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Title

Intestinal Schistosomiasis and Giardiasis Co-Infection in Sub-Saharan Africa: Can a One Health Approach Improve Control of Each Waterborne Parasite Simultaneously?

Running title

Co-infection with intestinal schistosomiasis and giardiasis in sub-Saharan Africa

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Abstract

Both intestinal schistosomiasis and giardiasis are co-endemic throughout many areas of sub-Saharan Africa, significantly impacting the health of millions of children within endemic areas. While giardiasis is not considered a neglected tropical disease, intestinal schistosomiasis is formally grouped within the NTD umbrella and, as such, receives significant advocacy and financial support for large-scale control, annually. Given the many epidemiological similarities between intestinal schistosomiasis and giardiasis, in this review, we critically discuss why disease surveillance and control activities for giardiasis are largely absent within low- and middle-income countries. With advances in new methods of parasite diagnostics and provision of existing anti-parasitic medications, better management of intestinal schistosomiasis and giardiasis co-infection could, not only be better understood but also, more effectively controlled. In this light, we appraise the suitability of a One Health approach for intestinal schistosomiasis, for if adopted more broadly, could also pave a way forward for more inclusive public health actions against giardiasis.

Key words: *One Health;* Schistosoma mansoni; Giardia duodenalis; *Sanitation and Hygiene (WASH); Uganda*

1. Introduction

Schistosomiasis is a debilitating neglected tropical disease (NTD) caused by infection with parasitic blood flukes of the genus *Schistosoma*. In sub-Saharan Africa and South America, infection with the trematode species *Schistosoma mansoni* gives rise to intestinal schistosomiasis. This disease compromises the general integrity of the small bowel *via* egg-induced perforations with associated local and systemic inflammation [1]. Giardiasis, another debilitating and underreported intestinal parasitic disease, is caused by infection with the

single-celled eukaryotic diplomonad *Giardia duodenalis*, a flagellated protist [2]. Notably, intestinal schistosomiasis and giardiasis are both waterborne parasitic diseases; they are highly prevalent and co-endemic throughout many tropical and sub-tropical lower-middle income countries (LMICs) where provision of water, sanitation and hygiene (WASH) infrastructure is inadequate [3–5]. Unlike schistosomiasis, however, giardiasis is currently not considered a NTD, despite previous discussions justifying its inclusion [6,7].

Since several epidemiological studies demonstrate similarities between intestinal schistosomiasis and giardiasis, we now consider a combined approach in control is needed. In doing so, we aim to diminish the detrimental effects of (co)infection and to raise the wellbeing of children in general. To do this an integrated, 'One Health' approach is needed that requires a detailed knowledge of the natural history of each parasite, alongside appropriate use of reliable point-of-care (POC) diagnostics, mitigation of environmental and zoonotic transmission and effective use of anti-parasitic chemotherapies are each needed. All of which are needed in careful co-ordination, to improve public health outcomes [8–11]. Here, we appraise integrated tactics, highlighting opportunities for potential synergies, and next steps to be taken.

2.0 Intestinal schistosomiasis and giardiasis: epidemiology and pathology

Intestinal schistosomiasis, like schistosomiasis more generally, disproportionally afflicts school-aged children between the ages of six and fifteen years old where pathology can be both acute and chronic [1]. As based on 'classic' age-infection profiles and measured using faecal egg counts, the intensity of infection typically begins to decline in late adolescence whilst morbidity associated with *S. mansoni*, such as multi-organ fibrosis, accumulates. This population decline in egg-patent prevalence is due to a variety of factors such as partial-

immunity to infection, notwithstanding extensive fibrosis of the bowel itself which can occlude egg exit sites, giving rise to granulomatous masses known as intestinal 'bilharzomas' [12–15].

Pathologies associated with intestinal schistosomiasis occur primarily as a result of the copious number of eggs produced by paired adult worms inhabiting the mesenteric veins surrounding the intestines. Rather than being passed in stool, or occasionally in urine, a large proportion of eggs will instead, by being swept away, become sequestered throughout the venous bloodstream of the intestinal and hepatoportal tracts, breaking out into general venous circulation and thence lodge in other major organs. Once eggs become sequestered, for example in the intestinal wall and/or liver sinuses, a range of clinical systemic and organ-specific morbidity ensues inclusive of acute abdominal pain, stunted growth, environmental enteropathy, presence of faecal occult blood and overt hepato/splenomegaly [16,17].

Human *Giardia*sis is caused by infection with *Giardia duodenalis* (syn. *Giardia intestinalis*, *Giardia lamblia*). Whilst *G. duodenalis* is the only human-infecting *Giardia* species, eight distinct evolutionary assemblages based on multi-locus genotyping, named A through H, are known to exist [18]. Of these eight, only assemblages A and B are able to successfully infect and are successfully transmitted by humans [18]. Unlike the distribution of *S. mansoni*, which is intrinsically linked to its *Biomphalaria* spp. intermediate freshwater snail host, the distribution of *G. duodenalis* is truly cosmopolitan. Prevalence in humans is particularly high, however, in LMICs lacking access to clean, safe drinking water and associated WASH infrastructures, including many areas of sub-Saharan Africa and South America where *S. mansoni* is endemic [3,19,20]. A notable feature of giardiasis, in humans, can be asymptomatic infections, however, acute and/or chronic and debilitating pathologies owing to infection are well described. These include diarrhoea, dehydration, malabsorption, tropical enteropathy, stunted growth, impaired cognitive development, anaemia and chronic fatigue [21,22]. The primary cause of these pathologies is a major disruption to the gut

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microbiota, a complex community of symbiotic microbes responsible for vitamin production, nutrient absorption and regulation of lipid metabolism, brought about through *G. duodenalis* invading, inhabiting and multiplying within the intestinal tract [23–25]. Importantly, severe morbidity is most often observed in certain high-risk groups including children, the elderly, those with physical/mental disability and the immunocompromised [26,27].

3. Common modes of environmental contamination

A major factor linking the transmission of both intestinal schistosomiasis and giardiasis is their transfaecal environmental contamination routes *via* the excretion of schistosome eggs (*S. mansoni*) or cysts (*G. duodenalis*) into a viable body of freshwater. Although not all eggs or cysts will successfully reach a viable freshwater habitat, in a disease-endemic setting, many environmental water bodies will undergo some extent of direct or indirect faecal contamination (Figure 1) [1,2,28,29].

<insert figure 1 near here please>

Once exposed to freshwater, referencing with Figure 1, schistosome eggs (***1***) will hatch to release free-swimming ciliated miracidia (***2***) that will then employ a range of morphological adaptations and host-seeking behaviours to locate and penetrate the soft tissues of its freshwater snail intermediate host, *Biomphalaria* spp. (***3***) [30–32]. Miracidia are ephemeral, living only a short period of time, typically less than six hours, then die as their glycogen energy reserves exhaust (Table 1).

Miracidia that successfully invade a suitable intermediate snail host metamorphose into mother sporocysts which, in turn, produce daughter sporocysts. These daughter sporocysts then differentiate upon sporogenesis, producing numerous cercariae that are shed from the snail,

approximately one month after initial invasion (***4***). Once established, cercarial production and shedding from *Biomphalaria* spp. snail hosts occurs daily and typically continues for the remainder of the snail's life. Over the course of an infected snail's life, tens of thousands of cercariae can be shed [1,33].

Shed cercariae will then go on to infect humans and other mammalian definitive hosts primarily *via* cutaneous penetration (***4***), but also occasionally through penetration of the buccal cavity when consuming contaminated water. Like miracidia, cercariae are ephemeral in freshwater as their glycogen energy reserves are quickly depleted, lasting no longer than 3 days, typically much shorter (Table 1). In addition, survival of both miracidia and cercariae is highly dependent on favourable biotic and abiotic environmental conditions. Freshwater too high in salinity or too polluted, for example, can prevent the hatching of eggs into miracidia and can be lethal to both miracidia and cercariae [31,32,34,35].

Unlike *S. mansoni*, which will asexually reproduce within an intermediate host, and although *Giardia* cysts may survive and even accumulate within certain freshwater invertebrates, *G. duodenalis* does not require an intermediate host for transmission and therefore has a direct, faecal-oral life cycle (Figure 1) [2,36]. Cysts passed in the stool are, however, extremely resilient and can remain viable in freshwater for up to eight weeks after excretion (5 and 6) (Table 1) [2,36,37]. Excystation occurs following ingestion by a suitable mammalian or fish host, typically *via* consumption of contaminated and unfiltered surface water, but also occasionally *via* the consumption of food, or use of utensils, washed using contaminated water without sufficient soaps (7 and 8) [38].

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Maintained transmission of both intestinal schistosomiasis and giardiasis is therefore dependant on the continued contamination of freshwater as well as the continued consumption of and exposure to contaminated/infested water. This may happen for a variety of reasons, for example, when it is used for sanitation purposes, income generation *via* fishing or farming and recreation [39,40]. As such, transmission of both diseases is exacerbated considerably in impoverished areas lacking adequate WASH infrastructures, such as access to functional pit latrines and clean drinking water, as well as behavioural impediments in their utilisation if available [41,42].

3.1 Zoonotic transmission and potentials

Transmission of both intestinal schistosomiasis and giardiasis is also exacerbated by a range of non-human definitive hosts acting as either major or minor reservoirs of infection (Table 2). Further to the significant health and economic impact of infection with African schistosomes and/or *Giardia* on, for example, livestock animals, animal reservoirs of both parasites also pose present challenges in controlling and reducing human transmission. The latter each follows similar routes of infection, contamination and ultimately environmental transmission [3,48,49].

<insert Table 2 near here>

To reduce human transmission effectively, animal reservoirs and the degree to which they contribute to and maintain disease transmission must therefore be carefully considered when developing, implementing and monitoring any disease control strategies. Moreover, special attention is needed on those animal hosts able to reintroduce parasites into viable bodies of freshwater following prior control or elimination campaigns [50]. Limiting contact of cattle and rodents with freshwater, for example, as well as limiting run-off from fields on which cattle

manure has been spread, and disposing of animal waste away from bodies of freshwater, are known to reduce transmission of both non-human-infecting and human-infecting *Schistosoma* species, as well as *Giardia* [50–52]. Doing so, however, can be extremely challenging to implement and maintain through time.

In light of recent findings, additional consideration should also be given to the potential emergence of schistosome hybrids and their impact on schistosomiasis transmission [53–55]. *Schistosoma mansoni*, for example, can form hybrids with its rodent sister species *S. rodhani*, which have been observed along the shoreline of East-African greater lake, Lake Victoria. However, *S. rodhaini* appears exclusive to rodents, together with the *S. mansoni-rodhaini* hybrids, but with many gaps in routine surveillance this appraisal may be incomplete [56, 57]. Uniquely among trematodes, schistosomes are dioecious so adult worms form inter-species copulatory pairs which facilitate permissive introgression(s). In nature, pre- and post-zygotic reproductive isolating barriers, such as host specificity, anatomical site of infection, distribution, mating preferences, competition and incompatibility, are thought to prevent prolific inter-species admixture. Recently, however, and owing to advanced methods of molecular analysis on schistosome larval stages from snail-intermediate and mammalian-definitive hosts, surprising inter-species hybrid forms are now being identified in several endemic African countries [56]. Such hybrids, resulting from interactions between human- and animal-infecting species, not only raise concerns about zoonotic transmission, but also the expanded host ranges and increased transmission potential acquired through heritable traits [57].

Changes to natural landscapes can readily lead to the formation of new freshwater bodies, snail habitats and multi-host transmission sites, breaking down the ecological barriers between species and leading to further inter species interactions. Though the full impact that these hybridization events may have on disease epidemiology and disease pathology is

currently unknown, hybridization certainly suggests that future schistosomiasis control may warrant an expanded One Health approach with more tailored interventions specific to local settings and schistosome epidemiology [54,56,58].

3.2. A case example of co-infection and morbidity surveillance in Uganda

Given the many similarities in disease transmission biology and inevitable high prevalence of co-endemicity throughout sub-Saharan Africa, co-infection with both intestinal schistosomiasis and giardiasis is likely commonplace, yet only little formal attention is given towards co-surveillance of both diseases. This is despite that each parasite may influence reciprocal infection susceptibilities and disease-associated pathologies, before and after anti-parasitic treatment(s). As a better known interaction of *Giardia* with a parasitic helminth is reported upon co-infection with the roundworm *Ascaris lumbricoides*, a gastrointestinal nematode responsible for ascariasis. The associated worm burden (i.e. intensity of helminth infection) is shown to play an important role in biasing Th1 and Th2 immune responses which influences an individuals' susceptibility to chronic *Giardia* infection [59]. The full extent to which mucosal Th1 and Th2 responses to infection with *S. mansoni* influence susceptibility to *Giardia* infection, however, is unknown. Nevertheless, it is clear that egg-induced perforations with mucosal bleeding, inflammation and bacterial translocation, compromise the bowel's integrity which likely increases an individual's susceptibility to chronic *Giardia* infection.

This lack of attention on co-infection and co-surveillance may be, in part, due to an unfortunate division within parasitology which often siloes macro-parasite (helminths) and micro-parasite (protists) research, as well as the exclusion of giardiasis from the NTD control agenda. Though sparse, recent epidemiological studies are now beginning to shed more detailed light on the prevalence of co-infection of intestinal schistosomiasis and *Giardia*sis, with detection of associated morbidities, throughout rural areas of sub-Saharan Africa

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[8,60,61]. A suitable example arises from two recent studies assessing co-infection in school-aged children along the shoreline of Lake Albert, Uganda, which, despite ongoing preventive chemotherapy for intestinal schistosomiasis, can still be considered hyper-endemic for *S. mansoni* today (Figure 2) [62]. Here, initial infection with *S. mansoni* occurs very soon after birth, with all ages vulnerable to infection and chronic disease [63].

<insert figure 2 near here please>

Beginning in 2015, Al-Shehri *et al.* [8] conducted a novel attempt to integrate surveillance for intestinal schistosomiasis, giardiasis and malaria using available point-of-care (POC) rapid diagnostic tests (RDTs) combined with later real-time (rt)PCR analysis of stool and finger-prick collected blood with parasite-specific TaqMan DNA® probes. This was the first attempt to quantify giardiasis with the POC Quik-Check RDT, finding 42% of children attending Runga and Bugoigo primary schools to be positive (Figure 2). Upon rtPCR analysis of ethanol preserved stool using an 18S rDNA *Giardia*-specific TaqMan® probe, up to 87.0% of children were found excreting *Giardia* DNA. Of note, the prevalence of heavy infection by real-time PCR ($Ct \leq 19$) was 19.5% and strongly associated with Quik-Check RDTs, as well as postively correlated with increasing intensities of egg-patent schistosomiasis and host anaemia [8].

Giardia species assemblages present were also later identified and characterised with specific triose phosphate isomerase (TPI) Taqman® probes and by sequence characterisation of the *β*-giardin gene [64]. Whilst less sensitive than the 18S rDNA assay, general prevalence by TPI probes was 52%, with prevalence by taxon assemblage of 8% (assemblage A), 36% (assemblage B) and 8% co-infection (A & B assemblages), and whilst assemblage B was dominant across the sample, proportions of assemblages A and B, and co-infections thereof,

varied by school and by age of child. Mixed infections were particularly common at Runga school and in children aged 6 and under. Most importantly, infection with assemblage B was associated with underweight children. The presence of each assemblage was also confirmed by sequence analysis of the *β*-giardin gene finding sub-assemblage AII and further genetic diversity within assemblage B; also of note, was the absence cryptosporidiosis, another pertinent water-borne disease, concurrently detectable by the same QuikCheck RDT.

To assess any changes through time, a repeat epidemiological survey was undertaken in 2017 which included reinspection of Bugoigo school and expanded POC testing with QuikCheck (Figure 2). The prevalence of giardiasis at Bugoigo primary school was shown to be identical with a third of children examined positive by QuikCheck, with even higher local prevalence in pre-school-age children (63%) and their mothers (55%), good evidence for pervasive nature of giardiasis across all ages. Away from the lake at Biiso and Busingiro, the prevalence of giardiasis and intestinal schistosomiasis declined, suggesting that the risk of infection is perhaps higher on the lake shoreline. This study also attempted to evaluate a new POC-RDT recombinase polymerase assay (RPA) onsite, as well as a pilot assessment of giardiasis in local livestock and companion animals [65]. Ultimately, the RPA assay did not perform as well as expected, in need of further optimisation of stool DNA extraction protocols.

4. Intestinal schistosomiasis and giardiasis: surveillance and control

Following our case example in Uganda, it is clear that with application of more sensitive diagnostic tools and inclusive surveillance protocols further interactions between these parasites will become clear. We therefore consider the following topics which ultimately require further attention as research methods develop and POC tools are rolled out.

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4.1 Diagnosis: from parasitological to molecular methods

Owing to its low cost and portability, light microscopy for the detection of *S. mansoni* ova and *G. duodenalis* cysts in faecal samples is widely regarded as the diagnostic gold standard to detect infection with both intestinal schistosomiasis and giardiasis in sub-Saharan Africa [66]. Using microscopy, routine parasitological surveillance to assess endemicity and prevalence of intestinal schistosomiasis, as well as other intestinal helminth infections, within a community is typically carried out *via* the Kato-Katz technique using faecal samples provided by school-aged children within schools. As the Kato-Katz technique is unsuitable for detection of *Giardia* cysts, only very rarely is the prevalence giardiasis or endemicity reported needing recourse to formalin/ether concentration techniques and/or (mini)FLOTAC, neither of which are straightforward or inexpensive to carry out under field conditions [8,67–69].

Moreover, whilst inexpensive and portable, sensitivity of light-microscopy is severely reduced in low-intensity and asymptomatic infections, hampering its use in areas of low disease endemicity or in areas having undergone successful disease control intervention [70–73]. For this reason, a variety of immunological and molecular diagnostic assays with improved sensitivity in low-intensity infections have been developed to detect infection of both intestinal schistosomiasis and *Giardia*sis using a range of bodily samples (Table 3).

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Though highly sensitive, immunoassays such as the enzyme-linked immunosorbent assay (ELISA) and molecular assays such as PCR/rtPCR require specialist laboratory infrastructure seldom available in disease-endemic areas, preventing their use at POC [85]. As such, a number of rapid and field-deployable RDTs, have also been developed to detect trace levels of anti-parasite antibodies, parasite-derived antigens and parasite-derived DNA within

urine and faecal samples. Some examples include straightforward lateral-flow dipsticks to detect *S. mansoni* circulating cathodic antigen within urine samples and *G. duodenalis* (and *Cryptosporidium* spp.) cyst antigen in stool samples, as well as loop-mediated isothermal amplification (LAMP) and RPA assays to detect species-specific *Schistosoma*- and *Giardia*-derived DNA within urine and stool samples [8,72,85].

Whilst POC-RDTs have many advantages over light-microscopy, microscopy remains less financially expensive to carry out and so is the favoured method of diagnosis during routine monitoring and control programmes with only limited financial resources available [69,77,81]. In addition, and though promising, assays such as LAMP and RPA to detect species-specific parasite DNA currently require further assessment and validation before their upscaled and routine use in such control programmes [65,85]. Nevertheless, continued development, assessment and validation of POC-RDT's is widely advocated as affordable and sensitive POC diagnostics, capable of detecting low-levels of infection within asymptomatic individuals able to maintain disease transmission, are sorely needed [86]. Given these challenges in reliably detecting infection within human samples, particularly in low-endemicity settings, alternative methods of detecting and monitoring disease transmission within a given foci such as parasite host surveillance and use of environmental DNA (eDNA) have also been explored.

4.2 Exploring the One Health interface with increased host surveillance

Intermediate hosts and definitive reservoir hosts, such as *Biomphalaria* freshwater snails (*S. mansoni*) and rodents or cattle (*S. mansoni* and *G. duodenalis*, respectively), offer an alternative means of detecting and monitoring disease transmission in areas where detecting transmission *via* human diagnosis may be unreliable [87]. Collecting freshwater snails capable of transmitting schistosomes and carrying out shedding analyses to assess cercarial emergence,

for example, may help identify active transmission sites [62,87]. This approach, however, can also be unreliable as very few snails are typically found to be shedding cercariae [88].

For this reason, molecular xenomonitoring approaches to detect *Schistosoma* DNA within snail hosts have also been developed and assessed [89]. Using PCR to detect *Schistosoma* DNA within snail hosts, for example, can detect prepatent infections and is not affected by diurnal fluctuations in cercarial shedding in the same way that cercarial shedding is; allowing a more reliable assessment of schistosome presence within a given locality than shedding analyses can allow. An additional advantage of molecular xenomonitoring by use of PCR is the ability to genotype parasite and snail DNA, providing valuable opportunities to better understand disease transmission and molecular epidemiology such as more reliable species identification of human-infecting cercariae and snail intermediate hosts than can be achieved using morphological analysis and the detection of *Schistosoma* hybridisation events [53]. Currently, however, mass collection and molecular screening of freshwater snail hosts using PCR remains logistically, technically and financially demanding, and so development of a high-throughput methodology, possibly incorporating use of rapid and point-of-care DNA amplification technologies such as LAMP or RPA, or pooling of snail samples, should also be further explored and assessed [90,91].

Similarly, molecular detection of *S. mansoni* and *G. duodenalis* DNA using PCR within faecal samples collected from definitive reservoir hosts capable of perpetuating transmission, such as rodents and cattle, has also been used to identify and monitor disease transmission and to better understand wild-type *Schistosoma* hybridisation events and zoonotic transmission of human-infecting *G. duodenalis* [46,53,92–94]. Again, however, this approach too requires significant financial and technological resources and so is unlikely to be widely integrated into control programmes undertaken within low-resource areas such as rural regions of sub-Saharan

Africa without further development and use of field-deployable DNA amplification technologies.

4.3 Detecting parasitic contamination by environmental DNA (eDNA) analysis

Assessing and monitoring disease transmission within a given foci *via* the detection of parasite-derived DNA within the environment (eDNA) rather than, or in conjunction with, using human bodily samples, has too been explored with respect to a range of waterborne pathogens, including both schistosomiasis and giardiasis [95–97]. Dependence of both parasites on freshwater provides an ideal target for sample collection and assessment using PCR/rtPCR, LAMP or RPA assays [84,98,99]. In addition, collection of water samples to detect eDNA derived from *Schistosoma* freshwater snail hosts to identify and monitor the presence of snail species capable of transmitting infection within a given waterbody has also been assessed [100,101].

Again, though promising, the upscaled and routine use of molecular assays to detect parasite- and/or parasite host-derived eDNA remains beyond the financial reach of most LMIC control programmes and too requires further methodological development, assessment and validation. Nevertheless, continued development of this approach to better understand the potential of eDNA as an effective monitoring tool and to reduce associated financial costs has been encouraged [77]. In particular, and like molecular xenomonitoring approaches, the monitoring of eDNA to identify disease transmission may prove extremely useful in areas of low disease endemicity where identifying infection within individual patients may be challenging.

4.4 Access to treatment and large-scale campaigns

In areas where schistosomiasis transmission is identified, preventive chemotherapy through repeated mass drug administration (MDA) of the donated anthelmintic drug praziquantel (40 mg /kg body weight) is the principal strategy for disease control [102]. Because the highest burden of infection is typically seen in children and young adolescents, MDA is customarily carried out within schools but aims to limit overall transmission within a community through a reduced human reservoir of infection whilst also reducing overall disease morbidity [103]. Though it's mechanism of action is not currently fully understood, significant reductions in disease prevalence and morbidity have been seen globally since MDA programmes began in 2001 [104]. Reinfection of schistosomiasis following treatment is, however, commonplace owing to a communities' reliance on freshwater and so MDA must be repeated annually or biannually, depending on disease prevalence, to achieve a sustained impact.

Severe adverse effects are seen only very rarely when distributing praziquantel, making it well suited for mass distribution. Praziquantel, however, typically does not achieve 100% infection clearance and because dosing is usually estimated based only on height, and so does not account for differences in body mass, treatment success can vary between individuals meaning many are still able to continue maintaining transmission [105]. In addition, and whilst local school-systems provide a viable means of mass-distributing praziquantel, important human reservoirs of infection, including pre-school-aged children and adults, typically remain untreated [63,106].

The need for repeated annual or biannual distribution of MDA in this way has also raised regular concerns about the development of praziquantel resistance in schistosomes; particularly as there is currently no-known efficacious alternative treatment to replace praziquantel if *Schistosoma* populations were to become more drug-tolerant or resistant [107,108]. A significant reduction in praziquantel efficacy, identified by a decreased reduction

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in *Schistosoma* egg-output from infected individuals pre- and post-praziquantel treatment, has already been reported in *S. mansoni* populations within many communities across sub-Saharan Africa that have undergone repeated rounds of MDA [105]. This reduced efficacy may be a direct result of selection pressure placed on schistosomes during repeated and prolonged MDA campaigns; highlighting an urgent need to consider alternative methods of disease control outside of MDA.

A variety of drugs can be used to treat *Giardia*sis [109,110]. Of these, metronidazole is the most predominantly used and most thoroughly studied owing to its straightforward oral administration and relatively low price. Like with schistosomiasis, reinfection with giardiasis is also commonplace, however, repeated mass drug administration to alleviate giardiasis transmission is not seen as a feasible strategy because the drug is not currently involved in any donation scheme, severe adverse effects of treatment are often seen, and metronidazole has only limited efficacy in clearing infection [109]. As an example, it has been reported that just one course of treatment has only an approximately 60% clearance rate and so repeated treatment is needed to significantly clear infection [111]. Repeated treatment, however, not only significantly increases the likelihood of severe adverse events but is difficult to carry out during MDA campaigns [3]. In addition, the potential emergence of giardiasis resistance to treatment with metronidazole has also recently been reported, and whilst alternative and more efficacious chemotherapies, such as tinidazole, exist, these are typically far more expensive and too can cause adverse events [109,111]. Albendazole, a broad-spectrum and efficacious anthelmintic treatment used in MDA campaigns to reduce transmission of soil-transmitted helminth and some filarial nematode infections, can also be used to treat *Giardia*sis [109,112,113]. To significantly reduce *Giardia* infection, however, a minimum dosage of 200 – 800 mg/day albendazole is needed for at least three concurrent days which, again, is difficult

to carry out in the context of MDA campaigns and, by having limited donated stocks, also diminishes albendazole availability for anti-helminth control programmes [109,110].

Though treatment of schistosomiasis and giardiasis using praziquantel and metronidazole are important components of disease control, it is now widely accepted that alternative methods of control to reduce transmission and overall prevalence must be implemented in tandem with treatment campaigns if disease elimination targets are to be met. One such example is the implementation of WASH initiatives into communities where both diseases are endemic. The extent to which WASH provision, when used in conjunction with MDA, can successfully reduce schistosomiasis transmission is now begging to be understood, and although only minimal data has been reported on the impact of WASH provision on *Giardia*sis transmission in sub-Saharan Africa, it is widely assumed that improved WASH infrastructure would help significantly reduce *Giardia*sis transmission [114–116].

4.5 A One Health approach to WASH

WASH provision and infrastructure is extremely inadequate throughout many areas of rural sub-Saharan Africa [117]. In 2012, the World Health Assembly (WHA) formally advocated for the integration of WASH provision and education initiatives into amenable NTD control and elimination programmes; subsequently publishing guidance on ways in which these can be integrated [118,119]. Since, much attention has been given towards how WASH initiatives can be tailored for use, specifically, in schistosomiasis control programmes and the impact such initiatives have had when used in tandem with routine strategies such as preventive chemotherapy [120–122].

WASH initiatives relevant to schistosomiasis control, such as the adequate provision of safe drinking water, fully functional and properly maintained pit latrines and improved community hygiene education, reduce disease transmission by minimising the need for infected

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individuals and animals to contaminate viable bodies of freshwater and come into contact with contaminated water, as well as helping communities better understand human and non-human disease transmission [122,123] (Figure 3).

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Despite numerous clear advantages of implementing WASH initiatives on reducing schistosomiasis transmission and despite many similarities between schistosomiasis and giardiasis with regard to disease transmission biology and epidemiology, surprisingly little attention has been given to the impact of improved WASH provision and education on giardiasis transmission in sub-Saharan Africa [42]. This oversight presents not only a missed opportunity with regards to better understanding, and reducing, giardiasis transmission and its associated pathological impact on some of the world's most disadvantaged communities, but also presents the question; why is giardiasis ignored in schistosomiasis, and NTD, monitoring and control programmes?

5. Why is giardiasis ignored in monitoring and control of intestinal schistosomiasis?

Like intestinal schistosomiasis and other NTDs, giardiasis is widely prevalent throughout many rural and impoverished regions of sub-Saharan Africa, intrinsically linked to contact with contaminated and unsafe water in areas lacking adequate water, sanitation and hygiene (WASH) infrastructure, and disproportionately afflicts hundreds of millions of children under the age of 15 years [2,40]. In spite of this, giardiasis is not currently considered a NTD and receives only relatively little attention with regard to disease control, surveillance and elimination throughout sub-Saharan Africa.

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Research funding opportunities for NTDs are limited. One way in which the impact of NTD control programmes can be significantly increased is by appropriate integration with other disease surveillance, control, research and policy efforts. Successful examples of this integrated One Health approach can be seen when integrating lymphatic filariasis surveillance and elimination efforts into malaria elimination programmes, as well as by integrating soil-transmitted helminth and schistosomiasis control and elimination efforts [121,124–127].

6. Intestinal schistosomiasis and giardiasis: towards a One Health approach

In keeping with this integrated One Health approach, here, we propose a variety of ways in which the transmission of, and pathologies associated with, co-infection of intestinal schistosomiasis and giardiasis can be better understood, monitored and reduced *via* the integration of giardiasis control efforts into existing schistosomiasis control programmes. These include:

- Integrating population screening of giardiasis endemicity and infection prevalence into existing schistosomiasis control programmes by utilising stool samples used for the microscopic detection of *S. mansoni* and other intestinal parasite eggs to also identify, record and report community levels of *Giardia* cysts, for example, within school-aged children using existing microscopy or POC-RDTs.
- The continued development, assessment and application of sensitive and straightforward POC-RDTs capable of detecting low-levels of *Giardia* infection within asymptomatic individuals capable of maintaining transmission of both parasites.
- Development and application of sensitive molecular assays to detect trace levels of species/assemblage-specific parasite DNA within freshwater snail intermediate hosts of human-infecting *Schistosoma*, and within faecal samples from non-human animal definitive hosts of both diseases.

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- Development and application of sensitive molecular assays to detect trace levels of species/assemblage-specific parasite DNA most likely from human-infecting *Schistosoma* cercariae and *Giardia* cysts within water samples easily collected from viable transmission sites.
- The up-scaled provision of WASH infrastructures with tailored education initiatives to afflicted communities to reduce environmental contamination events and to reduce contact with contaminated water; simultaneously reducing transmission of each diseases.
- Tailoring control measures to specific local settings with particular focus on controlling any potential non-human *S. mansoni* and *Giardia* reservoirs of infection, such as rodents and cattle.
- Monitoring *Giardia* disease prevalence and associated morbidities in tandem with schistosomiasis surveillance in school-aged children following any control programme intervention to better understand how giardiasis transmission and related pathologies can be reduced.
- An increased focus on a mechanistic understanding how the transmission of intestinal schistosomiasis and giardiasis, as well as immune responses and morbidities related to both diseases, interact and are potentially exacerbated by co-infection.

7. Conclusions

Here, we have highlighted the many similarities between intestinal schistosomiasis and *Giardia*sis with regard to disease transmission biology, epidemiology, surveillance and control. In addition, future steps needed to further advance an integrated One Health approach in intestinal schistosomiasis and giardiasis co-infection surveillance, control and elimination strategies, are also outlined. In adopting this One Health approach and by integrating giardiasis surveillance, control and elimination efforts into existing schistosomiasis elimination

programmes, not only can the debilitating pathological impacts of intestinal schistosomiasis/ giardiasis co-infection be better understood, but a reduction in co-infection and concurrent reduction in disease-related morbidities experienced by the worlds most disadvantaged communities can also be achieved.

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Concept of the study (JA, ALB & JRS). Literature searching and review (JA, LO'H, SS, MS). Fieldwork undertaken by (JA, HAL-S, NBK, AA, Mad, Mar, EJLaC, ALB, JRS). Molecular analyses performed by (HAL-S, TB, BLW). All authors have read and agreed to the published version of the manuscript.

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Abbreviations:

eDNA: environmental DNA

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ELISA: enzyme-linked immunosorbent assay

FGS: female genital schistosomiasis

LAMP: loop-mediated isothermal reaction

LMIC: lower- middle-income country

MDA: mass drug administration

NTD: neglected tropical disease

PCR: polymerase chain reaction

POC: point-of-care

rtPCR: real-time polymerase chain reaction

RDT: rapid diagnostic test

RPA: recombinase polymerase amplification

Th1: T-helper 1

Th2: T-helper 2

WASH: water, sanitation and hygiene

WHA: world health assembly

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Figure legends

Figure 1: Primary transmission routes of *S. mansoni* (red) and *G. duodenalis* (blue). Infection with *S. mansoni* cercariae (4) will also occasionally occur through penetration of the buccal cavity when consuming contaminated water. Adapted from [28,29].

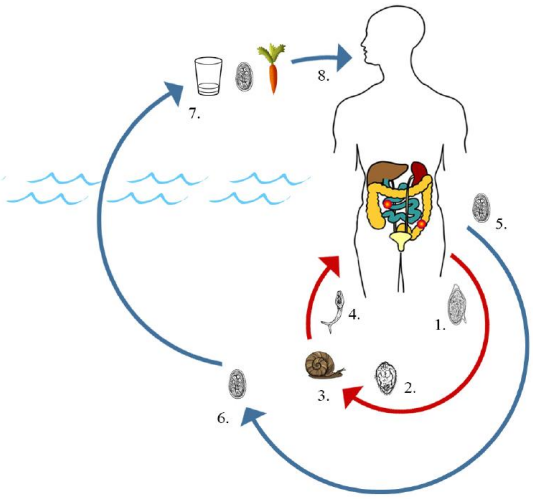
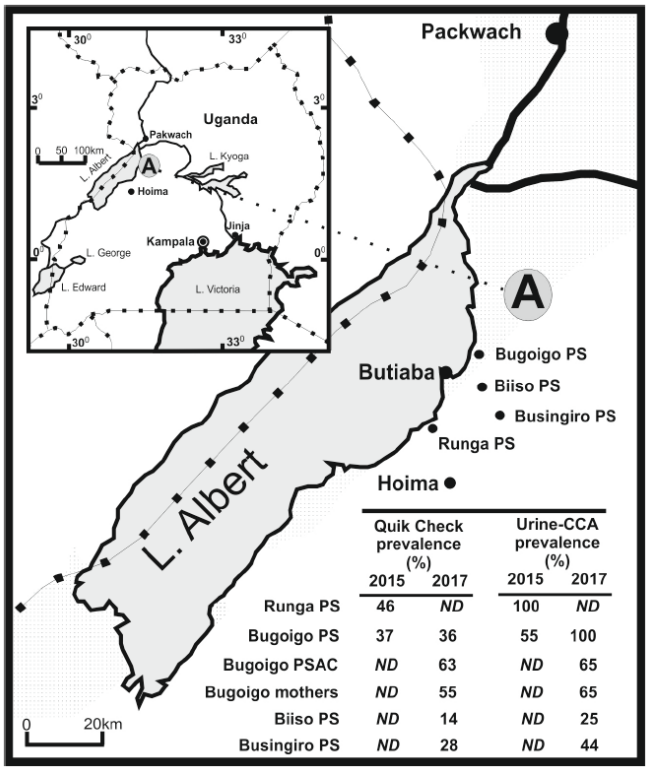


Figure 2: High prevalence of intestinal schistosomiasis (assessed using Urine-CCA POC-RDT) and giardiasis co-infection (assessed using QuikCheck POC-RDT) in school-aged children across multiple communities along the shoreline of Lake Albert, Uganda in 2015 and 2017 [8].



	Quik Check prevalence (%)		Urine-CCA prevalence (%)	
	2015	2017	2015	2017
Runga PS	46	*ND*	100	*ND*
Bugoigo PS	37	36	55	100
Bugoigo PSAC	*ND*	63	*ND*	65
Bugoigo mothers	*ND*	55	*ND*	65
Biiso PS	*ND*	14	*ND*	25
Busingiro PS	*ND*	28	*ND*	44

Figure 3: Examples of water, sanitation and hygiene (WASH) initiatives implemented to prevent the contamination of freshwater with *S. mansoni* eggs and *G. duodenalis* cysts, as well as to prevent contact with and consumption of contaminated water. Adapted from [28,29].

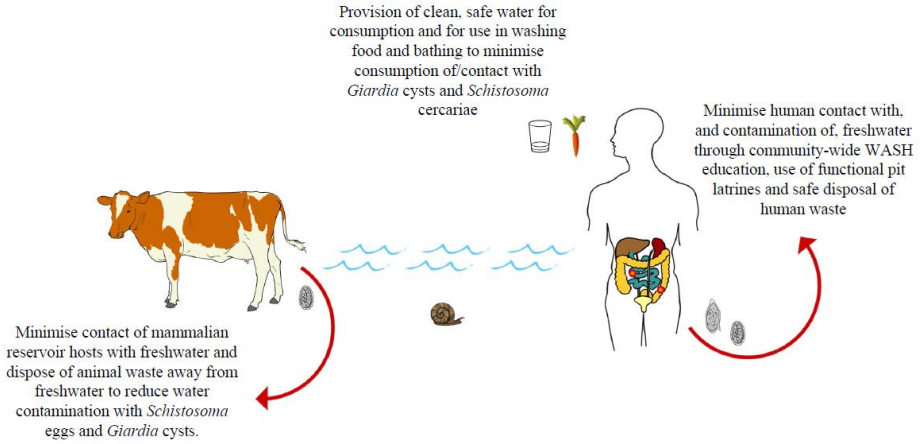


Table 1: Time spent by human-infecting *Schistosoma mansoni* and *Giardia* species in freshwater.

Species	Life-stage	Time viable in freshwater	Reference(s)
S. mansoni	Miracidia	<6 hours	[30–32]
	Cercariae	~1-3 days	[31,32,34,35]
G. duodenalis **(Assemblages A and B)**	Cyst	Up to eight weeks	[2,36,37]

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Table 2: Reservoir hosts of *S. mansoni* and *G. duodenalis* (assemblages A and B). *'+' denotes known-reservoir of infection; '-' denotes no known reservoir of infection.*

	Humans	Non-human primates	Ruminants	Rodents	Other mammals	Fish	References
S. mansoni	+	+	-	+	-	-	[43,44]
***G. duodenalis* (assemblage A)**	+	+	+	+	+	+	[45–47]
***G. duodenalis* (assemblage B)**	+	+	+	+	+	+	[45–47]

Table 3: Overview of diagnostic assays to detect infection with *Schistosoma mansoni* and *Giardia duodenalis. Point-of-care, rapid-diagnostic tests (POC-RDT's) are underlined.*

	Direct diagnosis	**Immunoassays**	**Molecular diagnosis**	**Other**
S. mansoni	Identification of ova in concentrated faecal smear *via* microscopy [74]	Detection of species-specific antigens and/or antibodies within urine, faecal or blood samples using ELISA or lateral-flow test strips [75,76]	Detection and amplification of species-specific DNA within urine or faecal samples using PCR/rtPCR, LAMP or RPA [75,77,78]	Detection of faecal occult blood and faecal calprotectin in faecal samples [79]
G. duodenalis	Identification of cysts in concentrated faecal smear *via* microscopy [69,80]	Detection of species-specific antigens and/or antibodies within faecal samples using ELISA or lateral-flow test strips [8,81,82]	Detection and amplification of species-specific DNA within faecal samples using PCR/rtPCR, LAMP or RPA [65,83,84]	