Monitoring schistosomiasis and sanitation interventions—The potential of environmental DNA

Teteh S. Champion1 | Stephanie Connelly2 | Cindy J. Smith2 | Poppy H. L. Lamberton1

1Institute of Biodiversity Animal Health & Comparative Medicine, University of Glasgow, Glasgow, UK
2Division of Infrastructure and Environment, James Watt School of Engineering, University of Glasgow, Glasgow, UK

Correspondence
Teteh S. Champion and Poppy H. L. Lamberton, Institute of Biodiversity Animal Health & Comparative Medicine, University of Glasgow, Glasgow, UK. Email: t.champion.1@research.gla.ac.uk (T. S. C.) and poppy.lamberton@glasgow.ac.uk (P. H. L. L.)

Abstract
Transmission of schistosomiasis, a human parasitic disease, is intrinsically linked to inadequate water, sanitation, and hygiene (WASH) facilities and/or their use. The mainstay of control is population-based chemotherapy. Globally, each year, 240 million people are estimated to require this preventative treatment. However, for long-term, sustainable control of this disease, supplementary WASH improvements are required to prevent (re)infection of humans (provision of safe water) and transmission from humans to the environment (improved sanitation). While there is established methodology for monitoring transmission in human populations, presently methods for monitoring the impact of WASH interventions, in particular sanitation, on environmental transmission are lacking. Development of such becomes paramount as integrated control programs combine drug treatments with enhanced WASH facilities and behavior change interventions, with uptake likely correlated to a reduction in fecal matter, and schistosome eggs, in the environment but any impact on infection levels in humans taking longer to become apparent. This article reports and critiques the methods currently used to monitor schistosomiasis in freshwater and soil environments and explores how environmental DNA could be used to better understand and monitor environmental contamination in relation to sanitation. Stronger evidence is required to understand how different sanitation interventions serve to limit the environmental transmission of the parasite and their relative effectiveness in preventing disease.

This article is categorized under:
Engineering Water > Methods

KEYWORDS
environmental monitoring, fecal contamination, NTD, Schistosoma mansoni, water, sanitation, and hygiene

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1 | INTRODUCTION

Human schistosomiasis is a chronic neglected tropical disease and transmission is intrinsically linked with inadequate water, sanitation, and hygiene (WASH) (Grimes et al., 2015). It is estimated that 240 million people worldwide are infected with this parasitic worm: with over 90% of cases occurring in underserved communities within sub-Saharan African countries (WHO, 2020b). Two species cause the majority of infection and disease on the African continent: Schistosoma mansoni, causing intestinal and hepatic schistosomiasis, and, Schistosoma haematobium which causes urogenital schistosomiasis. These parasites require a mammalian and aquatic snail host to complete their lifecycle (Figure 1). For S. mansoni, the focus of this review, humans typically serve as the mammalian host and snails of the Biomphalaria genus are the intermediate host. When access to safe water is limited, a person can become infected through contact with cercariae-infested water: these cercariae are shed from infected aquatic snails. Once humans are infected, the immature stage of the schistosome (schistosomula) develops in the human host and matures into adult parasitic worms which then sexually reproduce. Their eggs are released into the environment through their host’s excreta, feces in the case of S. mansoni. Inadequate WASH plays a key role in maintaining the transmission of the parasite, providing the opportunities for snail to human and human to snail transmission via the environment (Grimes et al., 2015).

Current control efforts focus on large-scale preventative chemotherapy treatment using mass-drug administration of praziquantel, a schistosomicidal drug, to at risk human populations (WHO, 2020b). In 2017, 98.7 million people were administered praziquantel (WHO, 2020b) and there have been successes in controlling and eliminating the parasite in low-transmission settings (Rollinson et al., 2013). However, in highly endemic areas without an integrated approach to control, the cycle of transmission remains unbroken (Freeman et al., 2013). An integrated strategy should encompass improved effectiveness of, and access to, WASH facilities, alongside community-led behavior change campaigns and where viable, snail control in the environment (Rollinson et al., 2013). Access to, and use of, WASH may be monitored via observations, surveys, and recall. Yet, confirming these qualitative results with quantitative values and monitoring the actual effectiveness of these WASH facilities in adequately containing feces and S. mansoni eggs, limiting environmental transmission, remains challenging (Campbell et al., 2018). This review builds on epidemiological and public health focused reviews (Table 1), by discussing the methods available for monitoring the various lifecycle stages of S. mansoni in soil and aquatic environments, and the potential for using environmental DNA (eDNA) as a tool to monitor the success of different sanitation interventions. eDNA monitoring techniques developed for monitoring soil transmitted helminths, other disease-causing parasites transmitted in the feces which are often co-endemic with schistosomiasis, are reported and an analogy is drawn with the schistosomiasis field for which such tools are presently lacking.

2 | SANITATION’S ROLE IN SCHISTOSOMIASIS TRANSMISSION

In the resource-poor settings where schistosomiasis is endemic, sanitation is inadequate, or sometimes non-existent (Campbell et al., 2014). In 2013, it was estimated that for 1.77 billion people worldwide (~25% of the global population

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**FIGURE 1** The lifecycle of Schistosoma mansoni. Exposure to the parasite occurs due to human contact with cercariae infested water, the cercariae are shed by infected snails. The host snail habitat can become contaminated by human feces contained S. mansoni eggs due to inadequate sanitation. The free-living lifestages of the parasite release environmental DNA (eDNA) into the environment. The transmission of S. mansoni can be disrupted with adequate water, sanitation and hygiene (WASH) measures, that break the parasite’s lifecycle.
| Review topic                                      | Title                                                                 | Key points                                                                                                                                                                                                 | Reference                  |
|--------------------------------------------------|----------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|
| WASH and schistosomiasis                         | Tailoring water, sanitation, and hygiene (WASH) targets for soil-transmitted helminthiasis and schistosomiasis control. | Helminth control needs to move beyond dependence on chemotherapy to be sustainable. Multicomponent strategies are required to prevent exposure and reinfection: adding WASH. Highlight of policy progress in endemic countries and summary of WHO reports. Summary of UN sustainable goals in relation to NTDs and WASH. | Campbell et al. (2018)     |
| The roles of water, sanitation, and hygiene in reducing schistosomiasis: a review. |                                                                       | Transmission of schistosomiasis is too deeply entrenched for control by preventative chemotherapy alone. Social and ecological factors at play. Review of literature relating to WASH control and their specific role in disrupting the schistosome's lifecycle. | Grimes et al. (2015)       |
| Integration of water, sanitation, and hygiene for the prevention and control of neglected tropical diseases: a rationale for inter-sectoral collaboration. |                                                                       | Conclusions of a roundtable discussion between stakeholders in WASH, NTD, and child health sectors. Identifies and discusses areas where inter-sector collaboration could take place. Identified that there are currently gaps in research that interrogate the impact of specific WASH interventions on NTD control. Research has been reliant on evidence from observational studies. | Freeman et al. (2013)      |
| Environmental monitoring of schistosomiasis | Diverse applications of environmental DNA methods in parasitology. | Practical insight into how eDNA methods can be adapted for parasites. Key advantages of eDNA: • Detection of “elusive” organisms. • Tool to study evolution and diversity of parasites by molecular genetics. • Opportunity for large scale sampling. Potential issues in policy context: if a disparity between infection data and eDNA detection. | Bass, Stentiford, Littlewood, and Hartikainen (2015) |
| Towards interruption of schistosomiasis transmission in sub-Saharan Africa: developing an appropriate environmental surveillance framework to guide and to support “end game” interventions. |                                                                       | Summary of snails’ role in schistosomes’ transmission and methods to monitor contamination and exposure events. Describes sampling of snails and considerations when designing snail sampling efforts. | Stothard et al. (2017)     |
| Molecular approaches for the detection of *Schistosoma mansoni*: possible applications in the detection of snail infection, monitoring of transmission sites, and diagnosis of human infection. |                                                                       | Review and summary of molecular detection methods: • Human infection (fecal and sera samples) • Snail infection • Infested water bodies | Abath, do Vale Gomes, Melo, Barbosa, and Werkhauser (2006) |
at that time), their primary means of sanitation was some form of pit latrine, a basic and often poorly managed sanitation solution (Graham & Polizzotto, 2013). Unimproved or basic sanitation services, as defined by the WHO/UNICEF Joint Monitoring Programme for Water Supply, Sanitation, and Hygiene, fail to hygienically separate excreta from subsequent human contact (WHO UNICEF, 2020). In sub-Saharan African countries, pit latrines are commonly employed, as they are often the cheapest and most logistically simple form of sanitation (WHO, 2017b). A basic pit latrine consists of a dug-out pit in the ground, a squat hole for the disposal of excreta, and a superstructure for privacy (WHO, 2017a). An improved pit latrine deploys a slab or platform covering the ground in which the squat hole is dug to serve as a physical barrier between the user and soil. If there is no lining to the dug-out pit, excreta within has the potential to leach into the groundwater. This has significant negative implications for human health, as it can result in the contamination of drinking water with fecal enteric pathogens (Graham & Polizzotto, 2013). In schistosomiasis endemic areas, if this contaminated ground water flows into the freshwater habitats of susceptible snails, the perpetuation of the schistosome’s lifecycle can also be facilitated. The transportation of schistosome eggs and microbes into the aquatic habitat of the snails will be affected by environmental conditions. Hydroecological factors such as rock strata, soil characteristics, and water table depth can affect the functionality of pit latrines, resulting in containment failure and water flowing freely in and out of the latrines (Graham & Polizzotto, 2013). In addition, the likelihood of groundwater contamination is affected by flooding of pit latrines during wet/rainy seasons, which can coincide with the peak of snail numbers (Ernould, Ba, & Sellin, 1999; Perez-Saez et al., 2016).

While the ultimate goal is to reduce schistosomiasis transmission and human infection intensity and prevalence, it is vital to be able to monitor the effect of sanitation interventions in the short term to assess their potential to reduce the level of *Schistosoma* in the environment, as well as to estimate the proportion of the population that have taken up the intervention. While in comparison to STH ova, schistosome eggs have a short lifespan in the environment, they have however been shown to survive for up to 3 weeks within drying pit latrine sludge (Newton, Figgat, Weibel, & Weibel, 1948) and when the environmental conditions became more favorable, the eggs were viable enough to hatch (M. F. Jones et al., 1947; Kawata & Krusé, 1966; Maldonado, Acosta-Matienzo, & Thillet, 1949; Newton et al., 1948). Thus, if the pit latrine does not effectively contain, or inactivate, the schistosome eggs, they have the potential to progress to their next lifecycle stages and contribute to onward transmission to humans. Unlike the transmission of bacteria, viruses (Graham & Polizzotto, 2013), and STH egg/ova (Baker & Ensink, 2012), the extent of schistosome egg containment by pit latrines is still an unknown. Methods for determining what is occurring in the environments facing with the sanitation system remain underdeveloped and primarily focus on non-nucleic acid-based methods.

3 | CONVENTIONAL ENVIRONMENTAL MONITORING TECHNIQUES

To assess endemicity of *S. mansoni* within a community, the presence of eggs in stool (enumerated by a standardized method called a Kato-Katz thick smear; Katz, Chaves, & Pellegrino, 1972) is used to inform the extent and frequency of praziquantel administration (WHO, 2020b). This gives indispensable information on individual parasite loads and community prevalence but provides limited information on the effectiveness of new sanitation interventions. Studies reporting the impact of sanitation on the infection rates in people show adequate sanitation is associated with lower odds of infection of *S. mansoni* (systematically reviewed by Grimes et al., Grimes et al., 2014). Historically, the impact of sanitation interventions has been measured by changes in human infection: not by direct evaluation of schistosome or fecal contamination related to sanitation interventions. These studies have relied on observational data (as opposed to cluster randomized controlled trials), often with weak statistical power (Grimes et al., 2014). As such there is a requirement for an evidence-based argument to enable and monitor an integrated approach to schistosomiasis control programs (Campbell et al., 2018). This requires robust and direct investigations into the impact of sanitation on *S. mansoni*, alongside the current monitoring of human schistosomiasis cases. Currently, there is no link up between the monitoring of WASH access with epidemiology data (Campbell et al., 2018) and despite the WHO 2020 roadmap calling for an investigation into environmental transmission, there are still no guidelines to enable robust and reproducible monitoring (WHO, 2020a). Furthermore, there is currently a lack of standardized tools fit for this purpose. Guidelines are also needed to inform the construction, maintenance, and evaluation of improved sanitation facilities specifically relating to schistosomiasis and other human-related helminths (Campbell et al., 2018). The current environmental monitoring options are summarized in Figure 2.
3.1 Monitoring of the soil environment

Both the terrestrial and aquatic environments are involved with transmission of the schistosome; however, investigations into freshwater environments and water treatment have been at the forefront of research (e.g., Braun, Grimes, & Templeton, 2018; Secor, 2014). Investigations quantifying eggs in environmental matrices such as wastewater, sludge, and soils are infrequently used and were adapted from centrifuge-based separation methods of helminth eggs/ova from feces (Faust et al., 1938). The centrifugal flotation techniques developed in the early 20th century enable egg separation from the bulk of an environmental sample, by the specific gravity of the helminth eggs/ova. However, techniques used to detect STH in wastewater, such as centrifugal sedimentation or flotation, are less effective for recovering *Schistosoma* eggs (Smith, 1999). *Schistosoma* eggs can become shriveled in flotation fluids, distorting them beyond the scope of microscopic identification, and the sedimentation process can induce egg hatching. In the context of STH detection in such environmental matrices, molecular approaches have by contrast proven to be sensitive, specific, and rapid alternatives to detection by microscopy. However, as with schistosomiasis monitoring more broadly, the uptake of eDNA methods for STH monitoring has been higher in clinical settings than for community level monitoring (Amoah, Singh, Stenström, & Reddy, 2017). The scientific literature is more limited to water-based investigations of the parasite regarding both microscopy- and nucleic acid-based methods to investigate the transmission of *Schistosoma* species in the environment.

3.2 Monitoring of the aquatic environment

3.2.1 Cercariometry and sentinel mice

Monitoring approaches for aquatic environments can be broadly categorized into collection of water samples, collection of the intermediate snail host, or sentinel rodent infections (Sokolow et al., 2018). Cercariometry, which requires filtering water from endemic areas, to collect and then count cercariae, can be used to infer the density of cercariae and transmission risk to humans in the environment (Aoki, Sato, Muhoho, Noda, & Kimura, 2003). These data can be analyzed with metadata such as hydrodynamic conditions, temporal variations related to seasonality, and pre-existing knowledge of the peak cercarial emissions from the snails to estimate cercarial densities (Théron, 1986). Counting is often performed using microscopy. As there are other species of trematode cercariae, highly skilled personnel are required to speciate the cercariae by microscopy. The cercariae’s furcocercous tail morphologies were the basis of species identification when the technique was first developed (Frandsen & Christensen, 1984) and as human and non-human schistosomes are often co-endemic, species identification is key to accurate xenomonitoring. Due to the similarities in tail morphology within the *Schistosoma* genus, identification by microscopy is challenging, and can impact the accuracy of this technique.

An alternative to identification by microscopy techniques is the sentinel rodent technique in which mice are exposed to waterbodies containing cercariae and maintained for 6 weeks before dissection to isolate and count adult worms in their bloodstream by perfusion. This technique is useful for directly assessing infection risk to humans from water contact, and was developed in China where *Schistosoma japonicum* is endemic, this species cercariae are stickier and more difficult to filter by methods used for *S. mansoni*. This technique raises ethical issues and is logistically challenging and costly due to both the technical staff, laboratory time, and resources required (Hung, Remais, & Webster, 2008). In addition to identifying cercariae by microscopy-based methods eDNA-based techniques to isolate cercarial DNA directly from transmission sites have been tested and are discussed below.

3.2.2 Snail surveys

Xenomonitoring, the surveillance of intermediate hosts (in this instance infected snails) can provide information on the dynamics of transmission between humans and snail vectors and are the mainstay of monitoring efforts after human infection surveys. Due to transmission focality and the resources available for surveillance, targeted sampling at transmission sites is favored over systematic sampling (Sokolow et al., 2018). The snails from these surveys are processed to determine their infection status, by the traditional method of cercarial shedding, crushing, or by molecular methods. To standardize snail collection a timed sampling effort should be carried out: the snails are commonly scooped with a sieve.
To determine the infection status of the collected snails, they are either placed in fresh water and exposed to light to test for cercarial shedding, or, the snails can be crushed and dissected to identify the sporocyst (the lifecycle stage prior to the cercarial stage) and immature cercariae. Cercarial shedding first occurs 28–42 days after the snail is exposed, therefore snails are maintained and observed daily for up to 6 weeks. Large numbers of snails are needed as there can be high mortality of snails during this observation period (Hanelt, Adema, Mansour, & Loker, 1997) and it is common to detect only a few infected snails among thousands of uninfected snails surveyed (Hamburger, Xin, Ramzy, Jourdane, & Ruppel, 1998). Identification of the shed cercariae by microscopy is most commonly used, alternatively polymerase chain reaction (PCR) assays from DNA extracts from snail tissue can be used (Table 2). As an alternative to microscopy, PCR assays targeting highly repetitive sequences were developed to detect *S. mansoni* (Table 2). PCRs were initially performed on DNA extracted directly from snail tissue (Hamburger, Xin, et al., 1998; Hanelt et al., 1997; Jannotti-Passos et al., 1997) and to increase sensitivity of DNA-based methods, DNA was then extracted from the tank water incubating snails (Hamburger, Yu-Xin, Ramzy, Jourdane, & Ruppel, 1998). Pooling snails collected from transmission sites for analysis can also reduce cost and increase the scale of the snail processing (Hamburger, Yu-Xin, et al., 1998). PCR has been the most widely applied and published molecular detection approach, however loop-mediated isothermal amplification (LAMP)-based methods using extracts from snail tissue have also been successfully employed more recently (Caldeira et al., 2017; Gandasegui et al., 2016; Hamburger et al., 2013). Advantages of LAMP over PCR, include simpler equipment requirements, such as a water bath or heat block due to simpler reaction conditions required, and visual readouts, where a positive reaction can be determined with the naked eye (Gandasegui et al., 2016). PCR by contrast requires thermocycler and fluorescence detection. It is noted that both LAMP and conventional PCR are presently limited in field use by the DNA extraction stage, which is often reliant on laboratory equipment, and is a pre-requisite for application of either method.

### 3.3 Indirect monitoring

In addition to traditional methods of estimating transmission risk from snails, mapping tools using remotely sensed environmental and climatic data are used to identify snail habitat niches and predict schistosomiasis hotspots (Wood et al., 2019). For monitoring sanitation interventions, a proxy for schistosomiasis “hotspots” in the environment could be microbial source tracking: the determination of fecal pollution sources. Fecal indicator bacteria, such as *Escherichia coli* and fecal coliforms can be used as a proxy for enteric pathogens. Environmental samples can be investigated by culturing samples and counting colony forming unit by eye or extracting the DNA from the samples for PCR. Research on the impact of sanitation, on enteric pathogens, has used fecal contamination to directly investigate transmission pathways (these techniques have been systematically reviewed by Sclar et al., 2016). In low-resource settings, where pit
latrines are commonplace, investigations into the presence of fecal makers in households (e.g., Boehm et al., 2016; Fuhrmeister et al., 2020; Pickering et al., 2012) as well as the contamination of groundwater in relation to pit latrines (Graham & Polizzotto, 2013) have used microbial source tracking. In the context of schistosomiasis, fecal indicator bacteria along with human prevalence data were used by Ponce-Terashima, Koskey, Reis, McLellan, and Blanton (2014) to explore the transmission dynamics of *S. mansoni* in water contact sites.

4 | ENVIRONMENTAL DNA

eDNA can be defined as the DNA retained by, or released, from organisms interacting in their surroundings, which is then extracted from environmental samples (Thomsen & Willerslev, 2015). In the context of schistosomiasis this eDNA could be extracted directly from an intermediate host organism (infected snail), samples from an infected organism (human excrement) and environmental matrices (soil, water, sewage) (Bass et al., 2015). The downstream analysis of eDNA can be contentious (Bohmann et al., 2014) and applications vary between aiming to capture the entire diversity of organisms within an environmental sample and those targeting a specific species or even strain (Thomsen & Willerslev, 2015). In the context of using eDNA as a monitoring tool to measure the successful uptake and impact of improved sanitation as a supplement to drug-based control strategies, detecting *Schistosoma* species specifically is likely of more value than a metagenomic survey of the environmental sample. As discussed, current risk models and targeting of control measures currently rely upon ecological surveillance of the environmental sample. As discussed, current risk models and targeting of control measures currently rely upon ecological surveillance of the environmental sample (R. A. Jones et al., 2018). Due to the parasite’s asexual reproduction in snails, a single miracidium from an egg can result in thousands of cercariae, shedding daily for the lifespan of the infected snail. As one miracidial can produce thousands of cercariae, uptake of current and/or improved sanitation is unlikely to be linearly associated with reduced prevalence in humans, or in snail water sampled through xenomonitoring. Sampling the environment interfacing with a sanitation intervention, for example soil surrounding an improved pit latrine or the reusable products from sanitation interventions (composted sludge), would provide an indication of how the sanitation in functioning and its effect on transmission.

4.1 | Application of eDNA in *S. mansoni* monitoring

Nucleic-acid based detection of *Schistosoma* species has been used on DNA extracts from snail tissue. In the last decade, eDNA has been successfully employed as a tool to specifically detect *S. mansoni* (Sato et al., 2018; Sengupta et al., 2019), *S. haematobium* (Akande, Odetola, Osamudien, Fowora, & Omonigbehin, 2012), and *S. japonicum* (the main species in Asia) (Fornillos, Sato, Kim, Tabios, & Sato, 2019) in water samples. These studies sampled water from known transmission sites in endemic settings to compare the arising data with that from traditional malacological surveys. In each case, water samples were filtered and retained material fixed with 70% ethanol or RNAlater to preserve DNA for extraction and analysis by real-time PCR. The PCR techniques demonstrated specificity to single *Schistosoma* (and/or snail) species, being able to differentiate between *S. mansoni* and *S. haematobium* in co-endemic areas (Sato et al., 2018). Both *S. mansoni* qPCR primers were designed to target a fragment of the mitochondrial cytochrome c oxidase gene (COI or COX1) and generate a short amplicon. A short PCR product targeting a high copy number gene can enhance the sensitivity of the assay and still detect fragments of the DNA that result from environmental degradation. The sensitivity of the eDNA methods for *S. mansoni* was interrogated in a lab setting. Sengupta et al. (2019) used water samples taken from tanks containing different densities of infected host snails to determine the lower limit of detection: one DNA copy per qPCR reaction. Traditional snail surveys were used as a comparison of the molecular assay’s sensitivity by both *S. mansoni* studies (Sato et al., 2018; Sengupta et al., 2019). The detection of *S. mansoni* by eDNA carried out by Sato et al. (2018) coincided with water contact sites where intermediate host snail species were found. Sengupta et al. (2019) maintained the surveyed snails to observe cercariae shedding and found the eDNA method more sensitive than the traditional methods of schistosomiasis detection. Although the eDNA studies detected specific *Schistosoma* species by qPCR, they determined presence/absence rather than relative or absolute quantification.

Estimating parasite numbers or densities in an environmental sample has its caveats. Aquatic stages of *Schistosoma* species, miracidia and cercariae, differ in size and cannot be distinguished by current published eDNA assays, as their DNA targets are expressed across the parasite’s lifecycle. Therefore, copy number determined by qPCR cannot currently be used to accurately infer the life-cycle stage or number of parasites from the amount of eDNA molecules detected in a
| Source of DNA extracts | Primer target | Method | Detection limit | Reference |
|-----------------------|---------------|--------|----------------|-----------|
| Snail survey          | Mitochondrial DNA | Multiplex PCR | 1 pg | Jannotti-Passos et al. (1997) |
|                       | 18s rRNA | Nested PCR | 10 fg | Hanelt et al. (1997) |
| Snail survey          | Sm1-7 (M61098) (repetitive sequence) | PCR | 1 fg | Hamburger, Yu-Xin, et al. (1998) |
|                       | Sm1-7 (M61098) (repetitive sequence) | PCR | — | Hamburger, Xin, et al. (1998) |
| Snail survey          | Sm1-7 (M61098) (repetitive sequence) | PCR | 1 fg | Hertel, Kedves, Hassan, Haberl, and Haas (2004) |
| Snail survey          | Small subunit (SSU) rRNA | PCR, two-step nested PCR, single-tube nested PCR | 10 pg, 0.1 fg, 1 fg respectively | Melo et al. (2006) |
| Snail survey          | Mitochondrial DNA | Multiplex PCR | — | Jannotti-Passos, Magalhães, Dos Santos Carvalho, and Vidigal (2006) |
| Snail survey          | Sm1-7 (M61098) (repetitive sequence) | LAMP | 0.1 fg | Abbasi, King, Muchiri, and Hamburger (2010) |
| Snail survey          | Sm1-7 (M61098) (repetitive sequence) | LAMP | — | Hamburger et al. (2013) |
| Snail survey          | Ribosomal intergenic spacer 28S-18S rRNA | LAMP | 0.1 fg | Gandasegui et al. (2016) |
| Snail survey          | Mitochondrial gene ND5 | PCR | >0.1 fg | Lu, Zhang, Mutuku, Mkoji, and Loker (2016) |
| Snail survey          | Internal transcript spacer (ITS) of rDNA gene | Low stringency-PCR and LAMP | 70 fg | Caldeira, Jannotti-Passos, and Dos Santos Carvalho (2017) |
| Snail survey          | Mitochondrial gene cytochrome oxidase I (COI) | Real-time PCR | — | Sato et al. (2018) |
| Snail survey          | Mitochondrial gene cytochrome oxidase I (COI) | Real-time PCR | 1 DNA copy per qPCR reaction | Sengupta et al. (2019) |
| Snail survey          | Mitochondrial 16s rRNA gene | Real-time PCR | 100 copies/μl | Alzaylæe et al. (2020) |

*Note:* Studies have successfully used polymerase chain reaction (PCR) or loop-mediated isothermal amplification (LAMP) assays to detect *S. mansoni* in the environment. DNA was extracted from samples collected by malacological survey, and more recently directly from water collected at transmission sites using environmental DNA (eDNA) methods. The lower limits of detection by each molecular method are stated as published.
water sample (Sengupta et al., 2019). During the validation studies however, in which water tanks were set up with increasing number of cercariae, Sengupta et al. (2019) observed a quantitative relationship between the number of eDNA molecules detected by qPCR and the number of cercariae in their tank experiments.

There is potential for quantification of schistosome eggs in matrices associated with sanitation, as the eggs require specific environmental conditions to hatch (Standen, 1951). DNA could be extracted from samples collected directly from the sanitation facilities themselves (sludge, wastewater) or from soil interfacing with sanitation interventions. As soil is the main reservoir of STH eggs/ova, eDNA techniques have been used to detect and enumerate these parasites, for example hookworm ovum from soil and wastewater samples (Gyawali, Ahmed, Sidhu, Jagals, & Toze, 2017; Gyawali et al., 2016). Copy number of a gene can vary for eggs (due to their maturity for example), therefore a range of gene copy numbers was estimated for a single hookworm ovum, this was then used to estimate the total number of ova in a wastewater sample (Gyawali et al., 2017).

4.2 Limitations of eDNA

As previously mentioned, eDNA cannot distinguish live and potentially infectious parasites from dead or dying organisms. RNA-based PCR assays have been used for molecular diagnostics of sampled snails (Table 2) and environmental RNA (eRNA) could allow for detection of viability. Cercarial DNA was found to decay within 7 days in a lab-based setting (decay is likely to be faster under natural conditions) and RNA is less stable than DNA. Investigations into the decay of schistosome RNA will be key to assessing the usefulness of an eRNA tool for schistosomiasis. Gyawali et al. (2016) used propidium monoazide qPCR (PMA-qPCR) to successfully differentiate between viable and non-viable helminth ova of a species of hookworm (Ancylostoma caninum). For both eDNA and eRNA, the environmental processes that will affect their movement and decay within the environment should also be noted. Studies into the spatial and temporal fate of eDNA in aquatic environments (Harrison, Sunday, & Rogers, 2019) and soil (Prosser & Hedgpeth, 2018) in relation to biodiversity have been carried out but there are still knowledge gaps of the fate of eDNA in tropical ecosystems (reviewed in the context of fecal indicator bacteria (Rochelle-Newall, Nguyen, Le, Sengtahaueuanghoung, & Ribolzi, 2015).

Cost, local laboratory capacity, and level of intervention monitoring are important factors to consider that will ultimately determine which tools are used to monitor schistosomiasis. Snail surveys are imperfect but are field applicable: they require skilled personnel but the equipment needed for both the snail surveying (scoop, container for collection) and shedding (beakers, microscope) are reusable. Sengupta et al. (2019) included a cost analysis of their eDNA study. The cost of eDNA-based survey is comparable with traditional methods if the number of samples taken from each site is reduced and therefore laboratory consumable costs lowered (PCR reagents, filters).

If monitoring sanitation interventions, eDNA techniques cannot distinguish where the schistosome eggs have originated from: for example an inadequate sanitation facility or from open defecation. Local information would complement the molecular data captured from the environment. Information on community uptake of an intervention would also help to understand if detection of the parasite in the wider environment is due to failure of sanitation to contain or treat the parasite or open defecation. Community engagement is also a key component to sustained adoption of any WASH intervention (Galvin, 2015; Schmidt, 2015).

5 CONCLUSION

Quantifying transmission risk of S. mansoni, by eDNA techniques to sample the aquatic environment, can provide valuable information on transmission risk related to water access and use (Sato et al., 2018; Sengupta et al., 2019). However, data on the soil environment may be more beneficial for the evaluation of sanitation interventions including their uptake and effectiveness. eDNA could provide timely information on the success (or failure) of interventions in containing the fecal material and locate any leakage or general inadequate containment. The installation of improved sanitation interventions will have a delayed effect on a reduction of parasite burden for the local community, and environmental surveillance could be used as an early indicator. Uptake of interventions is as important as their functioning and information for local communities regarding sanitation upgrades could increase acceptability of interventions.
Monitoring the function of a sanitation technology, and water contact sites by eDNA and human infection levels combined could provide valuable information on local transmission pathways and the schistosomiasis force of infection within a given environment and how it could be reduced by different intervention types. This in turn would provide accurate data relating directly to the efficacy of sanitation and other WASH interventions which are currently difficult to monitor, and also therefore to model their potential impact (Toor et al., 2018). eDNA has the potential to be a highly sensitive and high throughput tool, needed for enrolment and maintenance of sanitation for long-term and sustainable control for schistosomiasis.

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Stephanie Connelly: Conceptualization; funding acquisition; supervision; writing-review and editing. Cindy Smith: Conceptualization; funding acquisition; supervision; writing-review and editing. Poppy Lamberton: Conceptualization; funding acquisition; supervision; writing-review and editing.

ORCID
Teteh S. Champion https://orcid.org/0000-0001-7397-3186
Stephanie Connelly https://orcid.org/0000-0001-5261-2090
Cindy J. Smith https://orcid.org/0000-0003-4905-0730
Poppy H. L. Lamberton https://orcid.org/0000-0003-1048-6318

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