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Use of sentinel snails for the detection of *Schistosoma haematobium* transmission on Zanzibar and observations on transmission patterns

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ABSTRACT

Urogenital schistosomiasis is an important public health issue in Zanzibar. Transmission of the parasite to the human population is related to the distribution of the intermediate snail host, *Bulinus globosus*. We measured *B. globosus* abundance and *Schistosoma haematobium* prevalence within snails in a series of naturally occurring populations and compared prevalence detected by observing cercarial shedding for patent infections, and by PCR using *Dra*I repeat. A total of 2146 *B. globosus* were collected throughout the study period from 2003 to 2007; of these 85 (3.96%) were shedding cercariae. The levels of infection detected by PCR were consistently higher (40–100 percent). Levels of exposure to miracidia in the field were measured using sentinel snails. *B. globosus* (a susceptible host) and *B. nasutus* (a non-susceptible host) were placed in cages at transmission sites for 72 h to observe rates of penetration by miracidia. Both *B. globosus* and *B. nasutus* tested positive for *S. haematobium* by PCR (up to 24% infected) indicating frequent contamination of the waterbodies with *S. haematobium* miracidia. The use of sentinel snails coupled with PCR diagnostics could be a sensitive tool for mapping and monitoring transmission of schistosomiasis in areas of low prevalence.

1. Introduction

Urogenital schistosomiasis caused by *Schistosoma haematobium* is a common infection on the islands of Unguja and Pemba, collectively known as Zanzibar, where up to 6% of the school age population may be infected (Stothard et al., 2009). Ongoing control activities have reduced the prevalence and morbidity of urogenital schistosomiasis and a move towards elimination on both islands is now being formulated (Knopp et al., 2012, 2013). The intermediate snail host on Zanzibar is *Bulinus globosus*. Patent infections (i.e. emergence of cercariae) have not been observed in the other potential intermediate host on the islands, *B. nasutus* indicating that this snail does not play a role in transmission on Zanzibar, although it is recorded as a susceptible intermediate host on mainland Africa, for example in Kenya (Rollinson et al., 2001; Stothard et al., 2002; Kariuki et al., 2004).

Schistosome infection among snails is usually estimated by screening snails for patent infection, with prevalence varying, but typically being below 10% (Anderson and May, 1979). However, the observations of Hamburger et al. (2004), using a molecular screening approach, indicated that many more snails are penetrated, by *S. haematobium* miracidia, than go on to develop patent infections, thus raising questions about levels of exposure in the field, parasite prevalence and the factors that determine the outcome of initial infection.

A number of molecular tools exist for the detection of prepatent schistosome infections within snails. Successful use of the *Dra*I 121 base pair (bp) repeat polymerase chain reaction (PCR) assay developed for the detection of *S. haematobium* within *B. nasutus* was demonstrated in Kenya and a more specific, but less sensitive PCR assay for the detection of prepatent infections within the snail was later developed (Hamburger et al., 2004; Abbasi et al., 2007). The introduction of loop-mediated isothermal amplification (LAMP), using 121 bp repeated sequences *Dra*1 and Sm1-7, for the detection of *S. haematobium* and *Schistosoma mansoni* within the intermediate host has also been successful for laboratory infected snails (Abbasi et al., 2010).

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Table 1
Location of the monthly collections from six sites on Unguja and species of *Bulinus* found at each.

Site	Chaani	Kinyasini	Kilombero	Kidiki	Kidimni	Muyuni
Latitude/longitude	5°55′47.94″S 39°17′58.32″E	5°58′12.96″S 39°18′29.64″E	6°1′37.38″S 39°19′38.46″E	6°12′43.44″S 39°20′54.0″E	6° 07′91.0″S 37°17′95.0″E	6°22′70.0″S 39°27′85.0″E
Snail species	*B. globosus*	*B. globosus*	*B. globosus*	*B. globosus*	*None found*	*B. nasutus*

Of the six study sites observed, five are known habitats for *B. globosus* and one site, Muyuni, for *B. nasutus*. There are no mixed populations of *B. globosus* and *B. nasutus*. No snails were present at Kidmni.

The aim of the present study was to investigate the seasonal variation and relationships between snail abundance, *S. haematobium* patency rates, prepatent exposure and force of transmission in the field. Over a four-year period, monthly prevalence of patent (cercarial shedding) and prepatent (detected by PCR) *S. haematobium* infection in *B. globosus* and *B. nasutus* snails collected from transmission sites in Unguja, Zanzibar were identified. Force of transmission was investigated by measuring the rate of infection acquisition among schistosome-naïve sentinel snails artificially deployed within transmission sites.

2. Materials and methods

2.1. Snail collections and cercarial shedding (2003–2007)

To measure parasite prevalence in naturally occurring snail populations, monthly collections were made at 6 sites between July 2003 and February 2007 as part of an ongoing research programme into schistosomiasis control on Unguja (Table 1). Snails were collected by hand and/or by scoop for approximately 15 min by 3 collectors. The edges and the main part of the water body were investigated for snails, which were removed from the underside of water lilies, debris or leaves using forceps. When water levels were higher or less vegetation was present a scoop (mesh sieve or net on the end of a pole) was used. Snail species were identified morphologically, counted and stored in labelled plastic bottles with water from the site and returned to the laboratory.

During each collection, pH, salinity, total dissolved solids (TDS) and conductivity of the water were recorded using a handheld water meter at a depth of 40 cm. Notes were made on the water flow rate (flooded, fast, gentle, stagnant, dry), coverage of vegetation (grass, water lilies or rice) and any human impacts, such as soap lather contaminating the water.

To investigate the number of snails with patent infections, snails were placed individually in containers or 24-well plates, with bottled mineral water and exposed to sunlight for 2 h to induce cercarial shedding (Sturrock, 1993). Cercarial identification was based on morphology; the number of snails that were shedding *S. haematobium* cercariae was recorded. No other *S. haematobium* group schistosomes, such as *S. bovis*, have been recorded on Unguja (Stothard et al., 2002).

In addition to the observation of patent infections, all snails from two (Chaani and Kidiki) of the six sites collected from 2003 to 2007, were screened using the *Dra*I PCR assay (Hamburger et al., 2004) for the presence of immature and patent *S. haematobium* infections. Another detection method for early infections would be to look for immature cercariae or sporocysts within the snail tissue (Shiff, 1974). This was not performed here as the snails had been fixed in ethanol, and therefore the tissues were hard to manipulate.

2.2. Use of sentinel snails to estimate exposure to S. haematobium miracidia *in the field*

B. globosus and *B. nasutus* were used as target host and non-host snails in these experiments. Laboratory cultures were set up using *B. globosus* collected from Chaani, Unguja (NHM 3052) and *B. nasutus* collected from Muyuni, Unguja (NHM 3070). The external surface of each shell was cleaned with a paintbrush before experiments began, to remove any debris. Snails were dotted with paint to distinguish one species from the other. The cages were crafted from plastic piping 12 cm diameter and the sides were covered with gauze (50 μm mesh) and were held together with screws.

The sites chosen for the sentinel snail studies were Chaani and Tingatinga (Table 2). From past records both of these foci have high transmission; Chaani is located on a main road next to a school and is used by both students and general members of the public for washing (clothes, persons and bicycles). Tingatinga is deeper and water flow is generally slower than Chaani, human activities such as the washing of clothes and swimming are common. Sites were chosen upstream and downstream of the main transmission foci, at regular intervals (7 for Chaani and 5 for Tingatinga). At sites where human water contact was anticipated, signs were erected in an attempt to stop interference with the experimental cages.

At each of the sites a cage was positioned at approximately 50 cm depth, each cage containing 33 *B. nasutus* and 10 *B. globosus* (Table 2). Numbers were based on availability of uninfected snails from the laboratory cultures. The cages were submerged at all the sites and pinned to the riverbank or bottom with tent pegs.

Rates of parasite penetration were recorded at 24 and 72 h after deployment. At 24 h each site was revisited, the number of surviving snails was recorded and half of the surviving *B. nasutus* and *B. globosus* individuals (the numbers varied due to high mortality in some of the sites, see Table 4) were collected and fixed in ethanol. The cages were then sealed and redeployed. After a further 48 h the cages were removed, mortality was recorded and all of the snails that remained alive were fixed in 100% ethanol. To screen for prepatent infection, the snails were screened by PCR as detailed in Section 2.3.

To observe the level of infection within *B. globosus* at each of the snail cage sites, collections of naturally occurring snails were made on day 1. This involved collection by hand for approximately 10 minutes with three collectors or 15 min with two people.

Table 2
Latitude and longitude for sites 1–7 along Chaani stream and sites 1–5 from Mto Pweza to Tingatinga.

Collection point	Latitude/longitude	Percentage positive at 24 h	Percentage positive at 72 h
Chaani			
1	S5 56.248 E39 17.820	0	0
2	S5 56.111 E39 17.793	0	0
3	S5 56.227 E39 17.845	10	12
4	S5 56.041 E39 17.897	17	20
5	S5 55.855 E39 17.999	17	24
6	S5 55.855 E39 17.999	20	20
7	S5 55.798 E39 18.019	10	8
Tingatinga			
1	S5 57.250 E39 18.382	17	24
2	S5 57.288 E39 18.392	14	14
3	S5 57.299 E39 18.421	0	6
4	S5 57.374 E39 18.465	9	9
5	S5 57.406 E39 18.511	18	24

Percentage of *B. nasutus* and *B. globosus* ($n = 43$) that were PCR positive for *S. haematobium* at 24 h and 72 h post deployment of cages, upstream and downstream of Chaani and Tingatinga.