

A longitudinal study of men with male genital schistosomiasis (MGS) in Southern Malawi associated with human, zoonotic and hybrid schistosomes

Bright Mainga^{1,2,*}, Sekeleghe A. Kayuni^{1,3,4,*}, Fatima Ahmed^{3,*}, Guilleary Deles³, Lucas J. Cunningham³, Dingase Kumwenda^{1,5}, David Lally^{Jnr1}, Priscilla Chammudzi¹, Donales Kapira¹, Gladys Namacha¹, Alice Chisale¹, Tereza Nchembe¹, Louis Kinley^{1,6}, Ephraim Chibwana^{1,6}, Bazwell Nkhalema^{1,6}, Gilbert Chapweteka⁷, Henry Chibowa⁸, Victor Kumfunda⁸, Alexandra Juhasz^{3,9}, Sam Jones³, John Archer³, Angus M. O'Ferrall³, Sarah Rollason¹⁰, Abigail Cawley³, Ruth Cowlshaw³, Andrew Nguluwe¹¹, John Chiphwanya¹¹, Michael Luhanga¹¹, Holystone Kafaninkhale¹¹, Peter Makaula¹, E. James LaCourse², Janelisa Musaya^{1,4,#} and J. Russell Stothard^{3,#}

¹Malawi Liverpool Wellcome Programme, Kamuzu University of Health Sciences, Queen Elizabeth Central Hospital campus, 1 Chipatala Avenue, Private Bag 360, Chichiri, Blantyre 3, Malawi;

²Laboratory Department, Mangochi District Hospital, P.O. Box 52, Mangochi, Malawi;

³Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, CTID Building, Pembroke Place, Liverpool, Merseyside, L3 5QA, United Kingdom;

⁴Pathology Department, School of Medicine and Oral Health, Mahatma Gandhi campus, Private Bag 360, Chichiri, Blantyre 3, Malawi;

⁵Obstetrics and Gynaecology Department, Queen Elizabeth Central Hospital, 1 Chipatala Avenue, P.O. Box 95, Blantyre, Malawi;

This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted re- use, distribution and reproduction, provided the original article is properly cited.

⁶Radiology Department, Queen Elizabeth Central Hospital, 1 Chipatala Avenue, P.O. Box 95, Blantyre, Malawi;

⁷Nsanje District Hospital, Ministry of Health, Nsanje, Malawi;

⁸Mangochi District Hospital, Ministry of Health, Mangochi, Malawi;

⁹Institute of Medical Microbiology, Semmelweis University, H-1089 Budapest, Hungary;

¹⁰School of Biosciences, The Sir Martin Evans Building, Cardiff University, Cardiff, CF10 3AX, United Kingdom;

¹¹National Schistosomiasis and Soil-Transmitted Helminths Control Programme, Community Health Sciences Unit (CHSU), Ministry of Health, Area 3, Off Mtunthama Drive, Private Bag 65, Lilongwe, Malawi.

* Joint First Authors

Joint Senior Authors

Corresponding author: Dr Sekeleghe Kayuni, Email: sekekayuni@live.com

Abstract

In sub-Saharan Africa's endemic areas for urogenital schistosomiasis, Male Genital Schistosomiasis (MGS) can cause significant morbidity. As part of the *Hybridization in UroGenital Schistosomiasis* (HUGS) investigation, a MGS sub-study examined a cohort of adult men over a calendar year to better ascertain general infection dynamics and putative zoonotic schistosome transmission. During follow-up, demographic, health and socio-economic data were collected through individual questionnaire interviews. Collected urine and semen were analysed using urine filtration, urine and semen microscopy and molecular DNA analyses of semen. Ten participants with reported MGS-associated symptoms had *Schistosoma* eggs in their urine and semen at 6 months' follow up, with seven at 12 months. Ten out of eleven participants with *S. haematobium* eggs on semen microscopy at baseline

had persistent infection at 6 months follow-up, together with 6 new participants, giving an MGS prevalence of 84.2% (n=19). Two also had *S. mattheei* eggs co-infection. Four of the 13 participants at 12 months follow-up had *S. haematobium* eggs in their semen which were persistent at all the time-points. Using semen PCR, 14 participants (73.7%) had *Schistosoma* infection at 6 months, with only 2 participants being infected for first time. Upon DNA analysis, three participants also had hybrid co-infection at this time-point. At 12 months, only six participants had *Schistosoma* infection with no hybrids detected. In summary, like *S. haematobium* and despite praziquantel treatment, both zoonotic and hybrid schistosomes can continue to cause MGS, which pose a further tangible challenge in future management and control measures.

Keywords: *Schistosoma haematobium*, *Schistosoma mattheei*; hybrid; MGS; praziquantel; semen; Lake Malawi; Shire River.

Introduction

The WHO published a Road Map for control and elimination of neglected tropical diseases (NTDs) 2021–2030 of which one of their public health targets is specific for schistosomiasis (WHO 2022). Despite being targeted for elimination as a public health problem, schistosomiasis is one of the NTDs that continue to affect millions of people, particularly in resource-limited regions of the world (Buonfrate *et al.* 2025; McManus *et al.* 2018). Despite being a major public health issue in many tropical and subtropical countries, schistosomiasis has often been overlooked in global health discussions and funding priorities. In sub-Saharan Africa (SSA), male genital schistosomiasis (MGS), a gender-specific manifestation of urogenital schistosomiasis in men, has a long history of being an under-recognized and under-reported largely due to limited awareness and access to diagnostic tools, particularly resource poor rural areas where urogenital schistosomiasis is endemic (Bustinduy *et al.* 2022). This is in contrast with female genital schistosomiasis (FGS) which has been widely

studied, diagnostic, management and control strategies developed and advocated (WHO 2015).

The condition, MGS, was first described in 1911 (Madden 1911), though it likely existed much earlier in SSA and the Middle East regions, where *Schistosoma haematobium* was prevalent (Kayuni *et al.* 2019a). Men infected after exposure to schistosome cercariae-infested water bodies were observed to have genital lesions, including fibrosis in scrotum, prostate and seminal vesicles, testicular atrophy, and even infertility due to inflammatory response to tissue eggs deposition (Costain *et al.* 2018).

In recent years it has been demonstrated that *S. haematobium* has an innate capacity to form viable hybrids with other schistosome species, particularly with *Schistosoma mattheei*, a common species of animal schistosome found in livestock (Stothard *et al.* 2024; Stothard *et al.* 2020). Hybridization is a well-established evolutionary mechanism that contributes to genetic diversity, allowing species to adapt more quickly to environmental changes (Mawa *et al.* 2021). In light of the future challenge to interrupt schistosome transmission, there is growing interest in hybrid schistosomes, and their environmental dynamics, resulting from crossbreeding between zoonotic and human *Schistosoma* species. Furthermore, such introgressed or hybrid variants can potentially complicate the clinical picture of urogenital schistosomiasis and how the parasite is evolving and adapting to different hosts (Kayuni *et al.* 2024b; Rey *et al.* 2021).

Certainly, zoonotic schistosomes have contributed significantly to the overall burden of schistosomiasis in humans, with some causing fibrosis and calcification in prostate, seminal vesicles and testes (Zhong *et al.*, 2022). Advancements in molecular biology significantly improved modern diagnostic tools for schistosomiasis, including MGS where detection of *Schistosoma* eggs, DNA and antigens can be made directly from semen, prostate biopsies, and other tissue samples, offering more precise and reliable diagnosis (Cunningham *et al.*,

2024, Rinaldi *et al.*, 2015).

Following a baseline MGS survey within the longitudinal study entitled *Hybridization of UroGenital Schistosomiasis* (HUGS) in Mangochi and Nsanje districts, Southern Malawi (Kayuni *et al.* 2024a), a clinical 12-month follow-up was conducted on participating men to describe progression of schistosome infections either from human, zoonotic and hybrid worms undertaken between June 2023 and July 2024.

Materials and methods

Study sites

This MGS longitudinal study was conducted in the two sentinel communities at Samama and at Mthawira in Mangochi and Nsanje Districts respectively (Figure 1). Here, hybrid infections have previously been identified in school children in these communities, with a tenth of infected children shedding atypical eggs in urine, alongside ectopic egg patent *S. mansoni* and zoonotic eggs, at the baseline of the HUGS study in 2022.

Study population

The study population comprised of all males aged between 18 and 45 years old who had active egg-patent schistosomiasis at baseline in 2022 in the two study sites of Samama and Mthawira_areas. These were recruited as at baseline of the MGS study in June and July 2023 and underwent a 6- and 12-months' follow up. Written informed consent was sought after adequate awareness before enrolment into the study. All participants were provided a directly observed treatment with praziquantel standard dose, 40mg/kg body weight, at the end of each study visit.

Data collection

Demographic data were gathered through individual questionnaire interviews to collect details on participants' health, socio-economic status, water contact habits, livestock availability, and symptoms associated with MGS (Kayuni *et al.* 2024a).

Urine and semen microscopy

A 120 ml clear container was used to collect a urine sample during the study visit, which was then filtered and examined microscopically to detect *Schistosoma* eggs in 10 ml of the well-mixed sample (Cheesbrough 2005). Additionally, each sample underwent reagent dipstick testing and point-of-care circulating cathodic antigen (POC-CCA) analysis for detection of intestinal schistosomiasis. Participants were then requested to provide a semen sample, which was collected in a clear plastic bag for direct field microscopy (Kayuni *et al.* 2019b). The samples were subsequently centrifuged, and the sediments were re-examined microscopically. These sediments were preserved in 1 ml of 70% ethanol to prepare for shipment to Liverpool School of Tropical Medicine (LSTM) in the United Kingdom.

Molecular analyses

As previously described (Cunningham *et al.* 2024; Kayuni *et al.* 2024a), molecular analysis of the semen sediments included High-Resolution Melt (HRM) and TaqMan® real-time PCR for *Schistosoma* spp. DNA markers for viral infection with HPV were also screened for using the QIAscreen HPV PCR Test kit (Qiagen, Manchester UK), capable of screening for two high-risk genotypes, 16 and 18 alongside the others. Also, seminal sediments were analyzed for other STIs such as *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Herpes simplex virus* (HSV) 1, HSV 2, *Candida* spp., *Ureaplasma* spp., *M. hominis*, *S. agalactiae* using the EasyScreen™ STI Kit (SydPath, Sydney, Australia).

Data analyses

The results of the diagnostic tests were analysed using non-parametric tests due to the small sample size and non-normal distribution. These were tabulated and presented accordingly.

Ethical considerations

Ethical approval for the study was granted by the College of Medicine Research Ethics Committee (COMREC), Kamuzu University of Health Sciences (KUHeS), Malawi,

(Approval number: P.08/21/3381) and the LSTM Research Ethics Committee (LSTM REC) in the United Kingdom (registration number: 22-028). Informed consent to participate in the study was obtained from all of the participants. Privacy and confidentiality were maintained throughout the study. All participants including those who had *Schistosoma* eggs in urine and/or semen were provided a directly observed treatment with praziquantel standard dose, 40mg/kg body weight, at each time-point. Appropriate referral for treatment and management was provided to participants who had other infections.

Results

Of the 22 male participants recruited into the MGS sub-study at baseline in June 2023, 19 (7 in Nsanje, 12 in Mangochi) and 14 (3 in Nsanje, 11 in Mangochi) participants were available for follow-up at 6- and 12-months' time points respectively (Table 1).

Three participants (J, L and O) reported symptoms associated with MGS at baseline, namely blood in semen (haemospermia), sores and pain in the genitalia, pain during coitus and ejaculation (Table 2). Only one participant (J) with symptoms had *Schistosoma* eggs in their urine and semen. At 6 months' follow up, ten participants reported MGS symptoms while seven participants reported the symptoms at 12 months' follow up timepoint.

Five of the ten participants who had *Schistosoma* eggs in their urine at baseline, remained egg-patent at 6 months follow-up, with four participants (E, L, R and T) who were urinary egg-patent for the first time, giving a prevalence of 47.4% ($n = 19$). At 12-month follow-up, seven out of the ten participants with *Schistosoma* eggs in their urine at baseline were present and participant J was urinary egg-patent at baseline and this timepoint while participant M was infected at all 3 time points. In total, four (J, L, M and P) had eggs in their urine (28.6%, $n = 14$) at this timepoint.

On semen microscopy, 10 out of 11 participants with *S. haematobium* eggs at baseline

had persistent infection at 6 months follow-up, together with 6 participants who had eggs in their semen for the first time, showing a higher infection rate of 84.2% ($n = 19$). Two participants, G and M, also had *S. mattheei* eggs in semen.

Of the 13 participants who were available at 12-months follow-up, only 4 (A, D, G and M) had *S. haematobium* eggs in their semen which were persistent at all the time-points, with one (participant G) having persistent *S. mattheei* eggs from 6 months follow-up, depicting a dual human and zoonotic infection at both time-points. Two participants (A and D) had calcified *S. haematobium* eggs at this time point.

Only one of the 3 participants, D, who had changes in his semen observed at baseline, had normal features at subsequent follow-up time points. However, 4 additional participants (E, G, L and S) who also had *Schistosoma* eggs in their semen were observed to have abnormal changes ranging from loose semen consistency, few live or dead spermatozoa to azoospermia at 6- and 12-months' time points.

Using real time PCR at 6 months follow-up, 14 of the 19 participants (73.7%) were positive for *Schistosoma* infection, with only 2 participants (E and R) who were infected for first time at this time point (Table 3 and Supplementary Table 1). Three participants had a possible hybrid infection at this time-point. On 12 months' follow-up, only 6 out of 13 participants had positive *Schistosoma* infection with no possible hybrid infection. All in all, only 6 of all recruited participants (B, C, F, L, P and S) had no positive *Schistosoma* infection on PCR at the end of the study.

Of those participants who had *Schistosoma* eggs in semen during the study, 4 had none in urine (D, F, G and S). Furthermore, participants Q and T had no eggs in urine nor semen, also with negative PCR.

All the 12 (54.5%) participants who had detectable HPV serotypes 16 and 18 in semen, associated with penile and cervical carcinoma at baseline, had no HPV detected

during follow up at both time points (Table 4 and Supplementary Table 2). On STIs, particularly *T. vaginalis*, only one participant was positive at 6 months follow-up and none at 12 months follow-up, showing a downward trend of both infections.

Discussion

As described previously, here in these two southern communities of Malawi, MGS remains a seldomly recognised, undiagnosed condition in endemic areas, where it is as commonly prevalent as urogenital schistosomiasis, and with coinfection signatures with *S. mattheei* (Kayuni *et al.* 2024a). This study is a 12-months follow up to our initial study comprising a cohort of 22 men where *Schistosoma* eggs was detected in semen of eleven men (50.0%) with sixteen men (72.7%) positive on real-time PCR for *Schistosoma* infection, conducted at 2 time-points of 6 months each. It was noted that despite taking a directly observed standard dose of praziquantel treatment, 40mg/kg body weight, some participants had persistent or possible re-infections.

An important observation is that four participants had *Schistosoma* eggs only in semen during the study, which is consistent with similar studies, showing that the individuals can have MGS due to worms inhabiting the blood vessels around the genital organs, rather than contamination of the urethra from urinary schistosomiasis (Bustinduy *et al.* 2022; Kayuni *et al.* 2021; Kayuni *et al.* 2019a). Hence, the critical importance of analysing semen samples as a way of diagnosing MGS.

Previous reports on MGS associated with non-human schistosomes have been inadequate and mostly case reports of people visiting to endemic areas, despite studies done in Malawi and Madagascar describing significant burden of MGS (Kayuni *et al.* 2023a; Kayuni *et al.* 2021; Leutscher *et al.* 2000). Furthermore, the extent of the morbidity resulting from these zoonotic and hybrid MGS infections remains poorly understood although these schistosomes in SSA are increasingly becoming recognized as an emerging public health

problem (Stothard *et al.* 2020; Webster *et al.* 2019) and future challenge in gaining adequate environmental transmission control (Stothard *et al.* 2024).

The proximity of most study participants to domesticated livestock such as goats, sheep and cattle in the study area which have shown to harbour both human and zoonotic mixed infections (Juhász *et al.* 2024a; Juhász *et al.* 2024b), could have significantly contributed to the level of MGS. Certainly, some participants were observed to have hybrid infections during the follow up using our novel two-tube real-time PCR (Cunningham *et al.* 2024), which brings about the need for advanced molecular diagnostics to improve detection of such diseases. Furthermore, One Health initiatives can aid in addressing the emerging zoonotic and hybrid infections which is being worsened with adverse climate change thereby affecting efforts in the control and elimination of schistosomiasis as public health problem in endemic areas (Shukla *et al.* 2023; Mbabazi *et al.* 2011)

Although some participants had changes in their semen, namely haemospermia, changes in semen colour and consistency and azoospermia which have also been reported in other studies, only one had resolved these abnormalities during follow-up. This highlights the need for earlier treatment with praziquantel to experience its beneficial effects, apart from other interventions to prevent such infections as well as improving diagnostic services, healthcare professional awareness and capacity in disease management (Shukla *et al.* 2023; Mbabazi *et al.* 2011).

Certainly, most health facilities in the endemic areas use the syndromic approach in the STI management (WHO, 2021, WHO 2005). These genital symptoms utilised are similar to those attributed to MGS, also observed in our study, which can result in underdiagnosis and inappropriate management. Also, MGS has been observed to play a role in the progression of other infections such as Human immunodeficiency virus (HIV) among other factors, as previously observed in increasing seminal viral load of dually infected men

(Kayuni *et al.* 2023b; Midzi *et al.* 2017). Therefore, there is need for more advocacy for appropriate diagnostic and management resources together with awareness and capacity building among community people and healthcare providers in schistosomiasis-endemic areas.

As earlier alluded to, the study was limited in the low number of study participants owing to the sensitivity of the condition which calls for larger stratified studies in other endemic areas to provide a representative picture of the MGS outcome.

Conclusion

Human, zoonotic and hybrid schistosomes continues to being incriminated in causing MGS significantly, hence posing a One Health challenge in management and control measures in resource poor settings. This calls for more tailored public health education campaigns in the endemic communities, awareness of MGS among primary healthcare workers and local animal health experts and collaborations among these stakeholders and National Schistosomiasis Control Programme in handling this burden and address it adequately.

Data availability. The datasets generated and analysed for this study has been included in this manuscript.

Acknowledgements. The study team acknowledge the generous support rendered by the Directors of Health and Social Services, District Medical Officers, District Environmental and Assistant Environmental Health Officers, District Laboratory Managers, Schistosomiasis Coordinators, Management and staff of Nsanje and Mangochi District Health Offices; the In-charges and Nurse-Midwives of Tengani and Mpondasi Health Centres; the Headteachers and teachers of Mthawira and Samama Primary Schools; and the study participants who took part in the HUGS Human longitudinal study, especially MGS sub-study. Much appreciation to Professor John Ellis at University of Sydney, Australia for testing STIs on the semen samples using their EasyScreen™ kit (SydPath, Sydney, Australia). Also grateful to the local

community health workers: Abdul Salimu, Bossman Kutama, Christopher Thonje, Cynthia Issah, Grace Nyakamera, Harrison Makawa, Rester Fellow, Theresa Masauli, Witness Mapila, Angellina Mwenyewe, Flora Majawa, Caroline Mnthubula, Alice Kasonde, Doreen Chitsulo; and all traditional leaders and community members around Mthawira and Samama schools for their enthusiasm and support.

Author's contribution. The authors contributed to this work as follows: SK, JM and JRS conceived and designed the study. SK, BM, FA, GD, DK¹, AC¹, TN, DL, PC, DK², GN, LK, EC, BN, GC, WK, HC, VK, AJ, SJ, JA, AOF, SR, AC², RC, LC, JC, PM, E JL, JM and JRS conducted data gathering. SK, BM, FA and LC performed statistical analyses. SK, BM, FA, GD, DK¹, AC¹, TN, DL, PC, DK², GN, LK, EC, BN, GC, WK, HC, VK, AJ, SJ, JA, AOF, SR, AC², RC, LC, JC, PM, E JL, JM and JRS wrote the article.

Financial support. This research received funding from National Institute for Health Research (NIHR) and Wellcome Trust through a Joint Investigator Award, grant number WT 220818/Z/20/Z.

Competing interests. The authors declare there are no conflicts of interest. The funders had no role in the design of the study; in data gathering, statistical analyses and writing of the manuscript.

Ethical standards. The study was conducted in accordance with the Declaration of Helsinki and approved by the College of Medicine Research Ethics Committee (COMREC) of Kamuzu University of Health Sciences (KUHeS) in Malawi, (approval number: P.08/21/3381) and LSTM Research Ethics Committee (LSTM REC) in the United Kingdom (approval number: 22-028). Informed consent to participate in the study was obtained from all of the participants.

References

- Buonfrate D, Ferrari TCA, Adegnikaa AA, Stothard JR and Gobbi FG** (2025) Human schistosomiasis. *The Lancet* **405**(10479), 658-670.
- Bustinduy AL, Randriansolo B, Sturt AS, Kayuni SA, Leutscher PD, Webster BL, Van Lieshout L, Stothard JR, Feldmeier H and Gyapong M** (2022) An update on female and male genital schistosomiasis and a call to integrate efforts to escalate diagnosis, treatment and awareness in endemic and non-endemic settings: the time is now. *Advances in Parasitology* **115**, 1-44.
- Cheesbrough M** (2005) *District laboratory practice in tropical countries, Part 2*. Cambridge University Press.
- Costain AH, MacDonald AS and Smits HH** (2018) Schistosome egg migration: mechanisms, pathogenesis and host immune responses. *Frontiers in Immunology* **9**, 3042.
- Cunningham L, Kayuni S, Juhász A, Makaula P, Lally D, Namacha G, Kapira D, Chammudzi P, Mainga B and Jones S** (2024) A rapid DNA screening method using high-resolution melt analysis to detect putative *Schistosoma haematobium* and *Schistosoma mattheei* hybrids alongside other introgressing schistosomes. *Frontiers in Tropical Diseases* **5**.
- Juhász A, Makaula P, Cunningham LJ, Field L, Jones S, Archer J, Mainga B, Lally D, Namacha G and Kapira D** (2024a) Revealing caprine schistosomiasis and its One Health importance in Malawi: A molecular epidemiological investigation augmented with a praziquantel treatment and GPS animal tracking pilot sub-study. *One Health* **19**, 100918.
- Juhász A, Makaula P, Cunningham LJ, Jones S, Archer J, Lally D, Namacha G, Kapira D, Chammudzi P, LaCourse EJ, Seto E, Kayuni SA, Musaya J and Stothard JR**

(2024b) Revealing bovine schistosomiasis in Malawi: Connecting human and hybrid schistosomes within cattle. *One Health*, 100761.
<https://doi.org/https://doi.org/10.1016/j.onehlt.2024.100761>.

Kayuni S, Cunningham L, Mainga B, Kumwenda D, Jnr DL, Chammudzi P, Kapira D, Namacha G, Chisale A and Nchembe T (2024a) Detection of male genital schistosomiasis (MGS) associated with human, zoonotic and hybrid schistosomes in Southern Malawi. *BMC infectious diseases* **24**(1), 839.

Kayuni SA, Musaya J and Stothard JR (2024b) Highlighting male genital schistosomiasis in Malawi. *Trends in Parasitology* **40**(7), 546-548.
<https://doi.org/10.1016/j.pt.2024.05.003>.

Kayuni SA, Alharbi MH, Shaw A, Fawcett J, Makaula P, Lampiao F, Juziwelo L, LaCourse EJ, Verweij JJ and Stothard JR (2023a) Detection of male genital schistosomiasis (MGS) by real-time TaqMan® PCR analysis of semen from fishermen along the southern shoreline of Lake Malawi. *Heliyon* **9**(7).
<https://doi.org/10.1016/j.heliyon.2023.e17338>.

Kayuni SA, Abdullahi A, Alharbi MH, Makaula P, Lampiao F, Juziwelo L, LaCourse EJ, Kumwenda JJ, Leutscher PDC and Geretti AM (2023b) Prospective pilot study on the relationship between seminal HIV-1 shedding and genital schistosomiasis in men receiving antiretroviral therapy along Lake Malawi. *Scientific Reports* **13**(1), 14154.

Kayuni SA, Alharbi MH, Makaula P, Lampiao F, Juziwelo L, LaCourse EJ and Stothard JR (2021) Male genital schistosomiasis along the shoreline of Lake Malawi: Baseline prevalence and associated knowledge, attitudes and practices among local fishermen in Mangochi District, Malawi. *Frontiers in Public Health* **9**.
<https://doi.org/10.3389/fpubh.2021.590695>.

Kayuni S, Lampiao F, Makaula P, Juziwelo L, Lacourse EJ, Reinhard-Rupp J, Leutscher PD and Stothard JR (2019a) 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* **4**, e00077.

Kayuni SA, Corstjens PLAM, LaCourse EJ, Bartlett KE, Fawcett J, Shaw A, Makaula P, Lampiao F, Juziwelo L, de Dood CJ, Hoekstra PT, Verweij JJ, Leutscher PDC, van Dam GJ, van Lieshout L and Stothard JR (2019b) 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* **146**(14), 1785-1795. <https://doi.org/10.1017/S0031182019000969>.

Leutscher P, Ramarokoto C-E, Reimert C, Feldmeier H, Esterre P and Vennervald BJ (2000) Community-based study of genital schistosomiasis in men from Madagascar. *The Lancet* **355**(9198), 117-118.

Madden F (1911) Two rare manifestations of Bilharziosis. *The Lancet* **178**(4593), 754-755.

Mawa PA, Kincaid-Smith J, Tukahebwa EM, Webster JP and Wilson S (2021) Schistosomiasis morbidity hotspots: roles of the human host, the parasite and their interface in the development of severe morbidity. *Frontiers in Immunology* **12**, 635869.

Mbabazi PS, Andan O, Fitzgerald DW, Chitsulo L, Engels D and Downs JA (2011) Examining the relationship between urogenital schistosomiasis and HIV infection. *PLoS Neglected Tropical Diseases* **5**(12), e1396.

McManus DP, Dunne DW, Sacko M, Utzinger J, Vennervald BJ and Zhou XN (2018) Schistosomiasis. *Nature Reviews Disease Primers* **4**(1), 13. <https://doi.org/10.1038/s41572-018-0013-8>.

- Midzi N, Mduluzu T, Mudenge B, Foldager L and Leutscher PD** (2017) Decrease in seminal HIV-1 RNA load after praziquantel treatment of urogenital schistosomiasis coinfection in HIV-positive men—an observational study. In: *Open Forum Infectious Diseases*. Oxford University Press US.
- Rey O, Toulza E, Chaparro C, Allienne J-F, Kincaid-Smith J, Mathieu-Begn   E, Allan F, Rollinson D, Webster BL and Boissier J** (2021) Diverging patterns of introgression from *Schistosoma bovis* across *S. haematobium* African lineages. *PLoS Pathogens* **17**(2), e1009313.
- Shukla JD, Kleppa E, Holmen S, Ndhlovu PD, Mtshali A, Sebitloane M, Vennervald BJ, Gundersen SG, Taylor M and Kjetland EF** (2023) The association between Female genital schistosomiasis and other infections of the lower genital tract in adolescent girls and young women: A cross-sectional study in South Africa. *Journal of Lower Genital Tract Disease* **27**(3), 291-296.
- Stothard JR, Juh  sz A and Musaya J** (2024) *Schistosoma mattheei* and zoonotic schistosomiasis. *Trends in Parasitology*.
- Stothard JR, Kayuni SA, Al-Harbi MH, Musaya J and Webster BL** (2020) Future schistosome hybridizations: Will all *Schistosoma haematobium* hybrids please stand-up! *PLoS Neglected Tropical Diseases* **14**(7), e0008201.
- Webster BL, Alharbi MH, Kayuni S, Makaula P, Halstead F, Christiansen R, Juziwele L, Stanton MC, LaCourse EJ, Rollinson D, Kalua K and Stothard JR** (2019) Schistosome Interactions within the *Schistosoma haematobium* group, Malawi. *Emerging Infectious Diseases* **25**(6), 1245-1247. <https://doi.org/10.3201/eid2506.190020>.
- WHO** (2022) *WHO guideline on control and elimination of human schistosomiasis*. World Health Organization.

WHO (2021) Guidelines for the management of symptomatic sexually transmitted infections.

9789240024168. Geneva, Switzerland: World Health Organization.

WHO (2015) *Female genital schistosomiasis: A pocket atlas for clinical health-care professionals*. Geneva, Switzerland: World Health Organization.

WHO (2005) *Sexually transmitted and other reproductive tract infections: a guide to essential practice*. Geneva, Switzerland: World Health Organization.

Figure Legends

Figure 1. Map showing two study communities around Samama School in Mangochi District and Mthawira School in Nsanje District of Southern Malawi where participants came from.

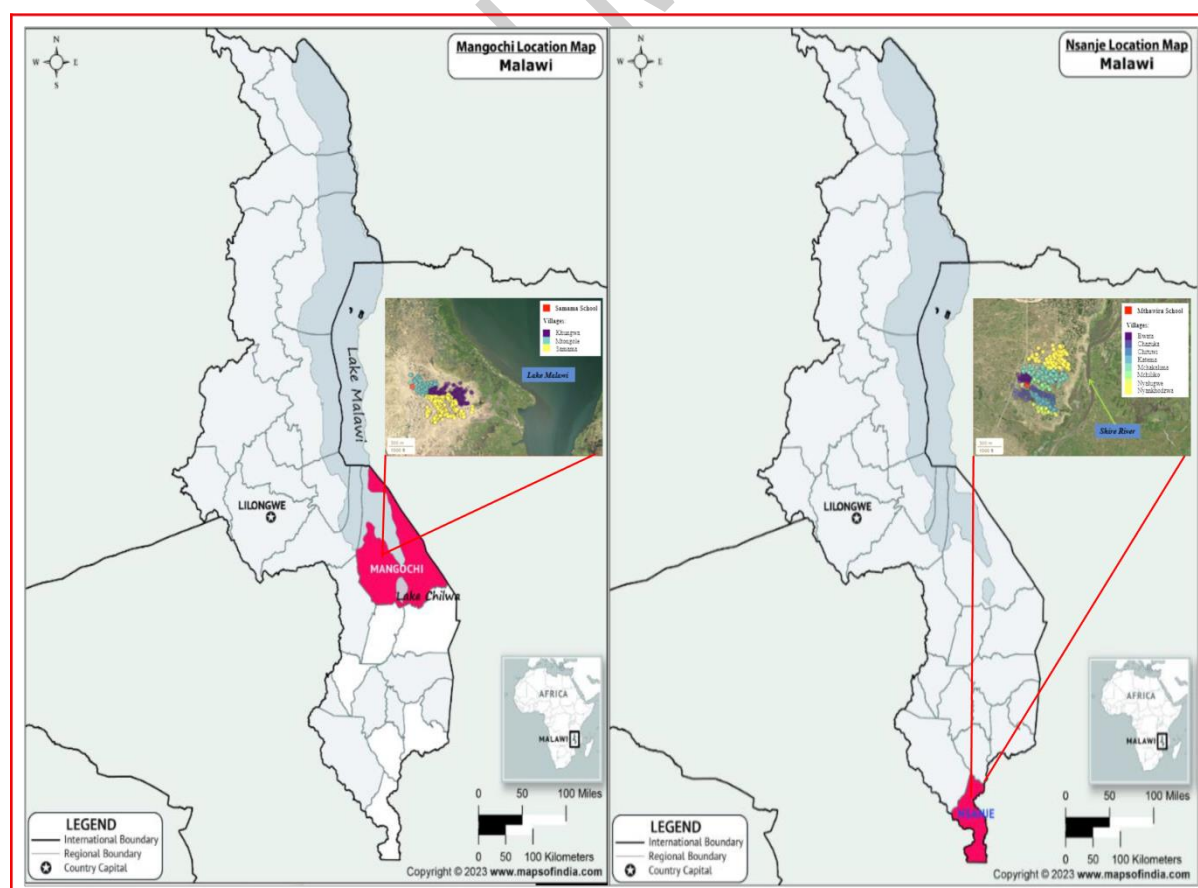


Figure 2. Map showing findings on urine and semen microscopy of the study participants in the two communities around Samama school in Mangochi district and Mthawira school in Nsanje district of Southern Malawi where participants came from.

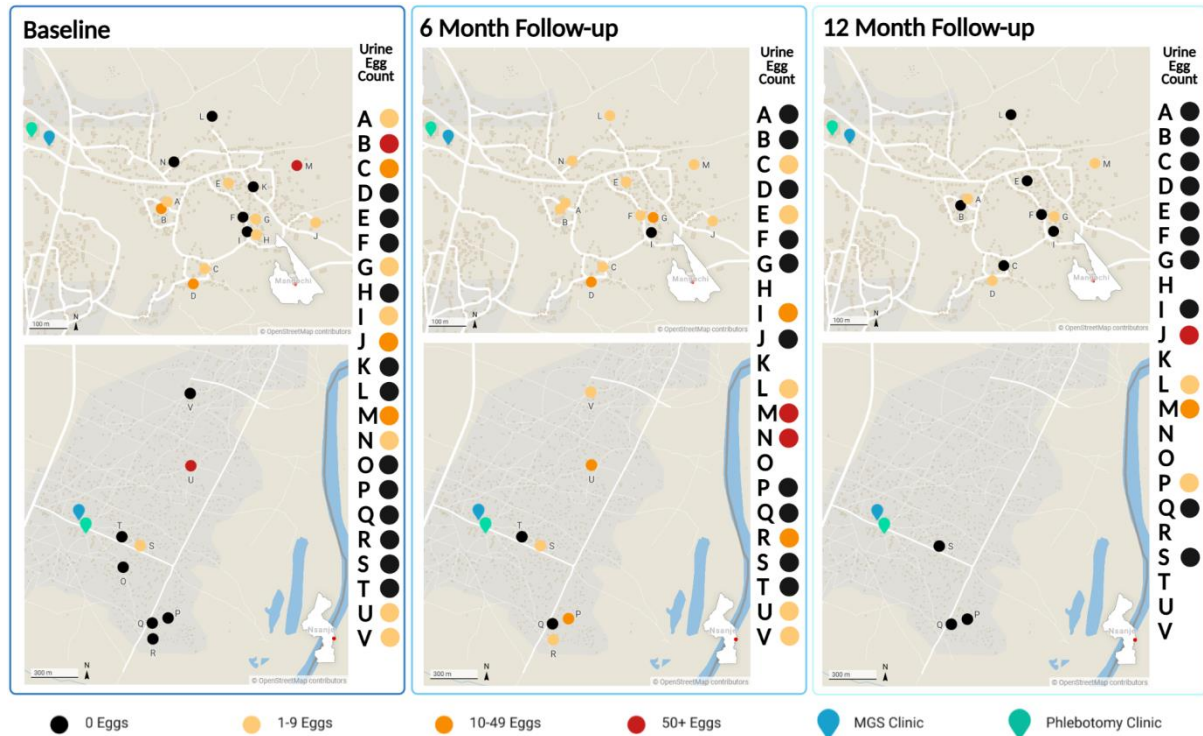


Table 1: Demographical information and laboratory findings of the participants at all the time points

ID	Age (years)	Urine filtration egg count			Semen microscopy						Observations on semen microscopy		
		Baseline	6-month	12-month	Baseline		6-month		12-month		Baseline	6-month	12-month
					<i>S. h.</i> eggs	<i>S. matt.</i> eggs	<i>S. h.</i> eggs	<i>S. matt.</i> eggs	<i>S. h.</i> eggs	<i>S. matt.</i> eggs			
A	19	1-9	0	0	8	1	9	0	2 (cal)	0	Normal	Normal	Normal
B	18	50+	0	0	25	0	5	0	0	0	Normal	Normal	Normal
C	18	10-49	1-9	0	6	0	8	0	0	0	Haemospermia (blood in semen), loose consistency, azoospermia	Loose consistency, azoospermia	Loose consistency, azoospermia
D	18	0	0	0	15	0	10	0	1 (cal)	0	Colour change, loose consistency, azoospermia	Normal	Normal

This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted re- use, distribution and reproduction, provided the original article is properly cited.

E	21	0	1-9	0	2	0	3	0	0	0	Normal	Few live spermatozoa	Some fragmented spermatozoa
F	28	0	0	0	0	0	6	0	0	0	Normal	Normal	Normal
G	27	1-9	0	0	1	0	8	2	2	1	Normal	Dead spermatozoa	Dead spermatozoa
H	19	0	N/A	N/A	5	0	N/A	N/A	N/A	N/A	Normal	N/A	N/A
I	30	1-9	10-49	0	0	0	0	0	0	0	Normal	Normal	Normal
J	22	10-49	0	50+	9	0	7	0	N/A	N/A	Normal	Normal	Normal
K	18	0	N/A	N/A	0	0	N/A	N/A	N/A	N/A	Normal	N/A	N/A
L	21	0	1-9	1-9	0	0	4	0	0	0	Normal	Dead spermatozoa	1/3 dead spermatozoa
M	18	10-49	50+	10-49	100	0	8	1	4	0	Haemospermia, loose consistency, dead spermatozoa	Azoospermia	Loose consistency, azoospermia
N	32	1-9	50+	N/A	0	0	7	0	N/A	N/A	Normal	Normal	N/A
O	32	0	N/A	N/A	0	0	N/A	N/A	N/A	N/A	Normal	Normal	Normal
P	39	0	0	1-9	0	0	10	0	0	0	Normal	Normal	Normal
Q	30	0	0	0	0	0	0	0	0	0	Normal	Normal	Azoospermia

R	26	0	10-49	N/A	0	0	7	0	N/A	N/A	Normal	Normal	N/A
S	19	0	0	0	1	0	3	0	0	0	Normal	Cloudy, loose consistency	Cloudy, loose consistency, azoospermia
T	22	0	10-49	N/A	0	0	0	0	N/A	N/A	Normal	Normal	N/A
U	22	1-9	1-9	N/A	500	0	10	0	N/A	N/A	Normal	Normal	N/A
V	25	1-9	0	N/A	0	0	8	0	N/A	N/A	Normal	Normal	N/A

Note: *S. h.* = *Schistosoma haematobium*, *S. matt.* = *Schistosoma mattheei*, N/A = Not Available

Accepted Manuscript

Table 2: Symptoms of MGS experienced by the participants at all the time points

		Symptoms	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	N in cohort
Timepoint 1 - June 2023	Urinary	Fever	1	1	1	0	0	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	1	1	9
		Headache	1	1	1	0	0	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	1	1	9
		Fatigue	1	1	1	0	0	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	1	1	8
		Abdominal pain	1	1	1	0	0	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	1	1	5
		Dysuria	1	1	1	0	0	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	1	1	7
		Polyuria	1	1	1	0	0	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	1	1	10
		Discoloured Urine	1	1	1	0	0	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	1	1	8
		Haematuria	1	1	1	0	0	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	1	1	6
		Blood in stool	1	1	1	0	0	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	1	1	2
	Genital	Haemospermia	1	1	1	1	1	0	1	1	0	1	0	0	1	0	0	0	0	0	1	0	1	0	1
		Dyspareunia	1	1	1	1	1	0	1	1	0	1	0	0	1	0	0	0	0	0	1	0	1	0	2
		Dysorgasmia	1	1	1	1	1	0	1	1	0	1	0	0	1	0	0	0	0	0	1	0	1	0	2
		Genital pain	1	1	1	1	1	0	1	1	0	1	0	0	1	0	0	0	0	0	1	0	1	0	2
		Genital sores	1	1	1	1	1	0	1	1	0	1	0	0	1	0	0	0	0	0	1	0	1	0	3
N sx experienced		0	3	0	0	6	4	1	0	1	13	0	6	6	2	11	2	3	7	1	4	1	3		

		Symptoms	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	N in cohort
Timepoint 2 - January 2024	Urinary	Fever	0	0	1	0	1	0	0	N/A	1	0	N/A	1	1	1	N/A	0	0	1	0	0	1	0	12
		Headache	0	0	1	0	1	0	0	N/A	1	0	N/A	1	1	1	N/A	0	0	1	0	0	1	0	17
		Fatigue	0	0	1	0	1	0	0	N/A	1	0	N/A	1	1	1	N/A	0	0	1	0	0	1	0	11
		Abdominal pain	0	0	1	0	1	0	0	N/A	1	0	N/A	1	1	1	N/A	0	0	1	0	0	1	0	16
		Dysuria	0	0	1	0	1	0	0	N/A	1	0	N/A	1	1	1	N/A	0	0	1	0	0	1	0	12
		Polyuria	0	0	1	0	1	0	0	N/A	1	0	N/A	1	1	1	N/A	0	0	1	0	0	1	0	13
		Discoloured Urine	0	0	1	0	1	0	0	N/A	1	0	N/A	1	1	1	N/A	0	0	1	0	0	1	0	15
		Haematuria	0	0	1	0	1	0	0	N/A	1	0	N/A	1	1	1	N/A	0	0	1	0	0	1	0	11

	Genital	Blood in stool	0	0	1	0	1	0	0	N/A	1	0	N/A	1	1	1	N/A	0	0	1	0	0	1	0	5
		Haemospermia	1	1	1	1	1	1	1	N/A	0	1	N/A	1	1	1	N/A	1	0	1	1	0	1	1	3
		Dyspareunia	1	1	1	1	1	1	1	N/A	0	1	N/A	1	1	1	N/A	1	0	1	1	0	1	1	3
		Dysorgasmia	1	1	1	1	1	1	1	N/A	0	1	N/A	1	1	1	N/A	1	0	1	1	0	1	1	5
		Genital pain	1	1	1	1	1	1	1	N/A	0	1	N/A	1	1	1	N/A	1	0	1	1	0	1	1	7
		Genital sores	1	1	1	1	1	1	1	N/A	0	1	N/A	1	1	1	N/A	1	0	1	1	0	1	1	3
	N sx experienced		4	12	7	0	4	8	8	0	3	7	0	11	10	5	0	5	10	12	6	5	3	8	

Timepoint 3 - June 2024	Urinary	Symptoms	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	N in cohort
		Fever	0	0	0	0	0	0	0	N/A	0	1	N/A	1	1	N/A	N/A	1	0	N/A	0	N/A	N/A	N/A	9
		Headache	0	0	0	0	0	0	0	N/A	0	1	N/A	1	1	N/A	N/A	1	0	N/A	0	N/A	N/A	N/A	12
		Fatigue	0	0	0	0	0	0	0	N/A	0	1	N/A	1	1	N/A	N/A	1	0	N/A	0	N/A	N/A	N/A	6
		Abdominal pain	0	0	0	0	0	0	0	N/A	0	1	N/A	1	1	N/A	N/A	1	0	N/A	0	N/A	N/A	N/A	8
		Dysuria	0	0	0	0	0	0	0	N/A	0	1	N/A	1	1	N/A	N/A	1	0	N/A	0	N/A	N/A	N/A	7
		Polyuria	0	0	0	0	0	0	0	N/A	0	1	N/A	1	1	N/A	N/A	1	0	N/A	0	N/A	N/A	N/A	8
		Discoloured Urine	0	0	0	0	0	0	0	N/A	0	1	N/A	1	1	N/A	N/A	1	0	N/A	0	N/A	N/A	N/A	8
		Haematuria	0	0	0	0	0	0	0	N/A	0	1	N/A	1	1	N/A	N/A	1	0	N/A	0	N/A	N/A	N/A	7
		Blood in stool	0	0	0	0	0	0	0	N/A	0	1	N/A	1	1	N/A	N/A	1	0	N/A	0	N/A	N/A	N/A	5
	Genital	Haemospermia	1	0	0	1	0	0	1	N/A	0	N/A	N/A	0	1	N/A	N/A	0	0	N/A	0	N/A	N/A	N/A	2
		Dyspareunia	1	0	0	1	0	0	1	N/A	0	N/A	N/A	0	1	N/A	N/A	0	0	N/A	0	N/A	N/A	N/A	3
		Dysorgasmia	1	0	0	1	0	0	1	N/A	0	N/A	N/A	0	1	N/A	N/A	0	0	N/A	0	N/A	N/A	N/A	4
		Genital pain	1	0	0	1	0	0	1	N/A	0	N/A	N/A	0	1	N/A	N/A	0	0	N/A	0	N/A	N/A	N/A	6
		Genital sores	1	0	0	1	0	0	1	N/A	0	N/A	N/A	0	1	N/A	N/A	0	0	N/A	0	N/A	N/A	N/A	4
	N sx experienced		0	8	8	4	14	11	8	0	14	8	0	1	3	0	0	7	0	0	3	0	0	0	

Note: Within the Table, Red colour = Symptom experienced, 1 = *Schistosoma* eggs in urine or semen, 0 = No *Schistosoma* eggs in urine or semen, N/A = urine or semen sample not available for analysis

Table 3: Results of molecular analysis on *Schistosoma* infection using real-time PCR of the participants' semen.

Participant ID	Age (years)	Baseline				6-months				12-months			
		Gen	Mito	HRM Tm1	HRM Tm2	Gen	Mito	HRM Tm1	HRM Tm2	Gen	Mito	HRM Tm1	HRM Tm2
A	19	Sh	Smat	Smat	0.0	Sh	0.0	Smat	0.0	Sh	Smat	Smat	0.0
B	18	Sh	Sm	Sm	0.0	Shyp	Shyp	Shyp	Shyp	0.0	0.0	0.0	0.0
C	18	Sh	Smat	Smat	0.0	Sh	0.0	0.0	0.0	0.0	0.0	0.0	0.0
D	18	Sh	0.0	0.0	0.0	Sh	Sc	Sc	0.0	Sh	0.0	0.0	0.0
E	21	0.0	0.0	0.0	0.0	Sh	Smat	Smat	0.0	Sh	0.0	0.0	0.0
F	28	Shyp	0.0	Shyp	Sm	Sh	Smat	Smat	0.0	0.0	0.0	0.0	0.0
G	27	Sh	Smat	Smat	0.0	Sh	Smat	Smat	0.0	Sh	Smat	Smat	0.0
H	19	Sh	0.0	0.0	0.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
I	30	Sh	0.0	0.0	0.0	Sh	0.0	0.0	0.0	Sh	0.0	0.0	0.0
J	22	Sh	0.0	0.0	0.0	Sh	Sc	Sc	0.0	N/A	N/A	N/A	N/A
K	18	0.0	0.0	0.0	0.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L	21	Sh	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M	18	Sh	0.0	0.0	0.0	Shyp	Shyp	Shyp	0.0	Sh	0.0	0.0	0.0
N	32	Sh	Sm	Sm	0.0	0.0	0.0	0.0	0.0	N/A	N/A	N/A	N/A
O	32	0.0	0.0	0.0	0.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P	39	Sh	0.0	0.0	0.0	Sh	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Q	30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R	26	0.0	0.0	0.0	0.0	Shyp	Shyp	Shyp	0.0	N/A	N/A	N/A	N/A
S	19	Sh	0.0	0.0	0.0	Sh	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T	22	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	N/A	N/A	N/A	N/A
U	22	Sh	0.0	0.0	0.0	Sh	Sc	Sc	0.0	N/A	N/A	N/A	N/A
V	25	0.0	Smat	Smat	0.0	0.0	0.0	0.0	0.0	N/A	N/A	N/A	N/A

Note: Gen = Generic, Mito = Mitochondrial, HRM = High-Resolution Melt, T_m = melting temperature, N/A = Not Available; *Sh* = *Schistosoma*

haematobium, *Sm* = *Schistosoma mansoni*, *Smat* = *Schistosoma mattheei*, *Sc* = *Schistosoma curassoni*, *Shyp* = *Schistosoma* hybrid, 0.0 = No

Schistosoma detected; Colours: Dark Blue = *Schistosoma haematobium*; Plum = *Schistosoma mansoni*; Dark Green = *Schistosoma mattheei*;

Orange = *Schistosoma curassoni*; Red = *Schistosoma* hybrid

Accepted Manuscript

Table 4: Results of the real time PCR for Human Papilloma Virus (HPV) serotypes and STIs - *Trichomonas vaginalis* in comparison with and *Schistosoma* species

Participant ID	Age (years)	Baseline					6-months					12-months				
		Gen	HRM Tm1	HRM Tm2	HPV type	STI Tv	Gen	HRM Tm1	HRM Tm2	HPV type	STI Tv	Gen	HRM Tm1	HRM Tm2	HPV type	STI Tv
A	19	Sh	Smat	Smat	16	0.0	Sh	Smat	0.0	-	0.0	Sh	Smat	Smat	-	0.0
B	18	Sh	Sm	Sm	Other	0.0	Shyp	Shyp	Shyp	-	0.0	0.0	0.0	0.0	-	0.0
C	18	Sh	Smat	Smat	Other	0.0	Sh	0.0	0.0	-	0.0	0.0	0.0	0.0	-	0.0
D	18	Sh	0.0	0.0	18	0.0	Sh	Sc	0.0	-	0.0	Sh	0.0	0.0	-	0.0
E	21	0.0	0.0	0.0	Other	0.0	Sh	Smat	0.0	-	0.0	Sh	0.0	0.0	-	0.0
F	28	Shyp	Shyp	Sm	16	0.0	Sh	Smat	0.0	-	0.0	0.0	0.0	0.0	-	0.0
G	27	Sh	Smat	0.0	18	0.0	Sh	Smat	0.0	-	0.0	Sh	Smat	Smat	-	0.0
H	19	Sh	0.0	0.0	Other	0.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
I	30	Sh	0.0	0.0	16	Tv	Sh	0.0	0.0	-	0.0	Sh	0.0	0.0	-	0.0
J	22	Sh	0.0	0.0	-	0.0	Sh	Sc	0.0	-	0.0	N/A	N/A	N/A	N/A	N/A
K	18	0.0	0.0	0.0	-	Tv	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
L	21	Sh	0.0	0.0	Other	Tv	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	-	0.0
M	18	Sh	0.0	0.0	-	Tv	Shyp	Shyp	0.0	-	0.0	Sh	0.0	0.0	-	0.0
N	32	Sh	Sm	0.0	16	Tv	0.0	0.0	0.0	-	0.0	N/A	N/A	N/A	N/A	N/A
O	32	0.0	0.0	0.0	-	0.0	N/A	N/A	N/A	-	0.0	N/A	N/A	N/A	N/A	N/A

P	39	Sh	0.0	0.0	Other	Tv	Sh	0.0	0.0	-	0.0	0.0	0.0	0.0	-	0.0
Q	30	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	-	0.0
R	26	0.0	0.0	0.0	-	0.0	Shyp	Shyp	0.0	-	Tv	N/A	N/A	N/A	N/A	N/A
S	19	Sh	0.0	0.0	-	0.0	Sh	0.0	0.0	-	0.0	0.0	0.0	0.0	-	0.0
T	22	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	-	0.0	N/A	N/A	N/A	N/A	N/A
U	22	Sh	0.0	0.0	-	0.0	Sh	Sc	0.0	-	0.0	N/A	N/A	N/A	N/A	N/A
V	25	0.0	Smat	0.0	-	0.0	0.0	0.0	0.0	-	0.0	N/A	N/A	N/A	N/A	N/A

Note: Gen = Generic, HRM = High-Resolution Melt, Tm = melting temperature, Tv = *T. vaginalis*; N/A = Not Available, Sh = *Schistosoma haematobium*, Sm = *Schistosoma mansoni*, Smat = *Schistosoma mattheei*, Sc = *Schistosoma curassoni*, Shyp = *Schistosoma* hybrid, 0.0 = No *Schistosoma* detected; Colours: Dark Blue = *Schistosoma haematobium*; Plum = *Schistosoma mansoni*; Dark Green = *Schistosoma mattheei*; Orange = *Schistosoma curassoni*; Red = *Schistosoma* hybrid; Yellow = HPV serotypes 16 or 18 or others; Purple = *T. vaginalis*

Graphical Abstract

