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

Authors

John Archer¹,², Lisa O’Halloran², Hajri Al-Shehri²,³, Shannan Summers⁴, Tapan Battacharyya⁴, Narcis B. Kabaterine⁵, Aaron Atuhaire⁵, Moses Adriko⁵, Moses Arianaitwe⁵, Martyn Stewart², E. James LaCourse², Bonnie L. Webster¹, Amaya L. Bustinduy⁴, J. Russell Stothard²,*

¹. Wolfson Wellcome Biomedical Laboratories, Department of Zoology, Natural History Museum, Cromwell Road, London SW7 5BD, UK.
². Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK.
³. Department of Tropical Infectious Diseases, Ministry of Health, Asir District. Kingdom of Saudi Arabia.
⁴. Department of Clinical Research, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK.
⁵. Vector Control Division, Ministry of Health, Kampala, Uganda

*Corresponding author (JRS): russell.stothard@lstmed.ac.uk; +44 0151 7053724.
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, disproportionately 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 hepatoporal 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* 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
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 (*I*) 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].

<please insert Table 1 near here>
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].

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 Giardiasis, with detection of associated morbidities, throughout rural areas of sub-Saharan Africa.
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 Bugsigo 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 ≤ 19) was 19.5% and strongly associated with Quik-Check RDTs, as well as positively 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.
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 *Giardiasis* using a range of bodily samples (Table 3).

*please insert Table 3 near here*

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 its 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
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 *Giardiasis* [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 *Giardiasis* [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 Giardiasis transmission in sub-Saharan Africa, it is widely assumed that improved WASH infrastructure would help significantly reduce Giardiasis 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
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).

<please insert figure 3 near here>

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.
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.
• 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 *Giardiasis* 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.

**Author Contributions:**

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.

**Funding:** JA is funded by a MRC-DTP studentship. Fieldwork reported here was supported by HEFC and a PhD studentship awarded by the Ministry of Health, Saudi Arabia to HAl-S.

**Conflicts of Interest:** The authors declare that they have no competing interests.

**Ethical Standards:** Approvals for the work conducted in Uganda were granted by The Ugandan Council for Science and Technology and the Liverpool School of Tropical Medicine, UK.

**Acknowledgments:** JA would like to thank Mr Michael Fowler of EH Studios for support with figures.

**Abbreviations:**

eDNA: environmental DNA
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

References

1. Colley, D.G.; Bustinduy, A.L.; Secor, W.E.; King, C.H. Human schistosomiasis. *Lancet* 2014, 383, 2253–2264, doi:10.1016/S0140-6736(13)61949-2.

2. Thompson, R.C.A.; Reynoldson, J.A.; Sciences, B. *Giardia* and *Giardiasis*. 1993.

3. Squire, S.A.; Ryan, U. *Cryptosporidium* and *Giardia* in Africa: current and future challenges. *Parasites and Vectors* 2017, 10, 1–32, doi:10.1186/s13071-017-2111-y.

4. World Health Organisation. WASH and Health Working Together, 2018. Geneva, Switzerland: World Health Organisation. Available online:
5. Waite, R.C.; Woods, G.; Velleman, Y.; Freeman, M.C. Collaborating to develop joint water, sanitation and hygiene (WASH) and neglected tropical disease (NTD) sector monitoring: An expert consultation. *Int. Health* 2017, 9, 215–225, doi:10.1093/inthealth/ihx008.

6. Chifunda, K.; Kelly, P. Parasitic infections of the gut in children. *Paediatr. Int. Child Health* 2019, 39, 65–72, doi:10.1080/20469047.2018.1479055.

7. Savioli, L.; Smith, H.; Thompson, A. *Giardia* and *Cryptosporidium* join the “Neglected Diseases Initiative.” *Trends Parasitol.* 2006, 22, 203–208, doi:10.1016/j.pt.2006.02.015.

8. Al-Shehri, H.; Stanton, M.C.; LaCourse, J.E.; Atuhaire, A.; Arinaitwe, M.; Wamboko, A.; Adriko, M.; Kabaterine, N.B.; Stothard, J.R. An extensive burden of giardiasis associated with intestinal schistosomiasis and anaemia in school children on the shoreline of Lake Albert, Uganda. *Trans. R. Soc. Trop. Med. Hyg.* 2016, 110, 597–603, doi:10.1093/trstmh/trw072.

9. La Hoz, R.M.; Morris, M.I. Intestinal parasites including *Cryptosporidium*, *Cyclospora*, *Giardia*, and *Microsporidia, Entamoeba histolytica, Strongyloides*, Schistosomiasis, and *Echinococcus*: Guidelines from the American Society of Transplantation Infectious Diseases Community of Pract. *Clin. Transplant.* 2019, 33, 1–16, doi:10.1111/ctr.13618.

10. Zhou, X.N. Prioritizing research for “One health - One world.” *Infect. Dis. Poverty* 2012, 1, 1–5, doi:10.1186/2049-9957-1-1.

11. Mackenzie, J.S.; Jeggo, M. *One Health and Zoonoses*; 2019; ISBN 9783039212958; https://doi.org/10.3390/books978-3-03921-296-5.
12. Mitchell, K.M.; Mutapi, F.; Savill, N.J.; Woolhouse, M.E.J. Protective immunity to Schistosoma haematobium infection is primarily an anti-fecundity response stimulated by the death of adult worms. Proc. Natl. Acad. Sci. U. S. A. 2012, 109, 13347–13352, doi:10.1073/pnas.1121051109.

13. Mutapi, F.; Mduluza, T.; Gomez-Escobar, N.; Gregory, W.F.; Fernandez, C.; Midzi, N.; Maizels, R.M. Immuno-epidemiology of human Schistosoma haematobium infection: Preferential IgG3 antibody responsiveness to a recombinant antigen dependent on age and parasite burden. BMC Infect. Dis. 2006, 6, 1–11, doi:10.1186/1471-2334-6-96.

14. Woolhouse, M.E.J.; Taylor, P.; Matanhire, D.; Chandiwana, S.K. Acquired immunity and epidemiology of Schistosoma haematobium. Nature 1991, 351, 757–759, doi:10.1038/351757a0.

15. Bustinduy, A.L.; Edielu, A.; Sturt, A.S. Could This Child Have Schistosomiasis?: When to Suspect It and What to Do About It. Pediatr. Infect. Dis. J. 2020, 39, e125–e129, doi:10.1097/INF.0000000000002706.

16. Costain, A.H.; MacDonald, A.S.; Smits, H.H. Schistosome Egg Migration: Mechanisms, Pathogenesis and Host Immune Responses. Front. Immunol. 2018, 9, 3042, doi:10.3389/fimmu.2018.03042.

17. Olveda, David Bilharzia: Pathology, Diagnosis, Management and Control. Trop. Med. Surg. 2013, 01, 1–19, doi:10.4172/2329-9088.1000135.

18. Heyworth, M.F. Giardia duodenalis genetic assemblages and hosts. Parasite 2016, 23, doi:10.1051/parasite/2016013.

19. Halliez, M.C.M.; Buret, A.G. Extra-intestinal and long term consequences of Giardia duodenalis infections. World J. Gastroenterol. 2013, 19, 8974–8985, doi:10.3748/wjg.v19.i47.8974.
20. Nkrumah, B.; Nguah, S. *Giardia lamblia*: A major parasitic cause of childhood diarrhoea in patients attending a district hospital in Ghana. *Parasites and Vectors* 2011, 4, 1–7, doi:10.1186/1756-3305-4-163.

21. Bartelt, L.A.; Sartor, R.B. Advances in understanding *Giardia*: Determinants and mechanisms of chronic sequelae. *F1000Prime Rep.* 2015, 7, 1–14, doi:10.12703/P7-62.

22. Naess, H.; Nyland, M.; Hausken, T.; Follestad, I.; Nyland, H.I. Chronic fatigue syndrome after *Giardia* enteritis: Clinical characteristics, disability and long-term sickness absence. *BMC Gastroenterol.* 2012, 12, doi:10.1186/1471-230X-12-13.

23. Fink, M.Y.; Singer, S.M. The Intersection of Immune Responses, Microbiota, and Pathogenesis in *Giardiasis*. *Trends Parasitol.* 2017, 33, 901–913, doi:10.1016/j.pt.2017.08.001.

24. Keselman, A.; Li, E.; Maloney, J.; Singer, S.M. The microbiota contributes to CD8+ T cell activation and nutrient malabsorption following intestinal infection with *Giardia duodenalis*. * Infect. Immun.* 2016, 84, 2853–2860, doi:10.1128/IAI.00348-16.

25. Allain, T.; Amat, C.B.; Motta, J.P.; Manko, A.; Buret, A.G. Interactions of *Giardia* sp. with the intestinal barrier: Epithelium, mucus, and microbiota. *Tissue Barriers* 2017, 5, 1–16, doi:10.1080/21688370.2016.1274354.

26. Mmbaga, B.T.; Houpt, E.R. *Cryptosporidium* and *Giardia* Infections in Children: A Review. *Pediatr. Clin. North Am.* 2017, 64, 837–850, doi:10.1016/j.pcl.2017.03.014.

27. Lane, S.; Lloyd, D. Current trends in research into the waterborne parasite *Giardia*. *Crit. Rev. Microbiol.* 2002, 28, 123–147, doi:10.1080/1040-840291046713.

28. Centers for Disease Control and Prevention (CDC), USA: Schistosomiasis, About, Life Cycle. Available online: https://www.cdc.gov/parasites/schistosomiasis/biology.html (accessed on Jul 1, 2020).
29. Centers for Disease Control and Prevention (CDC), USA: *Giardiasis*, About, Life Cycle. Available online: https://www.cdc.gov/dpdx/Giardiasis/index.html#:~:text=The spectrum varies from asymptomatic carriage to severe, include diarrhea%2C abdominal pain%2C bloating%2C nausea%2C and vomiting. (accessed on Jul 1, 2020).

30. Wright, C.. *Flukes and Snails. Chapter 4: Fluke life-cycles*; George Allen and Unwin LTD. London., 1971;

31. Galaktionov, K.V., Dobrovolskij, A.. *The Biology and Evolution of Trematodes. An Essay on the Biology, Morphology, Life Cycles, Transmission, and Evolution of Digenetic Trematodes. Chapter 2: The Trematode Life Cycle as a System of Adaptations*; Dordrecht, Boston: Kluwer Academic Publishers, 2003;

32. Fried, B., Graczyk, T.. *Advances in Trematode Biology. Chapter 7: Host Recognition by Trematode Miracidia and Cercariae*; CRC Press. New York, 1997;

33. Lockyer, A.E.; Jones, C.S.; Noble, L.R.; Rollinson, D. Trematodes and snails: An intimate association. *Can. J. Zool.* 2004, 82, 251–269, doi:10.1139/z03-215.

34. Théron, A. *Chronobiology of trematode cercarial emergence: From data recovery to epidemiological, ecological and evolutionary implications*; Elsevier Ltd, 2015; Vol. 88;.

35. Frandsen, F. Christensen, N. An Introductory Guide to the Identification of Cercariae From African Freshwater Snails With Special Reference to Cercariae of Trematode Species of Medical and Veterinary Importance. *Acta Trop.* 1984, doi:10.5169/seals-313293.

36. Einarsson, E.; Ma’ayeh, S.; Svärd, S.G. An up-date on *Giardia* and *Giardiasis*. *Curr. Opin. Microbiol.* 2016, 34, 47–52, doi:10.1016/j.mib.2016.07.019.

37. Ankarklev, J.; Jerlström-Hultqvist, J.; Ringqvist, E.; Troell, K.; Svärd, S.G. Behind the smile: Cell biology and disease mechanisms of *Giardia* species. *Nat. Rev. Microbiol.*
2010, 8, 413–422, doi:10.1038/nrmicro2317.

38. Mohammed Mahdy, A.K.; Lim, Y.A.L.; Surin, J.; Wan, K.L.; Al-Mekhlafi, M.S.H. Risk factors for endemic giardiasis: highlighting the possible association of contaminated water and food. *Trans. R. Soc. Trop. Med. Hyg.* **2008**, *102*, 465–470, doi:10.1016/j.trstmh.2008.02.004.

39. Huang, Y.; Manderson, L. Schistosomiasis and the social patterning of infection. *Acta Trop.* **1992**, *51*, 175–194, doi:10.1016/0001-706X(92)90037-X.

40. Ahmed, S.A.; Guerrero Flórez, M.; Karanis, P. The impact of water crises and climate changes on the transmission of protozoan parasites in Africa. *Pathog. Glob. Health* **2018**, *112*, 281–293, doi:10.1080/20477724.2018.1523778.

41. Esrey, S.A.; Collett, J.; Miliotis, M.D.; Koornhof, H.J.; Makhale, P. The risk of infection from *Giardia lamblia* due to drinking water supply, use of water, and latrines among preschool children in rural Lesotho. *Int. J. Epidemiol.* **1989**, *18*, 248–253, doi:10.1093/ije/18.1.248.

42. Campbell, S.J.; Nery, S. V.; D’Este, C.A.; Gray, D.J.; McCarthy, J.S.; Traub, R.J.; Andrews, R.M.; Llewellyn, S.; Vallely, A.J.; Williams, G.M.; et al. Water, sanitation and hygiene related risk factors for soil-transmitted helminth and *Giardia duodenalis* infections in rural communities in Timor-Leste. *Int. J. Parasitol.* **2016**, *46*, 771–779, doi:10.1016/j.ijpara.2016.07.005.

43. Martins, A. V. Non-human vertebrate hosts of *Schistosoma haematobium* and *Schistosoma mansoni*. *Bull. World Health Organ.* **1958**, *18*, 931–944.

44. Standley, C.J., Dobson, A.P., Stothard, R.J. *Out of Animals and Back Again: Schistosomiasis as a Zoonosis in Africa*. Schistosomiasis; InTech: ISBN: 978-953-307-852-6., 2012;

45. Ryan, U.; Cacciò, S.M. Zoonotic potential of *Giardia*. *Int. J. Parasitol.* **2013**, *43*, 943–
Yaoyu, F.; Xiao, L. Zoonotic potential and molecular epidemiology of *Giardia* species and *Giardiasis*. *Clin. Microbiol. Rev.* **2011**, *24*, 110–140, doi:10.1128/CMR.00033-10.

Thompson, R.C.A. The zoonotic significance and molecular epidemiology of *Giardia* and *Giardiasis*. *Vet. Parasitol.* **2004**, *126*, 15–35, doi:10.1016/j.vetpar.2004.09.008.

Robinson, M.W.; Dalton, J.P. Zoonotic helminth infections with particular emphasis on fasciolosis and other trematodiases. *Philos. Trans. R. Soc. B Biol. Sci.* **2009**, *364*, 2763–2776, doi:10.1098/rstb.2009.0089.

Chomel, B.B. Control and prevention of emerging parasitic zoonoses. *Int. J. Parasitol.* **2008**, *38*, 1211–1217, doi:10.1016/j.ijpara.2008.05.001.

Hanelt, B.; Mwangi, I.N.; Kinuthia, J.M.; Maina, G.M.; Agola, L.E.; Mutuku, M.W.; Steinauer, M.L.; Agwanda, B.R.; Kigo, L.; Mungai, B.N.; et al. Schistosomes of small mammals from the Lake Victoria Basin, Kenya: New species, familiar species, and implications for schistosomiasis control. *Parasitology* **2010**, *137*, 1109–1118, doi:10.1017/S0031182010000041.

De Bont, J.; Vercruysse, J. The epidemiology and control of cattle schistosomiasis. *Parasitol. Today* **1997**, *13*, 255–262, doi:10.1016/S0169-4758(97)01057-0.

Olson, M.E.; O’Handley, R.M.; Ralston, B.J.; McAllister, T.A.; Thompson, R.C.A. Update on *Cryptosporidium* and *Giardia* infections in cattle. *Trends Parasitol.* **2004**, *20*, 185–191, doi:10.1016/j.pt.2004.01.015.

Savassi, B.A.E.S.; Mouahid, G.; Lasica, C.; Mahaman, S.D.K.; Garcia, A.; Courtin, D.; Allienne, J.F.; Ibikounlé, M.; Moné, H. Cattle as natural host for *Schistosoma haematobium* (Bilharz, 1852) Weinland, 1858 × *Schistosoma bovis* Sonsino, 1876 interactions, with new cercarial emergence and genetic patterns. *Parasitol. Res.* **2020**, doi:10.1007/s00436-020-06709-0.
54. Sene-Wade, M.; Marchand, B.; Rollinson, D.; Webster, B.L. Urogenital schistosomiasis and hybridization between *Schistosoma haematobium* and *Schistosoma bovis* in adults living in Richard-Toll, Senegal. *Parasitology* **2018**, *145*, 1723–1726, doi:10.1017/S0031182018001415.

55. Standley, C.J.; Stothard, J.R. DNA Barcoding of Schistosome Cercariae Reveals a Novel Sub-Lineage within *Schistosoma rodhaini* From Ngamba Island Chimpanzee Sanctuary, Lake Victoria. *J. Parasitol.* **2012**, *98*, 1049–1051, doi:10.1645/ge-3091.1.

56. Catalano, S.; Sène, M.; Diouf, N.D.; Fall, C.B.; Borlase, A.; Léger, E.; Bâ, K.; Webster, J.P. Rodents as natural hosts of zoonotic schistosoma species and hybrids: An epidemiological and evolutionary perspective from West Africa. *J. Infect. Dis.* **2018**, *218*, 429–433, doi:10.1093/infdis/jiy029.

57. Steinauer, M.L.; Hanelt, B.; Mwangi, I.N.; Maina, G.M.; Agola, L.E.; Kinuthia, J.M.; Mutuku, M.W.; Mungai, B.N.; Wilson, W.D.; Mkoji, G.M.; et al. Introgressive hybridization of human and rodent schistosome parasites in western Kenya. *Mol. Ecol.* **2008**, *17*, 5062–5074, doi:10.1111/j.1365-294X.2008.03957.x.

58. Huyse, T.; Webster, B.L.; Geldof, S.; Stothard, J.R.; Diaw, O.T.; Polman, K.; Rollinson, D. Bidirectional introgressive hybridization between a cattle and human schistosoma species. *PLoS Pathog.* **2009**, *5*, doi:10.1371/journal.ppat.1000571.

59. Hagel, I.; Cabrera, M.; Puccio, F.; Santaella, C.; Buvat, E.; Infante, B.; Zabala, M.; Cordero, R.; Di Prisco, M.C. Co-infection with *Ascaris lumbricoides* modulates protective immune responses against *Giardia duodenalis* in school Venezuelan rural children. *Acta Trop.* **2011**, *117*, 189–195, doi:10.1016/j.actatropica.2010.12.001.

60. Coulibaly, G.; Ouattara, M.; Dongo, K.; Hürlimann, E.; Bassa, F.K.; Koné, N.; Essé, C.; Yapi, R.B.; Bonfoh, B.; Utzinger, J.; et al. Epidemiology of intestinal parasite infections in three departments of south-central Côte d’Ivoire before the
implementation of a cluster-randomised trial. *Parasite Epidemiol. Control* **2018**, *3*, 63–76, doi:10.1016/j.parepi.2018.02.003.

61. Fofana, H.K.M.; Schwarzkopf, M.; Doumbia, M.N.; Saye, R.; Nimmesgern, A.; Landouré, A.; Traoré, M.S.; Mertens, P.; Utzinger, J.; Sacko, M.; et al. Prevalence of *Giardia intestinalis* infection in schistosomiasis-endemic areas in south-central Mali. *Trop. Med. Infect. Dis.* **2019**, *4*, 4–9, doi:10.3390/tropicalmed4020086.

62. STOTHARD, J.R.; ARCHER, J.; GYAPONG, M.; TCHUEM-TCHUENTÉ, L.A.; BUSTINDUY, A.L.; KABATERINE, N.B.; AL-SHEHRI, H. A centenary of Robert T. Leiper’s lasting legacy on schistosomiasis and a COUNTDOWN on control of neglected tropical diseases. *Parasitology* **2016**, *144*, 1602–1612, doi:10.1017/s0031182016000998.

63. Stothard, J.R.; Sousa-Figueiredo, J.C.; Betson, M.; Bustinduy, A.; Reinhard-Rupp, J. Schistosomiasis in African infants and preschool children: Let them now be treated! *Trends Parasitol.* **2013**, *29*, 197–205, doi:10.1016/j.pt.2013.02.001.

64. Al-Shehri, H.; James LaCourse, E.; Klimach, O.; Kabatereine, N.B.; Stothard, J.R. Molecular characterisation and taxon assemblage typing of giardiasis in primary school children living close to the shoreline of Lake Albert, Uganda. *Parasite Epidemiol. Control* **2019**, *4*, e00074, doi:10.1016/j.parepi.2018.e00074.

65. Gonzalez, S.J.M.; Bhattacharyya, T.; Alshehri, H.R.; Poulton, K.; Allen, S.; Miles, M.A.; Arianitwe, M.; Tukahebwa, E.M.; Webster, B.; Stothard, J.R. Application of a recombinase polymerase amplification (RPA) assay and pilot field testing for *Giardia duodenalis* at Lake Albert, Uganda. *Parasit. Vectors* **2020**, *1*–9, doi:10.1186/s13071-020-04168-1.

66. World Health Organisation. Research Priorities for Helminth Infections. WHO. Technical Report Series No. 972. Geneva, Switzerland: World Health Organisation.
Available online: https://apps.who.int/iris/bitstream/handle/10665/75922 (accessed on Jun 30, 2020).

67. Barda, B.D.; Rinaldi, L.; Ianniello, D.; Zepherine, H.; Salvo, F.; Sadutshang, T.; Cringoli, G.; Clementi, M.; Albonico, M. Mini-FLOTAC, an Innovative Direct Diagnostic Technique for Intestinal Parasitic Infections: Experience from the Field. *PLoS Negl. Trop. Dis.* **2013**, *7*, doi:10.1371/journal.pntd.0002344.

68. Barda, B.; Ianniello, D.; Zephyrne, H.; Rinaldi, L.; Cringoli, G.; Burioni, R.; Albonico, M. Parasitic infections on the shore of Lake Victoria (East Africa) detected by Mini-FLOTAC and standard techniques. *Acta Trop.* **2014**, *137*, 140–146, doi:10.1016/j.actatropica.2014.05.012.

69. Hossein Hooshyar, Parvin Rostamkhani, Mohsen Arbabi, M.D. *Giardia lamblia* infection: review of current diagnostic strategies. *Gastroenterol. Hepatol.* **2019**, *95*, 347–349., doi:10.22037/ghfbb.v0i0.1414.

70. Le, L.; Hsieh, M.H. Diagnosing Urogenital Schistosomiasis: Dealing with Diminishing Returns. *Trends Parasitol.* **2017**, *33*, 378–387, doi:10.1016/j.pt.2016.12.009.

71. Lamberton, P.H.L.; Kabatereine, N.B.; Oguttu, D.W.; Fenwick, A.; Webster, J.P. Sensitivity and Specificity of Multiple Kato-Katz Thick Smears and a Circulating Cathodic Antigen Test for Schistosoma mansoni Diagnosis Pre- and Post-repeated-Praziquantel Treatment. *PLoS Negl. Trop. Dis.* **2014**, *8*, doi:10.1371/journal.pntd.0003139.

72. Adeyemo, F.E.; Singh, G.; Reddy, P.; Stenström, T.A. Methods for the detection of *Cryptosporidium* and *Giardia*: From microscopy to nucleic acid based tools in clinical and environmental regimes. *Acta Trop.* **2018**, *184*, 15–28, doi:10.1016/j.actatropica.2018.01.011.

73. Zahan, N. A Comparison of Microscopy and Enzyme Linked Immunosorbent Assay
for Diagnosis of *Giardia lamblia* in Human Faecal Specimens. *J. Clin. Diagnostic Res.* 2014, 8, 10–12, doi:10.7860/jcdr/2014/9484.5087.

74. Utzinger, J.; Becker, S.L.; van Lieshout, L.; van Dam, G.J.; Knopp, S. New diagnostic tools in schistosomiasis. *Clin. Microbiol. Infect.* 2015, 21, 529–542, doi:10.1016/j.cmi.2015.03.014.

75. Archer, J.; Lacourse, E.J.; Webster, L.B.; Stothard, J.R. An update on non-invasive urine diagnostics for human-infecting parasitic helminths: What more could be done and how? *Parasitology* 2019, doi:10.1017/S0031182019001732.

76. Coulibaly, J.T.; N’Goran, E.K.; Utzinger, J.; Doenhoff, M.J.; Dawson, E.M. A new rapid diagnostic test for detection of anti-*Schistosoma mansoni* and anti-*Schistosoma haematobium* antibodies. *Parasites and Vectors* 2013, 6, 1–8, doi:10.1186/1756-3305-6-29.

77. Minetti, C.; LaCourse, E.J.; Reimer, L.; Stothard, J.R. Focusing nucleic acid-based molecular diagnostics and xenomonitoring approaches for human helminthiases amenable to preventive chemotherapy. *Parasitol. Open* 2016, 2, doi:10.1017/pao.2016.13.

78. Lodh, N.; Mikita, K.; Bosompem, K.M.; Anyan, W.K.; Quartey, J.K.; Otchere, J.; Shiff, C.J. Point of care diagnosis of multiple schistosome parasites: Species-specific DNA detection in urine by loop-mediated isothermal amplification (LAMP). *Acta Trop.* 2017, 173, 125–129, doi:10.1016/j.actatropica.2017.06.015.

79. Bustinduy, A.L.; Sousa-Figueiredo, J.C.; Adriko, M.; Betson, M.; Fenwick, A.; Kabatereine, N.; Stothard, J.R. Fecal Occult Blood and Fecal Calprotectin as Point-of-Care Markers of Intestinal Morbidity in Ugandan Children with *Schistosoma mansoni* Infection. *PLoS Negl. Trop. Dis.* 2013, 7, 1–9, doi:10.1371/journal.pntd.0002542.

80. El-Nahas, H.A.; Salem, D.A.; El-Henawy, A.A.; El-Nimr, H.I.; Abdel-Ghaffar, H.A.;
El-Meadawy, A.M. *Giardia* diagnostic methods in human fecal samples: A comparative study. *Cytom. Part B Clin. Cytom.* **2013**, *84B*, 44–49, doi:10.1002/cyto.b.21048.

81. Koehler, A. V.; Jex, A.R.; Haydon, S.R.; Stevens, M.A.; Gasser, R.B. *Giardia* giardiasis - A perspective on diagnostic and analytical tools. *Biotechnol. Adv.* **2014**, *32*, 280–289, doi:10.1016/j.biotechadv.2013.10.009.

82. L. Alexander, C.; Niebel, M.; Jones, B. The rapid detection of *Cryptosporidium* and *Giardia* species in clinical stools using the Quik Chek immunoassay. *Parasitol. Int.* **2013**, *62*, 552–553, doi:10.1016/j.parint.2013.08.008.

83. Crannell, Z.A.; Cabada, M.M.; Castellanos-Gonzalez, A.; Irani, A.; White, A.C.; Richards-Kortum, R. Recombinase polymerase amplification-based assay to diagnose *Giardia* in stool samples. *Am. J. Trop. Med. Hyg.* **2015**, *92*, 583–587, doi:10.4269/ajtmh.14-0593.

84. Plutzer, J.; Karanis, P. Rapid identification of *Giardia duodenalis* by loop-mediated isothermal amplification (LAMP) from faecal and environmental samples and comparative findings by PCR and real-time PCR methods. *Parasitol. Res.* **2009**, *104*, 1527–1533, doi:10.1007/s00436-009-1391-3.

85. MINETTI, C.; LACOURSE, E.J.; REIMER, L.; STOTHARD, J.R. Focusing nucleic acid-based molecular diagnostics and xenomonitoring approaches for human helminthiases amenable to preventive chemotherapy. *Parasitol. Open* **2016**, *2*, doi:10.1017/pao.2016.13.

86. Amoah, A.S.; Hoekstra, P.T.; Casacuberta-Partal, M.; Coffeng, L.E.; Corstjens, P.L.A.M.; Greco, B.; van Lieshout, L.; Lim, M.D.; Markwalter, C.F.; Odier, M.R.; et al. Sensitive diagnostic tools and targeted drug administration strategies are needed to eliminate schistosomiasis. *Lancet Infect. Dis.* **2020**, *3099*, 1–8, doi:10.1016/S1473-
3099(20)30254-1.

87. Pennance, T.; Person, B.; Muhsin, M.A.; Khamis, A.N.; Muhsin, J.; Khamis, I.S.; Mohammed, K.A.; Kabole, F.; Rollinson, D.; Knopp, S. Urogenital schistosomiasis transmission on Unguja Island, Zanzibar: Characterisation of persistent hot-spots. *Parasites and Vectors* **2016**, *9*, 1–13, doi:10.1186/s13071-016-1847-0.

88. King, C.H.; Sturrock, R.F.; Kariuki, H.C.; Hamburger, J. Transmission control for schistosomiasis - why it matters now. *Trends Parasitol.* **2006**, *22*, 575–582, doi:10.1016/j.pt.2006.09.006.

89. Allan, F.; Ame, S.M.; Tian-Bi, Y.-N.T.; Hofkin, B. V.; Webster, B.L.; Diakité, N.R.; N’Goran, E.K.; Kabole, F.; Khamis, I.S.; Gouvras, A.N.; et al. Snail-Related Contributions from the Schistosomiasis Consortium for Operational Research and Evaluation Program Including Xenomonitoring, Focal Mollusciciding, Biological Control, and Modeling. *Am. J. Trop. Med. Hyg.* **2020**, *1*–*14*, doi:10.4269/ajtmh.19-0831.

90. Abbasi, I.; King, C.H.; Muchiri, E.M.; Hamburger, J. Detection of Schistosoma mansoni and *Schistosoma haematobium* DNA by loop-mediated isothermal amplification: Identification of infected snails from early prepatency. *Am. J. Trop. Med. Hyg.* **2010**, *83*, 427–432, doi:10.4269/ajtmh.2010.09-0764.

91. Gandasegui, J.; Fernández-Soto, P.; Hernández-Goenaga, J.; López-Abán, J.; Vicente, B.; Muro, A. Biompha-LAMP: A New Rapid Loop-Mediated Isothermal Amplification Assay for Detecting *Schistosoma mansoni* in *Biomphalaria glabrata* Snail Host. *PLoS Negl. Trop. Dis.* **2016**, *10*, 1–14, doi:10.1371/journal.pntd.0005225.

92. Buret, A.; denHollander, N.; Wallis, P.M.; Befus, D.; Olson, M.E. Zoonotic Potential of giardiasis in Domestic Ruminants. *J. Infect. Dis.* **1990**, *162*, 231–237, doi:10.1093/infdis/162.1.231.
93. Bartley, P.M.; Roehe, B.K.; Thomson, S.; Shaw, H.J.; Peto, F.; Innes, E.A.; Katzer, F. Detection of potentially human infectious assemblages of *Giardia duodenalis* in fecal samples from beef and dairy cattle in Scotland. *Parasitology* 2019, 146, 1123–1130, doi:10.1017/S0031182018001117.

94. Sawitri, D.H.; Wardhana, A.H.; Martindah, E.; Ekawasti, F.; Dewi, D.A.; Utomo, B.N.; Shibahara, T.; Kusumoto, M.; Tokoro, M.; Sasai, K.; et al. Detections of gastrointestinal parasites, including *Giardia intestinalis* and *Cryptosporidium* spp., in cattle of Banten province, Indonesia. *J. Parasit. Dis.* 2020, 44, 174–179, doi:10.1007/s12639-019-01179-3.

95. Bass, D.; Stentiford, G.D.; Littlewood, D.T.J.; Hartikainen, H. Diverse Applications of Environmental DNA Methods in Parasitology. *Trends Parasitol.* 2015, 31, 499–513, doi:10.1016/j.pt.2015.06.013.

96. Sengupta, M.E.; Hellström, M.; Kariuki, H.C.; Olsen, A.; Thomsen, P.F.; Mejer, H.; Willerslev, E.; Mwanje, M.T.; Madsen, H.; Kristensen, T.K.; et al. Environmental DNA for improved detection and environmental surveillance of schistosomiasis. *Proc. Natl. Acad. Sci. U. S. A.* 2019, 116, 8931–8940, doi:10.1073/pnas.1815046116.

97. Baque, R.H.; Gilliam, A.O.; Robles, L.D.; Jakubowski, W.; Slifko, T.R. A real-time RT-PCR method to detect viable *Giardia lamblia* cysts in environmental waters. *Water Res.* 2011, 45, 3175–3184, doi:10.1016/j.watres.2011.03.032.

98. Alzaylaee, H.; Collins, R.A.; Rinaldi, G.; Shechonge, A.; Ngatunga, B.; Morgan, E.R.; Genner, M.J. Schistosoma species detection by environmental DNA assays in african freshwaters. *PLoS Negl. Trop. Dis.* 2020, 14, 1–19, doi:10.1371/journal.pntd.0008129.

99. Lass, A.; Szostakowska, B.; Korzeniewski, K.; Karanis, P. Detection of *Giardia intestinalis* in water samples collected from natural water reservoirs and wells in northern and north-eastern Poland using LAMP, real-time PCR and nested PCR. *J.
100. Alzaylaee, H.; Collins, R.A.; Shechonge, A.; Ngatunga, B.P.; Morgan, E.R.; Genner, M.J. Environmental DNA-based xenomonitoring for determining *Schistosoma* presence in tropical freshwaters. *Parasites and Vectors* **2020**, **13**, 1–11, doi:10.1186/s13071-020-3941-6.

101. Mulero, S.; Boissier, J.; Allienne, J.; Quilichini, Y.; Foata, J.; Pointier, J.; Rey, O. Environmental DNA for detecting *Bulinus truncatus*: A new environmental surveillance tool for schistosomiasis emergence risk assessment. *Environ. DNA* **2020**, **2**, 161–174, doi:10.1002/edn3.53.

102. Tchuem Tchuenté, L.A.; Momo, S.C.; Stothard, J.R.; Rollinson, D. Efficacy of praziquantel and reinfection patterns in single and mixed infection foci for intestinal and urogenital schistosomiasis in Cameroon. *Acta Trop.* **2013**, **128**, 275–283, doi:10.1016/j.actatropica.2013.06.007.

103. Stothard, J.R.; Sousa-Figueiredo, J.C.; Navaratnam, A.M.D. Advocacy, policies and practicalities of preventive chemotherapy campaigns for African children with schistosomiasis. *Expert Rev. Anti. Infect. Ther.* **2013**, **11**, 733–752, doi:10.1586/14787210.2013.811931.

104. Lo, N.C.; Addiss, D.G.; Hotez, P.J.; King, C.H.; Stothard, J.R.; Evans, D.S.; Colley, D.G.; Lin, W.; Coulibaly, J.T.; Bustinduy, A.L.; et al. A call to strengthen the global strategy against schistosomiasis and soil-transmitted helminthiasis: the time is now. *Lancet Infect. Dis.* **2017**, **17**, e64–e69, doi:10.1016/S1473-3099(16)30535-7.

105. Wang, W.; Wang, L.; Liang, Y.S. Susceptibility or resistance of praziquantel in human schistosomiasis: A review. *Parasitol. Res.* **2012**, **111**, 1871–1877, doi:10.1007/s00436-012-3151-z.

106. Bustinduy, A.L.; Friedman, J.F.; Kjetland, E.F.; Ezeamama, A.E.; Kabatereine, N.B.;
107. Bergquist, R.; Utzinger, J.; Keiser, J. Controlling schistosomiasis with praziquantel: How much longer without a viable alternative? Infect. Dis. Poverty 2017, 6, 1–10, doi:10.1186/s40249-017-0286-2.

108. Doenhoff, M.J.; Cioli, D.; Utzinger, J. Praziquantel: Mechanisms of action, resistance and new derivatives for schistosomiasis. Curr. Opin. Infect. Dis. 2008, 21, 659–667, doi:10.1097/QCO.0b013e328318978f.

109. Hill, D.R.; Timothy.B., G. Treatment of giardiasis. Curr. Treat. Options Gastroenterol. 2005, 8, 13–17, doi:10.1007/s11938-005-0047-3.

110. Ce, G.; Reveiz, L.; Lg, U.; Cp, C. Drugs for treating giardiasis (Review). 2012, doi:10.1002/14651858.CD007787.pub2.www.cochranelibrary.com.

111. Carter, E.R.; Nabarro, L.E.; Hedley, L.; Chiodini, P.L. Nitroimidazole-refractory giardiasis: a growing problem requiring rational solutions. Clin. Microbiol. Infect. 2018, 24, 37–42, doi:10.1016/j.cmi.2017.05.028.

112. Vercruysse, J.; Behnke, J.M.; Albonico, M.; Ame, S.M.; Angebault, C.; Bethony, J.M.; Engels, D.; Guillard, B.; Hoa, N.T.V.; Kang, G.; et al. Assessment of the anthelmintic efficacy of albendazole in school children in seven countries where soil-transmitted helminths are endemic. PLoS Negl. Trop. Dis. 2011, 5, doi:10.1371/journal.pntd.0000948.

113. Hoerauf, A.; Pfarr, K.; Mand, S.; Debrah, A.Y.; Specht, S. Filariasis in Africa—treatment challenges and prospects. Clin. Microbiol. Infect. 2011, 17, 977–985, doi:10.1111/j.1469-0691.2011.03586.x.
114. Omarova, A.; Tussupova, K.; Berndtsson, R.; Kalishev, M.; Sharapatova, K. Protozoan parasites in drinking water: A system approach for improved water, sanitation and hygiene in developing countries. *Int. J. Environ. Res. Public Health* **2018**, *15*, 1–18, doi:10.3390/ijerph15030495.

115. Pickering, A.J.; Njenga, S.M.; Steinbaum, L.; Swarthout, J.; Lin, A.; Arnold, B.F.; Stewart, C.P.; Dentz, H.N.; Mureithi, M.; Chieng, B.; et al. Effects of single and integrated water, sanitation, handwashing, and nutrition interventions on child soil-transmitted helminth and *Giardia* infections: A cluster-randomized controlled trial in rural Kenya. *PLoS Med.* **2019**, *16*, 1–21, doi:10.1371/journal.pmed.1002841.

116. Aw, J.Y.H.; Clarke, N.E.; McCarthy, J.S.; Traub, R.J.; Amaral, S.; Huque, M.H.; Andrews, R.M.; Gray, D.J.; Clements, A.C.A.; Vaz Nery, S. *Giardia* duodenalis infection in the context of a community-based deworming and water, sanitation and hygiene trial in Timor-Leste. *Parasites and Vectors* **2019**, *12*, 4–13, doi:10.1186/s13071-019-3752-9.

117. Roche, R.; Bain, R.; Cumming, O. A long way to go - Estimates of combined water, sanitation and hygiene coverage for 25 sub-Saharan African countries. *PLoS One* **2017**, *12*, 1–24, doi:10.1371/journal.pone.0171783.

118. World Health Organization (WHO) Water, Sanitation & Hygiene for accelerating and sustaining progress on Neglected Tropical Diseases Available online: https://www.who.int/water_sanitation_health/publications/wash-and-ntd-strategy/en/ (accessed on Jul 2, 2020).

119. World Health Organisation. Integrating Neglected Tropical Diseases into Global Health and Development, 2017. Geneva, Switzerland: World Health Organisation Available online: https://apps.who.int/iris/bitstream/handle/10665/255011/9789241565448-eng.pdf
120. Campbell, S.J.; Biritwum, N.K.; Woods, G.; Velleman, Y.; Fleming, F.; Stothard, J.R. Tailoring Water, Sanitation, and Hygiene (WASH) Targets for Soil-Transmitted Helminthiasis and Schistosomiasis Control. *Trends Parasitol.* **2018**, *34*, 53–63, doi:10.1016/j.pt.2017.09.004.

121. Campbell, S.J.; Savage, G.B.; Gray, D.J.; Atkinson, J.A.M.; Soares Magalhães, R.J.; Nery, S. V.; McCarthy, J.S.; Velleman, Y.; Wicken, J.H.; Traub, R.J.; et al. Water, Sanitation, and Hygiene (WASH): A Critical Component for Sustainable Soil-Transmitted Helminth and Schistosomiasis Control. *PLoS Negl. Trop. Dis.* **2014**, *8*, 1–5, doi:10.1371/journal.pntd.0002651.

122. Grimes, J.E.; Croll, D.; Harrison, W.E.; Utzinger, J.; Freeman, M.C.; Templeton, M.R. The roles of water, sanitation and hygiene in reducing schistosomiasis: A review. *Parasites and Vectors* **2015**, *8*, 1–16, doi:10.1186/s13071-015-0766-9.

123. Rollinson, D.; Knopp, S.; Levitz, S.; Stothard, J.R.; Tchuem Tchuenté, L.A.; Garba, A.; Mohammed, K.A.; Schur, N.; Person, B.; Colley, D.G.; et al. Time to set the agenda for schistosomiasis elimination. *Acta Trop.* **2013**, *128*, 423–440, doi:10.1016/j.actatropica.2012.04.013.

124. Van den Berg, H.; Kelly-Hope, L.A.; Lindsay, S.W. Malaria and lymphatic filariasis: The case for integrated vector management. *Lancet Infect. Dis.* **2013**, *13*, 89–94, doi:10.1016/S1473-3099(12)70148-2.

125. Kelly-Hope, L.A.; Molyneux, D.H.; Bockarie, M.J. Can malaria vector control accelerate the interruption of lymphatic filariasis transmission in Africa; Capturing a window of opportunity? *Parasites and Vectors* **2013**, *6*, 1–12, doi:10.1186/1756-3305-6-39.

126. Knipes, A.K.; Lemoine, J.F.; Monestime, F.; Fayette, C.R.; Direny, A.N.; Desir, L.;
Beau de Rochars, V.E.; Streit, T.G.; Renneker, K.; Chu, B.K.; et al. Partnering for impact: Integrated transmission assessment surveys for lymphatic filariasis, soil transmitted helminths and malaria in Haiti. *PLoS Negl. Trop. Dis.* **2017**, *11*, 1–12, doi:10.1371/journal.pntd.0005387.

Bronzan, R.N.; Dorkenoo, A.M.; Agbo, Y.M.; Halatoko, W.; Layibo, Y.; Adjeloh, P.; Teko, M.; Sossou, E.; Yakpa, K.; Tchalim, M.; et al. Impact of community-based integrated mass drug administration on schistosomiasis and soil-transmitted helminth prevalence in Togo. *PLoS Negl. Trop. Dis.* **2018**, *12*, 1–23, doi:10.1371/journal.pntd.0006551.
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].
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]     |
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] | |