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Occurrence of *Schistosoma bovis* on Pemba Island, Zanzibar: implications for urogenital schistosomiasis transmission monitoring

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Abstract

The causative agent of urogenital schistosomiasis, *Schistosoma haematobium*, was thought to be the only schistosome species transmitted through *Bulinus* snails on Unguja and Pemba Island (Zanzibar, United Republic of Tanzania). For insights into the environmental risk of *S. haematobium* transmission on Pemba Island, malacological surveys collecting *Bulinus globosus* and *B. nasutus*, two closely related potential intermediate hosts of *S. haematobium* were conducted across the island in November 2016. Of 1317 *B. globosus*/*B. nasutus* collected, seven *B. globosus*, identified through sequencing a DNA region of the mitochondrial cytochrome oxidase subunit 1 (*cox*1), were observed with patent infections assumed to be *S. haematobium*. However, when the collected cercariae were identified through sequencing a region of the *cox*1 and the nuclear internal transcribed spacer (ITS1 + 2), schistosomes from five of these *B. globosus* collected from a single locality were in fact *S. bovis*. The identified presence of *S. bovis* raises concerns for animal health on Pemba, and complicates future transmission monitoring of *S. haematobium*. These results show the pertinence for not only sensitive, but also species-specific markers to be used when identifying cercariae during transmission monitoring, and also provide the first molecular confirmation for *B. globosus* transmitting *S. bovis* in East Africa.

Introduction

The snail-borne neglected tropical disease (NTD), schistosomiasis, is the most important freshwater parasitic disease of humans associated with poverty, poor sanitation and lack of safe water supplies (Steinmann *et al.*, 2006; Hotez *et al.*, 2014), with an estimated 180–200 million people primarily from low- and middle-income countries being infected (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017). Ambitious goals to eliminate schistosomiasis have been announced by the WHO as part of its roadmap to overcome the global impact of NTDs by 2020–2025 (WHO, 2012). Whilst mass drug administration, behavioural change through education and snail control are having a major impact on schistosomiasis, further research into schistosome transmission biology together with better tools for transmission monitoring and surveillance are required to help achieve and monitor the success of these ambitious goals (Stothard *et al.*, 2017). Schistosomiasis is also a disease of animals, with large numbers of domestic livestock affected worldwide but the actual veterinary and economic impact is largely unknown (De Bont and Vercruysse, 1997, 1998).

There are 25 recognized species of mammalian schistosomes that cause human and animal infections, which can be split into four *Schistosoma* species groups (Webster *et al.*, 2006). The largest group is the *Schistosoma haematobium* group containing nine species that are all transmitted through *Bulinus* snails (Brown, 1994) with two species, *S. haematobium* and *S. bovis*, being responsible for the majority of all human (Hotez and Kamath, 2009) and livestock infections (De Bont and Vercruysse, 1997), respectively. Central to this group is *S. haematobium*, a major human schistosome species being the most widespread and prevalent across Africa and solely responsible for human urogenital schistosomiasis with often severe pathology (Schwartz, 1981; Leutscher *et al.*, 2000; Bustinduy *et al.*, 2014; Kjetland *et al.*, 2014; Christinet *et al.*, 2016). *Schistosoma bovis* is a pathogen of domestic livestock and some artiodactylids (Standley *et al.*, 2012), with its distribution commonly overlapping with that of *S. haematobium* across mainland Africa (Moné *et al.*, 1999), and utilising a wide range of *Bulinus* (Southgate and Knowles, 1975*a*, 1975*b*; Stothard *et al.*, 2004). These two species, among others, are also able to hybridize and inter-specific hybridization is now recognized in West Africa with possible detrimental consequences on disease control (Huyse *et al.*, 2009; Webster *et al.*, 2013; Léger and Webster, 2017).

Pemba and Unguja Islands (Zanzibar Archipelago, United Republic of Tanzania) have been historically identified as 'model islands' for implementing multiple effective infectious disease control and elimination programmes in sub-Saharan Africa (Pennance *et al.*, 2016). For schistosomiasis control, Zanzibar also offers an advantage due to the allopatric transmission of *S. haematobium* through a single snail host, *Bulinus globosus*, on both Islands (Stothard

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Table 1. Showing the collection sites and genetic profiles of the *Bulinus* and schistosome cercariae analysed

				Schistosoma cercariae mitochondrial and nuclear genetic profile	
Bulinus globosus ID (*cox*1 haplotype)	Shehia	Site (water body type)	*Schistosoma* cercariae species	*cox*1	ITS1 + 2
Kin2.1 (a)	Kinyasini	Kinya2 (stream)	*S. haematobium*	*S.h* (i)	*S.h*
Kin6.1 (b)	Kinyasini	Kinya6 (stream)	*S. bovis*	*S.b* (i)	*S.b*
Kin6.2 (b)	Kinyasini	Kinya6 (stream)	*S. bovis*	*S.b* (i & ii)	*S.b*
Kin6.3 (b)	Kinyasini	Kinya6 (stream)	*S. bovis*	*S.b* (i & ii)	*S.b*
Kin6.4 (b)	Kinyasini	Kinya6 (stream)	*S. bovis*	*S.b* (i)	*S.b*
Kin6.5 (b)	Kinyasini	Kinya6 (stream)	*S. bovis*	*S.b* (ii)	*S.b*
Cham10.1 (b)	Chambani	Cham10 (pond)	*S. haematobium*	*S.h* (ii)	*S.h*

Two *Bulinus globosus cox*1 haplotypes [Genbank accessions: (a) MH014040 and (b) MH014041]. Two *S. haematobium* cercariae *cox*1 haplotypes, Genbank accessions: *S.h* (i) MH014046 and *S.h* (ii) MH01404 and the two *S. bovis cox*1 haplotypes, Genbank accessions: *S.b* (i) MH014042 and *S.b* (ii) MH014043. ITS1 + 2 profiles showed no intra species variation (Genbank accessions: *S.h* MH014047 and *S.b* MH014044).

et al., 2000), whereas across most of sub-Saharan Africa, multiple *Schistosoma* and *Bulinus* species occur in sympatry (Brown, 1994), complicating control interventions and surveillance. Urogenital schistosomiasis was highly endemic on both islands but is now targeted for elimination (Knopp *et al.*, 2012, 2013).

As we move towards or reach elimination, there becomes a need for more sensitive methods to monitor the levels of transmission when egg–patent human infections become scarce (Le and Hsieh, 2017; Stothard *et al.*, 2017), the risk of infection and also a way to prove transmission interruption when it is finally reached. Xenomonitoring is a nucleic acid-based molecular diagnostic used to monitor the transmission of several vector-borne diseases (Cunningham *et al.*, 2016; Minetti *et al.*, 2016; Cook *et al.*, 2017), including to some extent schistosomiasis where tools are being developed for the xenomonitoring of snails that could support schistosomiasis transmission and elimination monitoring (Hamburger *et al.*, 2004; Allan *et al.*, 2013; Lu *et al.*, 2016; Abbasi *et al.*, 2017). The first stage for snail xenomonitoring for schistosomiasis is the identification of patent schistosome infections within the snails and collecting cercariae shed from them. Here, we report on the molecular identification of these cercariae and the infected snails collected from Pemba Island (Zanzibar) and how the findings complicate the development of robust molecular xenomonitoring protocols for ongoing and future transmission monitoring.

Methods

Malacological surveys and Schistosoma *collection*

In November 2016, as part of a larger ongoing molecular xenomonitoring study on Pemba, *Bulinus* snails were collected, by scooping, from human freshwater contact sites in eight shehias (smallest division of administrative regions), examined and individually induced to shed cercariae following previous methods (Allan *et al.*, 2013). An experienced microscopist identified schistosome cercariae, which were individually pipetted in 3.5 μL aliquots onto Whatman FTA cards (Whatman, Part of GE Healthcare, Florham Park, USA) for long-term deoxyribonucleic acid (DNA) storage. After shedding, all infected snails were preserved in 100% ethanol for future morphological and molecular characterization.

Schistosoma *and* Bulinus *identification*

DNA from individual cercariae was eluted from the FTA cards (Webster *et al.*, 2015) and characterized by amplification and sequencing of the mitochondrial cytochrome oxidase subunit 1 (*cox*1) and partial nuclear internal transcribed spacer (ITS1 + 2) DNA regions (Webster *et al.*, 2012).

To determine the species of the infected snails, total genomic DNA was extracted from the whole snail tissue using the DNeasy Blood & Tissue Kit (Qiagen, Manchester, UK), with minor changes to the standard protocol in that quantities of the digest reagents were doubled and digests were incubated for at least 12 h. From each snail, a 623 base pair region of the mitochondrial *cox*1 gene was amplified and Sanger sequenced using primers BulCox1 and CO2 following previous protocols (Kane *et al.*, 2008). The sequence data were manually edited in Sequencher v5.1 (http://genecodes.com) before being compared with reference sequence databases for *Bulinus* (Kane *et al.*, 2008) and *Schistosoma* (Webster *et al.*, 2012, 2013) to confirm species.

Results

In total, 1317 *B. globosus* and *B. nasutus* were collected, seven of these snails (Table 1) from Kinyasini (6) and Chambani (1) shehia were shedding schistosome cercariae (Fig. 1). The infected snails were identified as *B. globosus* with two *cox*1 haplotypes recognized (GenBank accession numbers: MH014040 and MH014041) which matched those snails previously reported from Pemba (Kane *et al.*, 2008). Cercariae collected from these were assumed initially to be the human parasite *S. haematobium*; however, molecular characterizations of the cercariae from five of these snails, collected from a stream in Kinyasini (Kinya6), were identified as *S. bovis* (Table 1). Two different *S. bovis cox*1 haplotypes [Genbank accessions: *S.b* (i) MH014042 and *S.b* (ii) MH014043] (Table 1) were identified from these five snails; three snails producing *S. bovis* cercariae of a single haplotype and two snails producing *S. bovis* cercariae of both haplotypes suggesting that they had been infected by more than one miracidium.

The other two infected snails shed *S. haematobium* cercariae and were collected from a pond in Chambani (Cham10) and a different stream site in Kinyasini (Kinya2). The *S. haematobium* cercariae from Kinyasini and Chambani, respectively, were of two different *S. haematobium cox*1 haplotypes [Genbank accessions: *S.h* (i) MH014046 and *S.h* (ii) MH014045] with only single haplotypes produced from each snail. These haplotypes matched those identified as group 2 *S. haematobium cox*1 haplotypes found only in the Indian Ocean Islands (Webster *et al.*, 2012).

ITS1 + 2 profiles showed no intra-species variation (Genbank Accessions: *S.h* MH014047 and *S.b* MH014044) and were

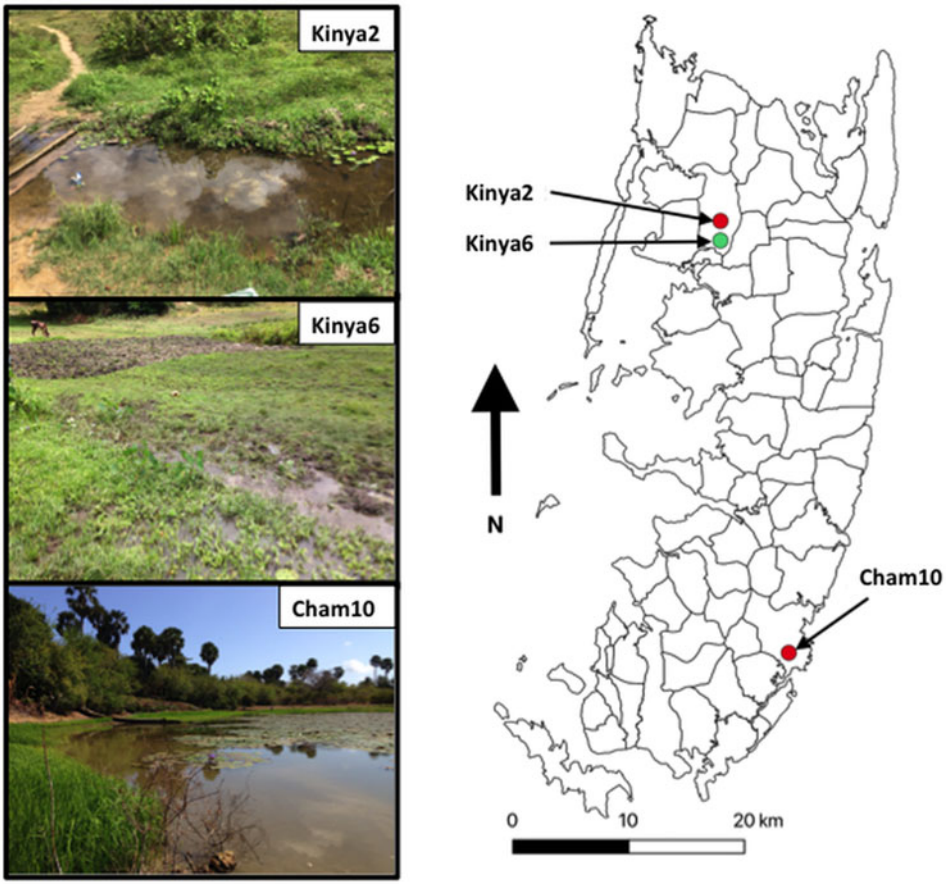

Fig. 1. Map outlining shehias (smallest division of administrative regions) on Pemba Island, Zanzibar (United Republic of Tanzania) showing the location and images of two freshwater bodies in Kinyasini (Kinya2 and Kinya6) and one in Chambani (Cham10) where *Schistosoma haematobium* (red) and *Schistosoma bovis* (green) cercariae were recovered from *Bulinus globosus*. GPS coordinates for sites (latitude and longitude in decimal degrees): Kinya2 (−5.02033°, 39.73855°); Kinya6 (−5.03560°, 39.73850°); Cham10 (−5.35805°, 39.79182°).

identified as either *S. bovis* or *S. haematobium* by the three inter-specific single nucleotide polymorphisms (Webster *et al.*, 2012).

Discussion

The detection of *S. bovis* on Pemba Island poses a potentially new threat to domestic livestock and wildlife health in Zanzibar (De Bont and Vercruysse, 1997, 1998; Standley *et al.*, 2012). The site where *S. bovis* transmission was identified had grazing cattle (see Fig. 1, Kinya6) in close proximity to the water where the shedding snails were collected; therefore, it is quite likely that ongoing transmission is being maintained. Moreover, the movement of infected cattle could enable the spread of the infection particularly as *B. globosus* are found throughout most of the island (Stothard *et al.*, 1997).

The presence of *S. bovis* complicates the monitoring of *S. haematobium* transmission since both parasites are shown here to infect the same intermediate snail host and cannot be distinguished from each other easily by microscopy. Therefore, *S. bovis*-infected *B. globosus* could be falsely identified as infected with *S. haematobium*, or *vice-versa*, complicating urogenital schistosomiasis transmission monitoring. This accentuates the need for routine molecular identification of schistosome infections in snails during malacological surveys (Minetti *et al.*, 2016), and the development of more species-specific xenomonitoring tools to differentiate *S. bovis* and *S. haematobium* transmission (Webster *et al.*, 2010; Abbasi *et al.*, 2017). The identification of schistosome cercariae shed from snails is often presumed to be of a particular species due to the snail host involved or the locality of the transmission. Our findings strongly emphasize that these assumptions are not accurate and transmission dynamics of different species may change over time and space. The assumed transmission of only *S. haematobium* by *B. globosus* on Zanzibar and the non-identification of these *S. bovis* infections would have led us to believe that the level of *S. haematobium* transmission is much higher than it actually is, hampering ongoing and future urogenital schistosomiasis transmission monitoring and surveillance.

Schistosoma haematobium and *S. bovis* hybridization has also been detected in sympatric West African areas (Webster *et al.*, 2013). Zanzibar was considered to be an allopatric area for *S. haematobium* (Webster *et al.*, 2012) but the identification of this sympatry with *S. bovis* could, in time, lead to inter-species hybridization. The potential consequences of hybridization include increased host associations of hybrids, possible zoonotic transmission and hybrid vigour (Huyse *et al.*, 2009; Webster *et al.*, 2013; Léger and Webster, 2017). Investigating the origin of *S. bovis* being transmitted on Pemba, by genetic comparison with other mainland strains of *S. bovis*, may help elucidate how this parasite has been imported to Zanzibar. Since the eradication of the tsetse fly, the vector of human and African animal trypanosomiasis, on Unguja Island (Vreysen *et al.*, 2000), there has been an increase of cattle farming (Mdoe, 2003) facilitated by the import of cattle under strict guidelines of the United Republic of Tanzania's Animal Resources Management Act (1999). Bovine schistosomiasis however is widely ignored/unknown as a veterinary health problem, and therefore is currently not included in these guidelines. This oversight could offer some explanation to how and within what time scale the introduction, or multiple introductions, of *S. bovis* may have occurred. Additionally, the prevalence and intensity of *S. bovis* in local cattle and other potential artiodactylid hosts (Standley *et al.*, 2012), such as the Ader's duiker (*Cephalophus adersi*) endemic to Zanzibar, should be determined to assess the impact on livestock and wildlife health. However, diagnosing *S. bovis* from the definitive host remains challenging, with the detection of *S. bovis* eggs in the stool being difficult

and the more sensitive method of observing adult worms in the host being only possible post-mortem *via* dissection. An antigen-based test with promising diagnostic performance has been developed (de la Torre-Escudero *et al.*, 2012), which could offer a sensitive method for judging the epidemiology of *S. bovis* in Pemba.

Due to the difficulty in classifying species within the *Bulinus africanus* species complex (Kane *et al.*, 2008), previous findings on snail–schistosome compatibilities should be treated with some caution. This molecular confirmation of *B. globosus* naturally transmitting *S. bovis* in East Africa gives credibility to a previous observation (Mwambungu, 1988), and dispels previous claims of *B. globosus* being naturally refractory (Christensen *et al.*, 1983) or only an intermediate host in West Africa (Diaw and Vassiliades, 1987; Ndifon *et al.*, 1988). Previous evidence for compatibility of *B. nasutus* with *S. bovis* in East Africa is also tainted with contradicting evidence, some showing natural infections (Dowdeswell, 1938; Kinoti, 1964*b*) going against failed experimental infections (Southgate and Knowles, 1975*a*, 1975*b*; Southgate *et al.*, 1980) and a lack of naturally infected *B. nasutus* in other endemic areas (Kinoti, 1964*a*; Southgate *et al.*, 1980; Mutani *et al.*, 1983). It is likely that *S. bovis* has a broad intermediate host range in East Africa utilising several *Bulinus* species, as it has also been identified from *B. ugandae* (Malek, 1969), *B. africanus* (McClelland, 1955; Teesdale and Nelson, 1958; Kassuku *et al.*, 1986) and *B. forskalii* (McClelland, 1955). Therefore, studies to confirm the intermediate snail host vectoral capacity and specificity of *S. bovis* to *B. globosus* or indeed other endemic *Bulinus* species on Pemba, including *B. nasutus* and *B. forskalii*, are required to determine the transmission potential and possible spread of this emerging schistosome in Zanzibar.

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References

Abbasi I, Webster BL, King CH, Rollinson D and Hamburger J (2017) The substructure of three repetitive DNA regions of *Schistosoma haematobium* group species as a potential marker for species recognition and interbreeding detection. *Parasites and Vectors* **10**, 364.

Allan FE, Dunn AM, Emery AM, Stothard JR, Johnston DA, Kane RA, Khamis AN, Mohammed KA and Rollinson D (2013) Use of sentinel snails for the detection of *Schistosoma haematobium* transmission on Zanzibar and observations on transmission patterns. *Acta Tropica* **128**, 234–240.

Brown DS (1994) *Freshwater Snails of Africa and Their Medical Importance*, 2nd Edn. London, UK: Taylor & Francis.

Bustinduy A, King C, Scott J, Appleton S, Sousa-Figueiredo JC, Betson M and Stothard JR (2014) HIV and schistosomiasis co-infection in African children. *The Lancet Infectious Diseases* **14**, 640–649.

Christensen NØ, Mutani A and Frandsen F (1983) A review of the biology and transmission ecology of African bovine species of the genus *Schistosoma*. *Zeitschrift Für Parasitenkunde* **69**, 551–570.

Christinet V, Lazdins-Helds JK, Stothard JR and Reinhard-Rupp J (2016) Female genital schistosomiasis (FGS): from case reports to a call for concerted action against this neglected gynaecological disease. *International Journal for Parasitology* **46**, 395–404.

Cook DAN, Pilotte N, Minetti C, Williams SA and Reimer LJ (2017) A superhydrophobic cone to facilitate the xenomonitoring of filarial parasites, malaria, and trypanosomes using mosquito excreta/feces [version 2; referees: 2 approved]. *Gates Open Research* **1**, 7. doi: 10.12688/gatesopenres. 12749.1.

Cunningham LJ, Lingley JK, Haines LR, Ndung'u JM, Torr SJ and Adams ER (2016) Illuminating the prevalence of *Trypanosoma brucei s.l.* in *Glossina* using LAMP as a tool for xenomonitoring. *PLoS Neglected Tropical Diseases* **10**, e0004441.

De Bont J and Vercruysse J (1997) The epidemiology and control of cattle schistosomiasis. *Parasitology Today* **13**, 255–262.

De Bont J and Vercruysse J (1998) Schistosomiasis in cattle. *Advances in Parasitology* **41**, 285–364.

de la Torre-Escudero E, Manzano-Román R, Pérez-Sánchez R, Barrera I, Siles-Lucas M and Oleaga A (2012) Molecular cloning, characterization and diagnostic performance of the *Schistosoma bovis* 22.6 antigen. *Veterinary Parasitology* **190**, 530–540.

Diaw OT and Vassiliades G (1987) Epidémiologie des schistosomoses du bétail au Sénégal. *Revue d'Elevage et de Médecine Vétérinaire Des Pays Tropicaux* **40**, 265–274.

Dowdeswell RM (1938) Schistosomiasis in the Kavirondo district of Kenya colony. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **31**, 673–688.

GBD 2016 Disease and Injury Incidence and Prevalence Collaborators (2017) Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet* **390**, 1211–1259.

Hamburger J, Hoffman O, Kariuki HC, Muchiri EM, Ouma JH, Koech DK, Sturrock RF and King CH (2004) Large-scale, polymerase chain reaction-based surveillance of *Schistosoma haematobium* DNA in snails from transmission sites in coastal Kenya: a new tool for studying the dynamics of snail infection. *American Journal of Tropical Medicine and Hygiene* **71**, 765–773.

Hotez PJ and Kamath A (2009) Neglected tropical diseases in sub-Saharan Africa: review of their prevalence, distribution, and disease burden. *PLoS Neglected Tropical Diseases* **3**, 1–10.

Hotez PJ, Alvarado M, Basáñez M-G, Bolliger I, Bourne R, Boussinesq M, Brooker SJ, Brown AS, Buckle G, Budke CM, Carabin H, Coffeng LE, Fèvre EM, Fürst T, Halasa YA, Jasrasaria R, Johns NE, Keiser J, King CH, Lozano R, Murdoch ME, O'Hanlon S, Pion SD, Pullan RL, Ramaiah KD, Roberts T, Shepard DS, Smith JL, Stolk WA, Undurraga EA, Utzinger J, Wang M, Murray CJ and Naghavi M (2014) The Global Burden of Disease Study 2010: interpretation and implications for the neglected tropical diseases. *PLoS Neglected Tropical Diseases* **8**, e2865.

Huyse T, Webster BL, Geldof S, Stothard JR, Diaw OT, Polman K and Rollinson D (2009) Bidirectional introgressive hybridization between a cattle and human schistosome species. *PLoS Pathogens* **5**, e1000571.

Kane RA, Stothard JR, Emery AM and Rollinson D (2008) Molecular characterization of freshwater snails in the genus *Bulinus*: a role for barcodes? *Parasites and Vectors* **1**, 15.

Kassuku A, Christensen NO, Monrad J, Nansen P and Knudsen J (1986) Epidemiological studies on *Schistosoma bovis* in Iringa Region, Tanzania. *Acta Tropica* **43**, 153–163.

Kinoti G (1964*a*) A note on the susceptibility of some gastropod molluscs to *Schistosoma bovis* and *S. mattheei*. *Annals of Tropical Medicine & Parasitology* **58**, 270–275.

Kinoti G (1964*b*) Observations on the transmission of *Schistosoma haematobium* and *Schistosoma bovis* in the Lake Region of Tanganyika. *Bulletin of the World Health Organization* **31**, 815.

Kjetland EF, Hegertun IEA, Baay MFD, Onsrud M, Ndhlovu PD and Taylor M (2014) Genital schistosomiasis and its unacknowledged role on HIV transmission in the STD intervention studies. *International Journal of STD & AIDS* **25**, 705–715.

Knopp S, Mohammed KA, Ali SM, Khamis IS, Ame SM, Albonico M, Gouvras A, Fenwick A, Savioli L and Colley DG (2012) Study and

implementation of urogenital schistosomiasis elimination in Zanzibar (Unguja and Pemba islands) using an integrated multidisciplinary approach. *BMC Public Health* **12**, 930.

Knopp S, Person B, Ame SM, Mohammed KA, Ali SM, Khamis IS, Rabone M, Allan F, Gouvras A and Blair L (2013) Elimination of schistosomiasis transmission in Zanzibar: baseline findings before the onset of a randomized intervention trial. *PLoS Neglected Tropical Diseases* **7**, e2474.

Le L and Hsieh MH (2017) Diagnosing urogenital schistosomiasis: dealing with diminishing returns. *Trends in Parasitology* **33**, 378–387.

Léger E and Webster JP (2017) Hybridizations within the genus *Schistosoma*: implications for evolution, epidemiology and control. *Parasitology* **144**, 65–80.

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

Lu L, Zhang S-M, Mutuku MW, Mkoji GM and Loker ES (2016) Relative compatibility of *Schistosoma mansoni* with *Biomphalaria sudanica* and *B. pfeifferi* from Kenya as assessed by PCR amplification of the *S. mansoni* ND5 gene in conjunction with traditional methods. *Parasites and Vectors* **9**, 166.

Malek EA (1969) Studies on bovine schistosomiasis in the Sudan. *Annals of Tropical Medicine & Parasitology* **63**, 501–513.

McClelland WFJ (1955) Two species of *Bulinus* found naturally infected with a bovine schistosome in Western Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **49**, 295.

Mdoe NSY (2003) Livestock and agriculture development in Zanzibar, post-tsetse eradication: a follow-up socioeconomic study. International Atomic Energy Agency confidential report, Vienna. https://doi.org/10.1371/journal.pntd.0002857.s002.

Minetti C, LaCourse JE, Reimer L and Storhard JR (2016) Focusing nucleic acid-based molecular diagnostics and xenomonitoring approaches for human helminthiases amenable to preventive chemotherapy. *Parasitology Open* **2**, e16.

Moné H, Mouahid G and Morand S (1999) The distribution of *Schistosoma bovis* Sonsino, 1876 in relation to intermediate host mollusc-parasite relationships. *Advances in Parasitology* **44**, 99–138.

Mutani A, Christensen NØ and Frandsen F (1983) Studies on the relationship between *Schistosoma* and their intermediate hosts. *Parasitology Research* **69**, 483–487.

Mwambungu JA (1988) Transmission of *Schistosoma bovis* in Mkulwe (Mbozi District, Mbeya Region, Southern Highlands of Tanzania). *Journal of Helminthology* **62**, 29–32.

Ndifon GT, Betterton C and Rollinson D (1988) *Schistosoma curassoni* Brumpt, 1931 and *S. bovis* (sonsino, 1876) in cattle in northern Nigeria. *Journal of Helminthology* **62**, 33–34.

Pennance T, Person B, Muhsin MA, Khamis AN, Muhsin J, Khamis IS, Mohammed KA, Kabole F, Rollinson D and Knopp S (2016) Urogenital schistosomiasis transmission on Unguja Island, Zanzibar: characterisation of persistent hot-spots. *Parasites and Vectors* **9**, 646.

Schwartz DA (1981) Helminths in the induction of cancer II. *Schistosoma haematobium* and bladder cancer. *Tropical and Geographical Medicine* **33**, 1–7.

Southgate VR and Knowles RJ (1975*a*) Observations on *Schistosoma bovis* Sonsino, 1876. *Journal of Natural History* **9**, 273–314.

Southgate VR and Knowles RJ (1975*b*) The intermediate hosts of *Schistosoma bovis* in western Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **69**, 356–357.

Southgate VR, Rollinson D, Ross GC and Knowles RJ (1980) Observations on an isolate of *Schistosoma bovis* from Tanzania. *Parasitology Research* **63**, 241–249.

Standley CJ, Mugisha L, Dobson AP and Stothard JR (2012) Zoonotic schistosomiasis in non-human primates: past, present and future activities at the human-wildlife interface in Africa. *Journal of Helminthology* **86**, 131–140.

Steinmann P, Keiser J, Bos R, Tanner M and Utzinger J (2006) Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *The Lancet Infectious Diseases* **6**, 411–425.

Stothard JR, Mgeni AF, Alawi KS, Savioli I and Rollinson D (1997) Observations on shell morphology, enzymes and Random Amplified Polymorphic DNA (RAPD) in *Bulinus africanus* group snails (Gastropoda: Planorbidae) in Zanzibar. *Journal of Molluscan Studies* **63**, 489–503.

Stothard JR, Loxton N, Rollinson D, Mgeni AF, Khamis S, Ameri H, Ramsan M and Savioli L (2000) The transmission status of *Bulinus* on Zanzibar Island (Unguja), with implications for control of urinary schistosomiasis. *Annals of Tropical Medicine and Parasitology* **94**, 87–94.

Stothard JR, Lockyer AE, Kabatereine NB, Tukahebwa EM, Kazibwe F, Rollinson D and Fenwick A (2004) *Schistosoma bovis* in western Uganda. *Journal of Helminthology* **78**, 281–284.

Stothard JR, Campbell SJ, Osei-Atweneboana MY, Durant T, Stanton MC, Biritwum N-K, Rollinson D, Ombede DRE and Tchuem-Tchuenté L-A (2017) Towards interruption of schistosomiasis transmission in sub-Saharan Africa: developing an appropriate environmental surveillance framework to guide and to support `end game' interventions. *Infectious Diseases of Poverty* **6**, 10.

Teesdale C and Nelson GS (1958) Recent work on schistosomes and snails in Kenya. *East African Medical Journal* **35**, 427–436.

Vreysen MJB, Saleh KM, Ali MY, Abdulla AM, Zhu ZR, Juma KG, Dyck VA, Msangi AR, Mkonyi PM and Feldmann HU (2000) The use of the sterile insect technique (SIT) for the eradication of the tsetse fly *Glossina austeni* (Diptera: Glossinidae) on the Island of Unguja (Zanzibar). *Journal of Economic Entomology* **93**, 123–135.

Webster BL, Southgate VR and Littlewood DTJ (2006) A revision of the interrelationships of *Schistosoma* including the recently described *Schistosoma guineensis*. *International Journal for Parasitology* **36**, 947–955.

Webster BL, Rollinson D, Stothard JR and Huyse T (2010) Rapid diagnostic multiplex PCR (RD-PCR) to discriminate *Schistosoma haematobium* and *S. bovis*. *Journal of Helminthology* **84**, 107–114.

Webster BL, Emery AM, Webster JP, Gouvras A, Garba A, Diaw O, Seye MM, Tchuente LAT, Simoonga C, Mwanga J, Lange C, Kariuki C, Mohammed KA, Stothard JR and Rollinson D (2012) Genetic diversity within *Schistosoma haematobium*: DNA barcoding reveals two distinct groups. *PLoS Neglected Tropical Diseases* **6**, e1882.

Webster BL, Diaw OT, Seye MM, Webster JP and Rollinson D (2013) Introgressive hybridization of *Schistosoma haematobium* group species in Senegal: species barrier break down between ruminant and human schistosomes. *PLoS Neglected Tropical Diseases* **7**, e2110.

Webster BL, Rabone M, Pennance T, Emery AM, Allan F, Gouvras A, Knopp S, Garba A, Hamidou AA, Mohammed KA, Ame SM, Rollinson D and Webster JP (2015) Development of novel multiplex microsatellite polymerase chain reactions to enable high-throughput population genetic studies of *Schistosoma haematobium*. *Parasites and Vectors* **8**, 432.

World Health Organization (2012) Accelerating work to overcome the global impact of neglected tropical diseases – A roadmap for implementation. Available at http://www.who.int/neglected_diseases/NTD_RoadMap_2012_Fullversion.pdf (accessed 25 June 2016).