The detection of *Schistosoma bovis* in livestock on Pemba Island, Zanzibar: A preliminary study

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ARTICLE INFO	ABSTRACT
	Schistosoma bovis is a parasitic trematode of ungulates transmitted by freshwater snails in Sub-Saharan Africa causing bovine intestinal schistosomiasis that leads to chronic morbidity and significant agricultural economic losses. The recently reported occurrence of *Bulinus globosus* infected with *S. bovis* for the first time on Pemba Island (Zanzibar, United Republic of Tanzania) is a cause of concern for livestock/wildlife health and complicates the surveillance of *Schistosoma haematobium*. To confirm that local cattle are infected with *S. bovis*, fresh faecal samples were collected from six adult cows surrounding two schistosomiasis transmission sites in Kinyasini, Pemba Island. Schistosome eggs were concentrated, egg hatching stimulated and miracidia were individually captured and identified by analysis of the partial mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) and the partial nuclear internal transcribed spacer region (ITS1+5.8S+ITS2). Two *S. bovis* miracidia were collected from one faecal sample with two *cox*1 haplotypes, one matching *cox*1 data obtained from *S. bovis* cercariae, collected previously at the same site in Pemba, the other matching *S. bovis cox*1 data originating from coastal Tanzania. The findings conclude that *S. bovis* transmission has been established on Pemba Island and is likely to have been imported through livestock trade with East Africa. Increasing the sensitivity of non-invasive diagnostics for bovine schistosomiasis, together with wider sampling, will enable a better assessment on the epidemiology of *S. bovis* on Pemba Island.

1. Introduction

Although the burden of human schistosomiasis is well reported, significantly less is known about infections in wildlife and domestic livestock, despite the high number of infections and species (host and *Schistosoma*) involved (Webster et al., 2006). When last estimated, at least 165 million cattle were predicted to be infected worldwide with schistosomes, although this was likely a gross underestimation (De Bont & Vercruysse, 1997). If not controlled through preventative chemotherapy, bovine schistosomiasis can cause significant health problems for cattle, such as anaemia, emaciation, haemorrhagic enteritis, and death, ultimately incurring economic costs by affecting local farming practices through loss of meat and milk products from infected livestock (De Bont

& Vercruysse, 1998; Legesse et al., 2014). The *Schistosoma haematobium* species group is diverse containing nine described species, five of which cause livestock schistosomiasis. Of these *Schistosoma bovis* is the most prevalent, pathogenic (along with *Schistosoma mattheei*) and widespread, found throughout northern, western and eastern Africa (except Egypt), the Middle East, Asia, and some countries bordering the Mediterranean Sea (Standley et al., 2012a, 2012b; Calavas & Martin, 2014; Gower et al., 2017; Pennance et al., 2018).

Extending schistosomiasis control strategies to include treatment of livestock and zoonotic schistosomiasis still requires a comprehensive evaluation to determine if this is economically and epidemiologically viable (Gower et al., 2017). A renewed interest in a One Health approach for tackling infections caused by species of the *S. haematobium* group,

including the sampling of livestock and understanding zoonotic species, has been brought about by the identification of hybrids involving human- and animal-infecting species of the *S. haematobium* group, such as *S. haematobium* and *S. bovis*, and more recently *S. mattheei* (see Léger & Webster, 2017; Catalano et al., 2018; Djuikwo-Teukeng et al., 2019; Léger et al., 2020; Rey et al., 2021; Savassi et al., 2021). Although *S. bovis* is endemic across a wide range, its presence has not been recorded on Indian Ocean Islands neighbouring the east coast of the African continent until the recent identification of *S. bovis*-infected *Bulinus globosus*, the intermediate host snail species, on Pemba Island, Zanzibar (Pennance et al., 2018).

Zanzibar, an archipelago of two islands situated off the east coast of Tanzania in the Indian Ocean is composed of two major islands, Unguja to the south and Pemba to the north, as well as several smaller isles. Pemba and Unguja have a long history of urogenital schistosomiasis research and control, which dates back almost a century (Crofton, 1928) and is now being targeted as the next elimination setting (Knopp et al., 2019). For urogenital schistosomiasis control and elimination on Zanzibar, the islands were thought to offer an advantage due to the allopatric transmission of *S. haematobium* predominantly through a single snail host, *B. globosus*, restricting areas where transmission can take place (Stothard et al., 2000). Recent observations on Pemba, however, demonstrated the occurrence of *S. bovis* transmission with the detection of shedding *B. globosus* snails collected from a stream that neighboured a cattle grazing area (Pennance et al., 2018).

The detection of *S. bovis* on Pemba not only complicates future transmission monitoring of *S. haematobium* but also poses a potentially new threat to domestic livestock and wildlife health in Zanzibar (Pennance et al., 2018). Schistosomiasis diagnosis in animals remains problematic, with most of our understanding of schistosomes infecting cattle derived from post-mortem autopsies (Léger & Webster, 2017). Methods relying on miracidial hatching or stool microscopy for eggs both lack sensitivity due to the difficulty in processing large amounts of cattle faeces (Giovanoli Evack et al., 2020). However, miracidial hatching ensures the viability of eggs and allows for the capture of miracidia for genetic analysis (De Bont et al., 1996; De Bont & Vercruysse, 1998; Savassi et al., 2020).

Here, we aimed to determine if cattle were infected with *S. bovis* on Pemba Island to confirm established transmission and to collect miracidia for genetic analysis and comparison to mainland African *S. bovis*.

2. Materials and methods

2.1. Study site and sampling

In mid-February 2019, fresh stool samples were collected from six adult cows (*Bos taurus*) across two sites in Kinyasini (Pemba Island). Three cows were sampled in the immediate area surrounding site Kinya6 (coordinates: −5.03560, 39.73972), a stream surrounded by marsh

previously reported for the occurrence of several *S. bovis*-infected *B. globosus* (see Pennance et al., 2018). Fresh stool samples were also taken from three cows in the immediate vicinity of a second stream site, Kinya9 (coordinates: −5.03077, 39.73333), 800 m north-west of the first site where only *S. haematobium* was known to be transmitted. A photo was taken of each cow and a 300 ml collection pot was filled using a spatula with freshly excreted faeces and transported back to the Public Health Laboratory (Chake Chake, Pemba Island). Faecal samples were stored at 4 °C upon return to the laboratory, and within 24 h of collection, each faecal sample was individually washed with 0.85% saline solution through four sieves of decreasing mesh size (1.4 mm; 710 μm; 355 μm; and 212 μm) to remove large debris and the sediment was collected in a final retention sieve of 125 μm (Fig. 1). This remaining sediment was subdivided in half to trial two different miracidia hatching methods, using either a Pitchford funnel or sedimentation flask (Fig. 1).

For the Pitchford funnel miracidial hatching method, the faecal solution was washed within the Pitchford funnel (Pitchford & Visser, 1975) (mesh sizes: inner sieve 200 μm; outer sieve 40 μm) using room temperature bottled water concentrated to provide a faecal sediment, which was then placed equally in three separate 90 mm Petri dishes (AB260; Appleton Woods Ltd., Birmingham, UK). Room temperature bottled water was added to each Petri dish and placed in indirect sunlight outside (28–34 °C) for at least 1 h to induce miracidia hatching. Petri dishes were checked for swimming miracidia at 4, 8, 20 and 24 h after plating.

For the sedimentation flask method, the other half of the sieved faecal solution was placed directly in individual 250 ml sedimentation flasks, filled with room temperature bottled water to the 250 ml mark, mixed using a wooden spatula and left in indirect sunlight outside (28–34 °C) for 1 h. Each sedimentation flask was then completely covered in a heavy microfibre piece of black fabric, except for the top 25 ml (i.e. between 225 and 250 ml) which was left exposed to light from a desk lamp to encourage any miracidia present to move to surface. Between 5 and 10 ml of liquid was removed from the top of the liquid surface and placed in a Petri dish to check for swimming miracidia following 4, 8, 20 and 24 h.

For both methods, any swimming *S. bovis* miracidia observed in Petri dishes, using a dissection microscope, were captured and individually pipetted in 3.5 μl onto Whatman FTA cards (Whatman, Part of GE Healthcare, Florham Park, USA) for subsequent molecular analyses (Gower et al., 2007; Webster et al., 2012).

2.2. Molecular analyses of Schistosoma specimens

Following elution of parasite DNA from Whatman FTA cards as described in Webster et al. (2015), schistosome species identification was performed by mito-nuclear analyses targeting a partial mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) region (956 bp) and the complete nuclear internal transcribed spacer (ITS1+5.8S + ITS2) rDNA region (967 bp) following previous methods (Webster et al., 2012, 2013a). Sequence data were manually edited and trimmed for both *cox*1 (750 bp) and
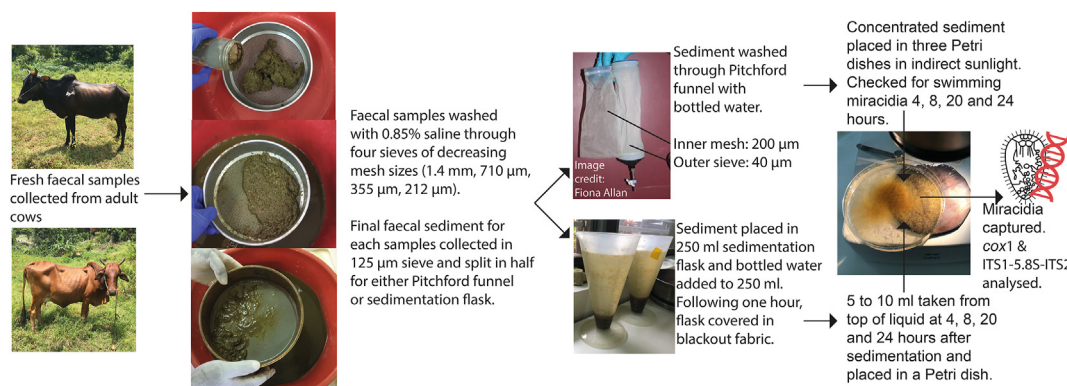

Fig. 1. Outline of two protocols for the detection, collection and identification of *Schistosoma bovis* miracidia from bovine faecal samples.

ITS1+5.8S+ITS2 (880 bp) in Sequencher v5.4.6 (GeneCodes Corp., Michigan, USA). The *cox*1 and ITS species identification was inferred by comparison to reference data as described in Webster et al. (2012, 2013b).

3. Results and discussion

From the six cow stool samples processed from the two sites in Kinyasini, miracidia were successfully hatched from one sample from site Kinya6 using the Pitchford funnel method. No miracidia were recovered from samples processed using the sedimentation flask method. From the positive sample, two swimming miracidia were recovered and molecularly identified as *S. bovis* with two *cox*1 haplotypes (Sb2; GenBank: OK484568 and Sb3; GenBank: OK484569), which differed from each other by nine single nucleotide polymorphisms. The ITS profiles from the two *S. bovis* miracidia were identical (GenBank: OK447652), and showed no intraspecies variation to the previously sequenced cercarial samples from Pemba Island (Pennance et al., 2018).

The adult cow, that two miracidia were recoved from, was from site Kinya6, where *S. bovis*-infected *B. globosus* have previously been identified (Pennance et al., 2018). Of the two *S. bovis cox*1 haplotypes identified from the miracidia, one (Sb2) was identical in its overlapping sequence (664 bp) to the *S. bovis cox*1 haplotype previously identified from *S. bovis* cercariae released from the *B. globosus* collected at this same site (Kinya6) in the previous study conducted in 2016 (GenBank: MH014043; see Pennance et al., 2018). The second *S. bovis cox*1 haplotype (Sb3) was identical to a *S. bovis* isolate originating from Iringa, coastal Tanzania (GenBank: AY157212; see Lockyer et al., 2003). The matching *S. bovis* haplotypes from the cow and also from the cercariae released by *B. globosus* confirmed direct and ongoing transmission at this site. The close relatedness of the haplotypes to *S. bovis* from Tanzania suggests introduction into Pemba from the African mainland, possibly *via* cattle importation, rather than being a distinct population originating in Pemba (see Supplement S2 in Vreysen et al., 2014; Pennance et al., 2018). Investigating the route of *S. bovis* transmission to Pemba by a more extensive genetic comparison with other mainland strains of *S. bovis*, such as those from coastal Kenya, may help elucidate how this parasite was introduced to Zanzibar. There has been a steady increase in cattle farming on Zanzibar since the late 20th Century (see Supplement S2 in Vreysen et al., 2014) that has been facilitated by the importation of cattle under strict guidelines of the United Republic of Tanzania's Animal Resources Management Act of 1999 (https://www.fao.org/faolex/resul ts/details/en/c/LEX-FAOC172321/). Although many veterinary concerns are covered in these nationwide guidelines, bovine schistosomiasis is not included here despite the risk to livestock/wildlife health.

The confirmed presence of *S. bovis* infecting cattle on Pemba Island is a cause for concern since increased transmission could lead to significant animal health and economic impact, as well as a potential risk for hybridisation with *S. haematobium* (Huyse et al., 2009; Savassi et al., 2020). Since many studies have investigated the larval schistosomes shed from the endemic *Bulinus* spp. collected from the Zanzibar archipelago (Stothard et al., 2002; Allan et al., 2013; Pennance et al., 2016), and none have identified *S. bovis* before 2016 (Pennance et al., 2018), it could be proposed that the introduction of *S. bovis* is recent. This is supported by the fact that in other endemic regions where *S. bovis* and *S. haematobium* occur in a well-established sympatry, the abundance of *S. bovis*-infected *Bulinus* spp. is often several times higher than that of *S. haematobium* (Pennance et al., 2020).

Our trial in recovering bovine schistosomes following miracidial hatching was successful in that we detected, collected and identified two *S. bovis* miracidia using a non-invasive sampling method. This low number of miracidia is expected (Giovanoli Evack et al., 2020), especially from such reduced sampling. In the immediate future on Pemba Island, a better assessment of *S. bovis* is required to determine the epidemiology of bovine infections. In addition to screening *Bulinus* snails for *Schistosoma* infections and differentiating *S. haematobium* and *S. bovis* (Pennance et al., 2018), one could also assess the prevalence and burden of disease in

cattle by dissections after animals are slaughtered for meat, as is the case in the majority of studies investigating bovine schistosomiasis (Léger & Webster, 2017).

4. Conclusion

To the best of our knowledge, we provide here the first evidence of *S. bovis* transmission on Pemba Island. Further faecal sampling on Pemba Island and trials to improve the sensitivity of *S. bovis* diagnosis are essential to identify the distribution and impact of bovine schistosomiasis on livestock, and assess the requirement for starting livestock treatment to curtail transmission. The latter goal is critical considering the importance of livestock farming to Zanzibar's economy (OCGS, 2019) and the potential, if any, of animal reservoirs of zoonotic *S. haematobium* group species and hybrid transmission.

CRediT author statement

Conception and design of the present study investigating the presence of bovine schistosomes on Pemba Island was done by TP, SMA, JC and BLW, field surveys and acquisition of data by TP, SMA, AKA, KRS and BLW and the analysis and interpretation of data by TP, JC and BW. TP produced the first draft, JC and BW contributed to subsequent drafts. TP and BW led the writing process. All authors read and approved the final manuscript.

Data availability

The datasets supporting the conclusions of this article are included within the article with additional nucleotide sequence data available in the GenBank repository (OK484568, OK484569 and OK447652).

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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