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Piloting an integrated approach for estimation of environmental risk of *Schistosoma haematobium* infections in pre-school-aged children and their mothers at Barombi Kotto, Cameroon

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Abstract

Within schistosomiasis control, assessing environmental risk of currently non-treated demographic groups e.g. pre-school-aged children (PSAC) and their mothers is important. We conducted a pilot micro-epidemiological assessment at the crater lake of Barombi Kotto, Cameroon with GPS tracking and infection data from 12 PSAC-mother pairs (n=24) overlaid against environmental sampling inclusive of snail, parasite and water-use information. Several high-risk locations or 'hotspots' with elevated water contact, increased intermediate snail host densities and detectable schistosome environmental DNA (eDNA) were identified. Exposure between PSAC and mother pairs was temporally and spatially associated, suggesting interventions which can benefit both groups simultaneously might be feasible. When attempting to interrupt parasite transmission in future, overlaid maps of snail, parasite and water contact data can guide fine-scale spatial targeting of environmental interventions.

Keywords: micro-epidemiology; urogenital schistosomiasis; GPS datalogging; snail survey; environmental DNA; Cameroon; pre-school-aged children; mothers

1. Introduction

Urogenital schistosomiasis is a waterborne parasitic disease, caused by infection with the trematode blood fluke *Schistosoma haematobium*, often common in impoverished rural sub-Saharan communities (Colley et al., 2014). The frontline intervention against this disease is with preventive chemotherapy (PC) by mass drug administration (MDA) of praziquantel (PZQ). In Cameroon, for example, whilst there has been regular annual treatment of school-aged children (Tchuenté and N’Goran, 2009), approaching the World Health Organization (WHO) treatment coverage targets (World Health Organization, 2017), treatment of other at-risk groups, such as pre-school aged children (PSAC) and their mothers, remains incomplete (Faust et al., 2020; Rollinson et al., 2013; Tchuem Tchuenté et al., 2017; World Health Organization, 2011, 2003). As the national control programme in Cameroon moves towards local interruption of schistosome transmission, the expansion of PC to currently excluded groups is needed alongside targeted interventions appropriate to disease foci (Stothard et al., 2017; Tchuem Tchuenté et al., 2017).

In 2017 the WHO published new guidelines on the use of molluscicides for schistosomiasis control but they lacked information on how to best identify local hotspots for most effective focal-targeting (World Health Organization, 2017). Today, with increasing investment specifically for snail control, this is an important requirement for disease surveillance (Xu et al., 2016). In contrast to general MDA campaigns where coarse disease prevalence mapping is sufficient, focal control requires precision mapping to capture spatial and temporal heterogeneities in schistosomiasis transmission (Tchuem Tchuenté et al., 2018). Therefore, when interruption of parasite

transmission is considered, a holistic mapping framework at fine scale is needed that integrates snail and schistosome populations with human water-contact patterns (Stothard et al., 2017).

Barombi Kotto, a crater lake in South-West Cameroon with an island-dwelling population, is a well-known transmission focus for urogenital schistosomiasis (Campbell et al., 2017). In June 2016, environmental and parasitological cross-sectional surveys were carried out in Barombi Kotto. They found a high egg-patent prevalence of 40.1% and a significantly higher level of water contact among infected participants than those who were uninfected (Campbell et al., 2017). These surveys were repeated in 2017 with a novel environmental DNA (eDNA) detection component, in which the DNA of a target organism which can be extracted from environmental samples (Taberlet et al., 2012). In this study, the top mid-layer of lake water was sampled since sediments may contain parasite DNA from a longer period. An additional study which aimed to improve understanding of fine-scale human mobility and exposure in MDA-excluded groups was carried out concurrently, tracking the movements of PSAC and mother pairs prior to an expanded treatment campaign (Macklin et al., 2018).

Using Barombi Kotto as an exemplar case, we attempted to pilot an integrated approach to identify potential transmission micro-hotspots by combining water, sanitation and hygiene (WASH) and human infection information, snail and eDNA sampling data as overlaid with individual GPS tracking data.

2. Materials and methods

Primary data available for this study were collected during malacological and parasitological cross-sectional surveys and a GPS survey conducted on the central

island of Barombi Kotto in June 2017. Additional information on the locations of known bathing sites was collected by supplementary questionnaire (Macklin et al., 2018).

For detailed malacological inspections, a total of 8 shoreline sites were selected based on observed human water-contact patterns and accessibility for the field team. Each site was sampled for aquatic snails by hand using metal collection sieves, with three collectors spending a total of 15 minutes searching. Following the methodology described by Campbell (Campbell et al., 2017), collected snails of medical importance were identified according to the morphological keys of Brown (Brown, 2002) and counted before being exposed to light for two hours on two occasions to check for schistosome cercarial shedding. At Sites 1-7 (Site 8 became unavailable for eDNA sampling during the study period), 1.5 litres of lake water were collected, approximately 50cm below the water surface, and passed through 5µm nylon water filter with subsequent DNA extraction and real-time PCR with TaqMan probes to detect *Schistosoma* environmental eDNA as described by Al-Shehri et al. (Al-Shehri et al., 2018).

Within the parasitological survey, a nested GPS study was carried out concurrently, as described (Macklin et al., 2018). A cohort of 12 mother and PSAC pairs (n=24) were randomly selected from the parasitological survey (n=180) to wear GPS dataloggers for 48 hours. The movements of each individual in the cohort were tracked using a GPS datalogger (I-gotU GT-120, Mobile Action, UK; dimension 44.5 x 28.5 x 13 mm, weight 20g) which was wrapped in a waterproof bag and attached to the arm or wrist with an elastic strap. The cohort was split into two 48-hour study periods and dataloggers were configured for their GPS location to be recorded at 1-minute intervals during the period 05:00-21:00, identified as the community's waking hours.

A water-contact risk area was defined as a buffer region of 10m inland from the shoreline and 20m into the lake to account for GPS uncertainty, the movement of individuals and swimming. To assess temporal trends in water contact, the number of different individuals entering the risk area was calculated for each 30-minute period across a day, collapsing all times from the two study periods into a single day. To map exposure hotspots, time-weighted activity heatmaps were produced using kernel density estimation in QGIS (QGIS Development Team, 2009) (with a bandwidth radius of 10m) for the GPS points within the risk area.

The proximity of mother-child pair movements was calculated as the Euclidean distance between the geometric centres of their locations in each ten-minute window period for i) all locations across the entire island space and ii) all locations within the risk area. The distance between locations in the risk area and estimated house location (identified from shared activity hotspots on the island for mother-PSAC pairs) was also calculated for each participant.

All analyses were carried out in the R statistical language version 3.6.3 (R Core Team, 2016) and maps were created in QGIS. Study protocols were approved by the Cameroon National Ethics Committee and the Liverpool School of Tropical Medicine Research Ethics Committee.

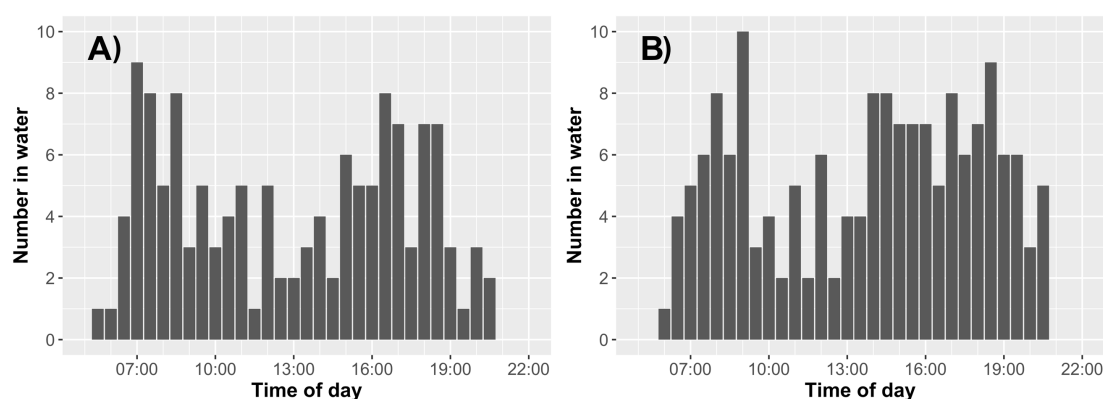
3. Results

The GPS cohort's pervasive water contact was clear, with at least one mother and one PSAC entering the risk area in every 30-minute window of the day after 05:45 (Figure 1). Group water contact was greater for mothers, while both mother and PSAC groups followed a similar temporal trend, peaking in the early morning (06:45-09:15) and afternoon/early evening. Peak water contact (>4 individuals) in the afternoon/evening period was longer for mothers (13:45-19:45) than PSAC (15:15-17:15 and 17:45-

18:45). As has been reported previously (Macklin et al., 2018), two mothers and one PSAC in the cohort were found to have egg-patent infections.

Across the entire island, PSAC and mother pair movements were consistently close during waking hours. Out of 12 pairs, 9 stayed within a distance of less than 35m between mother and child for at least 65% of all ten-minute periods. When within the risk area, mother-PSAC pairs were more consistently close together, with 9 pairs spending at least 83% of their time within 35m of each other.

Figure 1



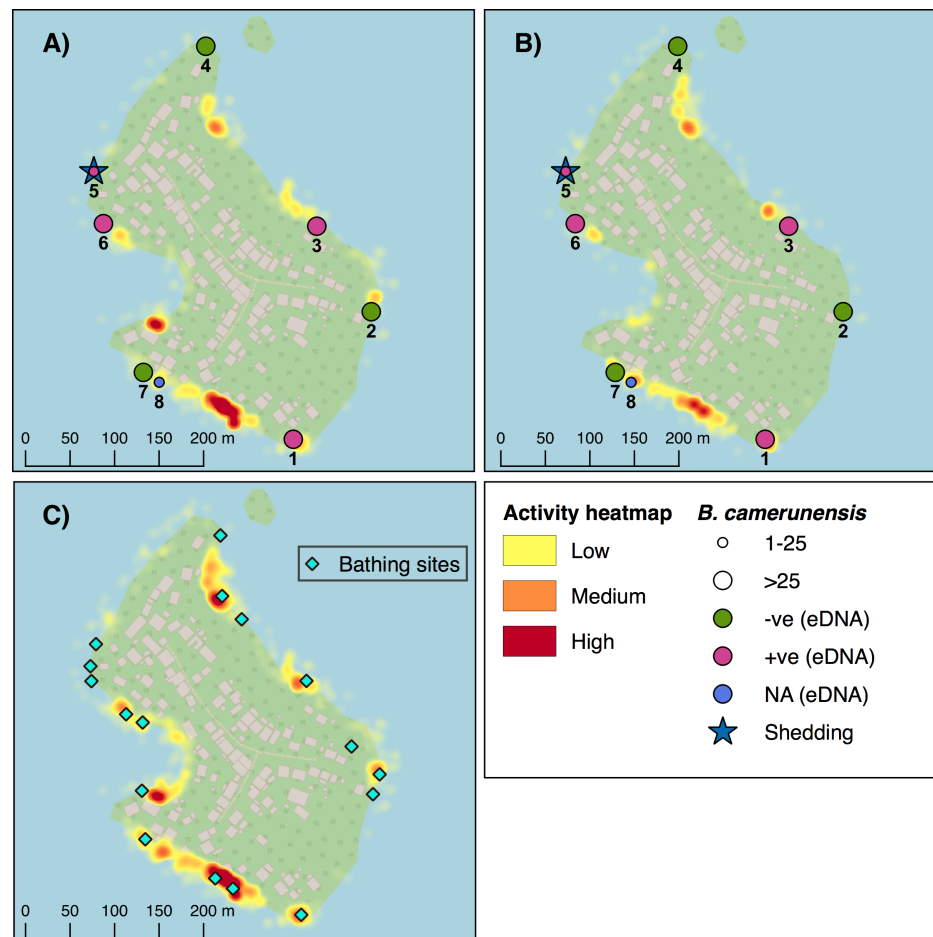
Title: The number of individuals with at least one water contact in each half hour period (n=12 in each group) for A) PSAC and B) mothers.

Mother and PSAC groups entered the risk area at a range of different locations around the island's shoreline (Figure 2). Figures 2A and 2B show that the two groups shared all contact sites except for Site 2. Both groups were particularly active across the length of the southwestern shore, with the highest intensity in the central section. The three members of the cohort with egg-patent infections almost exclusively entered the risk area in this section. All of the contact sites were independently identified as bathing sites (Figure 2C) except for three hotspots on the western bay and southwestern and north-eastern shores. Seven of the identified bathing sites were not visited by the

cohort. Individuals entered the risk area at a mean distance of 83.6m (range 18.0-140.0m) from their houses, showing that they generally, but not exclusively, chose contact sites near to their houses.

Environmental sampling found *Bulinus camerunensis* snails at all sites (Figures 2A and 2B) and a snail shedding schistosome cercariae at site. The mean number of snails found at all sites was 56 (range 21-107), with over 25 snails collected at 6 of the 8 sites. *Schistosoma* eDNA was detected in water samples for 4 out of 7 sites.

Figure 2 (in colour)



Title: Activity heatmaps of person-time in the risk area (yellow = low, red = high) for A) mothers (n=12), B) PSAC (n=12) and C) both groups together (n=24). In A) and B), the number of collected *Bulinus camerunensis* snails are shown by circle size (small

circle = 1-25 snails, larger circle = >25 snails) and water eDNA results are indicated by colour (green = negative, pink = positive, violet = unsampled) at each of the 8 numbered sampled sites. The identified shedding snail is marked (blue star) at Site 5. In C), known bathing sites are marked (diamonds).

There was a clear overlap of risk factors for transmission at the sampled sites. Three of the sites (1, 3 and 6) were high risk, with more than 25 snail hosts present, schistosome eDNA detected in the water and significant local water contact from both mothers and PSAC. Another three sites (2, 4 and 7) had a high number of snails present, were negative for eDNA and varied in water contact. Site 2 was only visited by two mothers, while members of both groups visited Site 7 and five individuals passed through Site 4 momentarily before spending time in the more southerly hotspots located nearby. Interestingly, Site 5, where the shedding snail was located, only had brief contact from a single mother-PSAC pair and a small number of snails present.

4. Discussion

Our pilot approach has demonstrated that this cohort of mothers and PSAC have substantial levels of at-risk water contact in areas where intermediate hosts were present and schistosome eDNA was detected. Whilst their relative contribution(s) to environmental contamination is debated, it is consistent with their now recognised position as particularly vulnerable groups for schistosomiasis globally (Poole et al., 2014; World Health Organization, 2011). Both group's similar daily exposure patterns and PSAC-mother movement patterns can be broadly explained by the mother's guardian role, an underappreciated epidemiological driver of the child's spatial and temporal risk of infection. An infected mother or PSAC will place their corresponding pair at high risk of infection and interventions should aim to target them jointly through either environmental control to their water contact points or from health education

towards mothers. The spatial heterogeneity of contact sites used by these groups is indicative of the increasing importance of human behaviour as a key driver of transmission at smaller spatial scales (Stothard et al., 2017).

In low-level transmission contexts with high heterogeneity, treatment or alternative interventions must be targeted at the 'most-at-risk' members of the population (Mari et al., 2017). GPS datalogging is a new tool which enables us to develop a detailed understanding of water-contact patterns within a target population and identify *who* has a high-risk water-contact profile and perhaps plays a disproportionate role in transmission (Campbell et al., 2017). It can collect individual-level data about frequency and intensity of water use at higher spatial and temporal resolutions than conventional methods, albeit for a short period of time. This allows future investigation of classic epidemiological questions of *where* and *when* transmission takes place.

For efficient use of GPS tracking, information on treatment status and current and historical infection status should be used to limit potential participants to those with the highest-risk profiles. In areas where transmission persists despite multiyear MDA, the priority is to identify potential 'contaminators' who may sustain transmission through intense daily water contact until they receive a curative dose of PZQ (Stothard et al., 2017). The untreated mothers and PSAC in this study are an example of a group at high-risk of playing this role due to their water contact in areas where snail populations were abundant and schistosome eDNA was present.

In this study, we included use of eDNA detection methods, which are being continuously refined (Alzaylaee et al., 2020b, 2020a), to complement water contact and snail survey data for the estimation of environmental risk. As has been found in other recent studies (Sato et al., 2018; Sengupta et al., 2019), these methods offer an alternative direct measure of recent infestation with schistosomes to other more laborious snail shedding testing methods, which can be insensitive (Opisa et al., 2011; Sengupta et al., 2019). These eDNA methods also allow direct detection of

schistosomes in water rather than infection in snails (Allan et al., 2013; Kane et al., 2013). In Barombi Kotto lake four sites were positive for eDNA, which we assume to be most likely of cercarial origin, although only a single shedding snail was collected. This may be indicative of insensitive snail shedding testing methods. With use of discriminatory DNA probes, eDNA collection should be considered more widely within micro-epidemiological studies. To ensure that eDNA testing and snail surveys are used most efficiently in the field, we recommend that GPS water contact information is collected first to help prioritise their use for sites regularly contacted by the target population.

In 'persistent hotspots' (Kittur et al., 2017) - areas which are resistant to multiyear MDA - there is a need for alternative control methods which can break transmission cycles through environmental control (Campbell et al., 2017; Kittur et al., 2017; Stothard et al., 2017; Tchuem Tchuente et al., 2017; World Health Organization, 2017). Several recent studies specifically recommend focal snail control as a cost-effective means of interrupting transmission in sub-Saharan Africa (King et al., 2015; Tchuem Tchuente et al., 2017; World Health Organization, 2017). However, successful application of focal mollusciciding that limits both cost and environmental damage (World Health Organization, 2017) is reliant on our ability to identify putative transmission hotspots. These hotspots must be identified at the fine spatial scale of schistosomiasis' focality, rather than the village-level scale at which hotspots are often defined with MDA distribution in mind (Kittur et al., 2017; Rollinson et al., 2013; Standley et al., 2013; Tchuem Tchuente et al., 2018).

Currently, there is no international or national guidance on how to formally investigate environmental transmission in sub-Saharan Africa (Stothard et al., 2017) and the WHO manual for field mollusciciding (World Health Organization, 2017) only provides minimal guidance about how to identify transmission hotspots. In this study we have demonstrated how NTD programme managers can collect and present data in accordance with the manual's recommendations that "simple maps of the local

transmission sites should be prepared” by “preparing risk maps of contact with water bodies by high-risk groups” (World Health Organization, 2017). By combining snail surveys with innovative new GPS and eDNA tools, more detailed maps of environmental risk can be produced for the identification of transmission hotspots for focal control.

Whilst we acknowledge the constrained setting and context-specificity of our study, it is a pertinent exemplar of schistosomiasis transmission more broadly in two understudied demographical groups, set within fine-scale spatial and temporal resolutions. In principle, our approach could be used to better target non-treated population groups and guide local intervention strategies when interruption of transmission is considered.

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Notes

Acknowledgments

We are grateful to the help and support of the community at Barombi Kotto, and we thank the donors for their support.

Funding

ME is in receipt of a studentship from the Medical Research Council, United Kingdom. ZB and LO'H were supported by LSTM Education Department MSc Degree Research Project Module funding. This study is funded as part of the COUNTDOWN research programme consortium, by the Research and Evidence Division of the Department for International Development, UK. The funders had no role in study design, decision to publish, or preparation of the manuscript. Study protocols were approved by the Cameroon National Ethical Committee of Research for Human Health and the Liverpool School of Tropical Medicine Research Ethics Committee.

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Conflicts of interest

All authors: No reported conflicts.