositive at baseline. We considered active infection as a contributor to the immune effect on HIV infections.

Re-infection arising from re-exposure to same cercariae infested waters could result in blips or rebound detectable VL after successful viral suppression, as observed in some study cases on long -term follow-up of 3-, 6- and 12-months' time-points. Repeated treatments as well as other schistosomiasis control strategies described earlier could be useful in preventing these rebound VL as this propagate viral mutations and development of resistant viral copies which could not be completely suppressed as observed in one study case who remained unsuppressed despite being on treatment. Coordinating and synergizing control strategies of both infections can contribute towards

morbidity control of schistosomiasis, maintain viral suppression and arrest HIV disease progression, since co-infections can occur earlier in life (Figure 54).

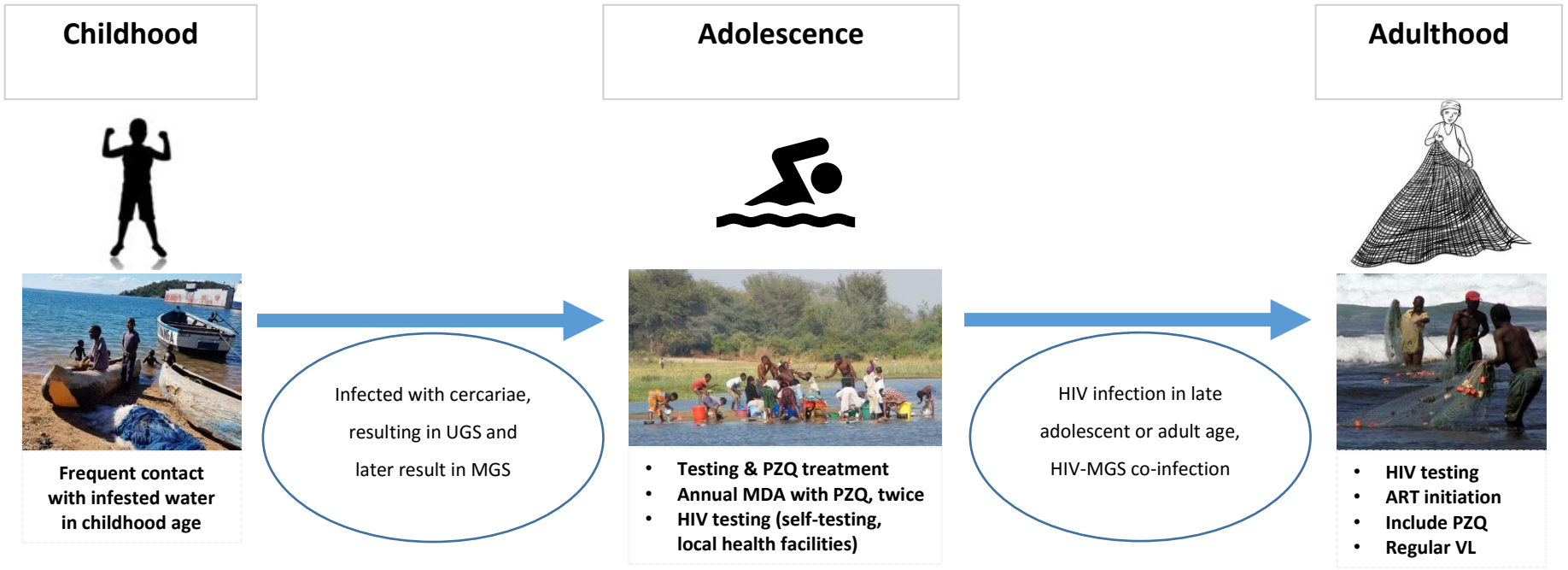


Figure 54: HIV and MGS co-infection and suggested management of an infected fishermen

Health education, community awareness and self-help campaigns of both HIV and schistosomiasis should be provided at all stages of life.

Photos credit: Dr Sekeleghe Kayuni, LSTM, June 2018.

9.5. Future work

As highlighted earlier, this longitudinal cohort study had limitations of sample size, affecting the power of generalising its results to the male population in the region. However, the novel findings in this study advocate for further multi-site research study in deeper understanding of the pathogenesis of the MGS which will help in identifying specific key components including proteins, glycans in blood, urine or semen which could be useful in development of sensitive and specific point-of-care diagnostic tool, to be accessible and available in endemic areas.

In addition, further research on point-of-care radiological diagnosis of MGS using accessible portable tools such android ultrasonography gadgets would be helpful to elicit pathological lesions earlier and drive earlier adequate treatment to clear the infection and achieve resolution of the lesions. This will aid in updating the available standard guidelines for ultrasonographic examination of MGS which is lacking despite huge advances in similar guidelines on FGS and acceptable Niamey protocol for urogenital and intestinal schistosomiasis where genital schistosomiasis in not included.

Treatment dosing of PZQ in MGS need to be explored further to define the optimal dosage for treating acute cases of MGS, in order to prevent and resolve early pathologies elicited by the schistosomes. Also, there is need to assess whether the determined dose could prevent development of MGS after re-infections with cercariae.

Furthermore, there is need to research psychosocial dynamics of MGS among at-risk and infected men, social determinants and economic implications in the communities in endemic areas, community perceptions on health with regards to MGS as well as FGS, applicable prevention and control strategies to the infection, and ascertain the best model on combined comprehensive strategies for managing both genital schistosome infections.

In relation with HIV infection, there is need for a larger, prospective study to validate the observations in our novel pilot study on MGS potentially impacting the HIV-1 shedding in men's plasma. Also, there is need to study further on the natural history of both infections, role of

preventive antihelminth treatments in HIV control by maintaining viral suppression by antiretroviral treatment, best time and frequency for PZQ treatment in prevention and / or resolution of early MGS morbidity and worsening especially in view of newer ART regimens like dolutegravir combined regimens recently incorporated in SSA countries like Malawi. Also, social determinants and community understanding of both infections and devise management approach form community to health facility level.

Finally, developing educational tools for training for key health professionals and raising more awareness on MGS especially in endemic areas is very critical in its control and management.

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Appendices

Appendix 1: Ethical clearance from LSTM REC

Dr Sekeleghe Kayuni
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
L3 5QA

LSTM
LIVERPOOL SCHOOL OF TROPICAL MEDICINE
Pembroke Place,
Liverpool, L3 5QA, UK
Tel: +44(0)151 705 3100
Fax: +44(0)151 705 3370
www.lstmed.ac.uk

Monday, 31 July 2017

Dear Dr Kayuni,

Re. Research Protocol (17-018) Multidisciplinary studies on Male Genital Schistosomiasis (MGS): Its prevalence, morbidity and management and interactions with HIV viral shedding among adult fisherman along Lake Malawi shores in Mangochi, Malawi

Thank you for your letter of 28 July 2017 providing the necessary in-country approvals for this project. I can confirm that the protocol now has formal ethical approval from the LSTM Research Ethics Committee.

The approval is for a fixed period of three years and will therefore expire on 30 July 2020. The Committee may suspend or withdraw ethical approval at any time if appropriate.

Approval is conditional upon:

- Continued adherence to all in-country ethical requirements.
- Notification of all amendments to the protocol for approval before implementation.
- Notification of when the project actually starts.
- Provision of an annual update to the Committee.
  Failure to do so could result in suspension of the study without further notice.
- Reporting of new information relevant to patient safety to the Committee
- Provision of Data Monitoring Committee reports (if applicable) to the Committee

Failure to comply with these requirements is a breach of the LSTM Research Code of Conduct and will result in withdrawal of approval and may lead to disciplinary action. The Committee would also like to receive copies of the final report once the study is completed. Please quote your Ethics Reference number with all correspondence.

Yours sincerely

Dr Angela Obasi
Chair
LSTM Research Ethics Committee

Appendix 2: Letter of Research sponsorship from LSTM REC

Dr Sekeleghe Kayuni
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
L3 5QA

LSTM
LIVERPOOL SCHOOL OF TROPICAL MEDICINE
Pembroke Place,
Liverpool, L3 5QA, UK
Tel: +44(0)151 705 3100
Fax: +44(0)151 705 3370
www.lstmed.ac.uk

Monday, 31 July 2017

Dear Dr Kayuni,

Re. Research Protocol (17-018) 'Multidisciplinary studies on Male Genital Schistosomiasis (MGS): Its prevalence, morbidity and management and interactions with HIV viral shedding among adult fisherman along Lake Malawi shores in Mangochi, Malawi'

I am pleased to confirm that LSTM has agreed to act as Sponsor for the above mentioned clinical research study.

Please note that LSTM approval to allow your study to proceed is conditional upon compliance with the relevant regulatory requirements.

All study staff should be given the appropriate training in Protocol, GCP, Consent and Data Protection, relevant to their responsibilities as defined within the study protocol.

LSTM Research Office should receive annual study progress and final close out reports via lstmgov@lstmed.ac.uk

Yours Sincerely,

Carl Henry
Research Governance Manager
Research Governance and Ethics Office

Appendix 3: Ethical clearance from NHSRC (2017-18 Approval)

Telephone: +265 789 400
Facsimile: +265 789 431

All Communications should be addressed to:

The Secretary for Health and Population

In reply please quote No.

MINISTRY OF HEALTH AND POPULATION
P.O. BOX 30377
LILONGWE 3
MALAWI

25th July, 2017

Sekeleghe Kayuni
University of Liverpool
UK

Dear Sir,

RE: PROTOCOL # 17/05/1805: MULTIDISCIPLINARY STUDIES ON MALE GENITAL SCHISTOSOMIASIS (MGS): ITS PREVALENCE, MORBIDITY AND MANAGEMENT AND INTERACTIONS WITH HIV VIRAL SHEDDING AMONG ADULT FISHERMAN ALONG LAKE MALAWI SHORES IN MANGOCHI, MALAWI

Thank you for the above titled proposal that you submitted to the National Health Sciences Research Committee (NHSRC) for review. Please be advised that the NHSRC has **reviewed** and **approved** your application to conduct the above titled study.

- **APPROVAL NUMBER : 1805**
- The above details should be used on all correspondences, consent forms and documents as appropriate.
- **APPROVAL DATE : 25/07/2017**
- **EXPIRATION DATE**
  This approval expires on **24/07/2018**. After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the NHSRC Secretariat should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING:** All serious problems having to do with subject safety must be reported to the NHSRC within 10 working days using standard forms obtainable from the NHSRC Secretariat.
- **MODIFICATIONS**: Prior NHSRC approval using forms obtainable from the NHSRC Secretariat is required before implementing any changes in the protocol (including changes in the consent documents). You may not use any other consent documents besides those approved by the NHSRC.
- **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the NHSRC using standard forms obtainable from the NHSRC Secretariat.
- **QUESTIONS**: Please contact the NHSRC on phone number *+265 888 344 443* or by email on mohdoccentre@gmail.com.
- **OTHER**: Please be reminded to send in copies of your final research results for our records (Health Research Database).

Kind regards from the NHSRC Secretariat

SECRETARY FOR HEALTH
2017 -07- 25
P.O. Box 30377, CAPITAL
LILONGWE 3

For: **CHAIRPERSON, NATIONAL HEALTH SCIENCES RESEARCH COMMITTEE**
Promoting Ethical Conduct of Research

Appendix 4: Ethical clearance from NHSRC (2018-19 renewal)

Telephone: + 265 789 400
Facsimile: + 265 789 431

All Communications should be addressed to:

The Secretary for Health and Population

In reply please quote No.

.....

MINISTRY OF HEALTH AND POPULATION
P.O. BOX 30377
LILONGWE 3
MALAWI

22nd July, 2018

Sekeleghe Kayuni
University of Liverpool
UK

Dear Sir,

Re: Protocol # 17/05/1805: Multidisciplinary Studies On Male Genital Schistosomiasis (Mgs): Its Prevalence, Morbidity And Management And Interactions With HIV Viral Shedding Among Adult Fisherman Along Lake Malawi Shores In Mangochi, Malawi

Thank you for the above titled proposal that you submitted to the National Health Sciences Research Committee (NHSRC) for review. Please be advised that the NHSRC has **reviewed** and **approved** your application for continuation of the above titled study.

- **APPROVAL NUMBER : 1805**
- The above details should be used on all correspondences, consent forms and documents as appropriate.
- **APPROVAL DATE : 23/07/2018**
- **EXPIRATION DATE**
  This approval expires on **22/07/2019**. After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the NHSRC Secretariat should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING:** All serious problems having to do with subject safety must be reported to the NHSRC within 10 working days using standard forms obtainable from the NHSRC Secretariat.
- **MODIFICATIONS:** Prior NHSRC approval using forms obtainable from the NHSRC Secretariat is required before implementing any changes in the protocol (including changes in the consent documents). You may not use any other consent documents besides those approved by the NHSRC.
- **TERMINATION OF STUDY:** On termination of a study, a report has to be submitted to the NHSRC using standard forms obtainable from the NHSRC Secretariat.
- **QUESTIONS:** Please contact the NHSRC on phone number *+265 888 344 443* or by email on mohdoccentre@gmail.com.
- **OTHER:** Please be reminded to send in copies of your final research results for our records (Health Research Database).

SECRETARY FOR HEALTH
2018 -07- 22
P.O BOX 30377, CAPITAL
LILONGWE 3

Kind regards from the NHSRC Secretariat.

.....
For: **CHAIRPERSON, NATIONAL HEALTH SCIENCES RESEARCH COMMITTEE**

Promoting Ethical Conduct of Research

Executive Committee: Dr B. Chilima (Chairperson), Dr B. Ngwira (Vice-Chairperson)
Registered with the USA Office for Human Research Protections (OHRP) as an International IRBIRB Number IRB00003905 FWA00005976

Appendix 5: Ethical clearance from NHSRC (2019-20 renewal)

Telephone: + 265 789 400
Facsimile: + 265 789 431

All Communications should be addressed to:

The Secretary for Health and Population

In reply please quote No. .....
MINISTRY OF HEALTH AND POPULATION
P.O. BOX 30377
LILONGWE 3
MALAWI

09th August, 2019

Dr. Sekeleghe Kayuni
University Liverpool and Liverpool school of tropical Medicine

Dear Sir/Madam,

Protocol # 17/05/1805: Multidisciplinary studies in male genital Schistosomiasis (MGS): its prevalence, morbidity and management and interactions with HIV viral shedding among fishermen along Lake Malawi shores in Mangochi, Malawi

Thank you for the above titled proposal that you submitted to the National Health Sciences Research Committee (NHSRC) for review. Please be advised that the NHSRC has **reviewed** and **approved** continuation of the above named study.

- **APPROVAL NUMBER : 1805**
- The above details should be used on all correspondences, consent forms and documents as appropriate.
- **APPROVAL DATE : 09/08/2019**
- **EXPIRATION DATE**
  This approval expires on **08/08/2020**. After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the NHSRC Secretariat should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING:** All serious problems having to do with subject safety must be reported to the NHSRC within 10 working days using standard forms obtainable from the NHSRC Secretariat.
- **MODIFICATIONS**: Prior NHSRC approval using forms obtainable from the NHSRC Secretariat is required before implementing any changes in the protocol (including changes in the consent documents). You may not use any other consent documents besides those approved by the NHSRC.
- **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the NHSRC using standard forms obtainable from the NHSRC Secretariat.
- **QUESTIONS**: Please contact the NHSRC on phone number +265 999397913 or by email on mohdoccentre@gmail.com.
- **OTHER**: Please be reminded to send in copies of your final research results for our records (Health Research Database).

Kind regards from the NHSRC Secretariat.

SECRETARY FOR HEALTH
2019 -08- 09
P.O. BOX 30377, CAPITAL CITY, LILONGWE 3

For: **CHAIRPERSON, NATIONAL HEALTH SCIENCES RESEARCH COMMITTEE**

Promoting Ethical Conduct of Research

Appendix 6: Participant information sheet for the MGS research study

Research Study of Male Genital Schistosomiasis among fishermen along shores of Lake Malawi

I am Seke Kayuni.

I would like to find out the level of genital schistosomiasis infection and possible HIV interactions in fishermen along Lake Malawi.

Schistosomiasis is an infection caused by flatworms, present in parts of Malawi especially along Lake Malawi. HIV is a virus which cause AIDS, affecting people and causing deaths in Malawi. Some people can be infected with the two diseases at the same time.

I will be working with fishermen in the area, to test for genital schistosomiasis in those HIV positive or negative. You are being requested to take part in the Study. Your participation in this study is voluntary.

What will you do?

You will be asked questions about your health, knowledge and practices related to schistosomiasis and HIV. You will be requested to provide urine and semen samples for testing of the genital schistosomiasis at the health centre and LSTM laboratory in the UK.

You will also be offered an ultrasound scan to see any effect of the schistosomiasis infection on your internal genital organs. You will be informed about the results and offered Praziquantel treatment for schistosomiasis.

Are there any costs of the Study?

The tests and treatment will be provided free-of-charge.

There will be no payment provided for participating in this study.

What are the benefits and risks of the Study?

The information you will provide, and the tests results are important in assisting the Ministry of Health and other organisations in improving treatment and control of schistosomiasis and HIV/AIDS.

There is no risk or harm in taking part in the Study and you can opt-out from the Study at any time.

Your name, answers and test results will be treated with privacy and confidentiality, will not be given to people not involved in the Study or medical care.

Participation in this Study will be of great value to the healthcare delivery in Malawi.

Further Information

You can get more information about the Study, your rights or to report any harm or injury because of participating in this Study, from the following:

District Health Officer, Mangochi District Hospital, P.O. Box, Mangochi.

Dr. Sekeleghe Kayuni (+265 995 645 647)

Appendix 7: Participant information sheet for the MGS research study (in Chichewa)

Kafukufuku Wofuna Kuona Chiwerengero Cha Asodzi Amene Ali Ndi Likodzo Okhala M'bali Mwa Nyanja Ya Malawi.

Ine dzina langa ndi Seke Kayuni.

Ine ndikupanga kafukufuku ofuna kuona kuchuluka kwa matenda a Likodzo yokhuza njira yaumuna ndi EDZI mwa asodzi okhala m'madera oyandikana ndi Nyanja ya Malawi. Likodzo ndi matenda amene amayamba ndi tinyongolotsi timene tili m'madera ena mu Malawi, kwambiri mbali mwa Nyanja ya Malawi. HIV ndi kachilombo koyambitsa matenda a EDZI amene afalikira mu Malawi. Matenda awiriwa amapezeka pamodzi ndi anthu nthawi zambiri.

Ine ndipanga Kafukufuku ndi asodzi omwe ali m'mderali, ndikuyeza za matenda a likodzo. Muli kufunsidwa kutenga nawo mbali pa Kafukufukuyu.

Kodi ndidzapanga chiani?

Inu mudzafunsidwa mafunso okhudza za umoyo wanu ndi zomwe mumadziwa za matenda a likodzo ndi EDZI. Kenako mudzapereka mkodzo ndi umuna kuti ukayedzedwe. Mudzafunsidwanso kujambulidwa ziwalo zachimuna poona za matendawa. Pamapeto mudzapatsidwa zotsatira ndi mankhwala.

Kodi pali mtengo pa Kafukufukuyu

Kuyezedwa ndi mankhwala ziperekedwa mwaulele.

Ndipo simulandira malipiro potenga mbali pa Kafukufukuyu.

Kodi pali mphindu kapena chiopsezo potenga mbali pa Kafukufukuyu

Zomwe munganene pakafukufukuyu zidzathandiza unduna wa za Umoyo ndi mabungwe kupititsa patsogolo chithandizo cha mankhwala ndi kuchepetsa Likodzo ndi EDZI.

Palibe choopsya chilichonse potenga nawo mbali pa Kafukufukuyu ndipo mutha kusiya kupanga nawo nthawi ina iliyonse.

Dzina lanu, mayankho ndi zotsatira za kuyezedwa kwanu zidzasungidwa mwachinsinsi ndipo sidzizaperekedwa kwa anthu omwe sakupanga nawo Kafukufukuyu kapena opereka chithandizo cha chipatala.

Kutenga mbali pa Kafukufukuyu kuthandiza kutukula ntchito za umoyo mu Malawi.

Zambiri za Kafukufukuyu

Ngati mukufuna kudziwa zambiri za Kafukufukuyu, ufulu wanu kapena kupereka chidandaulo mukutenga mbali, mutha kutero podziwitsa:

District Health Officer, Mangochi District Hospital, P.O. Box, Mangochi.

Dr. Sekeleghe Kayuni (+265 995 645 647)

Appendix 8: Participant written informed consent form

RESEARCH STUDY OF MALE GENITAL SCHISTOSOMIASIS AMONG ADULT FISHERMEN ALONG THE SOUTH SHORES OF LAKE MALAWI.

Principal Investigator: Dr Sekeleghe Kayuni (LSTM), MASM, P.O. Box 1254, Blantyre. +265 995645647

National Health Sciences Research Committee, Ministry of Health, P.O. Box 30377, Lilongwe 3.

Introduction: Schistosomiasis (snail-borne disease) and AIDS (caused by a virus called HIV) are diseases which affect most people in Malawi, living along Lake Malawi.

Purpose: This research study will look at the level of genital schistosomiasis and the effects of both diseases in adult fishermen males along Lake Malawi.

Procedure: Individual questionnaire interviews will be done to find out about your health and issues about schistosomiasis and HIV. Urine and semen will be requested to test for genital schistosomiasis. You'll be requested further if there will be needed to submit blood. Ultrasound scanning will be offered to you to look at your genital organs for effects of the infection. Some samples will be tested outside Malawi to get the results. You will be informed about the results and offered Praziquantel treatment.

Benefits and risks: The information collected, and tests results are important in assisting the Ministry of Health and other organisations in improving treatment and control of schistosomiasis and HIV/AIDS. Participation in this Study will be of great value to the healthcare delivery in Malawi. There is no risk or harm in taking part in the Study and you can opt-out from the Study at any time.

Privacy and confidentiality: You will be given a unique identifier (number) at the start of the study which will be used instead of your name. Your name, answers, testing including blood analysis and results will be treated with privacy and confidentiality, will not be given to people not involved in the Study or medical care.

Study Approval:

National Health Sciences Research Committee, Ministry of Health, P.O. Box 30377, Lilongwe 3. Malawi. Approval number: 1805

Liverpool School of Tropical Medicine, Research Ethics Committee, Pembroke Place, Liverpool L3 5QA, Merseyside, United Kingdom. Approval number: 17-018

I ..... have read the information leaflet which explains about schistosomiasis, what you are trying to find out, how you will find out and why you would like to talk to me.

Please mark in the box if you think the statement is true:

- I have asked all the questions I needed to and am happy with answers given ☐
- I understand that you will not tell anyone what I have told you ☐
- I allow you to write about what I have said and not using my real name ☐
- I understand that I don't have to answer questions that I don't want to talk about ☐
- I know that I can stop my participation at any time and without giving a reason ☐
- I understand that you will test my samples to a laboratory in the UK to get my results ☐
- I understand that I can get my test results and treatment where necessary if I want ☐
- I understand that I can look at the report for this study if I want to ☐
- **I would like to take part in the Study. I can still change my mind any time** ☐

My questions have been answered by .....

Participant (name in BLOCK CAPITALS) .....

Signed .....Date.....

Researcher (name in BLOCK CAPITALS) .....

Signed .....Date.....

THANK YOU VERY MUCH FOR YOUR PARTICIPATION.

Appendix 9: Participant written informed consent form (in chichewa)

KAFUKUFUKU WOFUNA KUONA CHIWERENGELO CHA MATENDA A LIKODZO MWA AMUNA OKHALA M'BALI YA NYANJA YA MALAWI.

Principal Investigator: Dr Sekeleghe Kayuni (LSTM), MASM, P.O. Box 1254, Blantyre. +265 995645647

National Health Sciences Research Committee, Ministry of Health, P.O. Box 30377, Lilongwe 3.

Mau oyamba: Likodzo ndi EDZI ndi matenda omwe amakhudza anthu ambiri m'madera a mbali mwa Nyanja ya Malawi.

Cholinga cha kafukufukuyu: Kafukufukuyu akufuna kupeza kuchuluka kwa matenda aLikodzo pakati pa asodzi m'mderali, ndikuona zokhuzana ndi matenda a EDZI.

Dongosolo la kafukufukuyu: mudzafunsidwa mafunso okhudza za umoyo wanu ndi zomwe mumadziwa za matenda a likodzo ndi EDZI. Kenako mudzapereka mkodzo ndi umuna kuti ukayedzedwe, kwa ena, muzafunsidwanso kepereka ndi magazi. Kuyezedwa kwina kudzapangidwa kunja kwa Malawi pofuna kupeza zotsatira zanu. Mudzafunsidwanso kuuninikidwa ziwalo zachimuna poona za matendawa. Pamapeto muzauzidwa zotsatila komanso mudzapatsidwa mankhwala.

Ubwino ndi chiopsezo pa kafukufukuyu: Zomwe munganene pakafukufukuyu zidzathandiza unduna wa za Umoyo ndi mabungwe kupititsa patsogolo chithandizo cha mankhwala ndi kuchepetsa Likodzo ndi EDZI. Kutenga mbali pa Kafukufukuyu kuthandiza kutukula ntchito za umoyo mu Malawi. Palibe choopsya chilichonse potenga nawo mbali pa Kafukufukuyu ndipo mutha kusiya kupanga nawo nthawi ina iliyonse.

Chinsinsi chanu pa kafukufukuyu: Muzapatsidwa nambala poyamba pa kafukufukuyu yomwe izagwiritsidwe mmalo mwa dzina lanu. Dzina lanu, mayankho, kuyezedwa ndi zotsatira zanu zidzasungidwa mwachinsinsi ndipo sizizaperekedwa kwa anthu omwe sakupanga nawo kafukufukuyu.

Mabungwe Opereka Chilolezo cha kafukufukuyu:

National Health Sciences Research Committee, Ministry of Health, P.O. Box 30377, Lilongwe 3. Malawi. Approval number: 1805

Liverpool School of Tropical Medicine, Research Ethics Committee, Pembroke Place, Liverpool L3 5QA, Merseyside, United Kingdom. Approval number: 17-018

Ine ..... ndawerenga chikalata chofotokoza za Likodzo, kafukufuku amene mukupanga, njira zomwe mugwiritse nchito ndi cholinga choyankhula nane. Chongani mu kabokosi ngati mwagwirizana ndi mawu ali m'munsimu:

- Ndafunsa zonse zimene ndinafuna kudziwa ndipo ndakhutira ndi mayankho ndapatsidwa ☐
- Ndamvetsa kuti simuwudza munthu aliyense zomwe ndakuwudzani ☐
- Ndikulolani kulemba zomwe ndanena ndipo musagwiritse dzina langa ☐
- Ndamvetsa kuti nditha osayankha mafunso amene sindikufuna kuyankhulapo ☐
- Ndikudziwa kuti nditha kusiya Kafukufukuyu nthawi iliyonse, osapereka chifukwa ☐
- Ndamvetsa kuti zoyeza zina zikapangidwa kunja kwa Malawi kufuna kupeza zotsatira ☐
- Ndamvetsa kuti nditha kulandira zotsatira za kuyezedwa ndi chithandizo moyenera nditafuna ☐
- Ndamvetsa kuti nditha kuwerenga lipoti la Kafukufukuyu nditafuna ☐
- Ine ndikufuna kutenga nawo mbali pa Kafukufukuyu. Nditha kusintha nthawi iliyonse ☐

Mafunso anga ayankhidwa ndi .....

Wopanga Kafukufuku (DZINA) .....

Saini .....Tsiku.....

Wopangitsa Kafukufuku (DZINA) .....

Saini .....Tsiku.....

ZIKOMO KWAMBIRI POTENGA NAWO MBALI PA KAFUKUFUKUYU.

Appendix 10: Registration form for MGS participants in the study

No.	Name of Participant	Study number	Village	Phone number	Other contacts
		BL/			

Appendix 11: Registration form for HIV participants in the MGS study

No.	Name of Participant	Study number	Village	Phone number	Other contacts
		H/			

Appendix 12: Individual participant questionnaire

Research study on Male genital schistosomiasis and HIV among Adult fishermen living in Southern shores of Lake Malawi (MGS Baseline & Follow-up Survey)

Explanation: Put a tick (√) for the participant's responses appropriately. If the boxes are not enough on one page, use another sheet. Return this and other sheets to the Study Investigator at the end.

Date: (dd/mm/yy): __ __ / __ __ / __ __ Interviewer Name_____

Consent checklist

Has written consent been obtained? Y ☐ N ☐ *Only proceed if Y*

Section A: Participant's details

1. Participant ID: _____
2. Village: _____
3. T/A: _____
4. Name of nearest Health centre: _____
5. Gender: Male ☐ Female ☐.
6. Date of birth (dd/mm/yy): __ __ / __ __ / __ __ Age: _____
7. Born where: _____
8. How long have you been staying here: Years _____ Months: _____ Weeks: _____ Days: _____
9. Weight (kg): _____
10. Height (cm): _____

Section B: General Health Information

Are you experiencing the following symptoms now? In the last month? Put a tick **(√)** for **Yes** on all items mentioned or demonstrated:

	Symptom	**Currently**	**In last month**	**Not in last month**	**Do not know**	**Refused**
11.	Fever					
12.	Headache					
13.	Fatigue					
14.	Abdominal cramping (pain)					
15.	Pain during urination (dysuria)					
16.	Frequency of urination					
17.	Colour of urine					

18.	Blood in urine (haematuria)					
19.	Blood in stool					
20.	Blood in semen					
21.	Pains during and / or after coitus					
22.	Pains on ejaculation					
23.	Pains of the genital organs					
24.	Other (please specify):					

Are you experiencing the following diseases now? In the last month? Put a tick **(√)** for **Yes** on all items mentioned or demonstrated:

	Disease	**Currently**	**In last month**	**Not in last month**	**Don't know**	**Refused**
25.	Malaria					
26.	Diarrhoea					
27.	Dysentery					
28.	Skin disease					
29.	Respiratory disease					
30.	Worm infestation					
31.	Schistosomiasis					
32.	Sexually transmitted infection (STI)					
33.	Other (please specify):					

34. Did you take deworming medicine (albendazole) in the last month? Y ☐ N ☐.

35. Did you take schistosomiasis medicine (praziquantel) in the last month? Y ☐ N ☐.

36. Did you take antimalarial medicine in the last month? Y ☐ N ☐.

37. Did you take any other medicine in the last month? Y ☐ N ☐. If Y, name _____

_____

The following questions are about HIV/AIDS:

38. Did you attend any meetings about health education in the last month? Y ☐ N ☐.

39. Were any of the meetings about HIV/AIDS? Y ☐ N ☐.

40. Do you know what is HIV? Y ☐ N ☐.

41. Explain your answer in question 40._____

_____

42. Do you know what is AIDS? Y ☐ N ☐.

43. Explain your answer in question 42._____

_____

44. Do you know how HIV is transmitted? Y ☐ N ☐.

45. Explain your answer in question 44._____

_____

46. Do you know how HIV is prevented? Y ☐ N ☐.

47. Explain your answer in question 46._____

_____

48. Have you been tested for HIV? Y ☐ N ☐.

49. If Y in question 48, can you tell us the results? Y ☐ N ☐.

If Y in question 49, tell us the HIV test results? Tick **(√)** for the relevant answer below:

50. Positive (+) ☐ .

51. Negative (-) ☐ .

52. Do not remember ☐ .

53. If N in question 48, will you be willing to go for an HIV test? Y ☐ N ☐.

54. If N in question 53, may you tell us the reason(s)?_____

_____

_____

<u>Section C</u>: <u>Hygiene and Sanitation Information</u>

55. Do you swim, walk or work in the Lake? Y ☐ N ☐. (*If N, go to question 57).*

56. If Y in question 55, how many times in a week?_____

57. Do you bathe or wash in the Lake? Y ☐ N ☐.

58. If Y in question 57, how many times in a week?_____

59. If Y in question 57, why do you bathe or wash in the Lake? _____

_____

_____

60. Do you wear shoes or long protective wear when in the Lake? Y ☐ N ☐.

61. If Y in question 60, how many times when in the Lake?_____

62. If N in question 60, why do you not wear them? _____

_____

_____

63. Where is the main place you go to urinate?_____

64. Where is the main place you go to defecate?_____

65. Does your house have a toilet? Y ☐ N ☐.

66. If Y in question 65, do you use it? Y ☐ N ☐.

67. If Y in question 66, how many times in a week?_____

68. If N in question 66, why do you not use it? _____

_____

_____

69. If N in question 66, where do you use the toilet?_____

_____

Do you have access to treatments for schistosomiasis? Tick **(√)** for the relevant answer below:

70. No, not at all ☐ .

71. Yes ☐ .

72. Do not know ☐ .

73. Refused to answer ☐ .

Section D: Socio-economic characteristics

What is your highest level of education? Put a tick **(√)** for **Yes** on only one option:

	Level of Education	**Option**
74.	Never went to school	
75.	Not finished primary school (6 years)	
76.	Completed primary school	
77.	Not finished secondary school	
78.	Completed secondary school	
79.	Not finished tertiary/professional school	
80.	Completed tertiary/professional school	
81.	*Don't know*	
82.	*Refused*	

83. Do you have a job or are you employed? Y ☐ N ☐.

If Y to question 83, can you tell us the job? Put a tick **(√)** for **Yes** on all items mentioned or demonstrated:

	Type of Employment / Job	**Yes**
84.	Self-employed. *Specify:*	
85.	Fishing	
86.	Farming/agriculture	
87.	Clerk/administration	
88.	Health worker	
89.	Selling at market	
90.	*Other. Specify:*	
91.	*Refused*	

Other employment status,

	Other Job	**Yes**
92.	Doing housework	
93.	Student	
94.	Retired	
95.	Long-term disabled	
96.	Unemployed	
97.	*Don't know*	
98.	*Refused*	

If Y to question 85 (fishing), can you tell us more about your work by answering the following:

99.How long have you been doing this job? _____

100. How often do you go for fishing? _____

101. What kind of fish do you catch? _____

102. Is the job (fishing) seasonal? Y ☐ N ☐.

103. If Y to question 102, when is the season? _____

104. If Y to question 102, what else do you do during off-season period? _____

_____

105. Do you migrate to other places during / after fishing? Y ☐ N ☐. _____

106. If Y to question 105, how often do you migrate in a year?_____

107. If Y to question 105, where do you migrate to? _____

108. If Y to question 105, why do you migrate? _____

_____

_____

109. Do you own a boat? Y ☐ N ☐.

110. What kind of boat? _____

111. Are you planning of getting a new boat? Y ☐ N ☐.

112. What kind of boat? _____

_____

Regarding marriage and family,

Can you tell us about your relationship status:

	Relationship	**Yes**
113.	Married	
114.	Co-habiting / engaged	
115.	Divorced	
116.	Single	
117.	*Other. Specify:*	
118.	*Refused*	

119. Do you have children? Y ☐ N ☐.

120. If Y to question 119, how many? _____

121. If N to question 119, why? _____

_____

_____

122. Has your spouse / partner experienced stillbirth / abortion? Y ☐ N ☐.

123. Have you or your spouse / partner had childlessness after marriage/engaged? Y ☐ N ☐.

124. Have you or your spouse / partner experienced infertility? Y ☐ N ☐.

125. What do you think about pains of genital organs or during / after coitus if you experience it?

_____

_____

_____

126. What do your spouse think about pains of your genital organs or during / after coitus?

_____

_____

_____

Does your spouse or partner experience the following symptoms:

127. Lower abdominal pain? Y ☐ N ☐. For how long _____

128. Pain during / after coitus? Y ☐ N ☐. For how long _____

129. Bleeding during / after coitus? Y ☐ N ☐. For how long _____

130. Pain during menstruation? Y ☐ N ☐. For how long _____

131. Changes of menstrual flow / flow? Y ☐ N ☐. For how long _____

132. If Y to any of the symptoms above, what does your spouse think about them? _____

_____

_____

_____

133. If Y to any of the symptoms above, what do you think about them? _____

_____

_____

_____

134. Any other comments related to issues above? _____

_____

_____

_____

THANK YOU FOR YOUR PARTICIPATION

Appendix 13: Individual participant questionnaire (in chichewa)

Kafukufuku wa matenda a Likodzo la amuna ndi HIV pakati pa Asodzi okhala m'mbali mwa nyanja ya Malawi (MGS Baseline & Follow-up Survey)

*Chidziwitso: Chongani **(√)** pa mayankho operekedwa ndi opangidwa kafukufuku. Pitilizani kulemba mayankho pa tsamba lina ngati malo oyankhira achepa. Mukamaliza, perekani mayankho onse kwa mkulu wa Kafukufukuyu.*

Tsiku: (dd/mm/yy): __ __ / __ __ / __ __ Opangitsa Kafukufuku _____

Chilolezo cha opangidwa Kafukufuku

Opangidwa kafukufuku apereka chilolezo cholemba? Inde ☐ Ayi ☐

Pitirizani Kafukufuku ngati avomera Inde

Gawo A: Mbiri ya Opangidwa Kafukufuku

1. Nambala ya Opangidwa Kafukufuku: _____
2. Mudzi: _____
3. Mfumu Yaikulu: _____
4. Chipatala chapafupi: _____
5. Ndinu: Mwamuna ☐ Mkazi ☐.
6. Tsiku lobadwa (dd/mm/yy): __ __ / __ __ / __ __ Zaka : _____
7. Munabadwira kuti: _____
8. Mwakhala nthawi yaitali bwanji kuno: Zaka _____ Miyezi: _____ Sabata: _____ Masiku: _____
9. Thupi kulemera (kg):_____
10. Msinkhu kutalika (cm): _____

Gawo B: Mbiri ya Zaumoyo wanu

Kodi mwakhala ndi zizindikiro monga izi? Tsopano? Kapena mu mwezi wapitawu? *(Kwa ofunsa: Chongani* **(√)** *ngati* **Inde** *pa zizindikiro zonse zimene zayankhidwa ndi kusonyezedwa)*:

	Chizindikiro	**Tsopano**	**Mwezi wapita**	**Osati mwezi wapita**	**Sindikud-ziwa**	**Sindiyan-kha**
11.	Kutentha thupi					
12.	Mutu kupweteka					
13.	Kufooka					
14.	Kupweteka mmimba					

15.	Kupweteka pokodza					
16.	Kukodza pafupi-pafupi					
17.	Kusinthika kwamtundu wa mkodzo					
18.	Magazi mu mkodzo					
19.	Magazi mu chimbudzi					
20.	Magazi mu umuna					
21.	Kupweteka pogonana					
22.	Kupweteka potulutsa umuna					
23.	Kupweteka kwa ziwalo za umuna					
24.	Zizindikiro zina (nenani)					

Kodi mwadwala matenda awa? Tsopano? Kapena mu mwezi wapitawu? *(Kwa ofunsa: Chongani* **(√)** *ngati* **Inde** *pa matenda onse zimene zayankhidwa ndi kusonyezedwa)*:

	Matenda	**Tsopano**	**Mwezi wapita**	**Osati mwezi wapita**	**Sindikudziwa**	**Sindiyankha**
25.	Malungo					
26.	Kutsegula m'mimba					
27.	Kutsegula kwa kamwazi					
28.	Matenda a pakhungu					
29.	Chifuwa					
30.	Njoka za mmimba					
31.	Likodzo					
32.	Matenda opatsana pogonana					
33.	Matenda ena (tchulani)					

34. Kodi munamwa mankhwala a njoka zammimba (albendazole) mu miyezi 12 yapita? Inde ☐ Ayi ☐.

35. Kodi munamwa mankhwala a likodzo (praziquantel) mu miyezi 12 yapita? Inde ☐ Ayi ☐.

36. Kodi munamwa mankhwala a malungo mu miyezi 12 yapita? Inde ☐ Ayi ☐.

37. Kodi munamwa mankhwala ena aliwonse mu miyezi 12 yapita? Inde ☐ Ayi ☐. Ngati Inde,

    tchulani dzina la mankhwala _____

Mafunso awa ndi okhudzana ndi kachilombo ka HIV ndi matenda a EDZI:

38. Kodi munakhalapo nawo pa misonkhano ya zaumoyo mu miyezi 12 yapita? Inde ☐ Ayi ☐.

39. Kodi mmisonkhanoyi ina inalipo yokhudza za matenda a EDZI? Inde ☐ Ayi ☐.

40. Kodi mukudziwa kuti HIV ndi chiani? Inde ☐ Ayi ☐.

41. Longosolani kayankhidwe kanu pa funsoli (40): _____

    _____

42. Kodi mukudziwa kuti EDZI ndi chiani? Inde ☐ Ayi ☐.

43. Longosolani yankho lanu pa funsoli (42). _____

_____

44. Kodi mukudziwa mmene HIV imafalikira? Inde ☐ Ayi ☐.

45. Longosolani yankho lanu pa funsoli (44). _____

_____

46. Kodi mumadziwa mmene HIV ingapewedwele? Inde ☐ Ayi ☐.

47. Longosolani yankho lanu pa funsoli (46). _____

_____

48. Kodi munayezetsa za HIV? Inde ☐ Ayi ☐.

49. Ngati Inde pa funsoli (48), mungafotokoze za zotsatira zake? Inde ☐ Ayi ☐.

Ngati Inde pa funsoli (49), fotokozani za zotsatira za kuyeza kwa HIV? Ikani **(√)** pa yaknho loyenera:

50. Ndili ndi HIV (+) ☐.

51. Ndilibe HIV (-) ☐.

52. Sindikukumbuka ☐.

53. Ngati Ayi ku funso 48 (simunayezetse HIV), mungalore kuyezedwa za HIV? Inde ☐ Ayi ☐.

54. Ngati Ayi pa kuyezedwa, mungalongosole zifukwa zake? _____

_____

_____

<u>Gawo C</u>: **<u>Zaukhondo ndi Kudzisamalira</u>**

55. Kodi mumasambira, kuyenda kapena kugwira ntchito mu nyanja? Inde ☐ Ayi ☐ *(pitani funso 57).*

56. Ngati Inde pa funsoli (55), mumatero kangati pa sabata? _____

57. Kodi mumasamba kapena kuchapa mu nyanja? Inde ☐ Ayi ☐ *(pitani funso 60)*.

58. Ngati Inde pa funsoli (57), mumatero kangati pa sabata? _____

59. Ngati Inde pa funsoli (57), chifukwa chiani? _____

_____

60. Kodi mumavala nsapato kapena malaya otalika mu nyanja? Inde ☐ Ayi ☐ *(pitani funso 63)*.

61. Ngati Inde pa funsoli (61), mumatero kangati mukakhala mu nyanja? _____

62. Ngati Ayi pa funsoli (61), chifukwa chiani simuvala? _____

_____

63. Kodi mumakodza kuti?_____

64. Kodi mumachita chimbudzi kuti?_____

65. Kodi nyumba yanu ili ndi chimbudzi? Inde ☐ Ayi ☐ *(pitani funso 70)*.

66. Ngati Inde pa funsoli (65), kodi mumagwiritsa ntchito Inde ☐ Ayi ☐ *(pitani funso 68)*.

67. Ngati Inde pa funsoli (66), mumagwiritsa ntchito kangati pa sabata? _____

68. Ngati Ayi pa funsoli (66), chifukwa chiani simugwiritsa ntchito? _____

_____

69. Ngati Ayi pa funsoli (66), mumagwiritsa ntchito chimbudzi chakuti? _____

_____

Kodi mumatha kupeza mankhwala a likodzo? *(Kwa ofunsa: Chongani* **(√)** *ngati* **Inde** *pa mayankho oyenera)*:

70. Ayi ☐ .

71. Inde ☐ .

72. Sindikudziwa ☐ .

73. Sindiyankha ☐ .

<u>Gawo D</u>: **<u>Zachikhalidwe ndi chuma</u>**

Kodi munaphunzira pakana kalasi yanji? *(Kwa ofunsa: Chongani* **(√)** *ngati* **Inde** *pa yankho limodzi loyenera):*

	Maphunziro	**Yankho**
74.	Sindinapiteko ku sukulu	
75.	Sindinamalize sukulu ya Pulaimale	
76.	Ndinamaliza sukulu ya pulaimale	
77.	Sindinamalize sukulu ya Sekondale	
78.	Ndinamaliza sukulu ya sekondale	
79.	Sindinamalize maphunziro a kukoleji	
80.	Ndinamaliza maphunziro a Koleji	
81.	*Sindikudziwa*	
82.	*Sindiyankha*	

83. Kodi muli pa ntchito? Inde ☐ Ayi ☐ *(pitani funso 92)*.

Ngati Inde pa funsoli (83), mungafotokoze ntchito yanu? *(Kwa ofunsa: Chongani* **(√)** *ngati* **Inde** *pa mayankho oyenera:*

	Mtundu wa ntchito	**Inde**
84.	Mumazilipila nokha. *Nenani:*	
85.	Usodzi	
86.	Ulimi	
87.	Ukalaliki	
88.	Ntchito ya chipatala	
89.	Kugulutsa mu msika	
90.	Ntchito zina, Nenani:	
91.	Sindiyankha	

	Ntchito ina	**Inde**
92.	Ntchito ya pakhomo	
93.	Mwana wa sukulu	
94.	Ndinapuma ntchito	
95.	Olumala	
96.	Sindili pantchito iliyonse	
97.	*Sindikudziwa*	
98.	*Sindiyankha*	

Ngati Inde pa funsoli (85) la zausodzi, mungafotokoze zambiri za ntchito yanu:

99. Kodi mwagwira nthawi yayitali bwanji pa ntchito ya usodzi? _____

100. Kodi mumachita usodzi kangati? _____

101. Mumagwira nsomba za tundu wanji? _____

102. Kodi mumapanga usodzi mwaka nyengo? Inde ☐ Ayi ☐.*(pitani funso xxx)*

103. Nyengo yake? _____

104. Ngati Inde pa funsoli (102), mumapanga chiani nthawi yomwe simuweza? _____

_____

105. Kodi mumapita madera ena nthawi ya usodzi? Inde ☐ Ayi ☐. _____

106. Ngati Inde pa funsoli (104), mumapita kangati pa chaka? _____

107. Ngati Inde pa funsoli (104), mumapita kuti? _____

108. Ngati Inde pa funsoli (104), mumayenda chifukwa chiani? _____

_____

109. Muli ndi bwato lanu? Inde ☐ Ayi ☐.

110. Mtundu wa bwato lanu? _____

111. Muli ndi ma pulani ogula bwato latsopano? Y ☐ N ☐.

112. Mtundu wa bwato lanu? _____

_____

Zokhudzana ukwati ndi banja lanu, mungafotokoze zambiri,

	Ukwati	**Inde**
113.	Okwatira	
114.	Unapanga unkhoswe	
115.	Banja linatha	
116.	Simunakwatirepo	
117.	Zina, nenani	
118.	Sindiyankha	

119. Kodi muli ndi ana? Inde ☐ Ayi ☐.

120. Ngati Inde, alipo angati? _____

121. Ngati Ayi pa funsoli (119), chifukwa chiani? _____

_____

122. Kodi akazi anu anapita padera ali woyembekezera? Inde ☐ Ayi ☐.

123. Kodi inu kapena akazi anu mukakhala opanda mwana m'banja? Inde ☐ Ayi ☐.

124. Kodi inu kapena akazi anu anakhalapo ndi vuto losabereka? Inde ☐ Ayi ☐.

125. Kodi munganene zotani pa kupweteka kwa ziwalo za umuna mukanamagonana? _____

_____

_____

_____

126. Kodi akazi anu anaganiza bwanji pa kupweteka kwa ziwalo za umuna kukamagonana? ____

_____

_____

_____

Kodi akazi anu amaona zizindikiro izi:

127. Kupweteka mmimba? Inde ☐ Ayi ☐. Ngati inde, Kwa nthawi yaitali bwanji _____

128. Kupweteka pogonana? Inde ☐ Ayi ☐. Ngati Inde, Kwa nthawi yaitali bwanji _____

129. Kutaya magazi pogonana? Inde ☐ Ayi ☐. Ngati Inde, Kwa nthawi yaitali bwanji _____

130. Kupweteka posamba? Inde ☐ Ayi ☐. Ngati Inde, Kwa nthawi yaitali bwanji _____

131. Kusintha kwa kusamba? Inde ☐ Ayi ☐. Ngati Inde, Kwa nthawi yaitali bwanji _____

132. Ngati Inde pa zizindikiro izi, akazi anu amaganiza chiani? _____

_____

_____

_____

133. Ngati Inde pa zizindikiro izi, inu amaganiza chiani? _____

_____

_____

_____

134. Muli ndi maganizo ena pa zomwe zakambidwa? _____

_____

_____

_____

ZIKOMO KWAMBIRI POTENGA NAWO MBALI PA KAFUKUFUKUYU.

Appendix 14: Individual HIV participant questionnaire

Research study on Male genital schistosomiasis and HIV among Adult fishermen living in Southern shores of Lake Malawi (MGS-HIV Baseline Survey)

Explanation: Put a tick (√) for the participant's responses appropriately. If the boxes are not enough on one page, use another sheet. Return this and other sheets to the Study Investigator at the end.

Date: (dd/mm/yy): __ __ / __ __ / __ __ Interviewer Name_____

Consent checklist

Has written consent been obtained? Y ☐ N ☐ *Only proceed if Y*

Section A: Participant's details

1. Participant ID: _____
2. Village: _____
3. T/A: _____
4. Name of nearest Health centre: _____
5. Gender: Male ☐ Female ☐.
6. Date of birth (dd/mm/yy): __ __ / __ __ / __ __ Age: _____
7. Born where: _____
8. How long have you been staying here: Years _____ Months: _____ Weeks: _____ Days:_____
9. Weight (kg):_____
10. Height (cm):_____

Section B: General Health Information

Are you experiencing the following symptoms now? In the last month? Put a tick **(√)** for **Yes** on all items mentioned or demonstrated:

	Symptom	**Currently**	**In last month**	**Not in last month**	**Do not know**	**Refused**
11.	Fever					
12.	Headache					
13.	Fatigue					
14.	Abdominal cramping (pain)					
15.	Pain during urination (dysuria)					
16.	Frequency of urination					
17.	Colour of urine					

18.	Blood in urine (haematuria)					
19.	Blood in stool					
20.	Blood in semen					
21.	Pains during and / or after coitus					
22.	Pains on ejaculation					
23.	Pains of the genital organs					
24.	Other (please specify):					

Are you experiencing the following diseases now? In the last month? Put a tick **(√)** for **Yes** on all items mentioned or demonstrated:

	Disease	**Currently**	**In last month**	**Not in last month**	**Don't know**	**Refused**
25.	Malaria					
26.	Diarrhoea					
27.	Dysentery					
28.	Skin disease					
29.	Respiratory disease					
30.	Worm infestation					
31.	Schistosomiasis					
32.	Sexually transmitted infection (STI)					
33.	Other (please specify):					

34. Did you take deworming medicine (albendazole) in the last month? Y ☐ N ☐.

35. Did you take schistosomiasis medicine (praziquantel) in the last month? Y ☐ N ☐.

36. Did you take antimalarial medicine in the last month? Y ☐ N ☐.

37. Did you take any other medicine in the last month? Y ☐ N ☐. If Y, name _____

_____

The following questions are about HIV/AIDS:

38. Did you attend any meetings about health education in the last month? Y ☐ N ☐.

39. Were any of the meetings about HIV/AIDS? Y ☐ N ☐.

40. Have you experienced any problems after starting ART? Y ☐ N ☐.

41. Did you had any problems also related to genitourinary organs in last 12 months? Y ☐ N ☐.

42. If Y in question 41, may you tell the problems?_____

_____

_____

43. Did you seek any help on the problems said in question 42? Y ☐ N ☐.

44. If Y in question 43, where did you get help?_____

45. If Y in question 43, what help were you given?_____

_____

_____

46. If N in question 43, may you tell the reason(s)?_____

_____

_____

47. Will you accept to do a full clinical examination including the genitourinary system? Y ☐ N ☐.

Section C: Hygiene and Sanitation Information

48. Did you swim, walk or work in the Lake? Y ☐ N ☐. (*If N, go to question 50).*

49. If Y in question 48, how many times in a week?_____

50. Did you bathe or wash in the Lake? Y ☐ N ☐.

51. If Y in question 50, how many times in a week?_____

52. If Y in question 50, why do you bathe or wash in the Lake? _____

_____

_____

53. Do you wear shoes or long protective wear when in the Lake? Y ☐ N ☐.

54. If Y in question 53, how many times when in the Lake?_____

55. If N in question 53, why do you not wear them? _____

_____

_____

56. Where is the main place you go to urinate?_____

57. Where is the main place you go to defecate?_____

58. Does your house have a toilet? Y ☐ N ☐.

59. If Y in question 58, do you use it? Y ☐ N ☐.

60. If Y in question 59, how many times in a week?_____

61. If N in question 59, why do you not use it? _____

_____

_____

62. If N in question 59, where do you use the toilet?_____

_____

Do you have access to treatments for schistosomiasis? Tick **(√)** for the relevant answer below:

63. No, not at all ☐ .

64. Yes ☐ .

65. Do not know ☐ .

66. Refused to answer ☐ .

Section D: Socio-economic characteristics

What is your highest level of education? Put a tick **(√)** for **Yes** on only one option:

	Level of Education	**Option**
67.	Never went to school	
68.	Not finished primary school (6 years)	
69.	Completed primary school	
70.	Not finished secondary school	
71.	Completed secondary school	
72.	Not finished tertiary/professional school	
73.	Completed tertiary/professional school	
74.	*Don't know*	
75.	*Refused*	

76. Do you have a job or are you employed? Y ☐ N ☐ .

If Y to question 76, can you tell us the job? Put a tick **(√)** for **Yes** on all items mentioned or demonstrated:

	Type of Employment / Job	**Yes**
77.	Self-employed. *Specify:*	
78.	Fishing	
79.	Farming/agriculture	
80.	Clerk/administration	
81.	Health worker	
82.	Selling at market	
83.	*Other. Specify:*	
84.	*Refused*	

Other employment status,

	Other Job	**Yes**
85.	Doing housework	
86.	Student	
87.	Retired	

88.	Long-term disabled	
89.	Unemployed	
90.	*Don't know*	
91.	*Refused*	

If Y to question 78 (fishing), can you tell us more about your work by answering the following:

92. How long have you been doing this job? _____

93. How often do you go for fishing? _____

94. What kind of fish do you catch? _____

95. Is the job (fishing) seasonal? Y ☐ N ☐.

96. If Y to question 95, when is the season? _____

97. If Y to question 95, what else do you do during off-season period?_____

_____

98. Do you migrate to other places during / after fishing? Y ☐ N ☐.

99. If Y to question 98, how often do you migrate in a year? _____

100. If Y to question 98, where do you migrate to? _____

101. If Y to question 98, why do you migrate? _____

_____

102. Do you own a boat? Y ☐ N ☐.

103. What kind of boat? _____

104. Are you planning of getting a new boat? Y ☐ N ☐.

105. What kind of boat? _____

Regarding marriage and family,

Can you tell us about your relationship status:

	Relationship	**Yes**
106.	Married	
107.	Co-habiting / engaged	
108.	Divorced	
109.	Single	
110.	*Other. Specify:*	
111.	*Refused*	

112. Do you have children? Y ☐ N ☐.

113. If Y to question 112, how many? _____

114. If N to question 112, why? _____

_____

115. Has your spouse / partner experienced stillbirth / abortion? Y ☐ N ☐.

116. Have you or your spouse / partner had childlessness after marriage/engaged? Y ☐ N ☐.

117. Have you or your spouse / partner experienced infertility? Y ☐ N ☐.

118. What do you think about pains of genital organs or during / after coitus if you experience it?

_____

_____

119. What do your spouse think about pains of your genital organs or during / after coitus?____

_____

_____

Does your spouse or partner experience the following symptoms:

120. Lower abdominal pain? Y ☐ N ☐. For how long _____

121. Pain during / after coitus? Y ☐ N ☐. For how long _____

122. Bleeding during / after coitus? Y ☐ N ☐. For how long _____

123. Pain during menstruation? Y ☐ N ☐. For how long _____

124. Changes of menstrual flow / flow? Y ☐ N ☐. For how long _____

125. If Y to any of the symptoms above, what does your spouse think about them? _____

_____

_____

126. If Y to any of the symptoms above, what do you think about them?_____

_____

_____

127. Any other comments related to issues above? _____

_____

THANK YOU FOR YOUR PARTICIPATION

Appendix 15: Individual HIV participant questionnaire (in chichewa)

Kafukufuku wa matenda a Likodzo la amuna ndi HIV pakati pa Asodzi okhala m'mbali mwa nyanja ya Malawi (MGS-HIV Baseline & Follow-up Survey)

*Chidziwitso: Chongani **(√)** pa mayankho operekedwa ndi opangidwa kafukufuku. Pitilizani kulemba mayankho pa tsamba lina ngati malo oyankhira achepa. Mukamaliza, perekani mayankho onse kwa mkulu wa Kafukufukuyu.*

Tsiku: (dd/mm/yy): __ __ / __ __ / __ __ Opangitsa Kafukufuku _____

Chilolezo cha opangidwa Kafukufuku

Opangidwa kafukufuku apereka chilolezo cholemba? Inde ☐ Ayi ☐

Pitirizani Kafukufuku ngati avomera Inde

Gawo A: Mbiri ya Opanga Kafukufuku

1. Nambala ya Opangidwa Kafukufuku: _____
2. Mudzi: _____
3. Mfumu Yaikulu: _____
4. Chipatala chapafupi: _____
5. Ndinu: Mwamuna ☐ Mkazi ☐.
6. Tsiku lobadwa (dd/mm/yy): __ __ / __ __ / __ __ Zaka : _____
7. Munabadwira kuti: _____
8. Mwakhala nthawi yaitali bwanji kuno: Zaka _____ Miyezi: _____ Sabata: _____ Masiku: _____
9. Thupi kulemera (kg):_____
10. Msinkhu kutalika (cm): _____

Gawo B: Mbiri ya Zaumoyo wanu

Kodi mwakhala ndi zizindikiro monga izi? Tsopano? Kapena mu mwezi wapitawu? *(Kwa ofunsa: Chongani* **(√)** *ngati* **Inde** *pa zizindikiro zonse zimene zayankhidwa ndi kusonyezedwa)*:

	Chizindikiro	**Tsopano**	**Mwezi wapita**	**Osati mwezi wapita**	**Sindikud-ziwa**	**Sindiyan-kha**
11.	Kutentha thupi					
12.	Mutu kupweteka					
13.	Kufooka					
14.	Kupweteka mmimba					

15.	Kupweteka pokodza					
16.	Kukodza pafupi-pafupi					
17.	Kusinthika kwamtundu wa mkodzo					
18.	Magazi mu mkodzo					
19.	Magazi mu chimbudzi					
20.	Magazi mu umuna					
21.	Kupweteka pogonana					
22.	Kupweteka potulutsa umuna					
23.	Kupweteka kwa ziwalo za umuna					
24.	Zizindikiro zina (nenani)					

Kodi mwadwala matenda awa? Tsopano? Kapena mu mwezi wapitawu? *(Kwa ofunsa: Chongani* **(√)** *ngati* **Inde** *pa matenda onse zimene zayankhidwa ndi kusonyezedwa)*:

	Matenda	**Tsopano**	**Mwezi wapita**	**Osati mwezi wapita**	**Sindikudziwa**	**Sindiyankha**
25.	Malungo					
26.	Kutsegula m'mimba					
27.	Kutsegula kwa kamwazi					
28.	Matenda a pakhungu					
29.	Chifuwa					
30.	Njoka za mmimba					
31.	Likodzo					
32.	Matenda opatsana pogonana					
33.	Matenda ena (tchulani)					

34. Kodi munamwa mankhwala a njoka zammimba (albendazole) mu miyezi 12 yapita? Inde ☐ Ayi ☐.

35. Kodi munamwa mankhwala a likodzo (praziquantel) mu miyezi 12 yapita? Inde ☐ Ayi ☐.

36. Kodi munamwa mankhwala a malungo mu miyezi 12 yapita? Inde ☐ Ayi ☐.

37. Kodi munamwa mankhwala ena aliwonse mu miyezi 12 yapita? Inde ☐ Ayi ☐. Ngati Inde,

    tchulani dzina la mankhwala _____

Mafunso awa ndi okhudzana ndi matenda a EDZI:

38. Kodi munakhalapo nawo pa misonkhano ya zaumoyo mu miyezi 12 yapita? Inde ☐ Ayi ☐.

39. Kodi mmisonkhanoyi ina inalipo yokhudza za matenda a EDZI? Inde ☐ Ayi ☐.

40. Kodi mwakumana ndi mavuto chiyambire kumwa ma ARVs? Inde ☐ Ayi ☐.

41. Munakumana ndi mavuto okhudza ziwalo za umuna pa miyezi 12 yapita? Inde ☐ Ayi ☐.

42. Ngati Inde pa funsoli (41), fotokozani mavutowo?_____

    _____

    _____

43. Munalandira chithandizo chilichonse oa funsoli (42)? Inde ☐ Ayi ☐.

44. Ngati Inde pa funsoli (43), munalindira chithandizo kuti? _____

45. Ngati Inde pa funsoli (43), thandizo munalira ndi lotani? _____

_____

_____

46. Ngati Ayi pa funsoli (43), fotokozani zifukwa zanu?_____

_____

_____

47. Kodi mukuvomera kuyezedwa mthupi limodzi ndi ziwalo za umuna? Inde ☐ Ayi ☐.

<u>Gawo C</u>: <u>Zaukhondo ndi Kudzisamalira</u>

48. Kodi mumasambira, kuyenda kapena kugwira ntchito mu nyanja? Inde ☐ Ayi ☐ *(pitani funso 50).*

49. Ngati Inde pa funsoli (48), mumatero kangati pa week _____

50. Kodi mumasamba kapena kuchapa mu nyanja? Inde ☐ Ayi ☐.

51. Ngati Inde pa funsoli (50), mumatero kangati pa week? _____

52. Ngati Inde pa funsoli (50), chifukwa chiani? _____

_____

53. Kodi mumavala nsapato kapena Malaya otalika mukakhala mu Nyanja? Inde ☐ Ayi ☐.

54. Ngati Inde pa funsoli (53), mumatero kangati mukakhala mu nyanja? _____

55. Ngati Ayi pa funsoli (53), chifukwa chiani simuvala? _____

_____

56. Kodi mumakodza kuti?_____

57. Kodi mumachita chimbudzi kuti?_____

58. Kodi nyumba yanu ili ndi chimbudzi? Inde ☐ Ayi ☐.

59. Ngati Inde pa funsoli (58), kodi mumagwiritsa ntchito? Inde ☐ Ayi ☐.

60. Ngati Inde pa funsoli (59), mumagwiritsa ntchito kangati pa week?_ _____

61. Ngati Ayi pa funsoli (59), chifukwa chiani simugwiritsa ntchito? _____

_____

62. Ngati Ayi pa funsoli (59), mumagwiritsa ntchito kuti chimbudzi?_____

_____

Kodi mumatha kupeza mankhwala a likodzo? *(Kwa ofunsa: Chongani* **(√)** *ngati* **Inde** *pa mayankho oyenera)*:

63. Ayi ☐ .

64. Inde ☐ .

65. Sindikudziwa ☐ .

66. Sindiyankha ☐ .

<u>Zachikhalidwe ndi chuma</u>

Kodi munaphunzira pakana kalasi yanji? *(Kwa ofunsa: Chongani* **(√)** *ngati* **Inde** *pa yankho limodzi loyenera):*

	Maphunziro	**Yankho**
67.	Sindinapiteko ku sukulu	
68.	Sindinamalize sukulu ya Pulaimale	
69.	Ndinamaliza sukulu ya pulaimale	
70.	Sindinamalize sukulu ya Sekondale	
71.	Ndinamaliza sukulu ya sekondale	
72.	Sindinamalize maphunziro a kukoleji	
73.	Ndinamaliza maphunziro a Koleji	
74.	*Sindikudziwa*	
75.	*Sindiyankha*	

76. Kodi muli pa ntchito? Inde ☐ Ayi ☐ *(pitani funso 92)*.

Ngati Inde pa funsoli (76), mungafotokoze ntchito yanu? *(Kwa ofunsa: Chongani* **(√)** *ngati* **Inde** *pa mayankho oyenera:*

	Mtundu wa ntchito	**Inde**
77.	Mumazilipila nokha. *Nenani:*	
78.	Usodzi	
79.	Ulimi	
80.	Ukalaliki	
81.	Ntchito ya chipatala	
82.	Kugulutsa mu msika	
83.	Ntchito zina, Nenani:	
84.	Sindiyankha	

	Ntchito ina	**Inde**
85.	Ntchito ya pakhomo	
86.	Mwana wa sukulu	
87.	Ndinapuma ntchito	

88.	Olumala	
89.	Sindili pantchito iliyonse	
90.	*Sindikudziwa*	
91.	*Sindiyankha*	

Ngati Inde pa funsoli (78) la zausodzi, mungafotokoze zambiri za ntchito yanu:

92.Kodi mwagwira nthawi yayitali bwanji pa ntchito ya usodzi? _____

93. Kodi mumachita usodzi kangati? _____

94. Mumagwira nsomba za mtundu wanji? _____

95. Kodi mumapanga usodzi mwaka nyengo? Inde ☐ Ayi ☐.

96. Nyengo yake? _____

97. Ngati Inde pa funsoli (96), mumapanga chiani nthawi yomwe simuweza? _____

_____

98. Kodi mumapita madera ena nthawi ya usodzi? Inde ☐ Ayi ☐. _____

99. Ngati Inde pa funsoli (97), mumapita kangati pa chaka? _____

100. Ngati Inde pa funsoli (97), mumapita kuti? _____

101. Ngati Inde pa funsoli (97), mumayenda chifukwa chiani? _____

_____

102. Mtundu wa bwato lanu? _____

103. Muli ndi ma pulani ogula bwato latsopano? Y ☐ N ☐.

104. Mtundu wa bwato lanu? _____

_____

Zokhudzana ukwati ndi banja lanu, mungafotokoze zambiri,

	Ukwati	**Inde**
105.	Okwatira	
106.	Unapanga unkhoswe	
107.	Banja linatha	
108.	Simunakwatirepo	
109.	Zina, nenani	
110.	Sindiyankha	

111. Kodi muli ndi ana? Inde ☐ Ayi ☐.

112. Ngati Inde, alipo angati? _____

113. Ngati Ayi pa funsoli (110), chifukwa chiani? _____

_____

_____

114. Kodi akazi anu anapita padera ali woyembekezera? Inde ☐ Ayi ☐.

115. Kodi inu kapena akazi anu mukakhala opanda mwana m'banja? Inde ☐ Ayi ☐.

116. Kodi inu kapena akazi anu anakhalapo ndi vuto losabereka? Inde ☐ Ayi ☐.

117. Kodi munganene zotani pa kupweteka kwa ziwalo za umuna mukanamagonana? _____

_____

_____

118. Kodi akazi anu anaganiza bwanji pa kupweteka kwa ziwalo za umuna kukamagonana? ____

_____

_____

Kodi akazi anu amaona zizindikiro izi:

119. Kupweteka mmimba? Inde ☐ Ayi ☐. Ngati inde, Kwa nthawi yaitali bwanji _____

120. Kupweteka pogonana? Inde ☐ Ayi ☐. Ngati Inde, Kwa nthawi yaitali bwanji _____

121. Kutaya magazi pogonana? Inde ☐ Ayi ☐. Ngati Inde, Kwa nthawi yaitali bwanji _____

122. Kupweteka posamba? Inde ☐ Ayi ☐. Ngati Inde, Kwa nthawi yaitali bwanji _____

123. Kusintha kwa kusamba? Inde ☐ Ayi ☐. Ngati Inde, Kwa nthawi yaitali bwanji _____

124. Ngati Inde pa zizindikiro izi, akazi anu amaganiza chiani? _____

_____

_____

125. Ngati Inde pa zizindikiro izi, inu amaganiza chiani? _____

_____

_____

126. Muli ndi maganizo ena pa zomwe zakambidwa? _____

_____

ZIKOMO KWAMBIRI POTENGA NAWO MBALI PA KAFUKUFUKUYU.

Appendix 16: Standard operating procedure for urine analysis

EXAMINATION OF URINE FOR EGGS OF *Schistosoma haematobium*

S.O.P. Reference number: MW-MGS-01

Version number: 02

Date of Issue: 1st November 2017

Authorised by: P.I. (Dr. Sekeleghe Kayuni)

This S.O.P. has been read and understood by Study team members:

NAME	POSITION	SIGNATURE	DATE
Dr Sekeleghe Kayuni	Principal Investigator		
Mr. Peter Makaula	Local Collaborator		
Mr. Bright Mainga	Laboratory Technician		

This S.O.P. must be reviewed regularly, where necessary and must be authorised by the P.I.

DATE OF REVIEW	REVIEWED BY	SIGNATURE

This S.O.P. has been superseded by S.O.P. number ……..

Revision……. Date…….....

The master copy was transferred to the superseded S.O.P. file

Signature……..Date…….

EXAMINATION OF URINE FOR EGGS OF *Schistosoma haematobium*

1. PRINCIPLE AND CLINICAL SIGNIFICANCE OF THE EXAMINATION

1.1 *Schistosoma haematobium* causes urogenital schistosomiasis, which is endemic in sub-Saharan African countries including Malawi, especially along water bodies like Lake Malawi. Infected people pass the parasites in urine, 12 weeks after exposure and present with symptoms like fever, fatigue, dysuria, haematuria

1.2 Laboratory diagnosis of *S. haematobium* infection is by detection of the *S. haematobium* eggs through microscopic examination of the urine. A quantitative report of the laboratory diagnosis is number of eggs per 10ml of urine.

1.3 The excretion of *S. haematobium* eggs in urine varies throughout the day with highest output between 10:00 hrs and 14:00 hrs, peaking around midday. It may also be necessary to examine several specimens collected on different days due to the irregular pattern of egg excretion.

2. SPECIMEN REQUIREMENTS

2.1 The type of specimen to be examined is urine, using filtration technique.

2.2 The materials required to conduct the urine examination include:

120ml urine container, 60ml syringe and forceps, Urine card for macrohaematuria, Urinalysis reagent strips, *Schistosoma* CCA strips, Filter holder and membrane, Microscope, slides and coverslips, Lugol's iodine, Examination gloves, Laboratory coat.

2.3 The participant will submit urine in the clean container between 10:00 hrs and 14:00 hrs and then immediately give it to the study team member.

2.4 The urine will be analysed immediately or where not possible, transferred to a designated laboratory in a cooler box to prevent *Schistosoma* eggs from hatching.

2.5 The results will be recorded on the data collection tool and thereafter given to the participant.

3. STEPS OF THE URINE FILTRATION TECHNIQUE

3.1. Safety precautions will be ensured always to prevent exposure to hazards. The study team will wear laboratory coats, gloves and acceptable protective wear throughout the technique.

3.2. Once the urine is submitted, the study team member will record the volume and then its appearance using the urine card to assess for macrohaematuria.

3.3. The urine will be analysed for microhaematuria using reagent strips and the result will be recorded.

3.4. Then a drop of urine will be drawn and put on the CCA strip, a drop of the buffer will be added, and the result will be recorded.

3.5. Thereafter, the entire amount of urine will be drawn by the 60ml syringe and filtered through a disinfected filter holder containing a clean polycarbonate filter membrane to trap as many eggs in the urine.

3.6. The filter membrane will be removed, placed on a microscope slide labelled with participant's unique identifier and examined under microscope. Iodine will be added to visualise *S. haematobium* eggs distinctly.

3.7. The number of eggs will be counted and recorded per 10ml of urine.

3.8. The microscope slide will be stored for quality control.

4. SPECIMEN AND WASTE DISPOSAL

4.1. The urine will be discarded in accordance with the aseptic practices and Infection prevention procedures set by Ministry of Health in Malawi.

4.2. The used gloves, disposable protective wear and other waste will be disposed in accordance with Infection prevention procedures set by Ministry of Health in Malawi.

5. QUALITY CONTROL PROCEDURES

5.1. The P.I. will perform microscopy on slides at random as part of quality control.

6. REFERENCES

- Cheesbrough, M. (2009). District Laboratory Practice in Tropical Countries, Part 1 - Second Edition updated. The Edinburgh Building, Cambridge, UK, Cambridge University Press.
- WHO (1991). Basic Laboratory methods in Medical parasitology. Geneva, Switzerland, World Health Organization.
- WHO (2013). Assessing the efficacy of anthelminthic drugs against schistosomiasis and soil-transmitted helminthiases. Geneva, Switzerland, World Health Organization.

Appendix 17: Standard operating procedure for semen analysis

EXAMINATION OF SEMINAL FLUID FOR EGGS OF *Schistosoma haematobium*

S.O.P. Reference number: MW-MGS-02

Version number: 02

Date of Issue: 1st November 2017

Authorised by: P.I. (Dr. Sekeleghe Kayuni)

This S.O.P. has been read and understood by Study team members:

NAME	POSITION	SIGNATURE	DATE
Dr Sekeleghe Kayuni	Principal Investigator		
Mr. Peter Makaula	Local Collaborator		
Mr. Bright Mainga	Laboratory Technician		

This S.O.P. must be reviewed regularly, where necessary and must be authorised by the P.I.

DATE OF REVIEW	REVIEWED BY	SIGNATURE

This S.O.P. has been superseded by S.O.P. number …….

Revision……. Date…….....

The master copy was transferred to the superseded S.O.P. file

Signature……..Date…….

EXAMINATION OF SEMINAL FLUID FOR EGGS OF *Schistosoma haematobium*

1. PRINCIPLE AND CLINICAL SIGNIFICANCE OF THE EXAMINATION

1.1 *Schistosoma haematobium* causes urogenital schistosomiasis, which is endemic in sub-Saharan African countries including Malawi, especially along water bodies like Lake Malawi. Infected people pass the eggs in urine and/or semen, and present with symptoms like fever, fatigue, dysuria, haematuria, haemospermia, pain of the genital organs, pain during or after sexual intercourse.

1.4 Laboratory diagnosis of *S. haematobium* infection is by detection of the *S. haematobium* eggs through microscopic examination of the semen. A quantitative report of the diagnosis is number of eggs per ejaculate (semen provided).

1.5 Like the variation of *S. haematobium* eggs in urine, excretion of eggs in semen is very variable and unpredictable, with eggs present in semen and not in urine. Therefore, it is essential to examine several specimens collected on different days to detect eggs in semen, even in absence of eggs in urine.

2. SPECIMEN REQUIREMENTS

2.1 The type of specimen to be examined is semen / seminal fluid.

2.2 The materials required to conduct the semen examination include:

- Clear, self-sealing plastic bag
- Heat sealer
- 2ml microtubes
- Centrifuge and Microscope
- Microscope slides and coverslips
- Pasteur pipettes
- Exam gloves
- Laboratory coat

2.3 The participant will be counselled to abstain from sexual activity for at least 2 days prior to semen submission.

2.4 On the day of submission, the participant will be given a clear, self-sealing plastic bag for submitting semen, labelled with their unique identifier. This bag will be used instead of the traditional container or nonspermicidal condoms commonly used in other similar studies.

2.5 The participant will be directed to a separate, quite private room where they submit semen through masturbation.

2.6 For those who will fail to submit semen, they will be asked to either try the following day or ejaculate into the bag during intercourse by coitus interruptus on the next morning and delivered to the study team within three hours of ejaculation.

2.7 The semen will be analysed within 3 hours of submission and results be given to the participant and recorded on the data collection tool.

3. STEPS OF THE SPECIMEN EXAMINATION

3.1. Safety precautions will be ensured always to prevent exposure to hazards. The study team will wear laboratory coats, gloves and acceptable protective wear throughout the technique.

3.2. Once the semen is submitted, the study team member will report the appearance (watery, lumpy) and colour (clear/colourless, white, yellowish/straw, red/bloody).

3.3. Thereafter, the semen should be placed on a bench at room temperature for 20-45 minutes to allow liquefication if not already done.

3.4. The semen should be pushed gently to one corner of the plastic bag and the bag should be heat sealed to evenly concentrate the semen for easy visualisation during microscopy.

3.5. Then the heat-sealed bag should be placed in a clean petri-dish and examined directly under microscope to check for *S. haematobium* eggs, presence of blood cells and other abnormalities. The results will be recorded.

3.6. The semen should be then transferred into 2ml microtube using the Pasteur pipettes and the volume measured and recorded.

3.7. The semen should be centrifuged at 3,000 x*g* for 5 minutes to collect the pellet and seminal plasma which should be stored at -80°c before shipment to Liverpool.

3.8. Normal saline (2.5ml) and ethanol (0.1 ml) should be added to the pellet in the microtube and mixed thoroughly well. Thereafter, a drop of the pellet should be placed on a slide and examined under microscope for *S. haematobium* eggs.

3.9. The seminal plasma should be analysed for molecular HIV viral load where necessary after shipment to Liverpool.

3.10. The microscope slide should be labelled with the participant's unique identifier and stored for quality control.

4. SPECIMEN AND WASTE DISPOSAL

4.1. The semen bag should be discarded in accordance with the aseptic practices and Infection prevention procedures set by Ministry of Health in Malawi.

4.2. The used gloves, disposable protective wear and other waste will be disposed in accordance with Infection prevention procedures of the Ministry of Health in Malawi.

5. QUALITY CONTROL PROCEDURES

5.1. The P.I. and/or Fieldwork Advisor will perform microscopy on slides of the semen, random selected on daily basis as part of quality control.

6. REFERENCES

- Cheesbrough, M. (2005). District Laboratory Practice in Tropical Countries - Part 2. The Edinburgh Building, Cambridge, UK, Cambridge University Press.
- WHO (1991). Basic Laboratory methods in Medical parasitology. Geneva, Switzerland, World Health Organization.

- WHO (2010). WHO Laboratory manual for the examination and processing of human semen - 5th ed. Geneva, Switzerland, World Health Organization.

Appendix 18: Post-semen submission questionnaire

INDIVIDUAL PARTICIPANT QUESTIONNAIRE

Research study on Male genital schistosomiasis and HIV among Adult fishermen living in Southern shores of Lake Malawi (Post-semen submission Survey)

Explanation: Put a tick (√) for the participant's responses appropriately. If the boxes are not enough on one page, use another sheet. Return this and other sheets to the Study Investigator at the end.

Date: (dd/mm/yy): __ __ / __ __ / __ __ Interviewer Name_____

Consent checklist

Has written consent been obtained? Y ☐ N ☐ *Only proceed if Y*

Section A: Participant's details

1. Participant ID: _____
2. Village:_____
3. T/A: _____
4. Name of nearest Health centre:_____
5. Gender: Male ☐ Female ☐.
6. Date of birth (dd/mm/yy): __ __ / __ __ / __ __ Age: : _____
7. Weight (kg):_____
8. Height (cm):_____

Section B: After Semen collection

9. Did you experience any challenges or problems in submitting the semen sample? Y ☐ N ☐.
10. If **Y** to question 9, may you tell us the challenges or problems_____

    _____

    _____
11. Would you have preferred a collection bottle instead of a collection bag given? Y ☐ N ☐.
12. If **Y** to question 11, may you tell us the reason(s)_____

    _____

    _____

THANK YOU FOR YOUR PARTICIPATION

Appendix 19: Post-semen submission questionnaire (in chichewa)

INDIVIDUAL PARTICIPANT QUESTIONNAIRE (in Chichewa)

Kafukufuku wa Matenda a Likodzo la njira yaumuna ndi Edzi mwa Asodzi okhala m'bali mwa Nyanja ya Malawi (Post-semen collection survey)

Chidziwitso: Ikani (√) pa mayankho operekedwa ndi opanga kafukufuku. Pitirizani mayaknho anu pa tsamba lina ngati malo oyankhira achepa. Perekani mayankho onse kwa mkulu wa Kafukufukuyu.

Tsiku: (dd/mm/yy): __ __ / __ __ / __ __ Opangitsa Kafukufuku _____

Chilolezo cha opanga nawo Kafukufuku

Opanga kafukufuku apereka chilolezo? Inde ☐ Ayi ☐ *Pitirizani Kafukufuku ngati avomera Inde*

Gawo A: Mbiri ya Opanga Kafukufuku

1. Participant ID: _____
2. Mudzi:_____
3. T/A: _____
4. Chipatala chapafupi:_____
5. Ndinu: Mwamuna ☐ Mkazi ☐.
6. Tsiku lobadwa (dd/mm/yy): __ __ / __ __ / __ __ Zaka :_____
7. Thupi kulemera (kg):_____
8. Thupi kutalika (cm):_____

Gawo B: Mutatha kupereka Umuna wanu

9. Munakumana ndi vuto lililonse popereka umuna wanu? Inde ☐ Ayi ☐.
10. Ngati **Inde**, fotokozani mavuto munakumana nawo _____

    _____

    _____
11. Mukanakonda kupereka umuna mu botolo mmalo mwa pepala munapatsidwa? Inde ☐ Ayi ☐.
12. Ngati **Inde**, fotokozani maganizo anu pa yankho lanu _____

    _____

    _____

ZIKOMO KWAMBIRI POTENGA NAWO MBALI PA KAFUKUFUKUYU.

Appendix 20: Standard operating procedure for ultrasonography

ULTRASOUND EXAMINATION FOR MALE GENITAL SCHISTOSOMIASIS (MGS)

S.O.P. Reference number: MW-MGS-03

Version number: 02

Date of Issue: 1st November 2017

Authorised by: P.I. (Dr. Sekeleghe Kayuni)

This S.O.P. has been read and understood by Study team members:

NAME	POSITION	SIGNATURE	DATE
Dr Sekeleghe Kayuni	Principal Investigator		
Mr. Peter Makaula	Local Collaborator		
Mr. Boniface Injesi	Radiography Technician		
Dr Elizabeth Joekes	Radiologist		

This S.O.P must be reviewed regularly, where necessary and must be authorised by the P.I.

DATE OF REVIEW	REVIEWED BY	SIGNATURE

This S.O.P. has been superseded by S.O.P. number …….

Revision……. Date…….....

The master copy was transferred to the superseded S.O.P. file

Signature…….Date…….

ULTRASOUND EXAMINATION FOR MALE GENITAL SCHISTOSOMIASIS (MGS)

1. PRINCIPLE AND CLINICAL SIGNIFICANCE OF THE EXAMINATION

1.1 *Schistosoma haematobium* causes urogenital schistosomiasis, which is endemic in sub-Saharan African countries including Malawi, especially along water bodies like Lake Malawi. Infected people pass the eggs in urine and/or semen, and present with symptoms like fever, fatigue, dysuria, haematuria, haemospermia, pain of the genital organs, pain during or after sexual intercourse.

1.2 Laboratory diagnosis of *S. haematobium* infection is by detection of the *S. haematobium* eggs through microscopic examination of the urine, semen and other affected tissues excised.

1.3 Ultrasonography of urinary and genital organs is an important examination for morbidity assessment related to *S. haematobium* infection, useful for appropriate participants' management.

2. REQUIREMENTS

2.1 The participant for this ultrasound examination is the fishermen in the study.

2.2 The requirements for the ultrasonography of the male genital organs include:

- Bed/Couch: participant will lie in supine position for the exam, with the examiner on his right side.
- Ultrasound machine.
- Probes: curved array transducer – 3.5 MHz.
- Ultrasound Gel: used every-time as conductive medium between the patient's skin and the ultrasound transducer.
- Tissue paper for cleaning the probe and participants after examination.
- Methylated spirit for cleaning the probe.

3. STEPS OF THE ULTRASONOGRAPHY EXAMINATION

3.1. Safety precautions will be ensured always to prevent exposure to hazards. The study team will wear gloves and appropriate protective wear during examination.

3.2. Participants will be asked to present to the examination room with a full bladder.

3.3. The ultrasound procedure will be explained to the patient and/or caregiver.

3.4. The lights in the room will be turned off and the room darkened, if possible.

3.5. The patient will be placed in a lying position and the ultrasound machine set up on the right side of the patient.

3.6. The ultrasound machine will be put on the appropriate urology preset for the examination.

3.7. The participant's study number will be registered in the ultrasound machine and report form prior to starting the examination.

3.8. A reasonable amount of ultrasound gel will be put on the probe.

3.9. The ultrasound examination will be conducted in the following procedure:

a. Urinary bladder:

i. Probe position: The probe is placed transverse (TS) above the pubic symphysis with probe orientation projecting the right side of the patient on the left side of the screen. Transverse sweeps through the bladder are performed to assess the shape (distension) and wall thickness of the bladder, as well as the distal ureters where possible. Care should be taken to adjust depth and gain settings appropriately for anterior and then posterior bladder wall/ureters to avoid artefacts and reduced visibility of bladder wall. Longitudinal (LS) sweeps will also be performed in similar way.

ii. Normal finding: The bladder is fully distended and has a regular, rectangular shape. The bladder wall is of regular thickness and not thicker than 5 mm. Normal distal ureters are not visible.

iii. Pathological findings: Schistosomiasis-related urinary pathologies include a rounded or irregular shape of the bladder, wall thickening with diffuse or focal thickening of > 5 mm, bladder wall calcifications and masses or pseudopolyps protruding in the bladder lumen. The distal ureters are considered pathological when dilated.

iv. Bladder wall thickness will be measured in mm and stored as a separate still image. Storing of images and clips: After having performed several sweeps through the bladder, the best representative sweep will be stored under the label "bladder". In case of any pathologic findings additional still images with relevant measurements will be stored.

v. Once the bladder wall thickness is abnormal, the kidneys will be scanned for evidence of hydronephrosis.

vi. Reporting: The visualization conditions will be documented first. Subsequently, the absence/presence of pathological findings is documented on the report form.

b. Prostate:

i. The prostate will be visualised with the probe directed deep into the pelvis, after scanning the bladder. Care should be taken to adjust depth and gain settings appropriately to clearly and fully visualise the prostate.

ii. Normal finding: The prostate is normal when volume is 30 mm^3 or less with smooth outline.

iii. Pathological findings: Schistosomiasis-related pathologies include nodules or masses above 1 cm, and calcifications of the prostate.

iv. The prostate will be measured in cm^3 or ml and stored as a separate still image. Storing of images and clips: After having performed several sweeps through the prostate, the best representative sweep will be stored under the label "prostate".

In case of any pathologic findings additional still images with relevant measurements will be stored.

v. Reporting: The visualization conditions will be documented first. Subsequently, the absence/presence of pathological findings is documented on the report form.

c. Seminal vesicles:

i. The seminal vesicles will be visualised adequately with several sweeps, just after scanning the prostate.

ii. Normal finding: The seminal vesicles will be symmetrical, measuring 15 mm or less in antero-posterior (AP) plane with smooth outline.

iii. Pathological findings: Schistosomiasis-related pathologies include enlarged and or asymmetrical vesicles with nodular, echogenic appearance.

iv. If the vesicles measure larger than 15 mm in AP plane, their measurement will be stored as a separate still image. Storing of images and clips: After having performed several sweeps through the vesicles, the best representative sweep will be stored under the label “SV”. In case of any pathologic findings additional still images with relevant measurements will be stored.

v. Reporting: The visualization conditions will be documented first. Subsequently, the absence/presence of pathological findings is documented on the report form.

d. Scrotum:

i. Probe position: The probe is placed transverse on the scrotum with probe orientation projecting the right side of the patient on the left side of the screen. Axial sweeps on the scrotum are performed to assess both testes. Care should be taken to adjust depth and gain settings appropriately.

ii. Testis: if abnormal, describe whether there are nodules, masses, atrophy or calcifications.

iii. Epididymis: describe whether the epididymis is normal or enlarged.

iv. Other: describe whether hydrocele is present or not; and other abnormalities not mentioned earlier.

v. Reporting: Pathological findings should be documented on the report form.

3.10. All clips and images saved as described above, will be transferred from the ultrasound machine to an external hard drive, for a random second reading by the P.I. and quality control.

3.11. The used gloves, disposable protective wear and other waste will be disposed in accordance with the Infection prevention procedures set by the Ministry of Health in Malawi.

3.12. Probe hygiene: the probe will be cleaned with tissue paper to remove the gel, and with methylated spirit after each participant.

4. QUALITY CONTROL PROCEDURES

4.1 The P.I. and/or Specialist Radiologist will review at least 15% of the stored images / clips, randomly selected as part of quality control.

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Appendix 21: Data collection form for field laboratory examinations

Multidisciplinary studies of Male genital schistosomiasis (MGA) along Lake Malawi

Date of Study	Name of the Study	Name of Health centre	Study Lab personnel

ID no.	Volume (ml)	Urine card	Dipstick result	CCA test	Urine filtration	Semen bag eggs	Semen bag leucocytes	Semen centrifuge

Appendix 22: Data collection form for field ultrasonography examinations

ULTRASOUND SCAN REPORT

Research study on Male genital schistosomiasis and HIV among Adult fishermen living in Southern shores of Lake Malawi (MGS Baseline & Follow-up Survey)

Explanation: Put a tick (√) for the appropriate result. If the boxes are not enough on one page, use another sheet. Return this and other sheets to the Study Investigator at the end.

Date: (dd/mm/yy): __ __ / __ __ / __ __ Sonographer name: _____

Consent: Has written consent been obtained? Y ☐ N ☐ *Only proceed if Y*

Participant ID: _____

Weight (Kg): _____ Height (cm): _____

Section A: Urinary Bladder

1. Filling: ☐ Adequate ☐ Inadequate (go to Section B).
2. Shape: ☐ Normal ☐ Abnormal.
3. Wall thickness: ☐ Normal (≤ 5 mm) ☐ Abnormal, mild (5-10 mm)
4. Wall thickness, abnormal (go to Kidneys): ☐ ≥ 11 mm/polypoid/flat; ☐ mass (possible cancer)
5. Kidneys: ☐ Normal ☐ hydronephrosis (transverse pelvis > 10 mm); ☐ Right ☐ Left

Section B: Prostate

6. Visualisation: ☐ Adequate ☐ Inadequate
7. Organ: ☐ Normal ☐ Abnormal
8. Size: **W**_____ X **H**_____ X **D**_____ X **0.52** = _____ **OR** _____ **ml**^{3}
9. Outline: ☐ Smooth ☐ Irregular
10. Nodules / Mass: ☐ Absent ☐ Present ☐ Multiple. If present,
    a. Hypo-echoic: ☐ : Size: ☐ ≤1 cm; **W**___ X **H**___ X **D**___ X **0.52** = _____ **OR** ____ **ml**.
    b. Hyper-echoic: ☐ : Size: ☐ ≤1 cm; **W**___ X **H**___ X **D**___ X **0.52** = _____ **OR** ____ **ml**.
    c. Mixed echogenicity: ☐; Size: ☐ ≤1 cm; **W**___ X **H**___ X **D**___ X **0.52** = ___ **OR** ___ **ml**.
11. Calcifications: ☐.

Section C: **Seminal vesicles**

12. Visualisation: ☐ Adequate ☐ Inadequate
13. Symmetry: ☐ Normal ☐ Abnormal.
14. Size: _____mm; ☐ Normal (≤15 mm) ☐ Enlarged (>15 mm). If enlarged,
    a. Right ☐, Size: _____mm; Left ☐, Size: _____mm.
    b. Hypo-echoic: ☐; Right ☐ Left ☐.
    c. Hyper-echoic: ☐; Right ☐ Left ☐.

Section D: **Scrotum**

Testis:

15. Right: Normal ☐ Abnormal ☐. If abnormal,
    a. Nodules ☐ ; Mass ☐ Size: _____cm; Atrophy ☐; Calcifications ☐.
16. Left: Normal ☐ Abnormal ☐. If abnormal,
    a. Nodules ☐ ; Mass ☐ Size: _____cm; Atrophy ☐; Calcifications ☐.

Epididymis:

17. Right: Normal ☐ Enlarged ☐.
18. Left: Normal ☐ Enlarged ☐.

Other:

19. Hydrocele: Present ☐ Absent ☐. If present,
    a. Right ☐ Left ☐ .

20. Other abnormalities: ☐. Describe_____

_____

THANK YOU FOR YOUR PARTICIPATION.

Appendix 23: Research output – Manuscript published in the American Journal of Tropical Medicine and Hygiene

Am. J. Trop. Med. Hyg., 101(6), 2019, pp. 1331–1335
doi:10.4269/ajtmh.19-0562

Case Report: Highlighting Male Genital Schistosomiasis (MGS) in Fishermen from the Southwestern Shoreline of Lake Malawi, Mangochi District

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Abstract. Urogenital schistosomiasis causes morbidity within the genitalia but is underreported and infrequently examined in men. To draw attention to male genital schistosomiasis (MGS), a longitudinal cohort study was conducted among fishermen along the southwestern shoreline of Lake Malawi. A case series of five participants is presented inclusive of questionnaire interviews, parasitological examinations, ultrasonography, and provision of a standard dose (40 mg/kg) of praziquantel (PZQ) treatment at baseline, 1-, 3-, 6-, and 12-month follow-up time points. Eggs of *Schistosoma haematobium* were observed in urine or semen across all time points; parasitological diagnostics were bolstered by real-time PCR for *Schistosoma* DNA in semen and by portable ultrasonography to document putative MGS-associated morbidity. We highlight the importance of developing standard diagnostic tests for MGS and increasing the accessibility of PZQ treatment to men, especially those in at-risk endemic areas.

CASE SERIES

We report on five most notable and more severe cases from our longitudinal cohort study investigating male genital schistosomiasis (MGS) among fishermen along the southwestern shoreline of Lake Malawi, Mangochi District, Malawi. Fishermen were recruited and interviewed, and they submitted a mid-morning urine sample for reagent strip analysis (using Siemens Multistix® 10 SG, Siemens Healthcare Diagnostics Inc., New York, NY), point-of-care circulating cathodic antigen (POC-CCA) analysis, and syringe filtration with microscopy[1]; a semen specimen was also provided for parasitological diagnosis.[2,3] Ethanol-preserved semen was also shipped to Elisabeth-TweeSteden Hospital in Tilburg, the Netherlands, for DNA extraction and real-time polymerase chain reaction (real-time PCR) detection of *Schistosoma* DNA.[4]

Study participants underwent transabdominal and scrotal ultrasonography examinations for genital pathologies before receiving praziquantel (PZQ) at standard dose (40 mg/kg). They were invited for follow-up studies at 1-, 3-, 6-, and 12-month time points. Research ethical clearance was granted by the Liverpool School of Tropical Medicine (LSTM) Research Ethics Committee in the United Kingdom and the National Health Sciences Research Committee in Malawi (NHSRC).

Overall characteristics of the participant cohort at baseline include the following: prevalence of *Schistosoma haematobium* egg patency in urine was 17.1% ($n = 210$, mean = 14.8 eggs per 10 mL) and in semen was 10.4% ($n = 114$, mean = 5.9 eggs per mL), whereas on real-time PCR, it increased to 26.6%. The prevalence of intestinal schistosomiasis was 3.8%, as estimated using POC-CCA tests.

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Case 1. LN, a 20-year-old man, weighing 66 kg, lives on Lake Malawi shores since birth and has been fishing for the past 10 years. He reported body weakness and increasing urinary frequency for a month and also noted delayed or no ejaculation during coitus, together with reduced semen volume. He reported terminal hematuria between age 9 and 14 years, which was treated. In the past year, he received PZQ as part of the annual mass drug administration (MDA) campaign in the district.

On examination, his urine sample was of normal color with no turbidity, and the reagent strip was negative for leukocytes and blood but with trace of protein. The POC-CCA test on urine was negative, and no *S. haematobium* egg was observed after filtration. In his 1.5-mL semen specimen, 14 eggs and 60 leukocytes were detected by direct clear-bag microscopy and 10 eggs after centrifugation. However, real-time PCR was subsequently negative. Ultrasonography was normal. He was given PZQ, and follow-up at 1, 3, and 12 months revealed no abnormalities or symptoms previously reported.

Case 2. AJ, a 44-year-old man, 57.9 kg body weight, has been fishing in the lake for 32 years, where he bathes and washes daily. He was stable on antiretroviral therapy for HIV infection for over 6 months. For a month, he experienced headache, dysuria, hematuria, blood in stool, hemospermia, and genital pain, sometimes on ejaculation. Together with his wife, he suspected sexually transmitted infection (STI) and had accessed treatment. He also suspected schistosomiasis but did not access treatment.

He was unable to submit a semen specimen for examination, although his urine was of normal color and reagent strip was positive for leukocytes (1+), protein (1+), and blood (microhematuria 3+), with a positive POC-CCA test. After filtration, 744 and 488 *S. haematobium* eggs were detected in 40 mL and 50 mL urine samples, respectively, submitted on 2 alternate days. Genital ultrasonography was normal (Figure 1A), and he was given PZQ on each of the 2 days. At 1-month follow-up, his symptoms had improved and submitted 1 mL semen which had no eggs, and real-time PCR

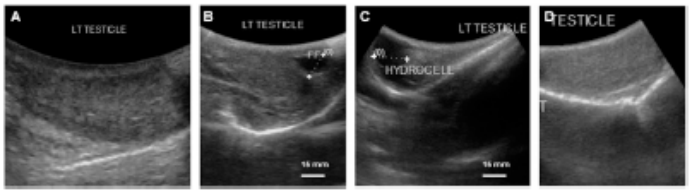

FIGURE 1. Pictorial illustration of the ultrasonography of the left testis for Case 2. (**A**) Normal testicular tissue at baseline; (**B**) left hydrocele noted at 1-month follow-up, measuring 12.7 mm; (**C**) left hydrocele persisted at 3-month follow-up, measuring 17.7 mm; (**D**) no hydrocele observed at 6-month follow-up, normal testicular tissue observed.

was positive (Ct value = 23.7). However, his 35-mL urine sample showed 81 *S. haematobium* eggs and a negative POC-CCA test. Ultrasonography revealed a 12.7-mm left hydrocele (Figure 1B). Praziquantel was given again.

At the 3-month follow-up, he complained of having had genital sores in the preceding month, although a venereal disease research laboratory serological test was negative for syphilis antibodies. His 160-mL urine sample contained 243 *S. haematobium* eggs, trace for protein and was negative for POC-CCA test. In his 1-mL semen specimen, seven and four eggs were observed on bag and centrifugation methods, respectively, and was positive by real-time PCR (Ct value = 26.7). The left hydrocele was still present on genital ultrasonography (17.7 mm), and PZQ was provided again (Figure 1C). At the 6-month follow-up, the only notable finding was a positive semen real-time PCR (Ct value = 32.3) (Figure 1D), while at 12 months, no abnormal results were observed, and real-time PCR was negative.

Case 3. SK, a 49-year-old man, 51.5 kg body weight, has lived along the lake since birth and has been fishing for the past 10 years. He had episodes of headache, dysuria, urine color changes, and blood in stool for over a month. He received PZQ in the past year during the annual MDA campaign.

His urine was of normal color with microhematuria (2+) and proteinuria (1+) but no leukocytes, and showed a negative POC-CCA test, and five *S. haematobium* eggs were observed after filtration. In his 4-mL semen specimen, two eggs and 12 leukocytes were observed using the bag method and three eggs after centrifugation. On ultrasonography, a calcified nodule in the left testis was noted (Figure 2).

Praziquantel was offered at this baseline presentation and at 1- and 3-month follow-up. No *S. haematobium* eggs were observed in urine or semen, and real-time PCR was negative, although ultrasonography showed mild bilateral hydroceles. After PZQ, he was lost to follow-up at 6- and 12-month time points.

Case 4. ARK, a 27-year-old man, 59 kg body weight, has been fishing daily in the lake for 5 years, where he bathes and washes his clothes regularly. He did not report any symptoms or receive PZQ during annual MDA campaigns.

His 110-mL urine sample was of normal color, and negative for leukocytes, protein, and POC-CCA, although the reagent strip test showed microhematuria (1+), and 12 *S. haematobium* eggs were observed. No eggs or leukocytes were observed on microscopy in his 2-mL semen specimen, although real-time PCR was positive (Ct value = 26.6). Ultrasonography was normal, and PZQ was given. At 1-month follow-up, where PZQ was given again, his semen remained positive by real-time PCR (Ct value = 25.8), although no eggs were seen in the urine and ultrasonography was normal. At 3-month follow-up, 18 eggs were observed in an 80-mL urine sample and two eggs in a 2.5-mL semen specimen (centrifugation only), with positive real-time PCR (Ct value = 25.0) and abnormal bladder shape and mild wall thickness (5–10 mm) revealed on ultrasonography. Urine, semen, and ultrasonography results were normal at 6 months, with negative real-time PCR.

Case 5. TF, a 47-year-old man, 60.3 kg body weight, lives near the lake and has been fishing for 5 years, where he bathes and washes his clothes daily. He experienced dysuria, urine frequency, and urine color change for a month, and episodes of malaria, dysentery, worm infestation, and STI for that duration.

His urine was of normal color, and the reagent strip test detected proteinuria (2+) and microhematuria (3+); however, POC-CCA was negative, with no *S. haematobium* eggs detected. His 4-mL semen specimen revealed 11 eggs on microscopy after centrifugation and was positive by real-time PCR (Ct value = 29.7). Ultrasonography was normal, and PZQ was provided. At the 3-month follow-up, no *Schistosoma* eggs were detected in urine or semen, with his semen now negative by real-time PCR. However, ultrasonography revealed a mildly enlarged bladder wall and asymmetrical, enlarged seminal vesicles (≥ 15 mm). Praziquantel was given

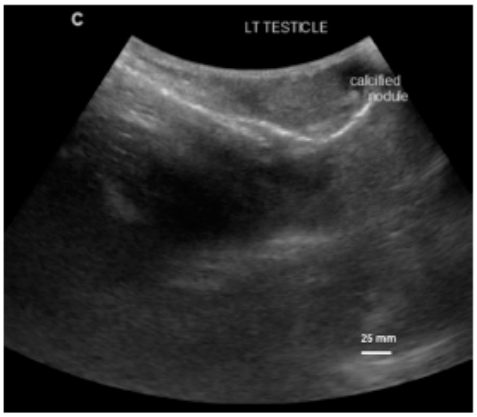

FIGURE 2. Ultrasonographic image showing genital abnormality, calcified nodule in the left testis for Case 3, observed baseline.

again, and he was lost to follow-up at 6- and 12-month time points.

DISCUSSION

Schistosomiasis, a waterborne parasitic disease associated with poverty, is prevalent in many tropical and subtropical countries, especially those in sub-Saharan Africa (SSA). The disease afflicts over 200 million people worldwide, and in SSA, *S. haematobium* and *Schistosoma mansoni* occur, causing urogenital and intestinal schistosomiasis, respectively.[5–7] Male genital schistosomiasis is a gender-specific manifestation of urogenital schistosomiasis, being first reported in 1911[8] and associated with the presence of schistosome eggs and pathologies in genital fluids and organs. Features described include genital or ejaculatory pain, hemospermia, infertility, abnormal enlarged organs,[9,10] as well as, granulomatous infiltration, fibrosis, and calcifications on postmortem, histopathological, and radiological examinations.[11–13] Despite these descriptions and significant schistosomiasis burden in SSA, MGS often remains undiagnosed and underreported within endemic regions like the shoreline of Lake Malawi.[14] It is also of note that some of these men also have intestinal schistosomiasis as evidenced by the POC-CCA test, which is consistent with local emergence of autochthonous transmission of *S. mansoni* in this part of the lake.[15]

To our knowledge, this case series from our longitudinal cohort study provides a unique description of MGS among local fishermen in an endemic setting of Lake Malawi shoreline in SSA. Our cases presented symptoms of MGS described in the literature, which can be mistaken for STI as in the example described in "Case 2," and, thus, incorrectly received routine syndromic STI management,[16] highlighting the mistaken diagnosis and mismanagement of MGS treatable with PZQ.[17,18]

The changing clinical presentations and parasitological results of our cases highlight the challenges associated with MGS diagnosis and management, requiring the development of better, low-cost, accessible, sensitive, and specific diagnostic tests. Although real-time PCR demonstrates greater ability to incriminate most cases, it remains relatively expensive and not always available in endemic areas. The downward trend in egg count and clinical improvement of our cases after standard PZQ treatment at 1 month and later show parasitological clearance of eggs in semen and putative cure of the infection (Figure 3). Repeated or increased PZQ doses could be further beneficial in individual patient management when cases of high egg intensity infection are seen, for example, in "Case 2," in addition to other prevention measures for schistosomiasis.

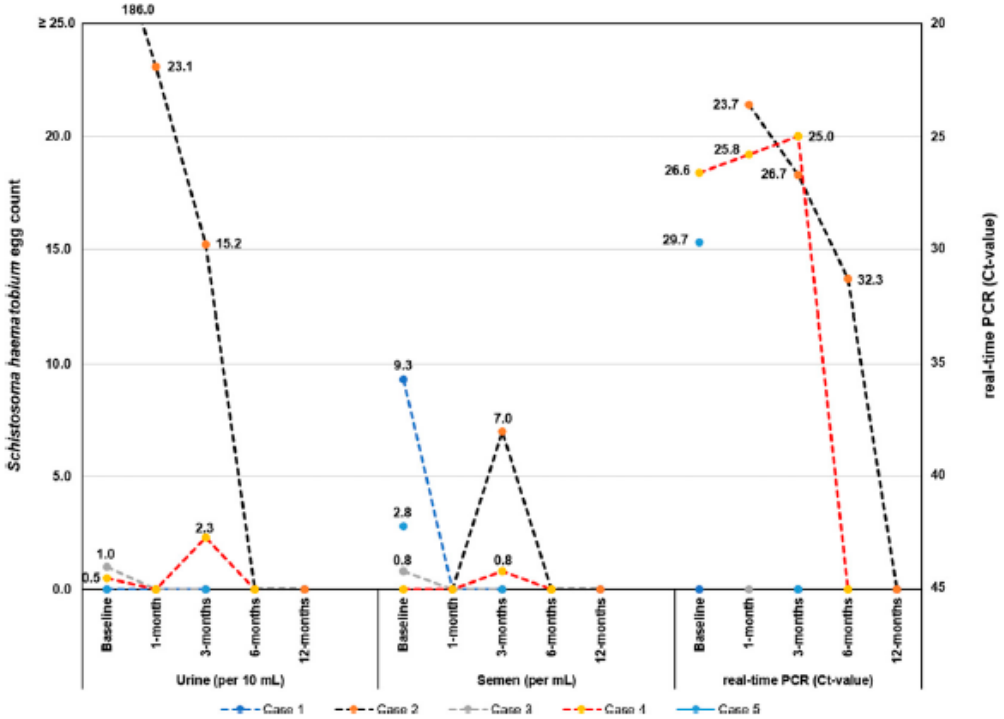

FIGURE 3. A line graph of the cases in the longitudinal cohort study showing results of *Schistosoma haematobium* egg counts in urine (per 10 mL) and semen (per mL), and Ct values for real-time polymerase chain reaction on semen at baseline, 1-, 3-, and 6-month follow-up studies. This figure appears in color at www.ajtmh.org.

Furthermore, organ-specific pathologies in seminal vesicles, testes, and prostrate alongside the urinary bladder, resulting from MGS, can be observed on ultrasonography. Encouragingly, these cases (Cases 2, 3, and 5) show a degree of some observed resolution after PZQ treatment. Comprehensive clinical assessment is essential to exclude other important diseases such as STI, tuberculosis, and malignancy which present similar symptomatology and pathologies as MGS.

Genital inflammation triggered by *Schistosoma* eggs has been shown to increase cytokine levels, such as interleukin-6 and tumor necrosis factor-α. These cytokines can facilitate HIV replication, which can increase seminal viral shedding, alluding to a plausible additional risk of HIV transmission from males infected with HIV and urogenital schistosomiasis.[19–21] Examples such as Case 2 describing the coinfection of these two diseases could in future provide opportunities for further virological and immunological analyses to illustrate the impact of routine preventive PZQ treatment on the potential risk of HIV transmission among dually infected fishermen and other high-risk populations in endemic areas. Furthermore, additional diagnostic investigations to exclude other diseases such as STIs, which could be present in this case, would be informative.

In conclusion, this case series better describes the occurrence of MGS among local fishermen from an area endemic for schistosomiasis along the shoreline of Lake Malawi where about just more than a fifth of all sampled men have active urogenital schistosomiasis. In Malawi, MGS has been underreported and remains overlooked by many medical professionals, highlighting the challenges relating to awareness of MGS in both health professionals and men at risk alongside their health-seeking behaviors, point-of-contact diagnostic limitations, and clinical management strategies. It is very clear that targeted future research on MGS and its coexistence with other common diseases, such as HIV, is needed in endemic areas.

Received July 29, 2019. Accepted for publication September 11, 2019.

Published online November 4, 2019.

Acknowledgments: We thank the district health officer and management team of Mangochi District Health Services for their overwhelming support toward the study; Dr. Sam Kamponderi and Dr. Elizabeth Joekes for their assistance in training on ultrasonography; and Dr. Joekes for quality control of the ultrasonography; Merck plc for donation of the portable ultrasound unit; Messrs Bright Mainga, Pilirani Mkambeni, Patrick Hussein, Mkonazi Nkhoma, Matthews Elias, and Boniface Injesi for technical assistance; and all the fishermen who participated in the longitudinal cohort study in Mangochi district. We are grateful to the head and staff of the laboratory department at Mangochi District Hospital; district ART coordinators, in-charge, and staff of Tikondane clinic; in-charges and staff of Billy Riordan Memorial Clinic; Monkey-Bay Community Hospital; Nkope and Koche Health Centres; Lumbua Katenda, Anwar, and staff of Mangochi LMJ clinic; local community health workers: Ambali Makochera, Alfred Kachikowa, Rodgers Wengawenga, Michael Tsatawe, Chikondi Mtsindula, Mambo Amin, Elias Matemba, Austin Kaluwa, Justin Mndala, Promise Mwawa, Dickson Tabu, George Matiki, Alfred Mdoka, and Charles Katandi; Brother Henry Chagoma and staff of Montfort Mission Guest House; and all traditional leaders in the hosting fishing communities and beach committee members for their enthusiasm and support during the study.

Financial support: S. A. K. was funded by a PhD scholarship from Commonwealth Scholarship Commission, United Kingdom, which supported the baseline and 1-month follow-up studies. He received an International Travel Fellowship from the British Society for Parasitology to conduct 3- and 6-month follow-up studies and from World Friendship Charity to conduct 12-month follow-up studies. J. F. and A. S. were supported by LSTM Education Department MSc Degree Research Project Module funding. This publication was supported by a grant from the African Research Network for Neglected Tropical Diseases (ARNTD) through United States Agency for International Development (USAID), UK aid from the British people (UK aid) and Coalition for Operational Research on Neglected Tropical Diseases (COR-NTD). Its contents are solely the responsibility of the authors and do not necessarily represent he views of ARNTD, COR-NTD, UK aid or the USAID.

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Parasitology

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Research Article

Cite this article: Kayuni S A *et al* (2019). How can schistosome circulating antigen assays be best applied for diagnosing male genital schistosomiasis (MGS): an appraisal using exemplar MGS cases from a longitudinal cohort study among fishermen on the south shoreline of Lake Malawi. *Parasitology* 1–11. https://doi.org/10.1017/S0031182019000969

Received: 20 February 2019
Revised: 30 June 2019
Accepted: 3 July 2019

Key words:
CAA; CCA; diagnostics; glycans; MGS; *Schistosoma haematobium*

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How can schistosome circulating antigen assays be best applied for diagnosing male genital schistosomiasis (MGS): an appraisal using exemplar MGS cases from a longitudinal cohort study among fishermen on the south shoreline of Lake Malawi

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Abstract

We provide an update on diagnostic methods for the detection of urogenital schistosomiasis (UGS) in men and highlight that satisfactory urine-antigen diagnostics for UGS lag much behind that for intestinal schistosomiasis, where application of a urine-based point-of-care strip assay, the circulating cathodic antigen (CCA) test, is now advocated. Making specific reference to male genital schistosomiasis (MGS), we place greater emphasis on parasitological detection methods and clinical assessment of internal genitalia with ultrasonography. Unlike the advances made in defining a clinical standard protocol for female genital schistosomiasis, MGS remains inadequately defined. Whilst urine filtration with microscopic examination for ova of *Schistosoma haematobium* is a convenient but error-prone proxy of MGS, we describe a novel low-cost sampling and direct visualization method for the enumeration of ova in semen. Using exemplar clinical cases of MGS from our longitudinal cohort study among fishermen along the shoreline of Lake Malawi, the portfolio of diagnostic needs is appraised including the use of symptomatology questionnaires, urine analysis (egg count and CCA measurement), semen analysis (egg count, circulating anodic antigen measurement and real-time polymerase chain reaction analysis) alongside clinical assessment with portable ultrasonography.

Introduction

Schistosomiasis remains a prevalent neglected tropical disease (NTD) in low and middle-income countries of tropical and sub-tropical regions (Colley *et al.*, 2014; McManus *et al.*, 2018). Each year, some 200 000 deaths occur from complications of this water-borne infection which is acquired by exposure to contaminated freshwater, often during household chores, recreational activities or income-generating activities such as fishing or agriculture (Hotez *et al.*, 2008; Christinet *et al.*, 2016). Following World Health Assembly resolutions to control schistosomiasis, the World Health Organization (WHO) and various stakeholders have continued urging countries in endemic areas to intensify morbidity control and strive towards interruption of schistosome transmission (WHO, 2001, 2012, 2013). A key intervention strategy is preventive chemotherapy by mass drug administration (MDA) with praziquantel (Cesol, Merck), integrated alongside complimentary measures inclusive of improved sanitation and hygiene, snail control and health education. In addition, an appropriate use of point-of-care (POC) diagnostic tests in high- and low-disease transmission settings and individual targeted treatment are vital in schistosomiasis control (Le and Hsieh, 2017).

Diagnosis of urogenital schistosomiasis

First reported by Theodor Bilharz in 1851, infection with schistosome blood flukes gives rise to schistosomiasis, with *Schistosoma haematobium*, as recognized today, responsible for

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urogenital schistosomiasis (UGS); here, adult female worms, as typically found in the vesicle plexus of the bladder, produce copious amounts of eggs each day that perforate and damage various internal organs (Gryseels *et al.*, 2006; Rollinson *et al.*, 2013). Schistosome eggs either cross the bladder wall to be voided in the urine, or become tissue-trapped in the lower abdominal organs, inclusive of the internal and external genitalia in both genders (Ross *et al.*, 2002). These incite local bleeding and induce fibrotic lesions leading to severe complications across the urogenital system. Early diagnosis in communities triggers appropriate praziquantel treatment campaigns and is paramount to maximizing the public health impact of preventive chemotherapy (Stothard *et al.*, 2013).

A range of parasitological, immunological and molecular methods have been used for the detection of UGS (Stothard *et al.*, 2014). The operational gold standard of diagnosing UGS is direct microscopy of filtered urine (Peters *et al.*, 1976), which has been widely used in high-transmission areas to estimate the morbidity upon enumeration of eggs in 10 ml of urine (i.e. ⩾50 eggs per 10 m). However, it lacks diagnostic sensitivity in light infection when the number of eggs shed in urine is very few (counting less than one egg in 10 ml) and repeated urine samples may need to be inspected. Other less expensive methods that can complement this test include the use of questionnaires in high-risk areas for the recognition of macrohaematuria (presence of red urine), and urine reagent test strips for microhaematuria as a diagnostic indicator and marker of bladder pathology, although these suffer from poor rates of sensitivity (<75%) and cannot detect sub-clinical or acute infections (Stothard *et al.*, 2014; Le and Hsieh, 2017).

Antibody-based tests such as enzyme-linked immunosorbent assay (ELISA) for IgG titres against schistosome soluble egg antigen present in human serum have been widely used for diagnosis (Le and Hsieh, 2017). Serological methods have much higher sensitivities than filtration and microscopy, especially in travellers originating from non-endemic regions, however they cannot distinguish active from past infections, nor discriminate between species of schistosome. Alternative highly sensitive approaches based on the detection of schistosome glycan antigens in blood or urine have been developed to diagnose active *Schistosoma* infections (Utzinger *et al.*, 2015; Le and Hsieh, 2017), which are described below. Nucleic acid amplification tests (NAAT) have increasingly been used as highly sensitive and specific diagnostic tools, utilizing several clinical specimens (i.e. stool, urine or tissue biopsy) in diagnosing the infection (Utzinger *et al.*, 2015). Currently NAAT are not widely available, owing to the need for skilled personnel, laboratory equipment and infrastructure which make roll out, especially in endemic areas, limited. Newer approaches based on loop-mediated isothermal amplification, POC magnetic bio-capture probes and microfluidic devices are being developed for resource poor settings (Minetti *et al.*, 2016; Candido *et al.*, 2018; Poulton and Webster, 2018).

Glycobiology of schistosome antigens and their applications

A number of different schistosome antigens are excreted and secreted into the human circulation, namely cercarial antigens, gut-associated antigens from living juvenile and adult worms and antigens secreted from eggs (van Lieshout *et al.*, 2000). Most of the described circulating genus specific antigens in humans are from gut-associated tissues of feeding worms, namely circulating cathodic antigen (CCA) and circulating anodic antigen (CAA). Both CCA and CAA, see Fig. 1A, are detectable in the host's serum as well as in urine although relative concentrations can differ and these are thought to have immunomodulating effects within the parasitized host (Dam and Deelder, 1996; van Dam *et al.*, 1996; van Diepen *et al.*, 2012; Hokke and van Diepen, 2017).

Glycoproteins containing CCA are produced by the gut epithelium of schistosomes presumably for its protection and are regurgitated into the human bloodstream upon digestion of the blood meal as worms have a blind-end gut. The structure of these positively-charged antigens consists of multiple trisaccharide units (Lewis-X) containing fructose, galactose and N-acetyl-galactosamine, Fig. 1. In addition, CCA epitopes are also present on *Schistosoma* egg secretions. They also evoke high titres of specific IgM/IgG antibodies, which may be responsible for the mild-moderate neutropenia during schistosome infection (van Dam *et al.*, 1996).

Making use of CCA, POC urine-based lateral-flow assays have been developed since the late 90s and have been commercially available since 2002 in the form of reagent dipsticks or cassettes, with carbon- or gold-labelled monoclonal antibodies and interpreted visually (van Dam *et al.*, 2004; Le and Hsieh, 2017). Detectable CCA-levels typically correlate with active schistosome infection, which become undetectable after successful praziquantel treatment. However, upon comparison with intestinal schistosomiasis (caused by *Schistosoma mansoni*) these tests perform poorly for UGS see Table 1, hence combining it with urine filtration is needed, and can help with simultaneous detection of co-infected cases (i.e. *S. haematobium* and *S. mansoni*). Since the first use of point-of-care circulating cathodic antigen (POC-CCA) tests, they have been subject to many evaluations of their performance, with the WHO now endorsing these tests as appropriate for estimating prevalence thresholds for intestinal schistosomiasis to guide preventive chemotherapy (Colley *et al.*, 2017; Bärenbold *et al.*, 2018). Current developments in POC testing include a prototype dual antigen cassette with both CCA and CAA strips included, enabling the detection and discrimination of intestinal and UGS simultaneously see Fig. 1B (see https://freebily.eu/about/).

The alternative CAA antigens are also gut-associated glycoproteins but are negatively charged. The structure of CAA is made up of carbohydrate chains which consist of multiple disaccharide units containing N-acetyl-galactosamine and glucuronic acid (Fig. 1). It binds to the collagen-like stalk of first complement component C1q, probably preventing host complement from attacking the schistosome gut (van Dam *et al.*, 1993). CAA is also present in urine or serum of actively infected people as shown by monoclonal antibody-based antigen detection ELISA's and more recently up-converting phosphor-lateral flow assay (UCP-LF CAA) (Corstjens *et al.*, 2008). This assay has shown to be more sensitive and specific especially in low-transmission areas but is unable to differentiate between urogenital and intestinal schistosomiasis (Corstjens *et al.*, 2015; Knopp *et al.*, 2015). The UCP-LF-CAA test therefore has future application in the general monitoring of schistosomiasis as disease control programmes move towards interruption of transmission or endgame scenarios (Corstjens *et al.*, 2017; Stothard *et al.*, 2017).

Focus on male genital schistosomiasis

Despite being known as urogenital disease, genital manifestations of *S. haematobium* infections in both genders have been underreported, ignored and less frequently diagnosed. Unlike the increasing awareness of female genital schistosomiasis (FGS) within endemic populations (Christinet *et al.*, 2016), in part due to the wider health-seeking behaviour of women, an appreciation of male genital schistosomiasis (MGS) remains limited. MGS describes a specific manifestation associated with the presence of schistosome eggs in seminal fluids and genital tissues in addition to various pathologies in the male genital system (WHO, 2018). Following

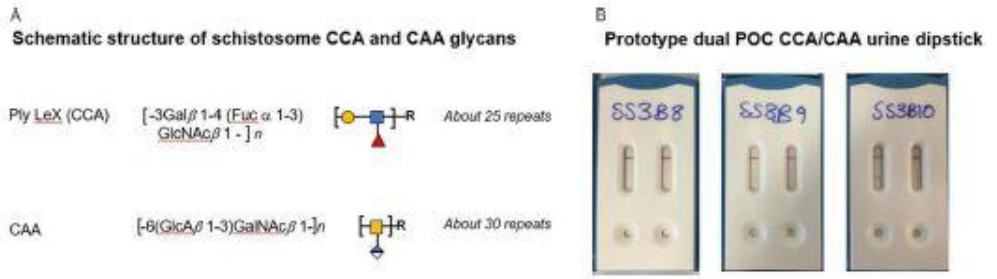

Fig. 1. (A) Schematic outline of the chemical and polymeric glycan structures of the two most common schistosome glycoproteins (CCA and CAA) using in rapid urine-antigen detection dipsticks. (B) An illustration of future developments in POC diagnostics with a prototype dual antigen urine-dipstick detecting each antigen separately (LHS CCA, RHS CAA). Having a dual design could detect and differentiate urogenital and intestinal schistosomiasis co-infection simultaneously, however, this prototype has inadequate sensitivity for the detection of urine-CAA and needs reformulation.

Table 1. Sensitivity and specificity of urine POC-CCA tests to diagnose *S. haematobium* infection, in comparison to urine filtration and microscopy as a routine standard test

Source	Prevalence (%)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Stothard *et al.* (2009)	31	9 (2–21)	98 (93–100)
Ayele *et al.* (2008)	Moderate	52 (42–62)	64 (54–73)
Midzi *et al.* (2009)	Moderate	79 (70–86)	44 (36–52)
Ashton *et al.* (2011)	Moderate	37 (26–49)	79 (72–84)
Obeng *et al.* (2008)*	Not stated	41 (not stated)	91 (not stated)
El-Ghareeb *et al.* (2016)*	5	88 (not stated)	96 (not stated)
Sanneh *et al.* (2017)*	23	48 (not stated)	76 (not stated)
Rubaba *et al.* (2018)*	40	68 (not stated)	46 (not stated)
Range	–	9–88	44–98

Data adapted from Ochodo *et al.* (2015), where intensities of infection are classed as 'moderate'. Additional sources marked by '*'. Where data is missing this is marked as 'not stated'.

its first description by Madden, (1911), several research studies and case reports have described the condition, unknowingly present in endemic areas, causing genital and pelvic pain, haemospermia, abnormal ejaculates, infertility among other abnormalities, also detectable by radiological methods (Vilana *et al.*, 1997; Squire and Stothard, 2014; Kayuni *et al.*, 2019). Furthermore, studies have shown increased levels of inflammatory cells and immunological mediators in semen harbouring *Schistosoma* eggs which necessitate HIV attachment and replication, and changes in seminal viral loads among co-infected males, highlighting the plausible link of increased risk of HIV transmission from co-infected males to their sexual partners (Leutscher *et al.*, 2000, 2005, 2008*b*; Stecher *et al.*, 2015; Midzi *et al.*, 2017).

Substantial progress has been made in developing a gold standard technique for a definite FGS diagnosis, namely colposcopy in gynaecological clinics (WHO, 2015) often with genital tissue biopsy for histopathology in the hospital laboratory. Conversely, MGS remains largely undefined, an orphan within disease syndromic triage (Kayuni *et al.*, 2019). At present, semen microscopy is considered as a standard technique for diagnosing active MGS infection and assessing its severity, since the schistosome eggs are directly visualized. Urine filtration has been used as diagnostic proxy markers in the presence of MGS symptoms; however, there have been reports of seminal schistosome eggs in urine negative patients (Schwartz *et al.*, 2002; van Delft *et al.*, 2007). In addition, genital tissue biopsy and ultrasonography can be applied as diagnostic tools relevant in diagnosing MGS through observation of pathologies associated with the disease in the absence of other genital diseases, which have successfully been studied and reported (Leutscher *et al.*, 2008*b*).

In light of the above, we describe the research study protocol of our longitudinal cohort MGS study among fishermen (with and without HIV infection) along the south shoreline of Lake Malawi in the Mangochi District. Preliminary results of the study at baseline are presented together with two exemplar clinical case reports, illustrating the diagnostic challenges for MGS.

Longitudinal cohort study of MGS along southern Lake Malawi shoreline

Malawi is one of the South Eastern African countries where both *S. haematobium* and *S. mansoni* are prevalent and highly focal around most water bodies (Teesdale and Chitsulo, 1985; Makaula *et al.*, 2014). The shoreline of Lake Malawi, the third largest lake in Africa, is endemic for urogenital schistosomiasis, with a high prevalence of urine-ova patent *S. haematobium* infections (Madsen *et al.*, 2011; Stauffer *et al.*, 2014). More recently, with the discovery of *Biomphalaria pfeifferi* there is also emergence of autochthonous transmission of intestinal schistosomiasis (Alharbi *et al.*, 2019). More broadly, children, women, farmers and fishermen are at greater risk of the disease due to more frequent water contact.

The prevalence of HIV in Malawi is considered high (10.6%), especially in this lakeshore region (11.8%), despite the control efforts contributing to reducing the incidence and mortality (NAC, 2015; UNAIDS, 2016). Despite the wide awareness for the significant burden of UGS in the area, MGS typically remains

undiagnosed and underreported among men. With no information about the burden of MGS on the south shoreline of Lake Malawi in Mangochi District, our research study set out to determine the current prevalence and morbidity of MGS among local fishermen on the shoreline and the potential risk of raised HIV transmission through viral load shedding in semen.

Study methodology

Study area, population and sampling

The research study was conducted among fishermen living in fishing communities (villages) identified and selected along the south shoreline of Lake Malawi in Mangochi District from October 2017 to December 2018. Mangochi is the largest district in the southern region of Malawi, covering 6729 km^2 of land with at least 1.1 million people (NSO, 2018). The district has a tropical continental climate with a longer dry season of cold weather from May to August and hot weather from September to November, and a relatively shorter wet season from December to April (NSO, 2011). Most fishermen in the area live in specific fishing villages, closer to the lake to carry out their routine fishing related activities.

This was a longitudinal cohort study, comprising baseline surveys of MGS among fishermen and follow-up studies after praziquantel treatment, conducted in villages and nearby health centres. Fishermen aged ⩾18 years willing to provide written informed consent were eligible to participate in the study. Using the estimated 20% prevalence of *S. haematobium* in adults from previous studies and assuming 10% having MGS, a minimum sample size of 275 fishermen (adjusted for assumed 10% loss to follow-up), was planned to be randomly selected for the study to measure the current prevalence of MGS and subsequent follow-up studies (Kirkwood and Sterne, 2006; CDC, 2014).

Data collection and analysis

The following are the data collection methods and analyses that were used in the study:

Individual questionnaires

After briefing about the study and obtaining written informed consent, fishermen were recruited in their communities and interviewed with individual questionnaires, collecting information on demographic, health, hygiene, sanitation and socio-economic characteristics. This information assessed their knowledge, perceptions, attitudes and practices on MGS and HIV. The questionnaires were developed from standardized questions administered elsewhere in a similar study (Ukwandu and Nmorsi, 2004). The questionnaires were piloted on the first 10 participants to assess the reliability of the questions. After the questionnaire interviews, the participants were invited to the nearby health facility to submit urine, semen and for ultrasonography examination.

Parasitological analyses

At the health facility, they were provided with a clean sample container to submit urine, between 10 am and 2 pm for filtration to examine for schistosome eggs (confirming UGS). Semen was submitted in a clear, transparent, self-sealing plastic bag, see Fig. 2, after abstaining from coitus for two days to examine for MGS, defined in the study as the presence of schistosome eggs in semen.

Urine analysis – filtration: Urine was analysed immediately for macrohaematuria by visual inspection using a urine colour card, and then for microhaematuria, leukocytes and proteinuria using reagent strips (Siemens multistix 10G) and scores were recorded in the following categories: negative, trace, +, ++ and +++. The POC-CCA test was conducted on the urine to assess for possible intestinal infection by *S. mansoni*, following manufacturer's instructions (Rapid Medical Diagnostics, South Africa; batch no. 171103130) and as described previously (van Dam *et al.*, 2004). Urine was measured and recorded accordingly, before conducting filtration following approved standard guidelines (WHO, 1991; Cheesbrough, 2009).

The entire volume of urine was filtered through a disinfected filter containing a clean polycarbonate membrane with 20 μm pores to trap all *S. haematobium* eggs in the sample. The membrane was removed, placed on a standard glass slide and examined under a microscope. Iodine was added to visualize the eggs distinctly. The number of eggs was calculated by first, dividing the total eggs observed by the total volume filtered and then multiplying by 10. The resultant egg count was recorded per 10 ml of urine. Highest infection intensity for UGS was defined as egg count of ⩾50 eggs per 10 ml urine as widely described (Cheesbrough, 2009).

Seminal microscopic analysis: After submission, the bag with semen was placed at room temperature on a clean bench surface to allow the semen to liquefy. Thereafter, the semen was pushed gently to one corner of the clear plastic bag. Then the bag was heat-sealed to evenly concentrate the semen for easy visualization during microscopy. The direct examination of the semen bag was conducted under a microscope to check for schistosome eggs and the presence of leukocytes (WHO, 2010), thereafter the results were recorded as per ml of ejaculate.

Afterwards, the semen was measured and centrifuged at 3000 rpm for 5 min to collect the seminal plasma. The sediment was re-dissolved in 0.5 ml normal saline for wet mount inspection using 2–3 drops and placed on a slide with a coverslip for microscopy, followed by recording of the results. Thereafter, 0.5 ml of ethanol was added to the remaining sediment for preservation and stored together with the seminal plasma at −80 °C in preparation for shipment to the United Kingdom for the real-time polymerase chain reaction (PCR) analysis of *Schistosoma* genus DNA and HIV viral load for those participants on antiretroviral therapy (ART).

UCP-LF CAA seminal analysis: A trichloroacetic acid (TCA) extraction was performed on the seminal plasma following standard methods used for serum with an equal volume of 4% w/v TCA (Corstjens *et al.*, 2008). Small volume extraction (50 μl seminal plasma with 50 μL TCA) in microfuge tubes resulted in a clear supernatant after centrifugation (5 min, 13 000 rpm). UCP-LF CAA analysis was performed according to standard methods with 20 μl of the clear supernatant, with a cut-off threshold of 10 pg ml^{-1}. High-volume extraction (0.5 ml seminal plasma with 0.5 ml TCA and a cut-off threshold of 1 pg ml^{-1}) required extended centrifugation time (30 min) before a clear supernatant was obtained; the resulting pellet was not rigid. Amicon 10 kDa centrifugal filtration devices (Merck Millipore) were used to concentrate 0.5 ml of the clear supernatant targeting concentration to 20 μl following standard methods used for serum undergoing centrifugation for 30 min at 13 000 rpm (Corstjens *et al.*, 2014).

Schistosoma *DNA real-time PCR analysis:* The ethanol preserved semen sediment was defrosted and centrifuged for 1 minute at 10 000 rpm. The ethanol layer was removed, and the pellet washed twice with 1 ml of phosphate buffered saline (PBS). The pellet was suspended in 0.4 ml of PBS containing 2% polyvinylpolypyrrolidone (Sigma, Steinheim, Germany). The suspension was heated for 10 min at 95 °c and stored frozen overnight at −20 °C. DNA was extracted using the QIA symphony DSP virus/pathogen midi kit and pathogen complex 400 protocol of the QIA symphony Sample Processing (SP) system (Qiagen, Hilden, Germany). In each sample, a fixed amount of Phocine Herpes Virus 1 (PhHV-1) was added within the isolation lysis buffer, to serve as an internal control for the isolation procedure and to monitor the inhibition of the real-time PCR. The *Schistosoma* genus-specific real-time PCR was performed using

Fig. 2. (A) pictorial methodology of visualization of schistosome ova in semen with a clean, non-sterile transparent plastic bag. (A) Semen is concentrated to one corner; (B) the bag is heat sealed to trap the liquid; (C) the bag is placed on microscope stage and inspected at ×40 or ×100 magnification; (D) an egg of *S. haematobium* with miracidium inside.

primers and probes as described previously (Obeng *et al.*, 2008; Kenguele *et al.*, 2014).

Ultrasonography examination

Participants were briefed on the transabdominal and scrotal ultrasonography procedures to be conducted on them using a portable Chison Q5 ultrasound scanner with 3.5 MHz probe supplied by Mount International United Services Ltd, Gloucester, United Kingdom. Participants were asked to present with a full bladder, before the procedure to increase the visualization and validity of the images. The participant was positioned supine on the examination couch with the scanner set up on their right side. Whenever possible, room lightning was turned off to maximize screen visibility.

The scanning procedure investigated the appearance, size and abnormalities of the following key pelvic and genital organs: urinary bladder (shape, thickness, calcifications, masses, polyps), seminal vesicles (symmetry, thickness, nodules, echogenicity) and scrotum (tests, epididymis: nodules, masses, calcifications, hydroceles), according to evidence-based recommendations (Vilana *et al.*, 1997; WHO, 2000; Martino *et al.*, 2014). The observations made during the procedure and degree of visualization were recorded accordingly.

All clips and images were stored on the device before transferring to the external hard drive for further analyses. A sample of 15% of the scan images were randomly selected and re-read by specialist radiologist for quality control, who conducted training of the study scanning personnel. All participants were notified of pathological findings that day, and further appropriate investigations and management were organized in accordance with standard clinical practice. Thereafter, praziquantel treatment at 40 mg kg^{-1} as a single dose was offered along with an invitation to follow-up studies after 1-, 3-, 6- and 12-months.

Data analyses

All the information collected during the study was screened and quality-controlled before entry into Microsoft Excel and SSPS computer packages. Screening for errors and cleaning were conducted, before commencing statistical analyses to present the results of the study.

Ethical considerations: Ethical clearance for the study was granted by both the Liverpool School of Tropical Medicine Research Ethics Committee (LSTM REC Approval number: 17-018) and the National Health Sciences Research Committee of Malawi (NHSRC Approval number: 1805). Utmost privacy and confidentiality were maintained in the study and where necessary, the information was anonymized to protect the identity of the participant. Participants were informed of their right to opt-out at any stage of the study if they wish to do so. No disruption was caused to their normal daily activities or seeking other services at any health facility. Since this was a test-to-treat study, participants were offered praziquantel treatment at the end of the visit before inviting them to the next follow-up study.

Preliminary baseline results of the study

A total of 376 fishermen were recruited at baseline into the study who were interviewed with questionnaires, 56 were HIV infected and receiving ART. The participants came from 39 villages located in two Traditional Authorities (T/A) of Mponda and Nankumba, along the shoreline within the study area. The median age of the participants was 30.0 years with a range of 18.0 to 70.0 years (interquartile range [IQR]: 13.0) and their duration of stay in the fishing village ranged from 1 month to 70 years (median: 20.0 years; IQR: 24.3). The mean weight of the participants was 59.1 kg (range: 43.0–85.0 kg; 95% C.I.: 58.1–59.9).

Out of the total recruited participants, only 210 submitted urine after questionnaires (55.9%) and 114 submitted semen (30.3%). Urine reagent dipstick showed that most of the urine was observed to be negative for leukocytes (82.4%), blood (72.9%), protein (63.8%) and glucose (100%). After urine filtration, 36 participants (17.1%) had *S. haematobium* eggs in urine (UGS), their mean egg count was 14.8 eggs per 10 ml and ranged from 0.1 to 186.0 eggs (median: 0.9, IQR: 5.4). The total urine volumes ranged from 10 ml to 240 ml and only three participants had the highest infection intensity (92, 137.8 and 186 eggs). Eight (3.8%) had a positive POC-CCA test, suggestive of possible *S. mansoni* intestinal schistosomiasis infection.

For those who submitted semen, 12 (10.4%) had *S. haematobium* eggs in semen (MGS). The median egg count was 2.9 per ml of ejaculate, ranging from 0.4 to 30.0 eggs and the volume of semen ranged from 0.1 to 4.5 mL (median: 1.4 ml). The semen bag method identified eight participants (66.7%) whose median egg count was 0.8, while the centrifuge method identified nine participants (75.0%) with a median of 2.9 eggs, and only five participants (41.7%) were observed to have MGS by both methods simultaneously. Eight participants (66.7%) with MGS had no eggs in urine, the median volume of which was 60.0 ml (range: 10–90 ml). Upon interview on the use of the collection bag, 71.0% ($n = 51$) men preferred the use of a bag method *vs* the use of the screw top sample container.

Transabdominal and scrotal ultrasonography was conducted on 125 participants at baseline and 25 abnormalities were noted in the gential organs. For UCP-LF CAA analyses of the first 14 semen samples, only five samples were noted to generate a supernatant that could be concentrated using the Amicon concentration devices. Further analyses are underway for the remaining samples collected in the study.

The real-time PCR conducted on 65 semen samples revealed that 18 (26.5%) were positive (Ct-value range: 18.6–36.6) of

which seven had no eggs in semen or urine while only six participants had eggs in urine only. For those participants with eggs in semen but negative on the PCR could explain an old infection with dead eggs which were migrating in the genital organs and then released into ejaculatory ducts and seminal fluid. Using the seminal schistosome PCR as a reference test for MGS diagnosis in comparison with semen microscopy, the latter had a sensitivity of 25.3% and a specificity of 70.6%, which is substantially lower than using urine filtration as a proxy for MGS diagnosis. Interestingly, the positive predictive value of semen microscopy was 40.6% while the negative predictive value was 77.3%, which highlights the further need to develop more sensitive and specific diagnostic tests to diagnose MGS.

Here we present two exemplar clinical case reports, summarized in Table 2, demonstrating the outcomes and potential challenges of different diagnostic tests for MGS used in the longitudinal study.

Case 1

This concerns a 24-year-old HIV-negative fisherman recruited into the study, 48.0 kg body weight, had been fishing in the lake for 14 years. He was experiencing occasional spontaneous pain in the scrotal region for over a month. He had no previous history of receiving praziquantel during annual MDA campaigns. After describing his symptoms during questionnaire interview, he was requested to submit urine and semen samples for parasitological diagnosis of egg-patent *S. haematobium* infections upon microscopy.

His 70 ml mid-morning urine was normal in colour, and the urine reagent strip showed a trace of leukocytes and protein, the presence of microhaematuria (+++ blood score), while the POC-CCA test was negative. After filtration, 150 *S. haematobium* eggs were detected on microscopy (21.4 eggs per 10 ml of urine). He submitted 2 ml of semen in which 5 eggs were observed by the bag method and 13 eggs after centrifugation (6.5 eggs per ml of ejaculate), however, no leukocytes were observed.

A CAA concentration of 5 pg ml^{-1} was found in the seminal plasma on UCP-LF CAA analysis, using the high-volume extraction procedure. Analysis of DNA extracted from the harvested semen sediment registered a strong positive output upon real-time PCR (Ct-value: 26.6). The results of the egg count and real-time PCR at baseline and follow-ups are shown in Fig. 3.

Transabdominal and scrotal ultrasonography showed a thickened bladder wall (⩾11 mm) and asymmetrically enlarged seminal vesicles (⩾15 mm). Case 1 was treated with praziquantel tablets and examined after 1 month and 3 months, where no schistosome eggs were detected in semen on both occasions. The real-time PCR on the semen sediment was negative at 1 month but became positive again at 3 months, the Ct-value was 30.8. A total of 11 and 187 schistosome eggs were observed in his urine at 1- and 3-months respectively (2.4 and 19.7 eggs per 10 ml of urine), with normal findings on ultrasonography.

Case 2

A 26-year-old HIV-negative fisherman recruited into the study, 59.7 kg body weight, had been fishing in the lake one day in a week for 11 years. He didn't report any symptoms or illness in the preceding months or receiving praziquantel during annual MDA campaigns.

He submitted 35 ml mid-morning urine, which was normal in colour, and the urine reagent strip showed a trace of protein, no leukocytes or blood, and the POC-CCA test was positive. No schistosome eggs were detected on urine filtration nor in his 2.5 ml ejaculate, which had no leukocytes. The real-time PCR analysis of his semen was negative, transabdominal and scrotal ultrasonography examination were normal and he was then given praziquantel treatment. Follow-up at 1-, 3-, 6- and 12-months showed negative POC-CCA results, no schistosome eggs in urine or semen, negative real-time PCR and normal ultrasonography examinations, except a seminal plasma CAA concentration of 1 pg ml^{-1} on UCP-LF CAA analysis at 6-months, Trace on POC-CCA and positive real-time PCR of 31.2 on his semen at 12-months.

On the detection of MGS

To our knowledge, this is a first longitudinal cohort research study and case description of MGS among local fishermen living along south shoreline of Lake Malawi, a schistosomiasis-endemic region in the South Eastern part of Africa. Previous studies have focussed on travellers and expatriates visiting the lake for recreation and sports. In this endemic setting of Malawi, it is clear that MGS remains an unrecognized, undiagnosed and underreported illness among fishermen, despite the more obvious burden of urogenital schistosomiasis, and we intend for our longitudinal cohort study to stimulate growing research interest into this condition. Our Case 1 presented symptoms of MGS previously described in the literature, resulting from granulomata and associated pathologies caused by schistosome eggs during their migration and entrapment through the walls of internal genital organs before being released in semen, which is pathognomonic of MGS. However, these symptoms are commonly mistaken for sexually transmitted infections, which result in poor diagnosis and management of this treatable and preventable condition. Praziquantel was generously donated and distributed to over 89 million people in 2016 through MDA campaigns although the focus is upon treatment of school-aged children rather than fishermen (Leutscher *et al.*, 2008*a*; Yirenya-Tawiah, Ackumey and Bosompem, 2016; WHO, 2018).

Our cases showed positive results on the UCP-LF CAA analysis on semen which was performed for the first time in its development, hence highlighting the need to optimize the semen sample concentration technique to allow better detection of CAA concentrations below 10 pg ml^{-1}. Our case illustrated a downward trend in egg count after praziquantel treatment at 1-month as well as a negative real-time PCR result, showing clearance of eggs in semen and a putative cure of *S. haematobium* infection. This demonstrates that praziquantel appears effective at the standard dose for MGS treatment, though repeated or increased doses could be beneficial in cases of heavy infestation and to counter reinfection (Schwartz *et al.*, 2002), as was seen in this case. It is worthy to note that at 3 months his urine egg-count increased together registering a positive real-time PCR result indicative of a newly acquired infection. To mitigate re-infection, other strategies such as public health education on avoidance of high-risk bathing areas, earlier diagnosis and stepped-up treatment of regularly patently infected people are needed, especially to regress any progressive morbidity.

Of particular interest is the only positive POC-CCA test in case 2 while all the other diagnostic tests were negative for schistosomiasis. This points towards the strong possibility of intestinal schistosomiasis caused by *S. mansoni*, as was recently discovered during the course of our longitudinal study (Alharbi *et al.*, 2019), which redefines the epidemiology of the disease along the shoreline. The negative POC-CCA result on follow up studies after praziquantel may further allude to the fact that this infection was cleared, owing to satisfactory cure rates, previously reported (Knopp *et al.*, 2013). Definitive results of this case could arise from further additional diagnostic tests for intestinal schistosomiasis and possible schistosome hybrids recently reported in the area (Webster *et al.*, 2019).

Table 2. Summary of the clinical cases from the longitudinal cohort research study on MGS among local fishermen along south shoreline of Lake Malawi

Study time-point	Parameter	Case 1	Case 2
Baseline	Symptoms/diseases experienced	Scrotal pain	No
	Previous MDA	No	No
	Urine		
	Volume (ml)	70	35
	Appearance	Clear	Clear
	Reagent dipstick	Trace (L, P) Positive+++ (B)	Trace (P) Negative (L, B)
	POC-CCA	Negative	Positive
	Filtration (eggs per 10 ml)	21.4	0
	Semen		
	Volume (ml)	2.0	2.5
	Semen bag (eggs per ml)	2.5	0
	Centrifuge (eggs per ml)	6.5	0
	UCP-LF CAA (pg ml^{-1})	5	0
	Real-time PCR (Ct value)	26.6	Negative
1-month Follow up	Urine		
	Volume (ml)	45	100
	Appearance	Clear	Clear
	Reagent dipstick	Positive+++ (L, P) Negative (P)	Negative (L, B, P)
	POC-CCA	Negative	Negative
	Filtration (eggs per 10 ml)	2.4	0
	Semen		
	Volume (ml)	2.5	0.5
	Semen bag (eggs ml^{-1})	0	0
	Centrifuge (eggs ml^{-1})	0	0
	UCP-LF CAA (pg ml^{-1})	N/A	0
	Real-time PCR (Ct value)	Negative	Negative
3-months Follow up	Urine		
	Volume (ml)	95	100
	Appearance	Clear	Clear
	Reagent dipstick	Positive++ (B), + (P), trace (L)	Negative (L, B, P)
	POC-CCA	Negative	Negative
	Filtration (eggs per 10 ml)	19.7	0
	Semen		
	Volume (ml)	2.6	2.0
	Semen bag (eggs per ml)	0	0
	Centrifuge (eggs per ml)	0	0
	UCP-LF CAA (pg ml^{-1})	N/A	0
	Real-time PCR (Ct value)	28.8	Negative
6-months Follow up	Urine		
	Volume (ml)	N/D	100
	Appearance	N/D	Clear
	Reagent dipstick	N/D	Negative (L, B, P)
	POC-CCA	N/D	Negative
	Filtration (eggs per 10 ml)	N/D	0

(*Continued*)

Table 2. (*Continued*)

Study time-point	Parameter	Case 1	Case 2
	Semen		
	Volume (ml)	N/D	0.8
	Semen bag (eggsper ml)	N/D	0
	Centrifuge (eggsper ml)	N/D	0
	UCP-LF CAA (pg ml^{-1})	N/D	1
	Real-time PCR (Ct value)	N/D	Negative
12-months Follow up	Urine		
	Volume (ml)	N/D	100
	Appearance	N/D	Clear
	Reagent dipstick	N/D	Trace (L), negative (B, P)
	POC-CCA	N/D	Trace
	Filtration (eggs per 10 ml)	N/D	0
	Semen		
	Volume (ml)	N/D	1.1
	Semen bag (eggs per ml)	N/D	0
	Centrifuge (eggs per ml)	N/D	0
	UCP-LF CAA (pg ml^{-1})	N/D	N/A
	Real-time PCR (Ct value)	N/D	31.2

Urine reagent dipstick test result: L, leukocytes; B, blood; P, protein; N/A, result not available, test currently underway; N/D, participant not available, test not done.

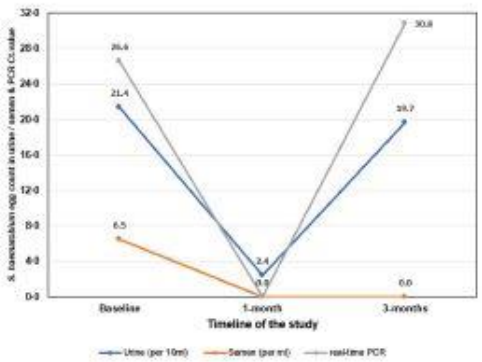

Fig. 3. A line graph of the clinical Case 1 in the longitudinal cohort study showing results of *S. haematobium* egg counts in urine (per 10 ml) and semen (per ml); and Ct-values for real-time PCR analysis of semen at baseline, 1- and 3-month follow-up studies.

Chronic pathologies in seminal vesicles, vas deferens, testes and prostrate alongside the urinary bladder such as calcifications, hyper-echogenicity, organ enlargements and among others can be observed on ultrasonography (Vilana *et al.*, 1997; Ramarakoto *et al.*, 2008). This provided key morbidity features aiding the MGS diagnosis and management, although this procedure is seldom available in most rural health facilities in Malawi. Our case 1 displayed similar MGS abnormalities in such organs described in previous publications (Corachan *et al.*, 1994; Vilana *et al.*, 1997), and showed some resolution after praziquantel therapy. Where such services are available, appropriate usage of such services could further aid in MGS management and avoid unnecessary invasive procedures which continue to be implored and reported.

Apart from ultrasonography, diagnosis of MGS using a gold standard technique of semen examination remains a substantive challenge among medical professionals as it requires the cumbersome task of semen collection using sterile specimen containers or non-spermicidal condoms which are costly and unavailable in endemic areas including Malawi (WHO, 2010; Kipandula and Lampiao, 2015). In addition, most facilities have poorly equipped laboratories, with limited-trained personnel to prepare and examine semen, hence the design of our research study to use a low-cost, clear self-sealing plastic bag to quickly and easily examine the semen by direct microscopy can improve opportunities for the diagnosis of MGS in resource poor settings.

The plastic bag is a readily available commodity used for various activities in households, workplaces and health facilities and is more affordable (costs 5 cents) than the sterile container or non-spermicidal condom (75 and 30 cents, respectively). Our Case 1 showed similar results between the bag method and the standard method of centrifugation. In addition, the preference for using the bag in comparison with the screw top sample container by participants, suggest the need for further validation of this method to determine its applicability in clinical practice, especially in limited-resource settings.

The real-time PCR on the harvested semen showed an increase in the prevalence of MGS (data not shown) which highlights the need to develop more sensitive and specific diagnostic tests to diagnose MGS. This is especially important since the current low-cost standard technique misses a substantial number of individuals at high-risk of the infection. Although urine filtration is used as a proxy for MGS diagnosis, its lower sensitivity in individuals with light infections and also in low-transmission areas, emphasizes the need to improve the diagnostic platform for MGS.

What outlook for MGS diagnostics?

Urogenital schistosomiasis remains a prevalent NTD in low- and middle-income tropical countries, particularly in sub-Saharan

Africa. As a consequence of MDA, the sensitivities of current urine-diagnostic tools will further reduce and pose a future challenge in the detection of lighter infections linked with clinical disease. MGS is an important but ignored complication of UGS, and there is as of yet no single diagnostic criteria entirely satisfactory. Indeed, greater effort should be made to improve specific POC diagnostics, to complement and monitor the progress of MDA programmes and integrated control strategies. We now therefore call for an all-inclusive diagnostic algorithm for MGS to be developed that accurately identifies infected men with delivery of best treatment.

Acknowledgements. We thank the District Health Officer and Management team of Mangochi District Health Services for their overwhelming support towards the study; Dr Sam Kampondeni and Dr Elizabeth Joekes for their assistance in training on ultrasonography; and Dr Joekes for quality control of the ultrasonography and to Merck plc for donation of the portable ultrasound unit; Messrs Mohammad Alharbi, Bright Mainga, Pilirani Mkambeni, Patrick Hussein, Mkonazi Nkhoma, Matthews Elias and Boniface Injesi for technical assistance; and all the fishermen who participated in the longitudinal cohort study in the Mangochi District. We are grateful to the Head and staff of Laboratory department at Mangochi District Hospital; District ART coordinators, In-charge and staff of Tikondane clinic; Officers in-charge and staff of Billy Rhodian Charity Clinic, Monkey-Bay Community Hospital, Nkope and Koche Health Centres; Dr Lumbua Katenda, Mr Anwar and staff of Mangochi LMJ clinic; local community health workers: Ambali Makochera, Alfred Kachikowa, Rodgers Wengawenga, Michael Tsatawe, Chikondi Mtsindula, Mambo Amin, Elias Matemba, Austin Kaluwa, Justin Mndala, Promise Mwawa, Dickson Tabu, George Matiki, Alfred Mdoka and Charles Katandi; Brother Henry Chagoma and staff of Montfort Mission Guest House; all traditional leaders in the hosting fishing communities, fishermen and beach village committee members for their enthusiasm and support during the study.

Financial support. S.A. Kayuni was funded by a PhD scholarship from Commonwealth Scholarship Commission, UK which supported the baseline and 1-month follow-up studies. He received an International Travel Fellowship from British Society for Parasitology to conduct 3- and 6-months follow-up studies, and from World Friendship Charity to conduct 12-month follow-up studies. K. Bartlet, J. Fawcett and A. Shaw were supported by LSTM Education Department MSc Degree Research Project Module funding.

Conflicts of interest. None.

Ethical standards. Ethical clearance for the study was granted by both the Liverpool School of Tropical Medicine Research Ethics Committee (LSTM REC Approval number: 17-018) and the National Health Sciences Research Committee of Malawi (NHSRC Approval number: 1805).

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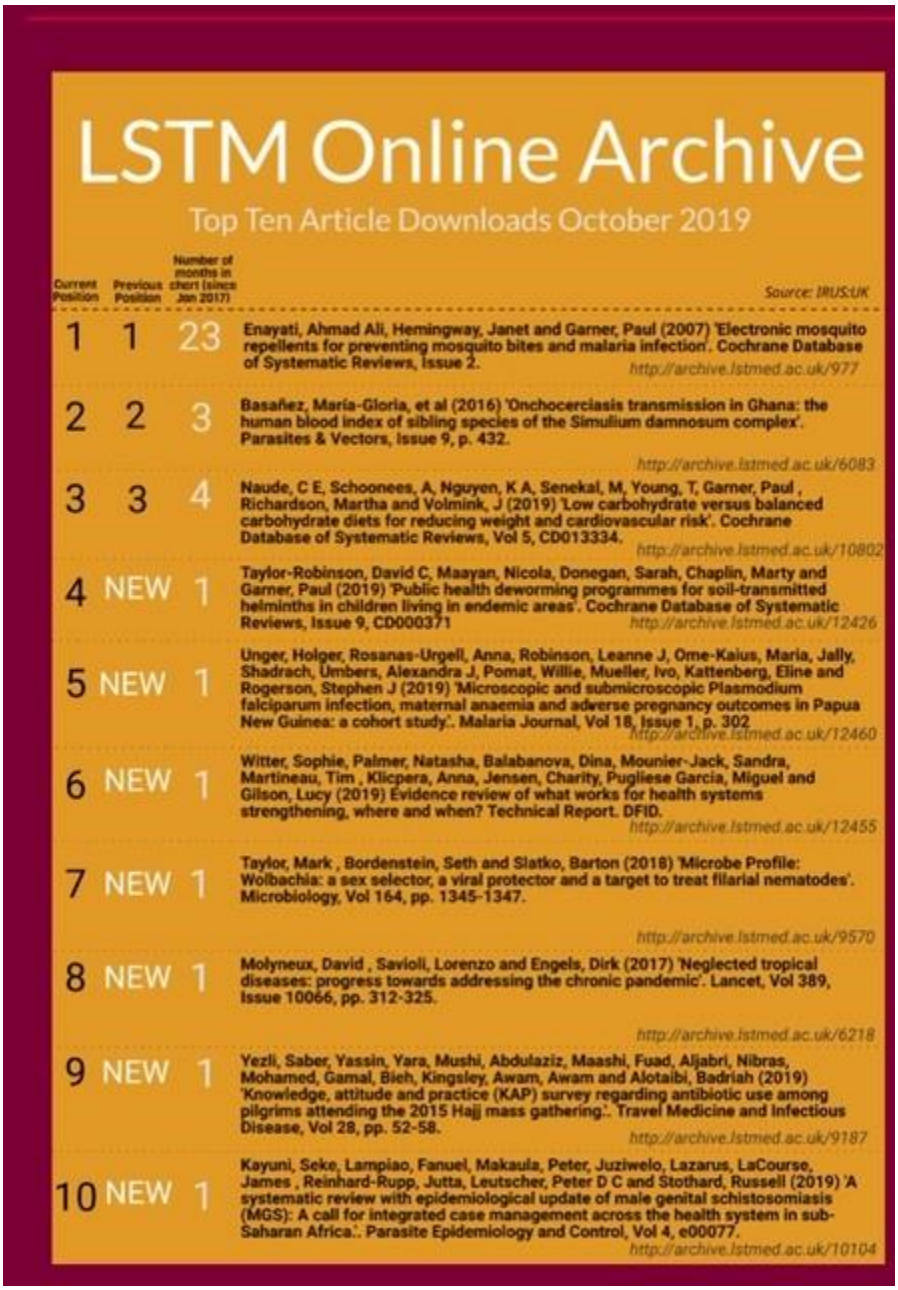
Appendix 25: Research output – Manuscript in Parasite Epidemiology and Control



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2	2	3	Basañez, María-Gloria, et al (2016) 'Onchocerciasis transmission in Ghana: the human blood index of sibling species of the Simulium damnosum complex'. Parasites & Vectors, Issue 9, p. 432. http://archive.lstmed.ac.uk/6083
3	3	4	Naude, C E, Schoonees, A, Nguyen, K A, Senekal, M, Young, T, Garner, Paul , Richardson, Martha and Volmink, J (2019) 'Low carbohydrate versus balanced carbohydrate diets for reducing weight and cardiovascular risk'. Cochrane Database of Systematic Reviews, Vol 5, CD013334. http://archive.lstmed.ac.uk/10802
4	NEW	1	Taylor-Robinson, David C, Maayan, Nicola, Donegan, Sarah, Chaplin, Marty and Garner, Paul (2019) 'Public health deworming programmes for soil-transmitted helminths in children living in endemic areas'. Cochrane Database of Systematic Reviews, Issue 9, CD000371 http://archive.lstmed.ac.uk/12426
5	NEW	1	Unger, Holger, Rosanas-Urgell, Anna, Robinson, Leanne J, Ome-Kaius, Maria, Jally, Shadrach, Umbers, Alexandra J, Pomat, Willie, Mueller, Ivo, Kattenberg, Eline and Rogerson, Stephen J (2019) 'Microscopic and submicroscopic Plasmodium falciparum infection, maternal anaemia and adverse pregnancy outcomes in Papua New Guinea: a cohort study'. Malaria Journal, Vol 18, Issue 1, p. 302 http://archive.lstmed.ac.uk/12460
6	NEW	1	Witter, Sophie, Palmer, Natasha, Balabanova, Dina, Mounier-Jack, Sandra, Martineau, Tim , Klicpera, Anna, Jensen, Charity, Pugliese Garcia, Miguel and Gilson, Lucy (2019) Evidence review of what works for health systems strengthening, where and when? Technical Report. DFID. http://archive.lstmed.ac.uk/12455
7	NEW	1	Taylor, Mark , Bordenstein, Seth and Slatko, Barton (2018) 'Microbe Profile: Wolbachia: a sex selector, a viral protector and a target to treat filarial nematodes'. Microbiology, Vol 164, pp. 1345-1347. http://archive.lstmed.ac.uk/9570
8	NEW	1	Molyneux, David , Savioli, Lorenzo and Engels, Dirk (2017) 'Neglected tropical diseases: progress towards addressing the chronic pandemic'. Lancet, Vol 389, Issue 10066, pp. 312-325. http://archive.lstmed.ac.uk/6218
9	NEW	1	Yezli, Saber, Yassin, Yara, Mushi, Abdulaziz, Maashi, Fuad, Aljabri, Nibras, Mohamed, Gamal, Bieh, Kingsley, Awam, Awam and Alotaibi, Badriah (2019) 'Knowledge, attitude and practice (KAP) survey regarding antibiotic use among pilgrims attending the 2015 Hajj mass gathering:'. Travel Medicine and Infectious Disease, Vol 28, pp. 52-58. http://archive.lstmed.ac.uk/9187
10	NEW	1	Kayuni, Seke, Lampiao, Fanuel, Makaula, Peter, Juziwelo, Lazarus, LaCourse, James , Reinhard-Rupp, Jutta, Leutscher, Peter D C and Stothard, Russell (2019) 'A systematic review with epidemiological update of male genital schistosomiasis (MGS): A call for integrated case management across the health system in sub-Saharan Africa.'. Parasite Epidemiology and Control, Vol 4, e00077. http://archive.lstmed.ac.uk/10104

Parasite Epidemiology and Control 3 (2018) e00077

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

journal homepage: www.elsevier.com/locate/parepi

A systematic review with epidemiological update of male genital schistosomiasis (MGS): A call for integrated case management across the health system in sub-Saharan Africa

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ARTICLE INFO

Article history:
Received 13 May 2018
Received in revised form 16 October 2018
Accepted 16 October 2018

Keywords:
Male genital schistosomiasis
Urogenital schistosomiasis
Praziquantel
HIV
Control

ABSTRACT

Male genital schistosomiasis (MGS) is a gender specific manifestation of urogenital schistosomiasis (UGS) first described in 1911 by Madden in Egypt. Today, while affecting millions of men and boys worldwide, MGS receives insufficient attention, especially in sub-Saharan Africa (SSA). To provide a systematic review with an epidemiological update of MGS, we inspected both online and hardcopy resources in our appraisal. A total of 147 articles were eventually identified, only 31 articles were exclusively focused on MGS with original or targeted research. From these, we discuss pertinent clinico-pathological features of MGS, highlight the possible connection and interplay with HIV, and assess current diagnostic techniques alongside consideration of their use and application in SSA. To appreciate the burden of MGS more fully, especially in endemic areas, there is a clear need for better surveillance and longitudinal population research to investigate the best point-of-care (POC) diagnostic and its performance through time. Furthermore, to optimise individual case management, exploration of alternative praziquantel dosing regimens is needed for MGS in men with or without HIV co-infection.

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https://doi.org/10.1016/j.parepi.2018.e00077

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1. Introduction

Schistosomiasis is a snail-borne disease of humans caused by parasitic helminths of the genus *Schistosoma* (Colley et al., 2014). It remains a major neglected tropical disease (NTD) and a significant public health challenge in low and middle-income countries (Chitsulo et al., 2000; Engels et al., 2002; Christinet et al., 2016). There it causes significant morbidity and in certain areas mortality (van der Werf et al., 2003), however, the burden of schistosomiasis is underestimated due to incomplete disease surveillance as undertaken by often stretched national healthcare systems and national control programmes (King et al., 2005; Gryseels et al., 2006). The latter is more focused on tracking the delivery and treatment coverage of mass treatment campaigns offering donated praziquantel (PZQ), typically to school-aged children (Savioli et al., 2017) rather than monitoring the disease in adults per se. The consequences and disability caused by gender specific manifestations of urogenital schistosomiasis (UGS) in adults often go unremarked at national and local levels. In contrast to female genital schistosomiasis (FGS), male genital schistosomiasis (MGS), as evidenced by schistosome eggs (usually those of *Schistosoma haematobium*) in male genital organs and reproductive tracts thereof, remains poorly reported, much understudied and often misunderstood. This review was conducted to draw attention to the current evidence on MGS and assess its public health importance across the world.

1.1. A brief history of schistosomiasis

Corroborated references to signs and symptoms ascribed to UGS can be traced back to 1900 BCE since haematuria (i.e. frank blood in urine) was described as a common occurrence and linked to 'menstruation' in Egyptian males (Davis and Ansari, 1973). Schistosomiasis is a proven disease of antiquity for *S. haematobium* ova have been found in kidney tubules of two Egyptian mummies from 1250 to 1000 BCE (Ruffer, 1910) and more recently *Schistosoma japonicum* ova retrieved within Chinese cadavers dated to 206 to 220 CE (Coon, 2005). Schistosomiasis itself was originally described in Egypt by the German pathologist Theodor Bilharz in 1851 who discovered male and female schistosome worms at autopsy, naming them all *Distomum haematobium*. This led him to ascribe, incorrectly, that UGS and hepato-intestinal disease were linked to this schistosome species alone (Rollinson, 2009). Some sixty years later, and again in Egypt, this unfortunate mistake and subsequent confusion was fully resolved by Robert T. Leiper who demonstrated the independent lifecycles of *S. haematobium* and *Schistosoma mansoni* (Leiper, 1916) and their respectively aetiology in urinary and hepato-intestinal disease (Leiper, 1916; Stothard et al., 2016).

Out of the 24 species of schistosomes recognised worldwide, only six cause human diseases, namely *S. haematobium*, *S. mansoni*, *S. japonicum*, *Schistosoma mekongi*, *Schistosoma intercalatum* and *Schistosoma guineensis* (Rollinson, 2009). The first three species are the most important from a public health perspective. Although there may be exceptions owing to ectopic egg laying sites, *S. haematobium* is exclusively associated with UGS which is widely distributed in Africa and adjacent regions, affecting more people (112 million) than all other species [(WHO) see http://www.who.int/schistosomiasis/epidemiology/table/en/]. *Schistosoma mansoni*, *S. japonicum* and the other species causes hepato-intestinal schistosomiasis, with *S. mansoni* prevalent in the Caribbean, South America and Africa and *S. japonicum* in Asia as South East Asia (Colley et al., 2014). Of note, *S. mansoni*, *S. japonicum*, *S. intercalatum* and *S. guineensis* have been reported to cause genital manifestations but even collectively can be considered as minor when compared against *S. haematobium* alone.

1.2. Focus on male genital schistosomiasis

Male genital schistosomiasis is a specific manifestation of schistosomal disease, associated with presence of ova and pathologies thereof in various genital organs and reproductive fluids. The original report of MGS was made by Professor Frank Cole Madden, Professor of Surgery at Kasr-el-Ainy Hospital in Cairo, Egypt. In 1911, he described a 14-year Egyptian boy having enlarged scrotum showing epididymal schistosomiasis and an English soldier complaining of haemospermia (blood in semen) concurrently with urinary schistosomiasis (Madden, 1911).

Other symptoms of MGS described in literature include pelvic pain appearing spontaneously, during coitus or on ejaculation, ejaculate changes, erection discomfort or dysfunction, infertility (Mabey et al., 2013; Farrar et al., 2014; Squire and Stothard, 2014). Although observations indicate that genital organs are frequently infested with schistosome eggs along with the urinary bladder (*S. haematobium*) or intestines (*S. mansoni*), the current extent of morbidity associated with MGS in endemic areas remains under-researched but is most clearly evidenced by post-mortem studies and case reports. By contrast, as colposcopy is

available for diagnosis, ongoing surveillance of PGS has been better reported particularly in light of its three-fold increased risk of association with human immunodeficiency virus (HIV) infection in women living in endemic areas of SSA (Kjetland et al., 2006; Kjetland et al., 2012). There is a similar plausibility of additional risk of HIV transmission among dually-infected males in schistosomiasis-endemic areas due to observed increase in inflammatory cells and immunological mediators in semen of people with MGS which might increase the viral copies (Leutscher et al., 2005). Hence treatment of MGS with PZQ could support the control of HIV/AIDS in overlapping prevalent areas of both diseases, especially in SSA.

This systematic review on MGS in endemic areas, has the following specific objectives:

1. update the epidemiology of MGS in endemic areas,
2. review the clinicopathological features of MGS including co-infections with other diseases,
3. assess the available diagnostic techniques and treatment of MGS, and
4. determine the existing gaps to develop future research agenda of MGS.

2. Methods of literature review

An online literature search was conducted systematically from January 2017 to April 2018 for publications made from 1900 up to 2017, using the main search term 'male genital schistosomiasis' in PUBMED, EBSCOhost (CINAHL Complete, MEDLINE Complete, Global Health, eBook Collection), COCHRANE LIBRARY and WEB OF KNOWLEDGE databases, following the stipulated guidelines of each database. The main search term was combined with terms for known symptoms of MGS retrieved from the main textbooks on Tropical Medicine (Mabey et al., 2013; Farrar et al., 2014; Squire and Stothard, 2014), which included 'haemospermia', 'haematospermia', 'ejaculate', 'erectile dysfunction', 'infertile', 'sterile', 'painful', 'discomfort', 'spermaturia', 'semen'. In addition, the main term was combined with terms of male genital organs, listed as 'prostate', 'seminal vesicle', 'spermatic cord', 'epididymis', 'vas deferens', 'testis' and 'reproductive organ'.

In the PUBMED database after inputting the main search term, it automatically searched the terms as Medical subject headings (MeSH) and all fields, to produce the total results which were narrowed to those of English language. The search of the main terms in the EBSCOhost database involved all possible forms of the terms, augmented using relevant syntax 'OR', 'AND'; for example, 'male+ OR male* OR man* OR man+' AND 'genital+ OR genital*' AND 'schistosomiasis+ OR schistosomiasis* OR *Schistosoma** OR *Schistosoma+* OR bilharzia+ OR bilharzia* OR bilharziosis* OR bilharziosis+'. These terms were automatically expanded for equivalent subjects and related words, also narrowed by English language. The search in the COCHRANE LIBRARY followed a similar pattern to the EBSCOhost database. For the WEB OF KNOWLEDGE, the main terms were searched using both field tags 'TOPIC' and 'TITLE' and then combined with Booleans 'OR', and 'AND' appropriately. The results were compiled together to produce the final list of articles. Additional articles from other sources such as references from the textbooks and known parasitologists were added to the final lists from these four databases. The final articles in French and Portuguese languages were translated into English (refer to Appendix 1 Supplementary Tables).

All the articles were screened in the following five stages:

- Stage 1: Lists of articles were checked for possible duplications, which were removed.
- Stage 2: Thereafter, the titles of the remaining articles were screened for relevance to MGS, and excluded accordingly.
- Stage 3: Then, the abstracts of those remaining were read and screened for relevance to MGS. Those articles not related to MGS were removed from the list.
- Stage 4: The full-text of the remaining articles was retrieved and read through to select those manuscripts on MGS to be included in the review.
- Stage 5: The references of the full-text articles included in the review were screened for additional articles not retrieved in the above database searches.

Furthermore, leading articles on PGS and texts from prominent textbooks describing schistosomiasis (Gelfand, 1967; Jordan and Webbe, 1969; Jordan et al., 1993; Kamel and Lumley, 2004; Weiss, 2004) were read to form background knowledge and comparison to MGS where necessary. Alerts were installed on all the databases searched for this review to capture new publications and additional articles relevant to MGS.

3. Analysis of assembled literature

The online database search produced a combined total of 3329 publications using the main search term (Fig. 1 & Table 1). Four articles were added from the alerts on the searched databases. After screening through the five stages described earlier, the final articles included in this MGS review are 151 (Appendix 1 Supplementary Table 2), of which 32 were original research studies, 96 case reports, 2 editorial papers, 3 systematic reviews, and 18 literature reviews on schistosomiasis with aspect of male genital pathology. The period of publications was from 1911 to 2018.

Thirty-two original research studies have been published from 1952, with half directly on genital schistosomiasis, while the other half focussed on schistosomiasis with brief descriptions on genital symptoms, pathologies or complications. Sixteen studies reported only on *S. haematobium*, 3 on *S. mansoni*, 9 on mixed *S. haematobium* and *S. mansoni* infections, while 4 had no mention of species (Fig. 3). In addition, 26 studies were conducted in Africa [Madagascar-6, Nigeria-6, Egypt-5, Zimbabwe-5, Zambia-2, Ghana-1] and one each in other continents except Australasia. There were 11 necropsy studies, 5 histopathological studies, 6

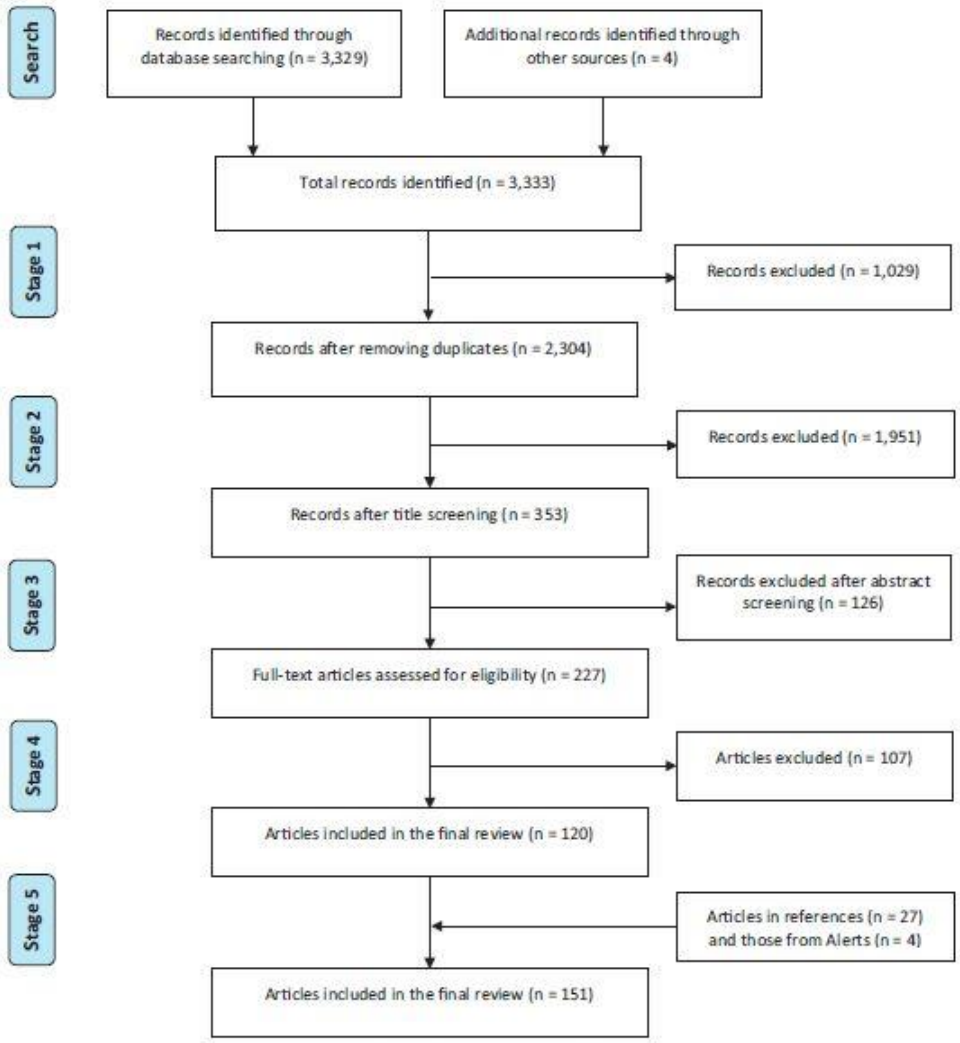

Fig. 1. Flow chart showing the results of the systematic literature search in the online databases.

longitudinal cohort studies, 2 qualitative studies, 1 radiography study and 1 hormonal analysis study. Seven studies involved examination of all genital organs, 2 on seminal vesicles, 1 on prostate only while other studies did not focus on specific genital organ (s).

Table 1
Results of literature search on the online databases conducted from January 2017 to April 2018.

Online database	Number of articles from each database		
	'male genital schistosomiasis'	Combined with 'symptoms'	Combined with 'symptoms' and 'organs'
EBSCOhost	680	837	1677
PUBMED	181	339	812
WEB OF KNOWLEDGE	140	275	570
COCHRANE LIBRARY	17	96	270
TOTAL	1018	1547	3329

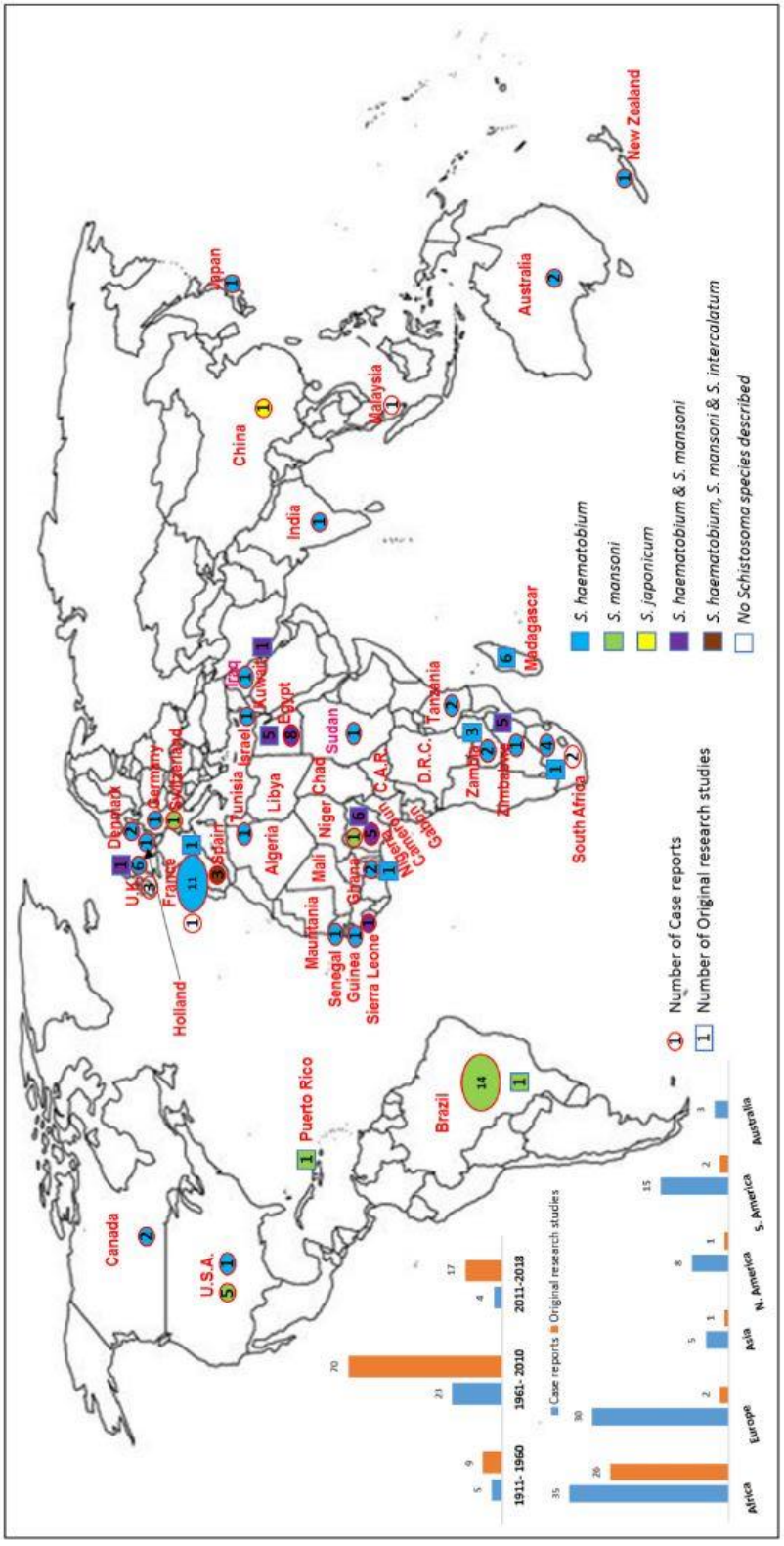

Fig. 2. Global map showing distribution of the publications on MGS from 1911 to 2018. The two charts displaying number of publications in the first and second 50 years and per continent. (The original research studies comprise post-mortem studies conducted in Africa and South America; prospective studies mainly in Africa.)

Ninety-six case reports were made between 1911 and 2018, with only five reports published prior to 1952. Fifty-five case reports were from endemic areas mostly in Africa [n = 35; 64%] while 40 reports were on travellers or people emigrating from endemic areas to non-endemic countries, especially in Europe [n = 30; 75%] (Fig. 2). Some travellers diagnosed in non-endemic countries in Europe, Asia and Australia, were infected after swimming or walking in Lake Malawi in SSA, which is endemic mainly for *S. haematobium*. In France, ten of the 12 case reports were of travellers to or emigrants from North, West and Central African countries of Algeria, Cameroun, Central African Republic, Chad, Democratic Republic of Congo (DRC), Egypt, Gabon, Libya, Mali, Mauritania, Niger and Tunisia.

Sixty case reports (63%) were on *S. haematobium*, 22 (23%) on *S. mansoni*, and the rest (5%) on mixed *S. haematobium* and *S. mansoni* (n = 2), on *S. haematobium*, *S. mansoni* and *S. intercalatum* (n = 2), on *S. japonicum* (n = 1), whereas 9 (10%) had no speciation. The pathological organs described in 74 case reports included scrotum (including testis and vas deferens) [n = 39], prostate [n = 17], seminal vesicles [n = 17], spermatic cord [n = 3], epididymis [n = 7] and penis [n = 1]. The main presenting symptoms or complaints were swelling of scrotum and other genital organs [n = 58], pelvic pain [n = 23], haemospermia [n = 14], hydrocele [n = 12], changes in semen/ejaculate [n = 11], infertility [n = 6] and urethral discharge [n = 2].

The 3 systematic reviews were published in 2011 and 2015, discussing the relationship between UGS and HIV (Mbabazi et al., 2011), prostate adenocarcinoma associated with prostatic *S. haematobium* infection (Figueiredo et al., 2015) and MGS treatment as a future HIV prevention tool (Stecher et al., 2015).

3.1. Update on the epidemiology of MGS

As described earlier, *S. haematobium* is endemic in Africa, particularly SSA, where most knowledge originates and the first recognised description of MGS was made by Madden a century ago (Madden, 1911). However, earlier literature by Chaker, Lortet, Vialleton, Letulle and Madden [1885–1909] described lesions in genital organs like seminal vesicles and prostate which were infiltrated by schistosome ova and granulomata formation (Madden, 1909; Mensah et al., 1972; Guirassay et al., 2008). Other genital organs have been described in the subsequent reports and research studies.

Post-mortem studies were among the earliest research in endemic areas especially in Africa, describing the epidemiology of genital schistosomiasis, four decades after the Madden report, giving the background knowledge to understanding MGS (Mohammed, 1952; Gelfand and Ross, 1953; Edington et al., 1970; Gelfand et al., 1970; Edington et al., 1975). Digestive methods were performed using potassium hydroxide (KOH) to harvest the ova from the genital organs with pathologies caused by *S. haematobium* and *S. mansoni*. Seminal vesicles were infected almost as much as the urinary bladders, ranging from 50% to 80% of vesicles with over 90% of bladders (Mohammed, 1952; Gelfand and Ross, 1953) with approximately 20,000 ova found in the vesicles [Table 2] (Edington et al., 1970; Gelfand et al., 1970; Edington et al., 1975). Histopathological examinations were also conducted, in other studies to compare with the digestive methods which showed that more ova were observed with the latter technique (Gelfand et al., 1970).

In endemic areas of *S. haematobium* and *S. mansoni*, the former predominates with more genital pathologies in literature than the latter, similarly to the case reports (Grace and Aidaros, 1952; Alves et al., 1955). From these studies, it has been described that MGS affects between 1% to 20% of those in endemic areas at risk and suffering from UGS (Ricosse et al., 1980; Fievet et al., 1984). This could be a gross underestimation, because several studies have reported that at least 50% of genital organs are infected by schistosome ova, emphasising that MGS is as common as urinary manifestations of schistosomiasis but with lower intensity of ova [Table 3] (Edington et al., 1970; Edington et al., 1975; Elem and Patil, 1987; Patil and Elem, 1988).

The first identified prospective study on MGS was conducted in Madagascar in 1999–2000, where 19 of 44 participants (43%) had MGS by *S. haematobium* ova in semen (Leutscher et al., 2000). Although the sample size of this study was small, subsequent longitudinal studies in the same country showed similar prevalence of MGS, ranging from 28% in 2005 to 53% in 2009 (Leutscher et al., 2005; Leutscher et al., 2008a; Leutscher et al., 2008b; Leutscher et al., 2009). *Schistosoma* ova were present in semen only in some cases, highlighting fact that the prevalence of MGS is quite significant, similarly to that of UGS in endemic areas, despite not having been studied as extensively.

Table 2
Total number of *Schistosoma* ova in pelvic organs in necropsy studies.

Study participants	Post-mortem studies	
	Gelfand et al., 1970	Edington et al., 1975
Total number	200	54
Pelvic organs	Intensity of *Schistosoma* egg distribution	
	Gelfand et al., 1970	Edington et al., 1975
Bladder	105,011	13,260–87,100
Seminal vesicles	19,801	4312–12,027
Vas deferens	2913	–
Prostate	34	169–9828

Table 3
Schistosoma ova in male genital organs seen in necropsy studies.

Year	Author(s)	Country	Autopsies	Species	Infected genital organs
1955	Alves et al.	Zimbabwe	50	*Sh, Sm*	18% vas deferens; 18% prostate; 4% tunica vaginalis; 2% epididymis
1956	Arban	Brazil	3233	*Sm*	10/3233 infected: 20% prostate; 30% testes
1970	Gelfand et al.	Zimbabwe	200	*Sh, Sm*	54% seminal vesicles; 39.9% spermatic duct; 20.5% prostate
1975	Edington et al.	Nigeria	54	*Sh*	Severe infections: 100% prostate; 100% seminal vesicles; 57% testes; 57% epididymis
1987	Elem & Patil	Zambia	50	*Sh*	62% bladder; 58% seminal vesicles; 50% prostate
1988	Patil & Elem	Zambia	100	*Sh*	62% bladder; 58% seminal vesicles; 50% prostate

Sh - *S. haematobium*; *Sm* - *S. mansoni*.

3.2. Clinico-pathological features of MGS including co-infections

From our search, genital organs with schistosomal pathologies have been recorded in case reports from Africa, namely prostate, seminal vesicles, vas deferens, testis and scrotum which were more associated with *S. haematobium* than *S. mansoni* (Cerqua, 1930; Mohammed, 1930; Makar, 1937; Gelfand and Davis, 1940). An early report from South America associated with *S. mansoni*, presented of enlarged scrotum, thickened seminal vesicles and hydrocele (Armbrust, 1951). Subsequent reports indicate that a higher burden of MGS is in *S. haematobium* - endemic areas of Africa than other schistosome - endemic areas in the world.

Although most of the MGS pathologies have been reported on *S. haematobium* in inhabitants and travellers to endemic areas, similar reports have been made on *S. mansoni*, *S. intercalatum* and *S. japonicum* (Corachan et al., 1994; Vilana et al., 1997; Yu et al., 2013). Infestation of genital organs results in several early symptoms of MGS. One major symptom observed in early stages is haemospermia resulting from egg penetration and release into seminal vesicle lumen, causing ulceration of mucosal lining, and pain during coitus and ejaculation (Madden, 1911; Makar, 1937; Mohammed, 1952; Kato-Hayashi et al., 2013; Lang et al., 2017). Haemospermia can occur as the only symptom or first symptom preceding haematuria, occurring within three months of exposure to infection (Becquet, 1966; Pedro Rde et al., 1973; Corachan et al., 1994; Schwartz et al., 2002).

Of interest, this symptom has been described more frequently among travellers than inhabitants of endemic areas, in 8 of the 12 case reports found in the search. This could be due to failure to recognise the symptoms, societal acceptance of condition as male menstruation and maturing from boyhood to adulthood, not knowing or making an association with MGS, being mistaken with sexually transmitted infections (STIs) or infidelity (Ukwandu and Nmorsi, 2004; Yirenya-Tawiah et al., 2016). In relation to haemospermia, other reported symptoms include alteration in semen quality and appearance with discolouration (McKenna et al., 1997; Torresi et al., 1997; Hawary et al., 2012), subjective change (Davies and Hamdy, 1998), lumpy semen (Lewis et al., 1996; Lang et al., 2017), rice grains with increased volume (Pedro Rde et al., 1973) and reduced viscosity or volume (Perignon et al., 2007; van Delft et al., 2007; Knapper et al., 2012).

The symptoms associated with mucosal thickening and enlargement of organs such as seminal vesicles cause irritation of the sympathetic nervous system leading to sexual hyperexcitability, night dreams and frequent painful erections (Mohammed, 1952). However, these symptoms have not been reported in the last four decades, raising the question of their reliability in the earlier studies or non-reporting in the recent studies, possibly due to the sensitive descriptive nature. The enlargement of genital organs has also been mistaken for other diseases such as tuberculosis or malignancy resulting in extraneous surgical interventions where PZQ treatment provided earlier might have prevented the surgery (Madden, 1911; Chippaux et al., 1957; Eltayeb, 1969; Kazzaz and Salmo, 1974; Fievet et al., 1984; Githae, 1992; Ferreira et al., 2015). Untreated, the organs chronically become nodular, firmer, smaller and non-functional.

More recently reported symptoms of MGS include spermaturia (sperm in urine) as a result of fibrosis and abnormal cystic dilatation of seminal vesicles (Etribi et al., 1967), hydrocele formation (Gelfand and Davis, 1940; Armbrust, 1951; Van Beukering and Vervoorn, 1956; Pawel et al., 2008; Ramarakoto et al., 2008; Rambau et al., 2011), epididymitis (Alves et al., 2004), funiculitis (Durand et al., 2004), orchitis (Mikhail et al., 1988; Ihekwaba, 1992; Al-Qahtani and Droupy, 2010), prostatitis (Cerqua, 1930; Alexis and Domingo, 1986; Patil and Elem, 1988; Cohen et al., 1995; Fender et al., 1996; Basilio-de-Oliveira et al., 2002; Al-Saeed et al., 2003; Lambertucci et al., 2006; Bacelar et al., 2007; Sharma et al., 2015), infertility from oligospermia, azoospermia either obstructive from blockage of vas deferens, spermatic cord, epididymis, tunica vaginalis or non – obstructive through infarction (Kini et al., 2009; Abdel-Naser et al., 2018a; Abdel-Naser et al., 2018b), fibrotic lesions (Edington et al., 1975) or functional lymphotic infiltration in testis (Adisa et al., 2012). While egg load in the bladder tissue has been found to correlate with pathological severity, few ova in seminal vesicles, prostate and other genital organs have been associated with severe extensive pathological changes (Edington et al., 1970; Edington et al., 1975).

On malignancies of genital organs, our search showed that MGS has been reported among travellers and those emigrating from endemic areas, apart from testicular or scrotal schistosomiasis simulating neoplasia (Alexis and Domingo, 1986; Cohen et al., 1995; Ma and Srigley, 1995; Basilio-de-Oliveira et al., 2002; Bacelar et al., 2007; Lopes et al., 2007; Figueiredo et al., 2015). Prostatic adenocarcinoma has been observed to occur together with *Schistosoma* ova, resulting in epithelial granulomata, marked fibrosis and organ enlargement, which have been described in reports of tissue histopathology and cancer spread to other genital organs affected by MGS. Despite an accepted link between chronic UGS and squamous cell carcinoma of the bladder (Honeycutt et al., 2014), the mechanism of association between prostatic cancer and schistosomiasis remains unknown.

Our search produced recent systematic reviews addressing extensively the interactions of both MGS and HIV and were included in this review (Mbabazi et al., 2011; Stecher et al., 2015). As one of the leading causes of morbidity and mortality in

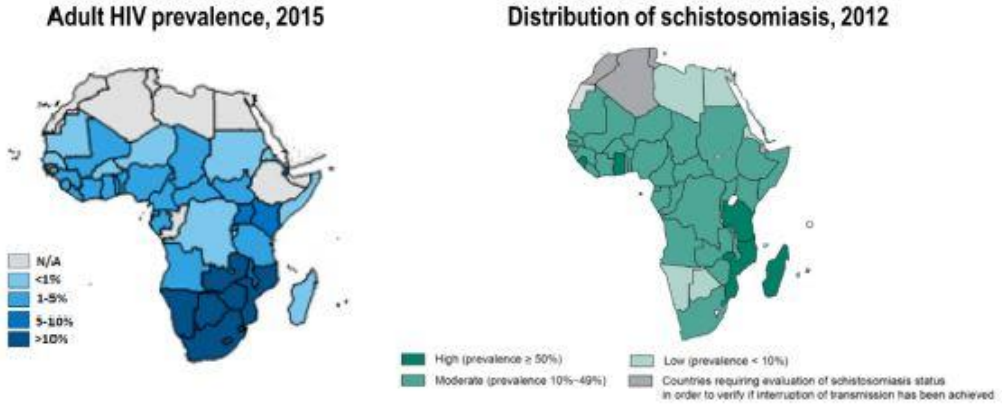

Fig. 3. Map of Africa showing the correlation of the prevalence of HIV and schistosomiasis. Produced from (WHO, 2014; Kaiser-Family-Foundation, 2016).

the world, HIV has its epicentre in the SSA region (UNAIDS, 2016) where coincidentally schistosomiasis is endemic (Fig. 3). Female genital schistosomiasis (FGS) has been observed in 33–75% of women having UGS living in endemic areas in SSA (Kjetland et al., 2012). In addition, FGS has been associated with a 3-fold increased risk of HIV infection with characteristic sandy-grainy patches present in egg-infected genital organs, abnormal blood vessel formation and increased levels of inflammatory cells expressing CD4+ receptors triggered by *Schistosoma* granulomata (Kjetland et al., 2006; Christinet et al., 2016). More research and gleamed knowledge on the risk and interplay of MGS with HIV infection is needed.

Various hypotheses have been proposed regarding the impact of MGS on HIV transmission. As described in Leutscher et al. (2005) study and the systematic reviews by Mbabazi et al. (2011) and Stecher et al. (2015), men with MGS have elevated levels of eosinophils and lymphocytes among other inflammatory cells expressing CD4+ receptors together with cytokines IL-4, 6, 10, TNF-α. These recruit more HIV-infected cells into semen, upregulating viral replication and increasing viral concentration (Leutscher et al., 2005; Mbabazi et al., 2011; Stecher et al., 2015). With the chronic inflammation and cell recruitment to the male genital tract, these may increase HIV viral load in semen, similar to that seen with STIs (Mabey, 2000). A recent observational pilot study in endemic SSA area demonstrated a reduction of viral load shedding in semen of HIV positive men coinfected with UGS 10 weeks after PZQ treatment (Midzi et al., 2017). Further case-cohort or randomized studies are needed to be conducted in endemic areas to explore these critical findings further.

3.3. Techniques for detection of MGS

Urine microscopy remains the definitive way for identifying schistosome ova (mainly of *S. haematobium*) to diagnose UGS. While it is the gold standard (Le and Hsieh, 2017) and also considered as a useful proxy for diagnosing MGS, our findings indicate challenges in its reliability due to presence of ova in semen or histological specimens in the absence of ova in urine (or stool), as well as other schistosome species that may on occasion cause MGS (Corachan et al., 1994; Torresi et al., 1997; Leutscher et al., 2000; Schwartz et al., 2002; Lang et al., 2017). As such, there is a direct need to conduct microscopy on semen and biopsy material from suspicious genital lesions/tissues to diagnose MGS. Also, semen should be analysed repeatedly with periods of abstinence to cater for the daily diurnal variations in excretion of ova from the genital organs and increase likelihood of maximum egg yield, similar to the recommended consecutive urine analyses (Leutscher et al., 2008b; Le and Hsieh, 2017). However, local perceptions, beliefs and sensitivity of people in endemic communities needs careful consideration with regards to ejaculation and handling semen samples. Combined this can affect the collection or submission and later analyses, thus to optimise the process there is usually a need for more health education and counselling of those required to submit as well as within the community to ensure acceptability and success in engagement alongside collection of the samples in the community (Price et al., 2005; Midzi et al., 2017).

Leutscher and colleagues report on use of eosinophil cationic protein (ECP), circulating anodic antigen (CAA) and soluble egg antigen (SEA) as blood-based markers of MGS which showed positive correlation to urine egg count, with ECP significantly correlating with urine count and declining after PZQ treatment, highlighting its importance in diagnosis (Leutscher et al., 2000; Leutscher et al., 2008b). However, other helminth, bacterial or viral infections and inflammatory conditions elevate ECP hence affecting the reliability in co-morbidities which are common in endemic areas. PCR and DNA-based tests on the other hand have shown to be highly sensitive and specific diagnostic tools, which can be used in urine, semen, and many other specimens (Le and Hsieh, 2017). These tests are still expensive for field use in endemic areas of Sub-Saharan Africa, hence the need to develop easier, accessible, low-cost tests.

Our findings showed that ultrasonography is useful in diagnosing lesions caused by MGS and monitoring morbidity of the pathologies (Richter, 2000; Al-Saeed et al., 2003; Ramarakoto et al., 2008). The pathological lesions seen in genital organs have been described as echogenic lesions and calcifications, with the former improving with treatment (Richter, 2000; Ramarakoto et al.,

2008). Availability of portable sonography machines could be more cost-effective in endemic areas since other radiological techniques such as computerised tomography (CT) and magnetic resonance imaging (MRI) are very expensive, not feasible and almost non-existent in these regions. However, there has been limited radiological research on MGS in endemic areas, hence there is a need for more field studies to study the resolution of pathologies after treatment.

3.4. Current treatment options for MGS

Praziquantel (typically offered at 40 mg/kg) has remained the mainstay treatment for most forms of schistosomiasis, including MGS (WHO, 2013). It is effective with population cure rates of over 90% and targets adult worms thereby reducing egg excretion and averting morbidity, however, praziquantel does not successfully kill juvenile worms (Rollinson, 2009). Most identified case reports and studies used the recommended traditional dosage of 40 mg/kg in treating MGS with some failure cases requiring further repeated doses or higher dose of 60 mg/kg (Schwartz et al., 2002; Alonso et al., 2006; Perignon et al., 2007). It has been suggested that higher doses are more efficacious in MGS treatment than the traditional dose alongside shorter intervals between retreatments, for example, 2–3 times a year (Lang et al., 2017).

Use of PZQ in most African programmes is based on morbidity control through mass drug administration versus specific case management. The former is an attempt to keep prevalence and intensity down to an acceptable level, below 10% in the endemic population and obtain the greatest cost-benefit outcome at population level. There are well-known gaps in this approach, for example, school-aged children are targeted with donated stocks of PZQ ring-fenced (restricted) for this use in school-based programmes. As an indirect consequence, many adolescents and adults rarely receive adequate treatment and PZQ is not always available in peripheral health clinics which further affects management of schistosomiasis in an individual case management setting (Christinet et al., 2016; McManus et al., 2018).

3.5. Existing gaps for further research of MGS

This review conducted a systematic search to elucidate the burden of MGS in endemic areas, a century after the first recognised report in 1911. Despite the detailed epidemiology of schistosomiasis in the world highlighting the enormous impact of UGS, much remains unknown of the burden of genital manifestations of schistosomiasis either FGS or MGS, specifically. More description and research studies of UGS especially in endemic areas have concentrated on urinary system and associated pathologies, however, with the growing interest in cervical cancer screening there are opportunities to integrate surveillance of FGS (Christinet et al., 2016). On the other hand, for men, no such screening programmes exist and therefore the prevalence and morbidity of MGS in endemic areas will remain under-reported.

In addition, the limited description of MGS is compounded by difficulties in diagnostic techniques and approaches, these include deficits in standardised protocols for analysis of semen. Indeed, future methods which involve molecular assays will be challenging to carry out in primary health facilities in SSA. Future research studies to explore the deployment of low-cost techniques and methods are urgently required. These would be particularly important regarding treatment and management of MGS, as currently there is a clear gap in our understanding of the optimal dose of PZQ to treat MGS, whether single, repeated (i.e. 2–3 times a year) or higher dosages (i.e. >60 mg/kg) would be effect a parasitological cure (Schwartz et al., 2002; Alonso et al., 2006; Lang et al., 2017), notwithstanding tracking the dynamics of lesions in the genital tract. This highlights the need for further prospective longitudinal studies in endemic areas and more clinical research exploring an agenda of how best to integrate preventive treatment and management of MGS alongside ongoing interventions for HIV in SSA.

4. Discussion

This review has revealed that genital organs are infested with schistosome ova in the early stages of the infection, similar to other forms of the disease. These organs are infected with substantial numbers of ova as much as urinary bladder or intestines, further indicating the higher levels of MGS in endemic areas. Clinical manifestations associated with MGS in this review have been described previously by Barlow after self-infection with cercariae (Barlow and Meleney, 1949) and are regarded as major symptoms and diagnostic for MGS. Symptoms like haemospermia can also present in other diseases such as hypertension, prostatitis or STIs (Feldmeier et al., 1999), raising the need to exclude other conditions before concluding the diagnosis of MGS. Underreporting and misconceptions of these symptoms which may have negative perception in the community, contribute to misdiagnosis and underestimation of MGS in these endemic areas (Ukwandu and Nmorsi, 2004; Yirenya-Tawiah et al., 2016). Furthermore, co-existence of MGS and prostatic metaplasia and malignancies require further research to understand the link, and develop diagnostic and therapeutic interventions.

Although urine microscopy has been considered as a proxy for diagnosing MGS, our findings observed challenges of ova found only in semen without any in urine or stool, hence the need to consider semen microscopy as a definitive way of diagnosing MGS. Accessible, low-cost molecular tests should be developed to address this diagnostic challenge. Similarly, radiological techniques like field-based ultrasonography should be rolled out into endemic areas to monitor the morbidity and resolution of MGS pathologies. MGS appears to be prevalent in areas endemic for UGS, which coincidentally are high prevalent areas for HIV. Some people in these areas in SSA have higher HIV prevalence and also at higher risk for schistosomiasis due to their lifestyles and daily activities, as reported about fishermen in Malawi (NSO, 2014; NAC, 2015). With some evidence of MGS potentially upregulating viral replication, increasing the concentration of HIV particles in the semen and exponentiating the infectiousness of dually infected males, treatment of MGS could be an importance tool in helping to avert new HIV infections in SSA (Stecher et al., 2015).

Interestingly, one of the current effective intervention of HIV prevention, male circumcision, was considered by ancient Egyptians around 2300 BCE as an intervention to prevent schistosomal infection among men bathing in infested waters, though later disputed (Allen, 1909; Madden, 1919; Jordan, 2000; Weiss, 2004).

5. Conclusion

MGS is an under-appreciated manifestation of UGS and has been reported worldwide but its current distribution is most tightly linked with areas endemic for *S. haematobium*. In SSA, MGS likely blights the lives of millions of men who currently do not have adequate access to point-of-care diagnostics or access to optimal praziquantel treatment regimes. We propose that MGS should be considered specifically in a new light of individual case management approaches as being used for other NTDs.

Acknowledgements

We wish to thank Mr. Hajri Al-Shehri, Dr. Suzy Campbell and Ms. Grace Macklin for their support in reading the manuscript. Huge appreciation to Alison Derbyshire of Liverpool School of Tropical Medicine (LSTM) Library for training and advice on systematic approaches to literature searching. Sekeleghe Kayuni is being supported by a Commonwealth PhD studentship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parepi.2018.e00077.

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Appendix 26: Research output – Contribution to Emerging Infectious Diseases (for *Schistosoma mansoni* infection)

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Biomphalaria pfeifferi Snails and Intestinal Schistosomiasis, Lake Malawi, Africa, 2017–2018

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DOI: https://doi.org/10.3201/eid2503.181601

Two surveys conducted in 2017 and 2018 demonstrated *Biomphalaria pfeifferi* snails in Lake Malawi in Africa. Epidemiologic examination of 175 local children at 3 primary schools confirmed emergence of intestinal schistosomiasis. These findings highlight autochthonous transmission of *Schistosoma mansoni* flukes in Lake Malawi and the need to revise international travel advice.

Throughout sub-Saharan Africa, *Biomphalaria pfeifferi* snails are freshwater intermediate hosts for *Schistosoma mansoni* blood flukes, which cause intestinal schistosomiasis (*1*). Geographic distribution of *B. pfeifferi* snails delineates actual or potentially active zones of *S. mansoni* fluke transmission (*2*). Other than a report of a single *Biomphalaria* shell at Karonga in the far northern portion of Lake Malawi (*3*), considered to be from a marginal swamp (*4*), *B. pfeifferi* snails have not previously been found in Lake Malawi (*5*). However, in November 2017, during malacologic surveillance for intermediate hosts of schistosomiasis in the Mangochi District, Malawi, along the southernmost tip of Lake Malawi, 2 discrete populations of *Biomphalaria* snails were unexpectedly encountered in submerged beds of *Vallisneria* spp. plants (Figure, panel A). DNA sequence analysis of the mitochondrial cytochrome oxidase subunit 1 (*cox*1) (*6*) indicated that the *cox*1 sequences (1,006 bp) of those snails differed from sequences of *B. pfeifferi* snails from Chiweshe, Zimbabwe (GenBank accession nos. DQ084829 [HCO/LCO region] and DQ084872 [Asmit1/2 region]) by only 3 synonymous single-nucleotide polymorphisms.

In May 2018, to confirm *B. pfeifferi* colonization within the lake and suspected risk for intestinal schistosomiasis, we undertook a conjoint malacologic and parasitologic survey with ethics approvals from the Liverpool School of Tropical Medicine, UK (application 17-018) and the Ministry of Health and Population, Malawi (application 1805). Reinspection of all prior malacologic sampling locations and another 43 sites found further populations of *B. pfeifferi* snails (Figure, panel A); large numbers (>50), along with innumerable dead shells, were again found at site 9. All snails were inspected for shedding cercariae, and although cercariae from snails at site 5 were seen, identification by microscopy (×100) was unsuccessful. Supplementary analysis indicated that *cox*1 sequences from 9 snails from sites 2, 5, 7, 10, and 11 were identical.

We conducted an epidemiologic survey of 175 schoolchildren, 5–15 years of age, equal numbers of boys and girls, from 3 primary schools closest to site 9 (Figure, panel B). Mean prevalence of intestinal schistosomiasis, calculated by detection of *S. mansoni* circulating cathodic antigen (CCA) on urine dipstick testing, was 34.3% (95% CI 27.9–41.3); prevalence rates by school were Samama, 46.7% (95% CI 36.7–56.7); Mchoka, 25.0% (95% CI 15.0–36.7); and Palm Beach, 9.1% (95% CI 0.0–22.7). We requested fecal samples from 60 *S. mansoni*–positive children and received samples from 46. Duplicate Kato-Katz examinations confirmed *S. mansoni* ova in 7 children; infection intensities were graded as light (<100 eggs/g feces). All urine samples were inspected for *S. haematobium* ova by syringe filtration (10 mL); general prevalence was 14.9% (95% CI 9.8–20.1); 52% of these samples were also positive by CCA urine dipstick, indicative of *S. mansoni* co-infection. To further determine autochthonous transmission of *S. mansoni* flukes, 2 egg-positive children from Samama and Mchoka took us, on foot, to the shoreline where they regularly swam, which corresponded to snail

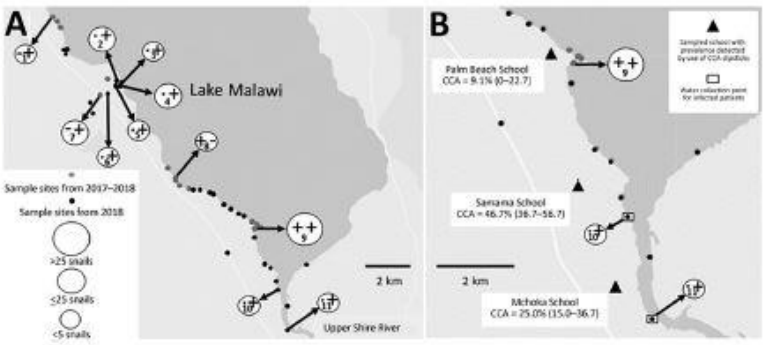

Figure. Locations sampled for *Biomphalaria pfeifferi* snails and of 3 primary schools where children were tested for intestinal schistosomiasis in the region of Lake Malawi, Africa. A) Locations sampled for *B. pfeifferi* snails in November 2017 (gray dots) and May 2018 (black dots), Lake Malawi, Africa. + indicates snails present, – indicates snails absent, and • indicates site not sampled; symbol position indicates year of sampling (left, 2017; right, 2018). Numbers within circles indicate site numbers. Collected snail numbers are indicated by circle size. In 2017, snails were collected at 2 sites and not collected at 12 sites; in 2018, snails were collected at 10 sites and not collected at 47 sites. On each sampling occasion, >50 *B. pfeifferi* snails were collected at site 9. Coordinates of *B. pfeifferi*–positive sites: site 1, 14.27752°S, 35.10419°E; site 2, 14.31371°S, 35.14174°E; site 3, 14.31424°S, 35.14383°E; site 4, 14.31354°S, 35.14424°E; site 5, 14.31568°S, 35.14030°E; site 6, 14.32033°S, 35.13613°E; site 7, 14.32100°S, 35.13072°E; site 8, 14.36919°S, 35.17629°E; site 9, 14.39363°S, 35.22104°E; site 10, 14.42708°S, 35.23349°E; and site 11, 14.44928°S, 35.23890°E. B) Location of the 3 sampled primary schools (Palm Beach, 14.391346°S, 35.215137°E; Samama 14.417465°S, 35.217580°E; Mchoka 14.439481°S, 35.220644°E) showing local prevalence (% [95% CI]) of intestinal schistosomiasis indicated by *Schistosoma mansoni* circulating cathodic antigen (CCA) detected by urine dipstick. Water collection sites pinpointed by 2 *Schistosoma* egg–positive children from Samama and Mchoka Schools are indicated.

collection sites 10 and 11 (Figure, panel B). Children who were positive for either *S. mansoni* CCA or *S. haematobium* eggs received praziquantel (40 mg/kg).

Colonization of *B. pfeifferi* snails in Lake Malawi and surrounding water is of concern, especially because active *S. mansoni* infections were found in local children. This finding highlights emergence of intestinal schistosomiasis, not previously documented here (*5*,*7*,*8*) or detected in this region by the most recent national survey (F. Fleming, Schistosomiasis Control Initiative, Imperial College London; 2017 Dec 20; pers. comm).

Intestinal schistosomiasis has been detected in children ≈150 km away, along the shoreline of the Lower Shire River (*9*). Finding snails and infected children in Mangochi District suggests recent ecologic and epidemiologic change. In May 2018, the lake was ≈75–80 cm higher than in November 2017, which perhaps favored detection of *B. pfeifferi* snails in the previously more accessible *Vallisneria* plant beds. Seasonal dynamics, such as lake level fluctuations, are well known, along with longer duration perturbations of the lake biota, either induced by climate change or mediated by anthropogenic activities. These changes have altered transmission of urogenital schistosomiasis (*10*); overfishing, particularly of the molluscivorous fish *Trematocranus placodon*, is changing the distribution of many freshwater snails (*5*).

Local aquaculture of fish (e.g., *Oreochromis* spp., called chambo) through use of water pumped inland from the lake has created novel, permanent water bodies colonized by *B. pfeifferi* snails (e.g., sites 2–7), which may now (re)seed snails into the lake for further establishment. Absence of *cox*1 genetic diversity in the *B. pfeifferi* snails we sampled implies a limited number or even a single founder event, but as conditions for autochthonous transmission became favorable, after introduction of *S. mansoni* flukes, intestinal schistosomiasis in local schoolchildren has emerged. This finding is of substantial public health concern in light of current control efforts, which consist only of annual praziquantel distribution in schools (*7*,*8*). We recommend increased surveillance of snails and characterization of schistosomes, along with intensified control interventions to arrest further spread of intestinal schistosomiasis. We also recommend revising and updating health and travel advice given to shoreline community residents and tourists who use the lake.

Acknowledgments

We thank Alexandra Shaw and Joanna Fawcett for assistance during the epidemiologic survey in the Mangochi District. We are grateful to the local health and education authorities of Malawi; district teachers; local community health workers Flora Jumbe, Caroline Nthubula, Angelina Mwenyewe, and Witness Mapira; and hosting communities for their enthusiasm and support. We are also indebted to Danie and Hazel Britz for assistance at Palm Beach School, to Paul and Stacey Kennedy for local boat hire, and to Anthony Butterworth and Liz Corbett for their kind hospitality in Blantyre.

M.H.A. was funded by a PhD scholarship from the Ministry of Health, Kingdom of Saudi Arabia, and S.K. by the Commonwealth Scholarship Commission. CCA urine dipsticks were supplied by Rapid Medical Diagnostics (http://www.rapid-diagnostics.com), South Africa (lot no. 171103130).

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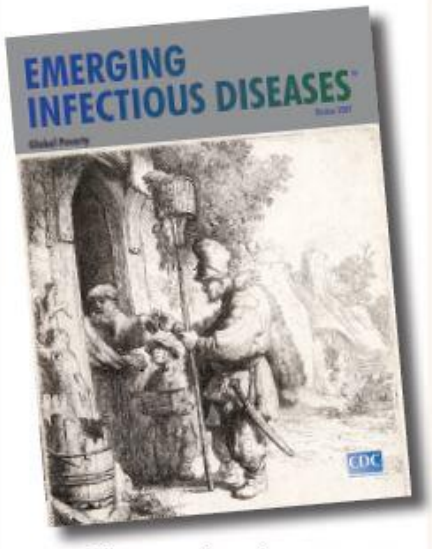

Originally published in October 2007

etymologia revisited

schistosomiasis

[shis"-, skis" to-so-mi'ə-sis],
from the Greek—*skhistos* (split) and *soma* (body)

Infection of the blood with a parasite of the genus *Schistosoma*. Originally thought a single organism with a split body, the parasite was eventually recognized as having male and female forms. Three main species cause human infection: *S. haematobium, S. mansoni,* and *S. japonicum*. Each species has its own range of host snails. The parasite releases eggs containing larvae through feces or urine; if the eggs reach water, the larvae are released and may penetrate a snail. A very large number of larvae are then produced inside the snail and released back into the water. Infection is acquired through skin contact with contaminated water.

Schistosomiasis, which leads to chronic hepatic and intestinal fibrosis of the urinary tract, was first identified in Egypt in 1851 by German pathologist Theodor Bilharz and is also called bilharzia. Approximately 160 million persons throughout the world are infected, particularly in Africa, the Middle East, South America, and Southeast Asia.

Source: Institute of Tropical Medicine of Antwerp: www.itg.be

https://wwwnc.cdc.gov/eid/article/13/10/e1-1310_article

Appendix 27: Research output – Contribution to the Emerging Infectious Diseases (for *S. haematobium*)

demarcated boundary of erythema with a tiny scab (Figure, panel D).

A new species of *Rickettsia* was detected from leeches in Japan (*5*,*6*). Furthermore, certain leech species, parasitizing frogs or fish, can complete the vertical transmission of *Rickettsia* spp. with possible horizontal transmission (*6*). The leech is reported to be a potential vector for human rickettsial infections (*7*,*8*). Slesak et al. described the case of a 39-year-old woman with *R. felis* infection confirmed by eschar PCR after a leech bite in northern Laos (*7*). Balcells et al. reported the case of a 54-year-old man with scrub typhus–like illness after a leech bite in southern Chile (*8*). In our previous study (*4*), 13% (4/31) of patients with Japanese spotted fever and 2% (4/188) of patients with scrub typhus diagnosed by serologic tests had a history of land leech bite before the symptom onset.

Our report is limited because we did not have the land leech for testing by PCR. The patient might have had rickettsia on his skin and then been inoculated by the leech bite or by scratching after the bite (*7*). Further investigations, including an experimental model, are needed to support the potential role of leeches in the transmission of *R. japonica* and other *Rickettsia* spp.

Acknowledgments

We thank the patient, who provided written consent for publishing this case report and the accompanying images; Satoshi Kobayashi for the patient's care and his assistance in collecting samples; and Shuji Ando for critical comments.

This study was supported by the Japan Agency for Medical Research and Development under grant no. JP18fk0108068.

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Schistosome Interactions within the *Schistosoma haematobium* Group, Malawi

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DOI: https://doi.org/10.3201/eid2506.190020

Molecular analysis of atypical schistosome eggs retrieved from children in Malawi revealed genetic interactions occurring between human (*Schistosoma haematobium*) and livestock (*S. mattheei* and *S. bovis*) schistosome species. Detection of hybrid schistosomes adds a notable new perspective to the epidemiology and control of urogenital schistosomiasis in central Africa.

Urogenital schistosomiasis is a waterborne disease transmitted by certain freshwater snails that occurs throughout much of sub-Saharan Africa. Until recently, this disease was attributed solely to *Schistosoma haematobium*, which was considered to have limited zoonotic potential (*1*). However, genetic analysis of natural infections with noninvasive larval sampling (*2*) has provided new evidence. In West Africa, for example, species interactions with hybrid combinations of *S. haematobium* and the bovine or ovine species of *S. bovis* and *S. curassoni* are commonly encountered in humans and snails (*3*). Although key biologic features of hybrids may not always be apparent, the risk for zoonotic transmission along with enhanced definitive and intermediate host compatibilities needs investigation (*2*,*3*). The recent emergence and persistent transmission of *S. haematobium–bovis* hybrids on the Mediterranean island of Corsica (*4*) demonstrates the public health impact of such genetic introgression.

Genetic analysis of *S. haematobium* group species in central and southern Africa is a high priority. Atypical egg morphologies suggest a capacity for natural hybridization of *S. haematobium* with the bovine species *S. mattheei*, later confirmed with biochemical markers and experimental infections demonstrating viable progeny (*3*). During ongoing surveillance of urogenital schistosomiasis in Chikhwawa District, Malawi, we encountered atypical *S. haematobium* eggs in urine samples from several infected children (*5*). We report the further genetic characterization of atypical eggs collected from epidemiologic surveys of children within Chikhawa, Nsanje, and Mangochi Districts (Figure, panel A).

Ethics approvals for the epidemiological surveys were granted by Liverpool School of Tropical Medicine, College of Medicine, Malawi, and Ministry of Health and Population, Malawi. All children found infected were treatment with praziquantel.

We filtered schistosome eggs from the urine of infected children, then photographed and measured them before storing them on Whatman FTA cards for molecular analysis (*2*). We alkaline-eluted and genotyped DNA from individual eggs using both the mitochondrial cytochrome oxidase subunit 1 (*cox*1) and the nuclear ribosomal internal transcribed spacer (rITS) DNA regions (*2*) (Appendix Table, https://wwwnc.cdc.gov/EID/article/25/6/19-0020-App1.pdf). In addition, for the samples from Mangochi District, we analyzed a partial region (300-bp) of the nuclear ribosomal 18S DNA to confirm the presence of *S. mattheei* nuclear DNA (*2*,*6*) (Appendix).

Of 6 atypical eggs from Chikhwawa, all had a pure *S. haematobium* genetic profile (Figure, panels B, C). Of 19 eggs from Nsanje, 18 had a pure *S. haematobium* genetic profile; 4 eggs had atypical morphology, but only 1 atypical egg had a discordant genetic profile (i.e., *cox*1 *S. bovis* and rITS *S. haematobium*). Of 20 eggs from Mangochi, 16 typical *S. haematobium* eggs had a pure *S. haematobium* genetic profile, whereas the 4 atypical eggs had the same discordant genetic profiles (*cox*1 *S. mattheei* and rITS *S. haematobium-mattheei*). Inspection of the partial 18S gene

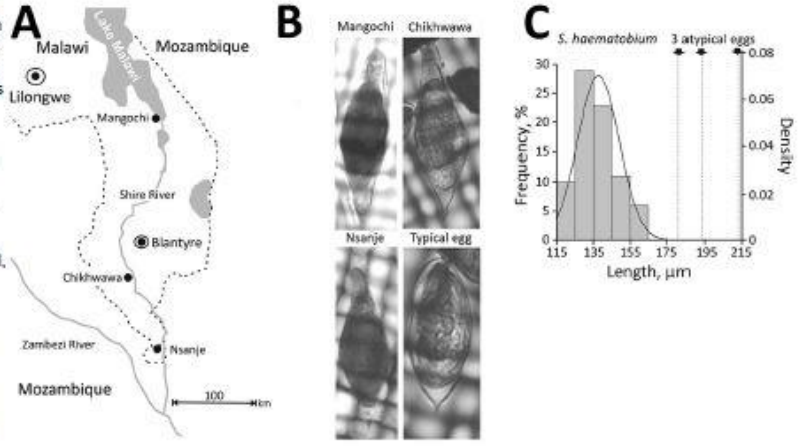

Figure. Investigation of atypical schistosome eggs retrieved from children in Malawi. A) Locations where urine samples containing *Schistosoma haematobium* eggs were collected from children in Mangochi (Samama village, 14°41'74.65"S, 35°21'75.80"E), Chikhwawa (Mpangani village, 16°03'62.99"S, 34°84'10.63"E), and Nsanje (Kastiano village, 16°90'63.98"S, 35°26'65.78"E) districts. Of the children sampled, ≈10% had atypical eggs in their urine, in an approximate atypical:typical ratio of 1:25. Note that the Shire River flows southward from Lake Malawi, linking the 3 sampled locations within the same drainage basin. B) Photomicrographs of a representative atypical egg from each location. Corresponding genotypes assigned for the mitochondrial *cox*1 and nuclear rITS loci: Mangochi, *cox*1 *S. mattheei* and rITS *S. haematobium-mattheei*; Chikhwawa, *cox*1 and rITS *S. haematobium*; Nsanje, *cox*1 *S. bovis* and rITS *S. haematobium*. A typical *S. haematobium* egg is shown for comparison. Sizes are not to scale. C) Histogram of length measurements for 83 typical *S. haematobium* eggs collected from Nsanje. Solid line indicates the associated density distribution. The mean length of this sample of typical eggs was 135 ± 28 µm (1 SD), with minimum 86 µm and maximum 180 µm. Arrows with dashed lines at right indicate the length of the 3 atypical eggs, which fall well outside the range of length variation of the 83 typical eggs as measured.

sequence confirmed *S. haematobium–mattheei* hybrids (Appendix). We deposited all sequence data into GenBank (accession nos. MK358841–MK358858).

Our genetic analysis demonstrated the presence of *S. haematobium* group hybrids in Malawi as introgressed forms of *S. haematobium–mattheei* and *S. haematobium–bovis*. Of note, an unusual egg morphology may not always correspond with the ability to detect introgression with the current combination of genetic markers used (*6*; Appendix). As described by Boon et al., successive backcrossings of hybrid progeny may obscure our ability to detect ancestral introgression, and the development of a wider panel of nuclear genetic markers is needed (*6*). Nonetheless, detection of these 2 hybrid schistosomes strongly suggests interactions of *S. haematobium* with the ungulate schistosomes *S. mattheei* and *S. bovis*. That *S. bovis* has not been reported in Malawi implies a changing species dynamic with possible zoonotic transmission along the drainage basin of Lake Malawi, adding a new dimension to the epidemiology and control of urogenital schistosomiasis in Malawi (*7*).

Because we did not attempt miracidial hatching during this study, we cannot confirm that these hybrids or introgressed forms are fully viable in autochthonous natural transmission. However, the process of ancestral introgression with subsequent natural selection may help explain unexpected shifts in local snail–schistosome relationships (e.g., the changing compatibility of *Bulinus nyassanus* snails in Lake Malawi with *S. haematobium* schistosomes) (*8*). Further studies are needed to better characterize schistosomes involved in human infection, investigate more thoroughly any zoonotic potential, and assess all possible combinations of interspecies introgressions.

Molecular evidence for ancestral hybridization between *S. haematobium* and *S. mansoni* schistosomes was presented recently (*9*); given autochthonous transmission of intestinal schistosomiasis in Lake Malawi (*10*), there may be sufficient epidemiologic opportunity for other introgression events to occur with the hybrids we report. We therefore advise heightened concurrent surveillance of urogenital and intestinal schistosomiasis, entailing a One-Health approach with molecular vigilance for interspecies interactions along with phenotypic assessments for any altered host pathogenicity or susceptibility to praziquantel treatment. Detection of the hybrid schistosomes we report adds a new perspective to the epidemiology and control of urogenital schistosomiasis in central Africa.

Acknowledgments

We are particularly grateful to the local health and education authorities of Malawi, district teachers, and community health workers involved in schistosome surveys in Chikhwawa, Nsanje, and Mangochi. We thank Jahashi Nzalawahe for assistance with measurements and analysis of schistosome egg morphology.

M.H.A. is funded by a PhD scholarship from the Ministry of Health, Kingdom of Saudi Arabia. S.K. received a scholarship from Commonwealth Scholarship Commission and a research fellowship from the African Research Network for Neglected Tropical Diseases (ARNTD).

About the Author

Dr. Webster is a researcher with the Natural History Museum, London, UK. She has specific expertise in medical helminthology and a longstanding interest in studies of schistosome hybridization in both natural and experimental settings.

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Article DOI: https://doi.org/10.3201/eid2506.190020

Schistosome Interactions within the *Schistosoma haematobium* Group, Malawi

Appendix

DNA Extraction

DNA preparation from FTA stored schistosome eggs and larvae:

1. Add 14 µL of Solution 1 (0.1M NaOH, 0.3mM EDTA, pH13.0) to the punchout 2 mm FTA disc.
2. Incubate at room temperature for 5 min.
3. Add 26 µL of Solution 2 (0.1M Tris-HCl, pH7.0).
4. Pulse vortex 3 times.
5. Incubate at room temperature for 10 min.
6. Pulse vortex 10 times.
7. Use 3 µL of the DNA elution in a 25 µL PCR.

PCR Analysis and Sequencing

We eluted DNA as described above from the schistosome eggs stored on the FTA cards. In separate PCRs, run on a Perkin Elmer 9600 Thermal Cycler, we amplified the *cox*1, ITS and 18S DNA regions (Appendix Table). We performed a 25 µL PCR reaction for each using illustraTM puReTaq Ready-To-Go PCR Beads (GE Healthcare, Hertfordshire, UK, https://www.gehealthcare.com) and 10 pmol of each primer (Appendix Table) and 3µL of the DNA elution.

We checked all PCR reactions for positive amplification of the correct band size by gel electrophoresis using 0.8% Gelred agarose gels (Biotium, https://biotium.com). We purified PCR amplicons and Sanger sequenced them in both directions using a dilution of original PCR primer.

We used Sequencher version 5.1 (Gene Codes Corp., http://www.genecodes.com) to visualize and manually edit all sequence data.

We confirmed mitochondrial *cox*1 sequence identity using the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). We analyzed the ITS and 18S sequence identity by visual comparison to personal reference sequences for each species (*S. haematobium*, *S. bovis*, and *S. mattheei*). We visually checked known interspecies SNP regions (Appendix Figure) to identify homogenous or heterogenous ITS and 18S DNA.

We inspected mitochondrial and nuclear genetic profiles to identify hybrids (and any discordance of mitochondrial and nuclear DNA data).

The mean egg length of this sample of 83 typical eggs was 135 ± 28µm (1 SD), which was very similar to the 137 ± 15µm (1 SD) reported by Boon et al (*1*). We referred to additional sources on unusual egg morphology (*2–4*).

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Appendix Table. PCR primers used to amplify each DNA region and their associated PCR thermal cycle in study of *Schistosoma haematobium* hybridization, Malawi

DNA region	Forward primer (name)	Reverse primer (name)	PCR thermal cycle
ITS1+2 rDNA	TGCTTAAGTTCAGCGGGT (ITS1)	AACAAGGTTTCCGTAGGTGAA (ITS2)	5 min at 95°C: 40 cycles of 30 s at 95°C, 30 s at 58°C, 1.30 min at 72°C: 10 min at 72°C.
Partial 18S rDNA	GCGAATGGCTCATTAAATCAG (WA)	TCCGGAGAGGGAGCCTGA (300R)	5 min at 95°C: 40 cycles of 30 s at 95°C, 30 s at 60°C, 1 min at 72°C: 10 min at 72°C.
Partial *cox*1 mt DNA	TAATGCATMGGAAAAAAACA (cox1Schisto5′)	TCTTTRGATCATAAGCG (cox1Schisto3′)	5 min at 95°C: 40 cycles of 30 s at 95°C, 30 s at 40°C and 1.30 min at 72°C 10 min at 72°C.

Marker	18S (300bp)			ITS (906bp)										
SNP	138	163	210	18	26	50	51	91	92	120	170	225	490	877
S. haematobium	T	C	T	C	T	G	T	C	G	C	G	C	T	T
S. bovis	C	T	C	C	T	A	T	C	G	T	A	T	T	C
S. mattheei	C	T	T	T	A	A	C	T	A	T	A	T	A	T

Appendix Figure. Comparison of DNA sequences from eggs of 3 *Schistosoma* species, Malawi.

Appendix 28: Research output – Invited blog related to Parasitology article

Seke Kayuni

From: Emily Louise Pascoe <elpascoe@ucdavis.edu>
Sent: 08 November 2019 19:17
To: Seke Kayuni
Subject: Article selected as Parasitology Paper of the Month

Dear Dr. Kayuni,

The editorial team at Parasitology would like to congratulate you, as your article that was recently published in our journal "How can schistosome circulating antigen assays be best applied for diagnosing male genital schistosomiasis (MGS): an appraisal using exemplar MGS cases from a longitudinal cohort study among fishermen on the south shoreline of Lake Malawi" has been selected by the editors to be featured as a Paper of the Month. We feel that it is important that the novel methods you describe for detecting *Schistosoma* ova should be highlighted and disseminated.

Parasitology would thus like to invite you to submit a blog post, before the **18th of December**, on this paper to be published on the Cambridge Core Blog, a widely-read and highly visible resource. In addition to the press associated with the Cambridge Core Blog, your paper would become Open Access for 30 days following the publication of the blog post. The blog would need to conform to the guidelines below. Please reply to this email by 13th of December to let us know as to whether you will be able to write the post.

Blog Post Guidelines
Blog posts should be approximately 300-500 words. However, if everything important can be written in less than this, or if a video is included, for example, then a shorter post is appropriate.

The post needs to be understandable to a layperson- we aim for a blog post that an educated person with no specific knowledge in the field could comprehend. A conversational blog style is best, as a less formal post is more likely to engage the audience. As an example, imagine explaining your work to a friend who doesn't work in the field. What would they find interesting to know? Is this something that could affect them or their friends? What is the broader impact of the material described in the blog post?

Media such as audio and video files can either be embedded or linked out as long as they are on an open site (e.g. YouTube). As a minimum, one appropriate image should accompany the post to make it visually appealing. For any images you select to accompany a blog post, permissions must have been obtained from the copyright holder to use the image in this way, and if appropriate an image credit should be included in the blog. Parasitology can provide a stock image if necessary.

Some recent examples of blog posts can be seen in the links below.
Link One: https://www.cambridge.org/core/blog/2019/05/20/giardiasis-in-the-united-kingdom-current-state-and-moving-forward/
Link Two: https://www.cambridge.org/core/blog/2019/06/19/malacosporean-myxozoans-exploit-a-diversity-of-fish-hosts/
Link Three: https://www.cambridge.org/core/blog/2019/06/28/parasitic-nematodes-simultaneously-suppress-and-benefit-from-coccidian-coinfection-in-their-natural-mouse-host/

I am happy to answer any questions or provide assistance. Thank you for submitting your work to Parasitology, we look forward to hearing from you soon.

Best wishes,

Emily L Pascoe Ph.D.

Parasitology Social Media Editor
Postdoctoral Researcher at Pacific Southwest Center of Excellence in Vector-Borne Diseases
School of Veterinary Medicine, Department of Medicine and Epidemiology

1

BLOG: Parasitology paper of the month

by

Dr. Sekeleghe Kayuni

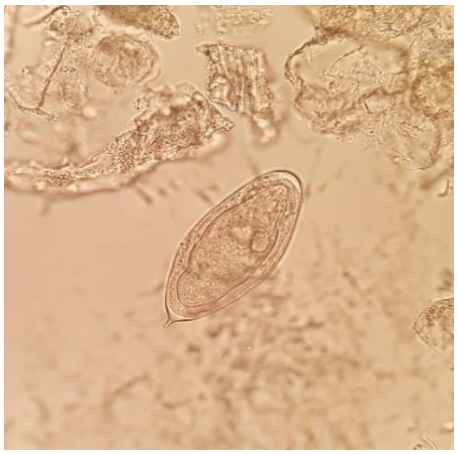
About the author: Seke is a medical doctor working with MASM Medi Clinics Limited in Blantyre City, Malawi and currently a Commonwealth PhD Scholar studying at the Liverpool School of Tropical Medicine and University of Liverpool. His twitter account is @sekekayuni.

Our paper on diagnostics originates from my soon-to-be-completed PhD study that has focused on developing a better understanding of the interplay between schistosomiasis and HIV in Malawian fishermen. In my country, Malawi, schistosomiasis is well known especially along the shoreline of Lake Malawi, where through my professional medical duties and research studies, I have encountered many people seeking for praziquantel treatment. The disease, also known as Bilharzia and grouped within the neglected tropical disease (NTDs), employs a preventative chemotherapy approach with praziquantel as its first foundation of disease control.

Across sub-Saharan Africa, schistosomiasis can be particularly common. Millions of young children, through to older adults, are affected by this disease, which may be life-threatening upon advanced disease progression like abdominal pain, blood or difficulties in passing urine, enlarged liver and spleen, liver fibrosis or bladder cancer. Schistosome infections are acquired through direct contact with freshwater sources that contains infective schistosome larvae, which penetrate the skin. Most often, daily activities such as bathing and swimming, or drawing water for domestic chores, places people of all ages at risk. Around Lake Malawi, adult men engaged in fishing are an especially well-known high-risk group, and having often had insufficient praziquantel treatment, have progressive disease.

In Africa, *Schistosoma haematobium* and *Schistosoma mansoni* are responsible for the two forms of schistosomiasis urogenital or intestinal, respectively. Accurate diagnosis of schistosomiasis in all its stages and forms remains a challenge despite scientific advances in general recognition of NTDs as a global public health concern. Several diagnostic methods for detection of urogenital schistosomiasis (UGS) have been developed over the years but we still do not have a reliable rapid point-of-care (POC) test. This is in contrast with intestinal schistosomiasis where a urine-based point of care circulating cathodic antigen (POC-CCA) strip assay test is commercially available and also advocated within WHO guidelines for disease mapping.

Although more efforts have been put into raising awareness in general, the treatment, control and prevention of schistosomiasis, and specific genital complications arising especially in male genital schistosomiasis (MGS) have been overlooked and underreported for decades. This perceived lack of interest has resulted in under-diagnosis, non-treatment and poor awareness in endemic areas; MGS is a gender-specific manifestation of schistosomiasis, associated with schistosome eggs and pathologies in genital fluids and organs most commonly observed with *S. haematobium* infection. This complication, first described in 1911 by Professor Madden in Egypt, causes genital or ejaculatory pain, abnormal ejaculates, infertility, enlarged organs, and tissue abnormalities observed on diagnostic examinations. Perhaps as the largest chronic parasitic public health burden in Africa, there is also a direct connection with HIV transmission as schistosome eggs in the genital tracts often are associated with raised viral loads in semen.



***S. haematobium* egg (x100) in semen**

Unlike the recent advances in defining a clinical standard protocol for female genital schistosomiasis (FGS), MGS remains inadequately defined as there is no 'gold-standard' diagnostic test. Semen microscopy remains the recommended test for MGS, although its acceptability and applicability in endemic areas with limited laboratory capacity poses a significant challenge in the

management of MGS. Urine filtration with microscopic examination for *S. haematobium* eggs has been utilised in such settings as a convenient but unfortunately error-prone proxy of MGS.

Through a recent longitudinal cohort study conducted among fishermen along the south shoreline of Lake Malawi in Mangochi district, my work described a novel low-cost sampling and direct visualisation method for enumeration of ova in semen, which helped to diagnose MGS, showing a prevalence of MGS was 10% using seminal microscopy and 27% on seminal real-time polymerase chain reaction (PCR), with UGS prevalence of 17%. With such diagnostic challenges regarding MGS highlighted, there is need to improve diagnostic tests as well as raise adequate professional awareness for comprehensive clinical assessment, treatment and inclusion of men in preventive chemotherapy programs like mass drug administration (MDA) campaigns with PZQ. I hope my paper on diagnostics draws attention to the importance of increased research on MGS diagnostics, and that an intricate knowledge on circulating schistosome glycans has application in better disease control.

Appendix 29: Research output - Invited to contribute through BMC BugBitten blog

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About this blog FOLLOW

Time to highlight the importance of Male Genital Schistosomiasis in Malawi

Medical doctor and PhD student at the Liverpool School of Tropical Medicine, Dr. Sekeleghe Kayuni describes his work in Malawi on Male Genital Schistosomiasis. He has recently published a systematic review on the topic and is conducting a field study looking at MGS in fishermen near lake Malawi.

Dr. Sekeleghe Kayuni 25 Jan 2019

Dr Seke carrying out an ultrasonography relieving a small hydrocele

As a medical doctor working in my home country of Malawi, I have seen the detrimental impact of many Neglected Tropical Diseases (NTDs) in our hospitals as well as on-the-ground in field-based clinics. NTDs comprise of 23 principally communicable diseases, prevalent in low- and middle-income countries, typically within tropical and sub-tropical environments. Over 1 billion people are affected by at least one NTD, and collectively these NTDs contribute to a substantial morbidity and disability burden globally, entrapping many in poverty.

Dr. Sekeleghe Kayuni

Dr. Sekeleghe Kayuni is a medical doctor working with MASM Medi Clinics Limited in Blantyre City, Malawi and currently a Commonwealth PhD Scholar studying at the Liverpool School of Tropical Medicine and University of Liverpool. His twitter account is @sekekayuni.

BugBitten: A blog for the parasites and vectors community

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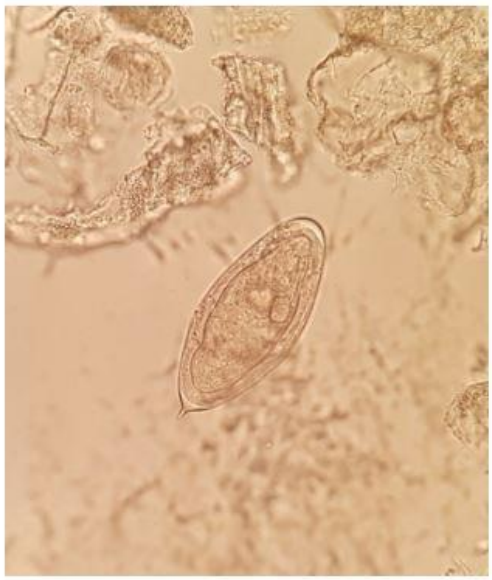
My PhD focus is on the epidemiology and pathology of schistosomiasis, specifically Male Genital Schistosomiasis (MGS) in fishermen along the shoreline of Lake Malawi. I have recently published a systematic literature review on the clinico-pathological features associated with MGS, highlighting a possible connection of the disease with HIV infection (also presented at 2018 International Congress of Parasitology). Our major findings were that 1) current diagnostics for epidemiological surveillance of MGS need to be improved; 2) infections are under reported and the burden is underestimated; and 3) HIV co-infection needs to be considered when optimising individual case management and reviewing Praziquantel dosing regimens.

Background



Schistosoma haematobium egg in semen (magnification x100)

Globally over 200 million people suffer from schistosomiasis every year, with over 90% of infections occurring in sub-Saharan Africa (SSA). As described in previous Bugbitten blogs, this snail-borne disease is predominantly caused by two species of schistosome flukes; *Schistosoma haematobium* and *Schistosoma mansoni*, responsible for urogenital or intestinal disease respectively. The infection is contracted when people come into contact with contaminated waters, during routine household, recreational, agricultural and income-generating activities in their communities. Despite efforts, accurate diagnosis of these blood vessel-dwelling flukes, the various species and the different life-cycle stages- remains a challenge.

MGS, described as the presence of schistosome eggs in semen or associated pathologies from entrapped eggs in male genital organs, causes various symptoms including spontaneous pelvic or ejaculatory pain, haemospermia, ejaculate irregularities, erectile dysfunction, infertility, enlarged genital organs, as well as histopathological and radiological abnormalities. Despite these horrific symptoms, MGS awareness is scarce even in endemic areas and many clinicians overlook the genital-associated complications of schistosomiasis, thereby affecting downstream diagnosis and treatment.

1 Parasitic and fungal infections play a significant role in porpoise strandings

2 World's largest rodent and its role in the emergence of Brazilian spotted fever

3 Achieving and sustaining elimination: How the Geshiyaro Project might shape the future of schistosomiasis and soil-transmitted helminths programmes

4 Mangy Mites Mess up the Mammalian Microbiome

ARCHIVES

Fieldwork in Malawi

As part of my PhD, I set out to investigate the prevalence and morbidity of MGS in an endemic area. We set up a longitudinal cohort study among fishermen on the shoreline of Lake Malawi, Mangochi District from November 2017 to December 2018.

Due to the challenging nature of collecting semen samples from participants, the first challenge was defining our methodologies for ethical review. After much discussion, an effective communication plan and pre-study sensitisation strategy was finalised in the hope that compliance would be as high as possible. 376 fishermen were recruited and interviewed using a standardised questionnaire. Mid-morning urine, semen and blood samples were collected for both field diagnosis of *S. haematobium* and further molecular analyses at the Liverpool School of Tropical Medicine, United Kingdom. Transabdominal and scrotal ultrasonography were also employed to investigate any genital pathologies. Subsequent praziquantel therapy was offered to participants, along with an invitation to follow-up studies at 1, 3, 6 and 12 months.

Seke interacting with fishermen in the MGS study, Malawi

Preliminary results

Of the 210 participants who submitted urine at baseline, 17.1% had *S. haematobium* egg-patent infections. Astonishingly, the presence of schistosome eggs in semen samples was confirmed in 10.7% of the 112 participants who provided semen samples. Of those, one third also had eggs in their urine. A fifth of the participants who underwent transabdominal and scrotal ultrasonography, had pathologies in the prostrate, seminal vesicles and testes. At the 1-month follow-up after praziquantel therapy, prevalence of urine egg-patent infection reduced to 11.1%, and no eggs were seen in the submitted

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semen. Further molecular analyses are underway to further investigate MGS characteristics of these samples. Excitingly, the full results will be presented at the Spring Meeting of the British Society for Parasitology and published soon after.

Given the disease has been overlooked for so long, I was surprised by these initial results- especially the high prevalence of MGS amongst the fishermen and other people exposed to the infectious water bodies in Malawi! Encouragingly, these preliminary results indicate that the current treatment can clear the MGS infections. Unfortunately, in many areas of Sub-Saharan Africa where access to diagnostics and treatment is limited, urogenital schistosomiasis (caused by *S. haematobium*) is endemic and millions of men suffer unduly from MGS. I would like to see that my research efforts are playing an influential role in raising greater awareness and surveillance as well as positively influencing better case management of men with MGS.

Topics:

- Biology
- Developing World
- Health
- Medicine

Tags:

fishermen malawi Male Genital Schistomsomiasis Praziquantel schistosomiasis

Appendix 30: Research output – Field report on the BSP online blog (2018)

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Home > Awards & Grants > International Training and Fieldwork Award

International Training & Fieldwork Award

This award aims to give parasitologists at an early stage in their careers (PhD students or recent graduates) the opportunity to undertake international fieldwork or to visit overseas institutions.

Applications are now being accepted. Up until 2015, IFTAs were assessed via two annual deadlines in Feburary and August but to increase the flexibility of these awards, we are now accepting applications on a 'rolling' basis for assessment at each meeting of the BSP council.

Several International awards will be available each year, but Council will only make an award when an application of great merit is received.

The intention of the Award scheme is to broaden the horizons of laboratory-based workers, by providing funds to allow travel in pursuit of their academic interests in parasitology, especially those who would not necessarily otherwise be able to gain international field and/or laboratory experience/tuition. Applications consistent with this intention are particularly encouraged.

Applicants should be PhD students or should have recently (within the last 2 years) completed their PhD. Applicants should be BSP members at the time of application (awardees must have been a member of the society for at least 6 months at the time of application). The Society will provide support of up to £1,500 which should cover the costs of travel and subsistence for not less than two weeks. Successful applicants will be expected to provide a report on their trip/visit for publication on the BSP website and that the support is acknowledged in any publications derived from the field work?.

Some previous reports are found here: Sabrina Lamour, Ciaran McCoy, Tapan Bhattacharyya, Rebecca Jones, Melanie Clerc, Luis Hernandez and Seke Kayuni.

Applications for The International award should be sent to the Hon. Treasurer by the given deadlines. Council will consider applications at the next appropriate meeting and applicants will be notified of the outcome within two weeks of the council meeting.

Applicants should include:

1. Completion of the relevant application form: download form
2. A CV of the applicant (on a single A4 page)

The applicant will also need to demonstrate that all appropriate formalities (e.g. Government clearance, health certificates) can be completed to allow the visit to go ahead. If clinical activities are planned ethical clearance must be obtained before making the application.

Disclaimer: The Award is made on the express condition that, save only as may be required by applicable law, the Society is not under any legal liability whatsoever that may arise from act or omission by the recipient or any third party.

BSP Travel Award 2017 recipient: Sekeleghe Kayuni

(Commonwealth PhD Scholar, Liverpool School of Tropical Medicine & University of Liverpool, UK).

A field study report from Mangochi in Malawi, funded by a BSP International Training and Fieldwork Award.

Discovering Male Genital Schistosomiasis, an ignored neglected tropical disease manifestation on the shorelines of Lake Malawi

Countries such as Malawi in sub-Saharan Africa, possess some of the world's most beautiful natural environments which attract global tourists and local peoples to visit them for pleasure, recreation, sports and nurture. Among such mountains, forests and historic sites, Lake Malawi (Figure 1) stands out as one of the most beautiful freshwater bodies in Malawi, the third largest in Africa, and ninth largest in the world. This African Great Lake flows along the East African Rift valley, across the length of the country Malawi, and is a natural habitat to more fish species than any other lake in the world. In addition to tourism, hydro-electricity generation, agriculture and aquaculture are key contributing economic factors supported by Lake Malawi, as well as serving as a source of water for daily household chores and subsistence income production for local inhabitants in shoreline communities.

However, the lake is known to harbour intermediate host molluscs of the *Schistosoma spp.* parasites, the causative agents of schistosomiasis, a neglected tropical disease (NTD), common in low and middle-income countries affecting over 200 million people with significant morbidity. In sub-Saharan Africa, this snail-borne helminthic disease is predominantly caused by two species of blood vessel-dwelling schistosomes; *Schistosoma haematobium* or *S. mansoni*, responsible for urogenital and intestinal diseases, respectively.

Figure 1: Lake Malawi shoreline showing local inhabitants conducting their daily routine activities involving water contact where risks of infection by *Schistosoma haematobium* are significant.

One of the specific manifestations of urogenital schistosomiasis is Male Genital Schistosomiasis (MGS), associated with various pathologies of the genital organs caused by entrapment of schistosome eggs. Since the first report of MGS in 1911, several studies have described symptoms (pelvic and ejaculatory pain, haemospermia, erection dysfunction, infertility, enlarged genital organs), histopathological and radiological findings associated with MGS. However, this male-specific manifestation of schistosomiasis remains under-diagnosed, under-treated and under-reported, thought to be due in part to the tendency of males to present at clinics less often to receive diagnoses and treatment, and in the difficulties in engaging males in standard community treatment programmes.

To further investigate the prevalence and morbidity of MGS, I conducted a longitudinal cohort study among fishermen along the shoreline of Lake Malawi (Figure 2), Mangochi District in November 2017 to June 2018, with ethical clearance from Liverpool School of Tropical Medicine Research Ethics

Committee (LSTM REC) and National Health Sciences Research Committee of Malawi (NHSRC). A total of 376 fishermen were recruited into the study, interviewed by questionnaire and asked to provide mid-morning urine and semen samples for parasitological diagnosis of egg-patent *S. haematobium* infections by microscopy.

Figure 2: Dr Seke Kayuni interacting with fishermen who came attended for the male genital schistosomiasis (MGS) a follow-up study in Mangochi district, Malawi.

A novel method was developed for examining semen for schistosome eggs, where ejaculate was collected into a transparent, self-sealing plastic bag; heat-sealed after liquefication, and thereafter examined directly under microscope (Figure 3). Afterwards, semen volume was measured, then centrifuged and wet mounts of sediments had microscopical examination. The supernatant harvested together with plasma collected were stored frozen at -80°c and shipped for further molecular analyses. Transabdominal and scrotal ultrasonography (Figure 4) were also conducted to

investigate presence of genital pathologies. Thereafter praziquantel treatment was offered to participants along with invite to follow-up studies.

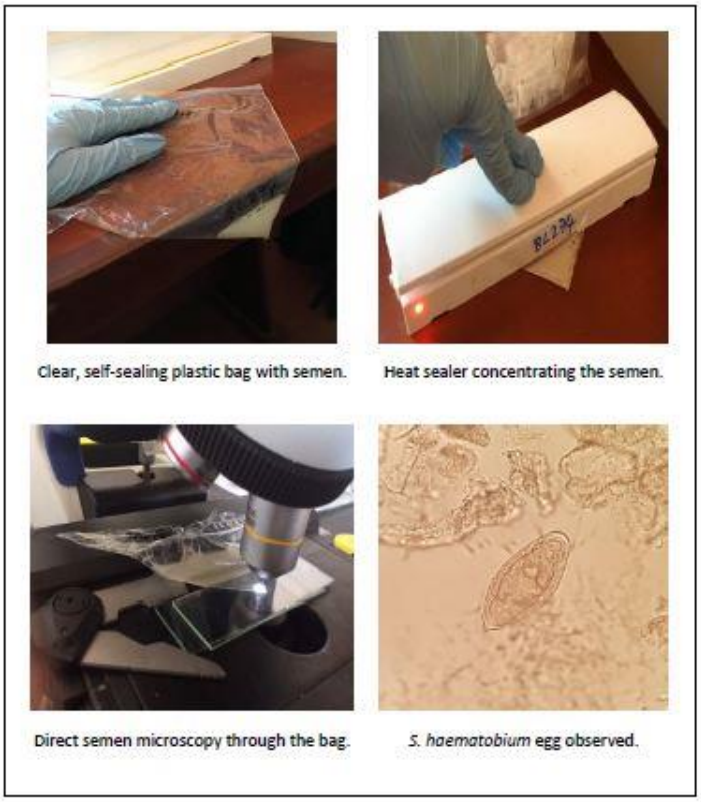

Figure 3: A novel, low-cost clear plastic bag method for semen examination of schistosome eggs.

The prevalence of urine egg-patent infection was 17.1% (n = 210) while MGS, defined by schistosome eggs in semen, was confirmed in 10.7% of the participants (n = 112) who submitted semen, with two-thirds having no eggs in urine. A fifth of the participants who underwent transabdominal and scrotal ultrasonography, had genital pathologies in their prostrates, seminal vesicles and testes. At 1-month follow-up study after praziquantel therapy, prevalence of urine egg-patent infection reduced to 11.1%, and no eggs were seen in the submitted semen. Further

molecular analyses are being conducted to describe parameters of MGS and the full results will be widely disseminated at the end of the study.

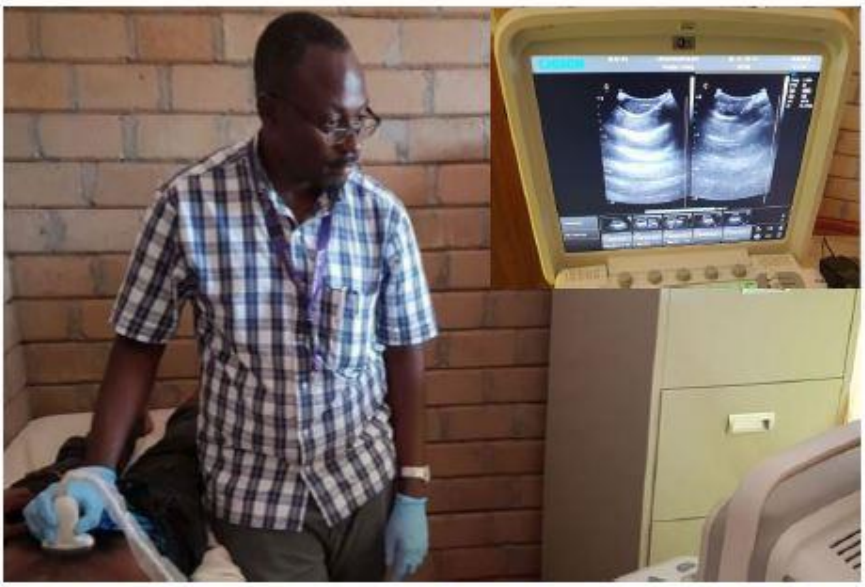

Figure 4: Dr Seke Kayuni performing transabdominal and scrotal ultrasonography on the genital organs of a study participant, insert showing a small hydrocele observed.

In summary, this initial study shows MGS is prevalent among fishermen along Lake Malawi, an area known to be endemic for schistosomiasis, and that the current treatment can clear the infection. These initial findings are encouraging and raise the need for availability and accessibility to appropriate diagnostics and treatment for all people in endemic areas, together with other control interventions.

I wish to thank and acknowledge the British Society for Parasitology (BSP) for the ITFA award which greatly assisted in conducting these essential follow-up studies of this unique longitudinal cohort research of MGS. I would also like to thank my supervisors – Professor J.R. Stothard and Dr. E.J. LaCourse, international and local collaborators, Programme Manager of National Schistosomiasis and STH Control in the Malawi Ministry of Health, the District Health Management Team (DHMT) of

Mangochi district, all Health centres' staff and community health workers, Chiefs, fishing committee members and fishermen who participated in the study for their overwhelming support, advice and assistance kindly given in making this important research successful.

Appendix 31: Research output – Description of the planned fieldwork studies through COUNTDOWN online blog (January 2017)

COUNTDOWN
Calling time on Neglected Tropical Diseases

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What about Male Genital Schistosomiasis: Developing a Research Perspective from the Shoreline of Lake Malawi

Guest Blog by *Dr Sekeleghe Kayuni (Liverpool School of Tropical Medicine PhD student)*

Prof. Sally Theobald has used her personal experience to highlight the importance of reporting female genital schistosom long term consequences, from Lake Malawi. What about male genital schistosomiasis (MGS) and its current situation wit fishermen?

Southern shore of Lake Malawi, inhabited by a dangerous parasite *Schistosome* causing MGS, the forgotten of a Neglected tropical disease (NTD) *(Photo courtesy of Dr Seke Kayuni).*

Described as "the jewel in the crown of the country's tourist attractions", this third largest lake in Africa is a renowned dest and water sport supporting the country's economy. Its beautiful fresh waters also offer local communities a place for hous income generation through fishing. However, the lake harbours various aquatic snail species which are intermediate host the *Schistosoma* parasite, a causative agent of schistosomiasis in humans.

Schistosomiasis is also known as Bilharzia or snail fever and its lifecycle was elucidated some a hundred years ago. Chr schistosomiasis is a debilitating disease causing severe damage to many internal organs and tissues and if untreated car As the second-most prevalent parasitic disease in the world after malaria, World Health Organisation (WHO) estimates th people are at risk of the disease globally, with 200 million infected of which 85% live in sub-Saharan Africa (SSA), and 20 severe consequences.

In Lake Malawi and surrounding water bodies, two major species of the *Schistosoma* parasite are present; *S. haematobiu* urogenital disease and *S. mansoni* which causes intestinal diseases. The parasite's distribution and thus risk of infection around these water bodies, with *S. haematobium* common in the southern areas of Lake Malawi and Shire valley, and *S.* Central plains and Northern areas.

Part of my future PhD research based at the Liverpool School of Tropical Medicine (LSTM); is to determine the local impc visited Malawi in December 2016 to start planning for my fieldwork later this year and appreciate the current situation of c within fishing communities along southern shores of Lake Malawi, especially within Mangochi district. These communities with higher prevalence of *S. haematobium* infection, average of 23.7%, some reaching as much as 94% and also having infection.

Local health services are provided by the District Health Office (DHO) and complemented by Faith-based and private clini have basic Outpatient departments, laboratories and dispensaries, with limited diagnostic resources resulting in limited cli

diseases like schistosomiasis. Preventive services are community-directed and provided by Health Surveillance Assistant a valuable multi-tasking human resource for health, supervised by the District Environmental Health Officer (DEHO).

Much emphasis of urogenital schistosomiasis in endemic areas is on urinary pathology, with less attention on its genital c despite its first description by Madden in vaginal tissue of a female Egyptian woman in 1899 and a young man's spermat This was echoed by health centre staff and people in fishing communities in the district.

FGS is now receiving at least some much-deserved focus in research and treatment, compared to MGS. This is despite tl and research studies describing *Schistosoma* eggs in male genital organs, its impact on reproductive health and possibili susceptibility to HIV infection, and transmission among infected males and females in schistosomiasis-endemic regions.

Dr Seke Kayuni discussed MGS with these Health Surveillance Assistants working in fishing communities of Mangochi district *(Photo courtesy of Dr Seke Kayuni)*

In preparation for my future PhD research with Professor Russell Stothard and Dr James LaCourse, I will be hoping to bri multidisciplinary study to determine and describe the prevalence and morbidity of MGS in the fishing communities of sout including assessing the co-morbidity of MGS with HIV infection. The aim is to raise the much-needed awareness and und MGS, expand access to regular treatment and holistic disease control interventions, by national control programmes to er So watch this space for further reports in future.

Appendix 32: Research output – Invited session speaker at the ECTMIH 2019

10:00 – 11:30 **Room 1A**

Track 2: Treatment/Patient Care **527**

Organised Session:
Schistosomiasis control through the ages.

Organiser:
Global Schistosomiasis Alliance (GSA)

Chairs:
Stothard J.R. (UK) and Reinhard-Rupp J. (Switzerland)

10:00 – 10:15
Paediatric praziquantel: Randomised clinical trials in Côte d'Ivoire.
N'Goran E.K (Cote d'Ivoire) on behalf of the Pediatric Praziquantel Consortium

10:15 – 10:30
Innovative approaches to schistosome control in pre-school age children: Applying lessons from immunology
Osakunor D.N.M. (UK)

10:30 – 10:45
Possible mechanisms of HIV susceptibility in reproductive-aged women with schistosome infections
Downs J.A. (USA & Tanzania)

10:45 – 11:00
The contribution of pre-school-aged children and adults to Schistosoma mansoni transmission in high endemic communities
Faust C.L. (UK)

11:00 – 11:15
Pathologies associated with male genital schistosomiasis and efficacy of praziquantel treatment in Malawian fishermen
Kayuni S.A. (UK & Malawi)

11:15 – 11:30
Discussion

10:00 – 11:30 **Room 1B**

Track 1: Prevention **530**

Organised Session:
Next Generation Nets.

Chair:
Janet Hemingway (UK)

10:00 – 10:15
Second generation LLIN to control resistant mosquitoes: Interceptor G2.
Susanne Stutz, Germany

10:15 – 10:30
Evaluation of next generation of insecticide treated nets: The Tanzanian experiences.
Jacklin Mosha, Tanzania

10:30 – 10:45
The impact of PBO LLINs in area of high transmission and resistance: An operationally embedded RCT in Uganda.
Amy Lynd, UK

10:45 – 11:00
Critical decision making and fighting insecticide resistance in malaria prevention.
Sherratt P. (UK)

11:00 – 11:15
Indoor residual spraying for malaria control: Past, present and ….does it have a future?
Invest J. (UK)

11:15 – 11:30
Discussion

Methods: A cross-sectional study design was used to collect samples from 209 calves, 269 lambs, 258 goat kids, 221 children and adults, 55 vegetables and 37 water samples that were analysed for occurrence of *Cryptosporidium* oocysts and *Giardia* cysts, with subsequent genotyping of positive samples. Questionnaires were also used to collect information on possible risk factors for infection from 221 animal owners and 41 non-animal owners.
Results: Laboratory analysis has started but not yet finalized. We would like to present preliminary data on the occurrence of infection with these two parasites among domestic animals and their owners, and also people who do not own animals, in this region.
Conclusion: Although diagnosis and treatment remain important touch-stones in intestinal parasitic infections, prevention is equally important. A One Health approach can be used to provide indicators of the most important sources and routes of infection.

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PATHOLOGIES ASSOCIATED WITH MALE GENITAL SCHISTOSOMIASIS AND EFFICACY OF PRAZIQUANTEL TREATMENT IN MALAWIAN FISHERMEN

Kayuni S.A.[1,2], Makaula P.[3], Lampiao F.[4], Juziwelo L.[5], LaCourse E.J.[1], Stothard J.R.[1]
[1]*Dept. of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool, UK;*
[2]*MASM Medi Clinics Limited, Medical Society of Malawi (MASM), Blantyre, Malawi;*
[3]*Research for Health, Environment and Development (RHED), Mangochi, Malawi;*
[4]*Physiology Dept., College of Medicine, University of Malawi, Blantyre, Malawi;*
[5]*National Schistosomiasis and STH Control Programme, Community Health Sciences Unit, Ministry of Health, Lilongwe, Malawi*

Introduction: Urogenital schistosomiasis (UGS), an important disease caused by the blood fluke *Schistosoma haematobium*, is endemic along the shoreline of Lake Malawi. Male genital schistosomiasis (MGS), an under-reported manifestation of UGS, is defined by the presence of schistosome eggs within seminal fluids, genitalia and associated pathologies[1]. In a cohort study among fishermen along southern lakeshores of Malawi's Mangochi District, the baseline prevalence of UGS was 17.1% (eggs in urine) while for MGS (eggs in semen) was 10.7%. There is need to assess whether recommended praziquantel treatment for UGS also clears eggs in semen and averts MGS pathologies observable by ultrasonography.
Aim: To investigate the morbidity associated with MGS as observed by ultrasonography, and the efficacy of praziquantel treatment in clearing egg-patent infection in semen.
Methods: Participants (fishermen) recruited in the cohort MGS study and treated with praziquantel at baseline, were invited to follow-up studies after 1-, 3-, 6- and 12-months. Mid-morning urine and semen were examined for parasitological diagnosis of schistosome ova. Transabdominal and scrotal ultrasonography were conducted on the participants to investigate for genital pathologies. Praziquantel treatment at 40 mg / kg was offered to all the participants.
Results: At 1-month follow-up study, prevalence of UGS reduced to 11.1%, with mean egg count of 13.5 per 10 ml whilst that of MGS was 0% (no eggs seen in semen). At 3-months follow-up, UGS prevalence rose to 11.7%, with higher mean egg count of 15.6 per 10 ml and MGS prevalence was 8.9%, mean of 3.6 eggs per ml. Follow-up at 6-months, revealed UGS prevalence was reduced further to 4.3%, mean egg count of 0.4 per 10 ml and MGS was 4.8% and mean of 0.9 eggs per ml. MGS pathologies was detected in 20% of the participants, which were seen to reduce on follow-up studies.
Conclusion: MGS is prevalent among fishermen along Lake Malawi, known to be endemic for UGS, and is associated with pathologies observed by ultrasonography. The current treatment of choice, praziquantel, can clear the infection which raises the need for wider availability and accessibility to diagnostics and treatment for all people in endemic areas, together with other complementary control interventions.

Reference:
1. Kayuni, S., Lampiao, F., Makaula, P., Juziwelo, L., Lacourse, E.J., Reinhard-Rupp, J., Leutscher, P.D.C. and Stothard, J.R. '**A systematic review with epidemiological update of Male Genital Schistosomiasis (MGS): A call for integrated case management across the health system in sub-Saharan Africa**', *Parasite Epidemiology and Control*, 2019; 4; doi: 10.1016/j.parepi.2018.e00077

070

EVALUATION OF NEXT GENERATION OF INSECTICIDE TREATED NETS: THE TANZANIAN EXPERIENCES

Mosha J.F.[1], Lukole E.[1], Mosha F.W.[2], Manjurano A.[1], Martin J.[2], Kulkarni M.[3], Mwalimu C.D.[4], Rowland M.[5], Protopopoff N.[5]
[1]*National Institute of Medical Research, Mwanza, Tanzania;*
[2]*Kilimanjaro Christian Medical University college, Moshi, Tanzania;*
[3]*University of Ottawa, Ottawa, Canada;*
[4]*Ministry of Health Community Development Gender Elderly and Children, National Malaria Control Program, Dar es Salaam, Tanzania;*
[5]*London School of Hygiene and Tropical Medicine, London, UK*

Insecticide Treated Nets (ITNs) are the primary method of malaria control in Sub-Sahara Africa. New types of mosquito nets, that combine a pyrethroid and either a second insecticide or a synergist, have been developed to respond to the expanding threat of insecticide resistance in malaria vectors. The Pan African Malaria Vector control research Consortium (PAMVERC), a research partnership between LSHTM, NIMR and KCMUCO, is supporting the development and evaluation of several of these new vector control products from laboratory assessment to large scale community trials. Recently we completed a cluster randomized controlled trial (RCT) comparing an ITN combining a pyrethroid and the synergist piperonyl butoxide (PBO) to a standard pyrethroid Long Lasting Insecticidal Net (LLIN), in an area of insecticide resistance in Tanzania, The study showed a reduction in malaria prevalence of 44%, 33% and 17% after one, two and three years of use in the intervention compared to the control arm. This finding led to a policy recommendation by WHO and the deployment of PBO-ITN in areas with pyrethroid resistance in Sub-Sahara African countries. Following WHO requirements on generating epidemiological evidence for novel vector control product class, we are conducting a new RCT to evaluate the efficacy of two dual ITNs combining pyrethroid with a second adulticide chlorfenapyr or with a hormone growth regulator; pyriproxyfen, conferring sterility to the mosquitoes.

071

CRITICAL DECISION MAKING AND FIGHTING INSECTICIDE RESISTANCE IN MALARIA PREVENTION

Sherratt P.
Against Malaria Foundation, UK

Global malaria rates are no longer falling. Strong evidence has been building over the last 15 years of increasing resistance to the three primary pyrethroids used in nets.

11TH EUROPEAN CONGRESS ON TROPICAL MEDICINE AND INTERNATIONAL HEALTH

DR. SEKELEGHE KAYUNI
CTID BUILDING, 3RD FLOOR,
PEMBROKE PLACE
L3 5QA LIVERPOOL
UNITED KINGDOM

CERTIFICATE OF ATTENDANCE

We hereby certify the participation of

SEKELEGHE KAYUNI

in the European Congress on Tropical Medicine and International Health on September 16 until September 20, 2019 in Liverpool, United Kingdom.

20/09/2019, Liverpool

Tamar Ghosh
CEO RSTMH and Director
ECTMIH 2019

Appendix 33: Research output – Poster presentation of study supported by SGP II award

Seke Kayuni

From: ARNTD Secretariat <secretariat@arntd.org>
Sent: 24 October 2018 10:37
To: Seke Kayuni
Subject: Re: *URGENT RESPONSE REQUIRED* You have been shortlisted for the ARNTD SGP II grant award

Dear SGP II Applicant,

The ARNTD Secretariat is pleased to inform you that following multiple reviews of your application, you have been shortlisted as a junior awardee for the ARNTD Small Grants Program II scheme funded by the USAID and DFID through the COR-NTD. The grant award amount that stands to be approved for your proposed project is USD 14,936.00.

In order to proceed with the process of confirming you as an awardee, you are required to respond to this message with a signed formal acceptance letter (from you), as well as a letter from the head of your institution/department/college/school (whichever is most appropriate in your setting) indicating institutional support towards hosting your work. Both letters are expected to be on your institutional letter head. Once we receive these letters, a formal announcement of the award will be made, and we shall commence administrative processes to secure further documentation from you, culminating in the signing of a contact between you (together with your institution) and the ARNTD which will mark the formal grant award.

We sincerely apologize, but owing to circumstances beyond our control resulting in very tight timelines, you are requested to respond to this email with the requisite letters at the very latest by Thursday October 25, 17:00 GMT. Failure to do so may result in the offer being withdrawn.

Congratulations once more, we look forward to receiving your letters and to engaging further with you.

Yours sincerely,

John H. Amuasi (MBChB. MPH. MS. PhD)
Executive Director, African Research Network for Neglected Tropical Diseases
--

The Secretariat

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1

Assessment of Male Genital Schistosomiasis (MGS) and co-infection with Human Immunodeficiency Virus (HIV-1) among men in fishing communities on the southern shoreline of Lake Malawi, Mangochi District, Malawi.

Kayuni S.A.N.[1, 2,3], Abdullahi A.[3], Makaula P.[4], Lampiao F.[5], Juziwelo L.[6], LaCourse E.J.[1], Geretti A.M.[3], Stothard J.R.[1]

Liverpool School of Tropical Medicine, UK [1]; MASM Medi Clinics Ltd, Malawi [2]; University of Liverpool, U.K. [3]; RHED, Malawi [4], Malawi College of Medicine, Malawi [5], NSCP, Ministry of Health, Malawi [6]

Introduction

Background

- Schistosomiasis is prevalent in sub-Saharan Africa (SSA), MGS, its gender consequence, is associated with eggs and pathologies in genital fluids and tissues [1].

Daily activities on the shores of Lake Malawi, with fishermen increasing their risk of MGS

- Increased seminal inflammatory markers with MGS, could facilitate HIV acquisition and transmission [2].
- Reduction in seminal viral load (VL) of men with HIV and urogenital schistosomiasis (UGS) 10 weeks after praziquantel (PZQ) treatment [3].
- HIV epidemic in SSA coincidentally overlaps with schistosomiasis, PZQ could be helpful with HIV control [4].
- Malawi endemic for schistosomiasis, with a generalised HIV epidemic, 10.6% adult prevalence.

Objective

- Determine the increased risk of HIV-1 transmission in men with schistosomiasis

Specific objective:

- Assess the seminal and plasma VL in men with HIV and UGS/MGS (study cases), compared to those with HIV only (control).

Awareness and sensitisation meeting with Study participants

Methods

Study setup

- A longitudinal cohort study conducted from Nov 2017 to Dec 2018,baseline UGS prevalence: 17.1%, MGS:: 10.4% (microscopy) and 26.6% (real-time PCR)

Study population and data collection

- Sample size: 20 study cases and 20 study control.
- Submitted semen and blood at baseline, 1-, 3-, 6- and 12-months' time-points, centrifuged in the field, harvesting plasma and shipping at -80°c to LSTM.

Xpert® HIV-1 Viral Load

Validation of the Cepheid HIV-1 assays [5]

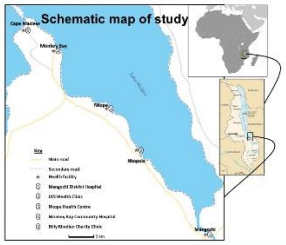
- HIV negative semen without UGS/MGS were spiked with 3rd WHO reference standard for HIV-1 RNA (NIBSC 10/152) for use of commercially available Cepheid HIV-1 assay.

Quantification of HIV-1 in blood and semen

- HIV-1 RNA detected in blood and seminal plasma using Cepheid Xpert HIV-1 VL assay, range of 40 - 10^7 copies / ml.

Ethical clearance

- NHSRC of Malawi and LSTM Research Ethics Committee (LSTM REC) of U.K.



Results

Key findings

- Only 33/48 recruited gave paired blood and semen samples.
- 15/16 with UGS had MGS (study cases) and 17 had no UGS/MGS (controls, with one starting ART during study).
- Mean age of cases was 42.7 years (S.D.:10.4, C.I.:37.2-48.2) while for controls was 43.5 years (S.D.:12.6, C.I.:37.1-50.0), with no statistical difference (t (31) = 0.21, p = 0.84).
- Median time since HIV diagnosis was 91.0 months (IQR: 133.3, range:2.1-175.8, n = 13 (cases)) and 105.4 months (IQR: 91.2, range:18.0-153.8, n = 11 (controls)).
- ART median duration for cases was 58.6 months (IQR: 125.1, range:1.7-149.6, n = 14); and 80.5 months for controls (IQR: 110.6, range: 1.0-148.2, n = 12), U = 68.0, p = 0.41.

Viral load (VL) status	Cases	Control
Total participants	16	16
Undetectable VL throughout the study time-points	11 (68.75%)	12 (75.0%)
Undetectable VL at baseline but detectable VL at the follow-up time-points	0 (0.0%)	1 (6.25%)
Detectable VL at baseline but undetectable VL at follow-up time-points	1 (6.25%)	0 (0.0%)
Detectable VL throughout the study time-points	3 (18.75%)	1 (6.25%)
Blips (slight detectable VL at single time-point)	1 (6.25%)	2 (12.5%)

Study cases (samples from 16 participants)

- 5 had detectable VL in blood, 1 in semen at least 1 time-point

Blood plasma

- Undetectable (<22 cps/ml) = 27; detectable (22-39 cps/ml) = 9; detectable, quantifiable = 10

Seminal plasma

- Undetectable (<55 cps/ml) = 38, undetectable (<110 cps/ml) = 3, undetectable (<220 cps/ml) = 0; detectable (55-100 cps/ml) = 3; detectable (220-400 cps/ml) = 1; detectable, quantifiable = 1.

Study controls (samples from 16 participants)

- 3 had detectable VL in blood, only 2 in semen at 1 time-point

Blood plasma

- Undetectable (<22 cps/ml) = 21; detectable (22-39 cps/ml) = 2; detectable, quantifiable = 3

Seminal plasma

- Undetectable (<55 cps/ml) = 21; undetectable (<110 cps/ml) = 3; undetectable (<220 cps/ml) = 4; detectable (55-100 cps/ml) = 0; detectable (220-400 cps/ml) = 0; detectable, quantifiable = 2

Results

Patient ID	Age (years)	Time point	HIV-1 RNA levels (copies/mL) Blood plasma[a]	Seminal plasma[b]	ART Regimen	Time on ART (years) at entry	Time on follow-up (months)
A	49	T1	Undetected	Detected btw 220-400	TDF+3TC+EFV	8.0	[c]
B	47	T1	Undetected	Detected btw 55-100	TDF+3TC+EFV	12.0	[c]
		T2	Undetected	Undetected <55			2.0
		T3	Undetected	Undetected <55			4.0
		T4	Undetected	Undetected <55			14.2
C	43	T1	Undetected	Undetected <55	TDF+3TC+EFV	11.9	[c]
		T2	Undetected	Detected btw 55-100			2.0
		T3	Undetected	123			4.0
		T4	Undetected	Detected btw 55-100			7.3
		T5	Undetected	Undetected			14.2
D	52	T1	48800	17500	TDF+3TC+EFV	N/D	[c]
		T2	Undetected	1293			4.0
		T3	Undetected	Detected btw 55-100			7.9
		T4	Undetected	Undetected			14.5

[a] Detection threshold of HIV in plasma was 22 copies/mL. [b] Detection threshold of HIV-1 varied based on the dilution factor which was subject to sample availability; [c] Only available timepoint ; [d] timepoint of entry into study. ART=antiretroviral therapy; TDF=tenofovir disoproxil fumarate; 3TC=lamivudine; EFV=efavirenz; N/D=not documented.

Conclusion

- MGS is as prevalent as UGS in endemic areas of Malawi
- Undetectable HIV RNA in both blood and seminal fluids.
- Detectable VL was observed more in men with UGS/MGS, more than those without.
- Other factors may drive intermittent viral shedding in semen despite undetectable HIV RNA in plasma

Future Directions

- Further studies to understand the infectiousness and transmissibility of HIV from semen in plasma suppression.
- Prospective studies with larger cohort with MGS at ART initiation and during longer follow-up, suppressed ART, to understand effect of MGS and PZQ on HIV progression.

References

1. Kayuni, S., *et al.* (2019). "A systematic review with epidemiological update of male genital schistosomiasis (MGS): A call for integrated case management across the health system in sub-Saharan Africa." PE&C. 4: e00077.
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This research was supported by the African Researchers' Small Grants Program, which is funded by USAID and UK aid via the Coalition for Operational Research on Neglected Tropical Diseases (COR-NTD) and administered by the African Research Network for Neglected Tropical Diseases (ARNTD).

Appendix 34: Research output - Poster presentation at BSP Spring meeting 2019

British Society for Parasitology
87 Gladstone Street
Bedford, MK41 7RS
United Kingdom
Tel: +44 (0) 1234 211015
Fax: +44 (0) 1234 481015
Email: info@bsp.uk.net
Web: www.bsp.uk.net

Letter of Invitation

31-Mar-2019

To Whom It May Concern:

Dr Sekeleghe Kayuni
Liverpool School of Tropical Medicine
PhD student
United Kingdom

The BSP Secretariat has received and processed an application for **BSP Spring Meeting 2019**. This meeting will be held at University of Manchester, Manchester, United Kingdom between Monday, 15 April 2019 and Wednesday, 17 April 2019.

I can confirm that the above named individual has been accepted to attend and present at the meeting. Dr Sekeleghe Kayuni is aware that the cost of travel, accommodation and attendance at the conference is covered by himself. Please let me know if you require any further information.

Yours truly

Julian Fuller

Head of Secretariat
BSP Secretariat

Diagnostic challenges in male genital schistosomiasis (MGS): Preliminary real-time PCR results of a longitudinal cohort study in Malawi.

S.A. Kayuni*, P. Makaula, J. Fawcett, A. Shaw, F. Lampiao, L. Juziwelo, E.J. LaCourse, J.J. Verweij and J.R. Stothard.

*Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, United Kingdom. (seke.kayuni@lstmed.ac.uk)

Male genital schistosomiasis (MGS)

- **Male Genital Schistosomiasis (MGS)**, an under-reported schistosomiasis manifestation, associated with schistosome eggs and pathologies in seminal fluids and genital tract
- **MGS increases inflammatory cells** in the genital tract and is known to elevate semen viral load in men with HIV
- **Schistosomiasis is treated with praziquantel (PZQ)** and HIV controlled by combination anti-retroviral therapy (ART)
- **Semen microscopy** is currently considered as a standard technique for diagnosing active MGS infection
- **Urine filtration with microscopy** in presence of MGS symptoms serves as a convenient but error-prone diagnostic proxy of MGS.
- **Unlike the diagnostic advances made in female genital schistosomiasis (FGS)**, MGS remains inadequately defined.

Aims

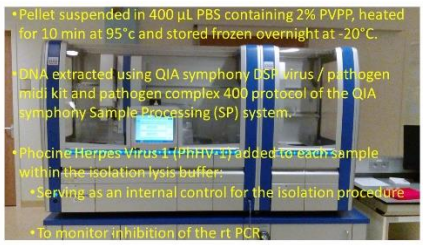
Investigate the current prevalence of MGS among fishermen using parasitological and molecular diagnostic techniques including real-time (rt) PCR analysis of semen.

Study fieldwork

- **Cohort study** LSTM REC and NHSRC Malawi ethical clearance, fishermen ≥ 18 years old recruited
- **Questionnaires** for KAP and MGS/HIV understanding
- **Urological** analysis by reagent strips, urine-CCA and filtration
- **Ultrasonography** (abdominal & scrotal) for morbidity
- **Semen** analysis for *Schistosoma* eggs by microscopy
- **Blood** analysis for HIV viral load analyses (also on semen)
- **Treatment and follow-up surveys**, PZQ given at baseline, same protocol followed at 1-, 3- and 6-months

Molecular analyses of semen

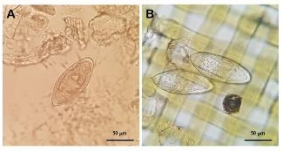
- The preserved semen pellet washed with 1,000 µl of phosphate buffered saline (PBS) to remove the ethanol.



- *Schistosoma* genus-specific rt PCR was performed using primers and TaqMan probes.

Fig. 1. Map of the study area in Mangochi district showing the location of study sites (★) on shores of Lake Malawi in sub-Saharan Africa.

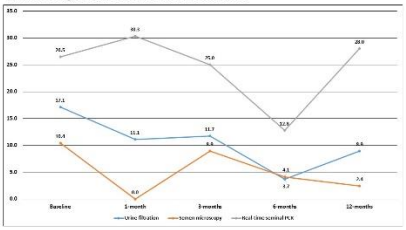
Fig. 2. *Schistosoma haematobium* eggs found in the study in semen (A) and urine (B).



Baseline results of the cohort MGS study

- 376 fishermen recruited,
  - 320 HIV -ve
  - 56 HIV +ve (stable on combination ART)
- Age range: 18 to 70 years (mean = 31.8 years)
- 36 (17.1%, n = 210) egg-patent *S. haematobium* in urine; mean egg count of 15/10 ml, range from 0 - 186 eggs
- 8 (3.8%) POC-CCA +ve indicative of *S. mansoni* co-infection
- 12 (10.4%, n = 114) egg-patent *S. haematobium* in semen mean = 5 eggs / ml, range = 0 - 30 eggs / ml
- 18 (26.5%, n = 68) positive on rt PCR on semen

Fig. 3. Prevalence of *S. haematobium* in the cohort study using parasitological and molecular techniques.



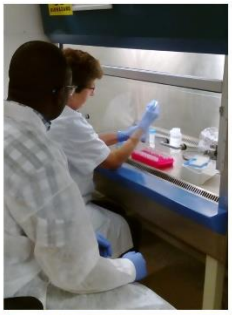
Follow-up results

- **One-month follow-up:**
  - 11.1% (n = 56) egg-patent urine, mean = 13.5 eggs / 10 ml; no positive POC-CCA
  - None had positive CCA nor eggs in semen
  - 30.3% (n = 33) rt PCR on semen
- **Three-months follow-up:**
  - 11.7% (n = 65, mean = 15.6 / 10 ml) urine egg-patency
  - 8.9% (n = 60, mean = 3.6 / ml ejaculate) MGS prevalence
  - 25.0% (n = 48) rt PCR on semen
- **Six-months follow-up:**
  - 3.7% (n = 54; mean = 0.4 /10 ml) urine egg-patent
  - 4.1% (n = 49, mean = 0.9 /ml ejaculate) MGS prevalence
  - 12.8% (n = 39) rt PCR on semen
- **Twelve-months follow-up:**
  - 8.9% (n = 45) egg-patent *S. haematobium* in urine
  - 2.4% (n = 41) egg-patent *S. haematobium* in semen
  - *rt PCR on semen currently in progress*

Conclusions

- **At 1-month** – short-term clearance of egg-patent infections
- **At 3-month** – evidence of re-infection in some patients
- **At 6-months** – continued suppression of egg-patent infection
- **At 12-months –** further suppression of egg-patent infection

Much appreciation to study participants, local community leaders, district health officials, study team, collaborators and supervisors



Appendix 35: Research output – Training report on BW Travel award in August 2019

UC San Diego
SKAGGS SCHOOL OF PHARMACY AND PHARMACEUTICAL SCIENCES

May 10, 2019

Re: 2019 Burroughs-Wellcome Travel Award

Dear Sekeleghe Kayuni:

We are pleased to inform you that you have been selected to receive a 2019 Burroughs-Wellcome Fund Collaborative Research Travel Award, generously funded by the Burroughs-Wellcome Fund to assist graduate students and post-doctoral scholars with their research on the biology and pathology of parasitic roundworms and flatworms at collaborating institutions. The goal of this award is to advance the field using new technologies and molecular tools, to foster new collaborations and transfer technology.

We experienced strong interest in this year's Burroughs Wellcome announcement and in order to broadcast the benefit as broadly as possible within the parasite worm community the amount of each award has been factored according to each applicant's estimated travel expense. Therefore, the amount of your award is $1,314, with an additional $400 bench fee payable to the supervisor of your host institution Laboratory for Medical Microbiology and Immunology to cover the cost of supplies.

Typically the award is paid upon completion of the fellowship, after submission of a 2-page report (Arial font 11; 0.75 inch margins) summarizing the results of the research. Please note that you will be required to provide information for UC San Diego's tax review process, which will determine whether any tax reporting or withholding requirements apply. You may also be required to submit supporting documentation, such as travel receipts.

For any questions, please contact Adrienne Rebollo, BWF Coordinator, at arebollo@ucsd.edu.

In publications resulting from the support, please acknowledge the "Burroughs-Wellcome Fund Collaborative Research Travel Award".

Once again, congratulations on being selected for the 2019 Burroughs-Wellcome Fund Collaborative Research Travel Award. We look forward to a productive research relationship with you!

Sincerely yours,

James H. McKerrow, PhD, MD
Dean, Skaggs School of Pharmacy &
Pharmaceutical Sciences
Associate Vice Chancellor of Health Sciences

Conor R. Caffrey, PhD
Associate Professor
Skaggs School of Pharmacy &
Pharmaceutical Science

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REPORT ON THE REAL-TIME POLYMERASE CHAIN REACTION (PCR) FOR MALE GENITAL SCHISTOSOMIASIS (MGS) AT LABORATORY FOR MEDICAL MICROBIOLOGY AND IMMUNOLOGY, ST. ELISABETH TWEESTEED HOSPITAL IN TILBURG, NETHERLANDS.

1. Background introduction

Schistosomiasis is caused by snail-borne parasites *Schistosoma spp*, causing urogenital disease (*S. haematobium*) and hepato-intestinal disease (*S. mansoni*, etc) (McManus *et al.*, 2018). Male genital schistosomiasis (MGS), a specific manifestation due to eggs and pathologies in genital fluids and tissues, remains underreported despite its first report in 1911 and possible association with Human immunodeficiency virus (HIV) (Kayuni *et al.*, 2019a). Advanced diagnostics tests for schistosomiasis using circulating cathodic antigen (CCA) and circulating anodic antigen (CAA), include point-of-care (POC) CCA urine dipsticks (van Dam *et al.*, 2004), up-converting phosphor-lateral flow assay (UCP-LF CAA) (Corstjens *et al.*, 2008) and real-time polymerase chain reaction (PCR) (Kenguele *et al.*, 2014). Despite these diagnostic advances, MGS remain undiagnosed and untreated among men in endemic areas.

2. Cohort study on Male genital schistosomiasis (MGS) in Malawi

A longitudinal cohort study was conducted among fishermen along south-western shores of Lake Malawi from October 2017 to December 2018. At baseline, 17.1% of the study participants had urogenital schistosomiasis (n = 210), 3.8% were positive on POC-CCA test (possible hepato-intestinal schistosomiasis), 35.7% had detectable CAA levels on UCP-LF CAA while 10.4% had MGS, thus *S. haematobium* eggs in semen (n = 114). Preserved semen samples in ethanol were shipped to Laboratory for Medical Microbiology and Immunology (LMMI) in St. Elisabeth Tweesteed Hospital in Tilburg, Netherlands, for real-time PCR, showing a higher MGS prevalence of 26.5%.

3. Specific aims

I visited the LMMI from 25th to 30th August 2019 to:

a. Undergo training in real-time PCR for detecting low-intensity schistosome infections.

b. Novel application of semen real-time PCR to diagnose undetectable MGS infection.

4. Activities and outcomes of the visit to LMMI

The following activities were done during the period of my visit to LMMI:

a. Training by Dr Jaco Verweij on real-time PCR and novel semen application for MGS.

b. Tutorial on developing primers for real-time PCR.

c. Oral presentation on the Cohort study on Male genital schistosomiasis in Malawi.

d. Compilation of real-time PCR results from 12-months follow-up of our cohort study.

e. Preliminary analysis, interpretation and discussion of the preliminary PCR results.

5. Outcomes of the visit to LMMI

The training on real-time PCR and its novel semen application emphasised the role of PCR in improving MGS diagnosis in our study. Real-time PCR was conducted on the samples which showed rise in MGS prevalence from 2% observed in the field, to 28% (Table below). We observed that some positive PCR participants had no *Schistosoma* eggs in semen and urine, which highlights the diagnostic challenges in MGS and need for improved, multiplex diagnostic platform for MGS to pick up more possible cases.

Sample	**Microscopy**			**Real-time PCR**		
	Number	**Positive**	**Prevalence**	**Number**	**Positive**	**Prevalence**
Urine	61	6	**9.8%**	61	4	**6.6%**
Semen	50	1	**2.0%**	50	14	**28.0%**

6. Conclusion

I am very grateful to Burroughs-Wellcome Fund for the Collaborative Research Travel Award, enabling my visit to LMMI in Tilburg, Netherlands for the short training on real-time PCR and its

novel application in MGS diagnosis. Also, to Dr Jaco Verweij and his team at LMMI for the warm reception, training, expertise and advice on the cohort study and real-time PCR results. Finally, to my supervisors Professor J.R. Stothard and Dr E.J. LaCourse for their overwhelming support throughout the cohort MGS study in Malawi.

Appendix 36: Research output – Invited speaker at the National MDA workshop by Ministry of Health in Malawi, December 2018

Female (FGS) and Male Genital Schistosomiasis (MGS):

A cohort MGS study in fishermen along the southern shoreline of Lake Malawi.

Dr. Sekeleghe Kayuni
(MBBS, DTM&H, MSc TMIH)
Liverpool School of Tropical Medicine and University of Liverpool;
MASM Medi Clinics Ltd, Medical Aid Society of Malawi (MASM)

Appendix 37: Research output – Invited speaker at the isNTD conference, November 2018

Seke Kayuni

From: Seke Kayuni
Sent: 03 October 2018 14:24
To: Russell Stothard; Marianne Comparet; Lucas Cunningham
Subject: RE: WASH & NTDs

Dear Marianne,

I would be delighted to present some of the findings on male genital schistosomiasis.

Keep me informed of the meeting on 15th November 2018.

Regards,

Seke

From: Russell Stothard
Sent: 02 October 2018 14:13
To: Marianne Comparet <comparetm@isntd.org>; Lucas Cunningham <Lucas.Cunningham@lstmed.ac.uk>; Seke Kayuni <Seke.Kayuni@lstmed.ac.uk>
Subject: RE: WASH & NTDs

Thanks Marianne – I am busy I am afraid but Dr Seke Kayuni my PhD student would be a good choice for a presentation on male genital schistosomiasis.

Trust all is well.

Russ

From: Marianne Comparet <comparetm@isntd.org>
Sent: 02 October 2018 13:05
To: Lucas Cunningham <Lucas.Cunningham@lstmed.ac.uk>; Russell Stothard <Russell.Stothard@lstmed.ac.uk>
Subject: WASH & NTDs

Dear Russell & Lucas,

I hope that all is well. Just a quick reminder that our annual WASH & NTDs meeting is coming up on November 15th at the Natural History Museum - we have kept open a speaking slot for LSTM colleagues and as we have the Flett Theatre we can open up LSTM guest passes to cover staff & students. There will be a schisto focus given the participation of NHM and Imperial College as an update on last year's workshop.

Would you be able to attend, and should I send over a flyer for staff/students who might be interested to secure a seat?

And another date for the diary is our Festival (March 11-12th at the Wellcome Trust) - watch this space & submissions are open for film, photo, apps, campaigns etc!

Happy to send over any additional information.

With bets regards,
Marianne

1

Urogenital schistosomiasis in men along the southern shoreline of Lake Malawi:

A cohort study of Male Genital Schistosomiasis (MGS) in fishermen

Dr. Sekeleghe Kayuni
(MBBS, DTM&H, MSc TMIH)
Liverpool School of Tropical Medicine & University of Liverpool;
MASM Medi Clinics Ltd, Medical Aid Society of Malawi (MASM)

Appendix 38: Research output – Poster presentation at BSP Autumn symposium 2018

Male Genital Schistosomiasis (MGS) in fishermen along southern shores of Lake Malawi.

Sekeleghe A.N. Kayuni*, J. Russell Stothard, E. James LaCourse

Department of Parasitology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, United Kingdom

(*seke.kayuni@lstmed.ac.uk)

Male Genital Schistosomiasis (MGS)

- **Male Genital Schistosomiasis (MGS)**, first described in 1911, is caused by schistosome eggs within male genital organs
- **MGS increases inflammatory cells** in the genital tract and is known to elevate semen viral load in men with HIV
- **Schistosomiasis is treated with praziquantel (PZQ)** and HIV controlled by combination anti-retroviral therapy (ART)

Aims

To investigate dynamics of schistosomiasis (i.e. MGS) after PZQ treatment by conducting a longitudinal cohort study in adult fishermen from Mangochi District, Lake Malawi

Methodology

- **Cohort study** LSTM REC and NHSRC Malawi ethical clearance, fishermen ≥ 18 years old recruited **(Figs. 1-4)**
- **Questionnaires** for KAP and MGS/HIV understanding
- **Urological** analysis by reagent strips, urine-CCA and filtration
- **Ultrasonography** (abdominal & scrotal) for morbidity
- **Semen** analysis for *Schistosoma* eggs/molecular biomarkers
- **Blood** analysis for HIV viral load analyses (also on semen)
- **Treatment and follow-up surveys**, PZQ given at baseline, same protocol followed at 1-, 3- and 6-months

Fig. 1. Study area: map of Mangochi District showing the location of the study sites along shores of Lake Malawi in sub-Saharan Africa, labelled with a ★

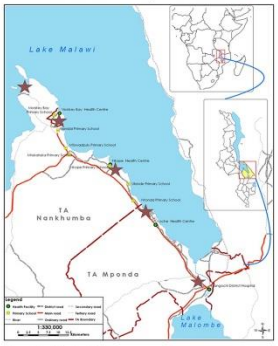
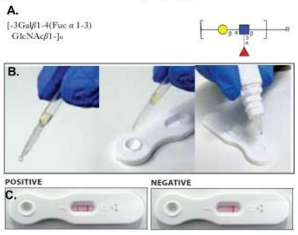


Fig. 2. Urine CCA dipsticks were also used to screen for intestinal schistosomiasis, **(A)** glycan structure of schistosome Circulating Cathodic Antigen (CCA), **(B)** protocol for urine screening & **(C)** typical RDT results



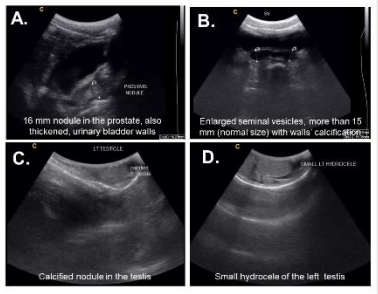
Key findings at study baseline

- 386 fishermen recruited,
  - 320 HIV-ve
  - 56 HIV +ve (on continued combination ART)
- Age range: 18 to 70 years (mean = 30.6 years)
- 31 (14.8%, n = 209) egg-patent *S. haematobium* urines

  mean egg count of 2.3/10 ml, range from 0 - 137.8
- 8 (3.8%) CCA-positive indicative of *S. mansoni* co-infection
- 11 (12%, n = 92) egg-patent with *S. haematobium* semen

  mean = 1 egg / ejaculate, range = 0 - 14 eggs / ejaculate
- 25 (20%, n = 125) with abnormalities by ultrasound

Pathologies observed on abdominal and scrotal ultrasonography



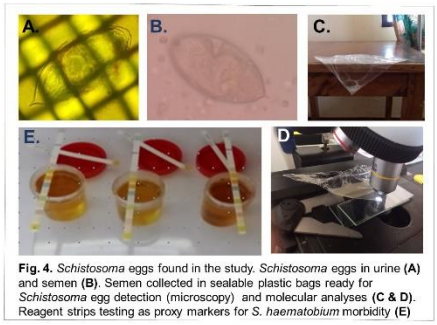


Fig. 4. *Schistosoma* eggs found in the study. *Schistosoma* eggs in urine **(A)** and semen **(B)**. Semen collected in sealable plastic bags ready for *Schistosoma* egg detection (microscopy) and molecular analyses **(C & D)**. Reagent strips testing as proxy markers for *S. haematobium* morbidity **(E)**

Preliminary results at follow-ups

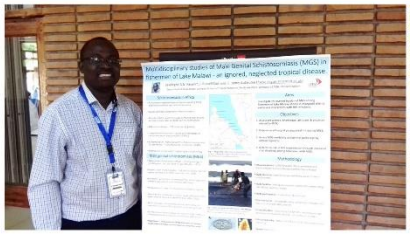
- **One-month follow-up:**
  - Only 4 still had UGS but 2 were new infections
  - Reduced mean egg count of 1.4 / 10 ml urine, range: 0 to 29.6
  - None had positive CCA nor eggs in semen
- **Three-months follow-up:**
  - 16.9% had UGS (n = 65); mean egg count: 1.9 / 10 ml urine, range: 0 to 69.0
  - 8.2% with MGS (n = 61); mean egg count: 0.3 / ejaculate, range: 0 to 9
- **Six-months follow-up:**
  - 4 participants had UGS (6.5%, n = 62; mean = 0.03 eggs / 10 ml)
  - 3 participants had 1 *S. haematobium* egg in semen (5.6%, n = 51)

Ongoing work and future outlook

- **At 1-month** – short-term clearance of egg-patent infections
- **At 3-month** – evidence of re-infection in some patients
- **At 6-months** – continued suppression of egg-patent infection
- **Laboratory investigation** - molecular analysis started

Many thanks to BSP for the international travel award

Scientific and public engagement opportunities



Presentation at 2017 Malawi Doctors Conference

Discussing MGS with fishermen groups

Male Genital Schistosomiasis (MGS) in Malawian fishermen

Dr. Sekeleghe Kayuni

Speed Poster Talks

- Male Genital Schistosomiasis (MGS) is a gender specific manifestation of the snail-borne disease schistosomiasis, first described in 1911 by F.C. Madden
- Today there is insufficient clinicopathological surveillance and awareness of MGS and its interplay with the HIV epidemic in Africa
- Men with MGS have raised inflammatory markers and numbers of immune cells in semen, which has been shown to increases shed viral load in men with HIV
- AIM: **To investigate the current burden of MGS among fishermen of Lake Malawi shores in Mangochi District and assess interactions with HIV infections**

Appendix 39: Research output – Presentations at the 2017 Doctors conference in Malawi

Multidisciplinary studies of Male Genital Schistosomiasis (MGS) in fishermen of Lake Malawi - an ignored, neglected tropical disease.

Sekeleghe A.N. Kayuni*, J. Russell Stothard, E. James LaCourse (*seke.kayuni@lstmed.ac.uk)
Department of Parasitology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, United Kingdom.

Schistosomiasis in Africa

- A snail-borne neglected tropical disease caused by blood-dwelling *Schistosoma* spp. helminth parasites,
- Causes significant urogenital or intestinal disease,
- Parasites hatch in water from eggs in infected stool or urine, develop in snails, later penetrate skin to infect humans,
- 400 million infected .... 779 million at risk globally,
- Urogenital schistosomiasis - caused by *S. haematobium*, causes most worm disease burden in Africa,
- *S. haematobium* - highly prevalent in shoreline communities of Lake Malawi - > 50% prevalence, re-infection: 30-40%,
- Emphasis on urinary system, neglecting genital pathology.

Male genital schistosomiasis (MGS)

- Male genital schistosomiasis (MGS) – *Schistosoma* eggs in genital organs - first described in Egypt (Madden, 1911),
- Reports / post-mortem studies - highlight pathology in genital organs (Gelfand, *et al.* 1970, Leutscher, *et al.* 2000),
- Symptoms - genital pain, haematospermia, altered ejaculates, infertility: granulomata and immuno-pathologies,
- HIV prevalence - higher in schistosomiasis endemic regions,
- Female genital schistosomiasis (FGS) - associated up to 3-fold higher risk HIV acquisition (Kjetland, *et al.* 2006, 2012),
- MGS – increases inflammatory cells and immunological mediators in semen of MGS patients; associated with HIV,
- Schistosomiasis treatment with praziquantel (PZQ) in HIV co-infected reduce HIV replication and increase CD4+ count.
- ***Hypotheses*....increased risk of HIV transmission among dually-infected males in schistosomiasis-endemic areas?**

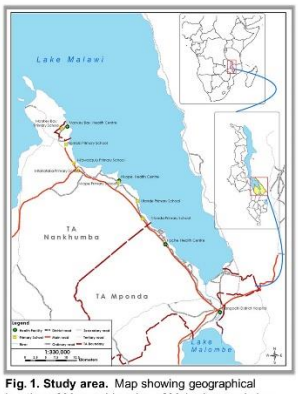


Fig. 1. Study area. Map showing geographical location of Mangochi region of Malawi around shores of Lake Malawi in sub-Saharan Africa.

Fig. 2. Daily activities of fishermen in Lake Malawi. Despite increased risk of schistosomiasis infection, MGS remains poorly described and monitored.

Fig. 3. Testing for *Schistosoma* eggs. *Schistosoma* eggs in urine (A) and semen (B). Urine is also tested for blood and protein as proxy for *S. haematobium* infection (C). Semen collected in sealed plastic bags ready for *Schistosoma* egg detection and molecular analyses (D).

Raising the profile of Male genital schistosomiasis – a blog

What about Male Genital Schistosomiasis: Developing a Research Perspective from the Shoreline of Lake Malawi

Guest Blog by ***Dr Sekeleghe Kayuni*** *(Liverpool School of Tropical Medicine PhD student)*

Southern shore of Lake Malawi, inhabited by a dangerous parasite *Schistosome* causing MGS, the forgotten of a Neglected tropical disease (NTD) *(Photo courtesy of Dr Seke Kayuni).*

http://q-r.to/bakOyu

COUNTDOWN
Calling time on Neglected Tropical Diseases

UNIVERSITY OF LIVERPOOL

Aims

Investigate the current burden of MGS among fishermen of Lake Malawi shores in Mangochi district and assess interactions with HIV infections.

Objectives

1. **Assess prevalence, knowledge, attitudes & practices related to MGS;**
2. **Determine efficacy of praziquantel in treating MGS;**
3. **Assess MGS morbidity and genital pathology by ultrasonography;**
4. **Determine risk of HIV transmission through increased viral shedding among fishermen with MGS.**

Methodology

- **Ethical clearance** - LSTM Research Ethics committee and National Health Sciences Research committee in Malawi,
- **Study duration** - baseline survey and follow-up at 1 month and 3 months,
- **Study location** - fishing communities and health centres along Lake Malawi southern shores.
- **Study participants** – fishermen ≥ 18 years old,
- **Questionnaires** - demographic data, knowledge, attitudes and practices related to MGS and HIV,
- **Urine collection** - parasitological *Schistosoma* analyses (egg detection and molecular testing); on 3 consecutive days,
- **Ultrasonography** - abdominal & transrectal scanning to assess morbidity,
- **Semen collection** - *Schistosoma* egg-count analyses and molecular testing; submission into transparent self-sealing plastic bag; repeat after 3 days,
- **Clinical assessment** - on participants taking antiretroviral therapy (ART), recruited for MGS/HIV study,
- **Blood collection** - for CD4+ count and HIV viral load analyses (also to be conducted on semen),
- **Treatment** - PZQ to all participants following baseline survey, and at follow-up 1 month and 3 months.

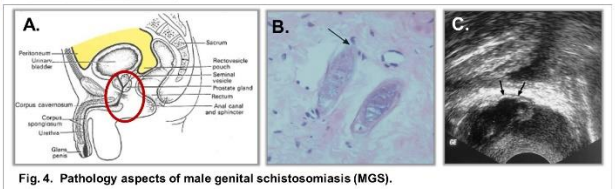


Fig. 4. Pathology aspects of male genital schistosomiasis (MGS).

A. Schematic diagram of male reproductive system highlighting genital organs typically affected by *S. haematobium* {circled}. (Wilson, Waugh *et al.* 1996)

B. Photomicrograph of biopsy specimen of seminal vesicle showing *S. haematobium* eggs trapped in tissues. Note terminal spine of egg {arrow}. (Al-Saeed, Sheikh *et al.* 2003)

C. Transrectal sonogram showing schistosomiasis granuloma in the seminal vesicle (calipers) with rim calcification {arrows}. (Al-Saeed, Sheikh *et al.* 2003)

COMMONWEALTH SCHOLARSHIPS

Male Genital Schistosomiasis (MGS) along shores of Lake Malawi - an ignored aspect of a Neglected tropical disease

Dr Sekeleghe Kayuni (MBBS, DTM&H, DLSHTM, MScTMIH)

Liverpool School of Tropical Medicine, University of Liverpool.

MASM Medi Clinics Ltd, Medical Aid Society of Malawi (MASM).

Appendix 40: Research output – Reports in the local media in Malawi

Survey issues bilharzia scare

Joseph Mwale March 8, 2019

Two recent surveys on infectious diseases in the country's lakeshore areas conducted in 2017 and 2018 have demonstrated the presence of Biomphalaria pfeifferi snails, hosts of some parasites which cause bilharzia (intestinal schistosomiasis).

Schistosomiasis is an acute and chronic disease caused by parasitic worms. People are infected during routine agricultural, domestic, occupational, and recreational activities, which expose them to infested water.

Biomphalaria pfeifferi snails in Lake Malawi and surrounding water are of concern

According to the survey, epidemiologic examination of 175 local children at three primary schools in Mangochi confirmed emergence of intestinal schistosomiasis, which it warns, is of substantial public health concern in light of current control efforts.

Carried out by researchers Mohammad Alharbi, Peter Makaula, Lazarus Juziwelo and Seke Kayuni, warns that the current control efforts which consist only of annual praziquantel distribution in schools, are not enough.

According to the report, colonisation of Biomphalaria pfeifferi snails in Lake Malawi and surrounding water is of concern, especially because active Schistosoma mansoni infections were found in local children.

"This finding is of substantial public health concern in light of current control efforts, which consist only of annual praziquantel distribution in schools.

"We recommend increased surveillance of snails and characterisation of schistosomes, along with intensified control interventions to arrest further spread of intestinal schistosomiasis. We also recommend revising and updating health and travel advice given to shoreline community residents and tourists who use the lake.

The survey, published the the Centre for Disease Control (CDC) comes just days after Malawi was ranked second among 49 African countries that are meeting targets to eliminate tropical diseases (NTDs).

MASM CORNER

Chikoti thanks donors

for scanning machine donation to MASM Medi Clinics Kanjedza

WILLIE ZINGANI

MASM Medi Clinics Kanjedza has received donation of a K4 million ultrasound scanning machine courtesy of Liverpool School of Tropical Medicine's project on Male Genital Schistomiasis and HIV Study.

Malawian Liverpool University PhD student/researcher Dr Sekeleghe Kayuni who handed over the equipment recently said funds for the machine were sourced through internationally renowned drug manufacturers MERCK plc, who also donate free bilharzia tablets to Malawi and other countries in need.

Dr Kayuni (MBBS, DTM&H, MSs), a Commonwealth scholar in Tropical Medicine, University of Liverpool School of Tropical Medicine (LSTM) in the United Kingdom, said while in the initial stages the scanning machine was meant specifically for Bilharzia/ HIV related research in some parts of the southern region, the team to which he is a member decided to make it available for full diagnosis for extended medical purposes, citing kidney and pregnancy cases among others.

"The portable scanning machine can take video, for instance, to establish the functioning of a patient's bladder as well as measurements on prostate diagnosis," said Kayuni. "Our hope is that it will be of great use to the clinic as it operates power with a memory space for 300 to 1,000 scans."

He explained that in Malawi Schistomiasis, (a type of bilharzia infection) is caused by parasitic flat worms that live in fresh waters and transmitted through direct contact whose diagnosis requires lab tests, hence the focus on Mangochi as one of Lake Malawi's tourist destinations, being a possible frequently source of contraction.

Dr Kayuni hands over new scanning machine keys to MASM CEO Mr Sydney Chikoti (centre) as Dr Kasonya of MASM Medi Clinics Kanjedza looks on

Chikoti: switches on the ultrasound scanning machine

Kayuni said the on-going study focusing on male human category in Mangochi aims to establish if Bilharzia increases the transmission of HIV since its symptoms include blood in the urine or stool.

MASM CEO and director in the Medi Clinics, Sydney Chikoti expressed gratitude to the donors for the timely donation which while still being available for research will be used in direct life-saving diagnosis at the Medi Clinics Kanjedza.

"We lose lives simply because of poor diagnosis which sometimes result in wrong prescriptions and medication," said Chikoti. "I, Therefore, appeal to other donors to come to our rescue, not just MASM Medi Clinics, but the entire health sector in Malawi."

Chikoti described MASM Medi Clinic Kanjedza as one of the pillars of the Society which under historical background was the first point of call for MASM members in Blantyre and other districts.

MASM Medi clinics form part of leading health care providers in Malawi, operating in Blantyre, Lilongwe, Zomba and Mzuzu serving both medically insured/ non-insured patients and clients. In the area of excellence the clinics have in the past won Customer Care Management award from the Chartered Institute of Management.