Schistosomes make captives of chimpanzees
Stopping schistosomes from ‘monkeying-around’ in chimpanzees

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Ngamba Island Chimpanzee Sanctuary (NICS) in Lake Victoria, Uganda is currently home to 44 wild-borne, semi-captive chimpanzees. Despite regular veterinary health checks, it only came to light recently that many animals, and sanctuary staff, were naturally infected with Schistosoma mansoni. Indeed, local schistosome transmission appears firmly engrained for intermediate snail hosts can be found along almost the entirety of Ngamba’s shoreline. Here, the epidemiology of infection is a dynamic interplay between human and chimpanzee populations, as revealed by genetic analyses of S. mansoni. In this review, our present understanding of this complex and evolving situation is discussed, alongside general disease control activities in Uganda, to highlight future interventions towards stopping schistosome morbidity and transmission within this conservation sanctuary setting.

Definitive hosts of Schistosoma mansoni

Intestinal schistosomiasis caused by the trematode parasite S. mansoni is a neglected tropical disease of global public health importance [1]. Although a scourge of many communities across sub-Saharan Africa, especially to those living on the shoreline of Lake Victoria [2], what is less well recognized is that this disease can be an (anthro)zoonosis within animals other than humans, with infections potentially exhibiting similar clinical signs and symptoms [3,4]. The most well-known non-human hosts of S. mansoni are perhaps rats, and in some parts of the world this parasite is almost completely sustained in rodent transmission cycles [5].

With our close phylogenetic proximity, however, it may come as no surprise that given sufficient epidemiological opportunity, non-human primates (NHPs) can become infected and sometimes act as fully competent definitive hosts; studies conducted in the 1960s and 1970s comprehensively demonstrated that several NHPs were capable of maintaining transmission of schistosomiasis [6,7]. When sampling opportunities have arisen, wild primates, particularly bangoons and chimpanzees, have been examined parasitologically and sometimes found with disease [8–10]. Nonetheless, prior to the introduction of molecular DNA tools for detecting and genotyping parasites, no studies have been able to clarify fully the levels of schistosome population crossover, precisely striking the balance between zoonotic and(or) anthropo-zoonotic transmission cycles [4].

Lake Victoria and intestinal schistosomiasis

Lake Victoria is particularly famous as the largest tropical freshwater body in the world, but is also infamous in the past half century for the extent of major ecological changes it has undergone. Entirely apart from the burgeoning human population along its shorelines, Lake Victoria hosted two of the most notorious human-mediated species introductions in modern history: the catastrophic introduction of Nile Perch in the 1950s [11,12], followed by the accidental invasion of water hyacinth in the 1980s [13,14]. Both introductions and invasions have extensively perturbed the aquatic flora and fauna. It has been hypothesized, for example, that intermediate hosts of S. mansoni, freshwater snails of the genus Biomphalaria, have particularly thrived and dispersed on floating vegetation mats provided by the water hyacinth [14] and that their natural fish predators, small cichlids which kept snail populations in check, were decimated by Nile Perch. At the same time, the expansion of the Nile Perch fishing industry has fostered numerous fishing landing sites creating shanty-like habitations and marginalized communities characterized by both poor hygiene and sanitation. Unfortunately, intestinal schistosomiasis is almost universal within such fisher-folk and associated populations [15,16].

Of the countries surrounding Lake Victoria, Uganda is perhaps the most afflicted by this disease [17,18], In 2003, a National Control Program was launched with the intention of providing mass drug administration of the anthelmintic praziquantel to school-aged children and adults living within disease endemic areas, such as along the shorelines of Lakes Victoria and Albert as well as along the Nile itself [19,20]. During this time many of the communities living on the numerous islands within Lake Victoria were somewhat overlooked owing to the considerable expenses in accessing and reaching these locations [2,21]. Moreover, certain communities on the Sese Islands were traditionally thought to be largely free from intestinal schistosomiasis and thus were not initially considered high priority for control. This appraisal, however, was recently overturned by targeted surveys showing there to be foci of hyperendemic disease [22–24]; for example, at a quarter of
the sites surveyed, local prevalence of infection was in excess of 50%, although mean prevalence of intestinal schistosomiasis was 34.6% (95% confidence intervals = 31.0–38.3) by stool sampling methods [23].

From additional surveying, the wide range in prevalence or overdispersion of disease across the Sesse Island group requires drug treatment regimes tailored to each island setting [23]. Confirmation that active transmission was occurring locally was later revealed by the observations of high abundance of *Biomphalaria* as well as field-caught snails shedding *S. mansoni* cercariae [24]. Using molecular DNA methods, inspection of parasite genetic diversity by DNA barcoding showed that previously encountered and common genotypes were present, inclusive of novel ones, which when taken together was consistent with the high known genetic diversity of *S. mansoni* within this lake [1,24–26]. More broadly, the situation on the Bufumira Island group demonstrated the existing challenges well faced by the Ugandan National Control Program in reaching these rather remote, inaccessible and largely itinerant populations. In addition, the local malarial fauna was then largely unknown with a poorly sampled and recorded freshwater snail species list. Thus, the remoteness and isolation of many of the islands in Lake Victoria have, until very recently, confounded surveying and hampered control efforts not only for schistosomiasis but also for other diseases such as malaria [21].

**Ngamba Island and a new epidemiological opportunity**

Within the above context, Ngamba Island presented itself as somewhat of a long-term exception (Figure 1 and Box 1). This island first hosted a small fishing community during the 1970s and 1980s, remaining almost fully-forested, until 1998 when it was taken over by the Chimpanzee Sanctuary and Wildlife Conservation Trust (CSWCT) to become a conservation area under the auspices of the NICS. The sanctuary was originally intended to act as a convenient off-shore reserve with natural boundaries for holding rescued chimpanzees and was particularly timely as the existing sanctuary in Entebbe was becoming increasingly overburdened with the need to care for these animals *ex situ*. Upon handover, the 8000 m² clearing left by the fishing village on its northern shore was subsequently divided up into a chimpanzee feeding station and animal accommodation pens for the soon-to-arrive chimpanzees, as well as an associated island camp for NICS staff, and then subsequently for visitors and tourists [24].

Although the remainder of the island remained a protected forested area for the chimpanzees to rove and forage within, it was unable to sustain large numbers of animals and was therefore partitioned from the feeding/accommodation station and staff camp by a tall electrified fence. More broadly, NICS also acts as an active conservation area and refuge zone for other flora and fauna native to Lake Victoria; around the island there is an enforced no-fishing exclusion

![Figure 1](image-url) Ngamba Island Chimpanzee Sanctuary (NICS) on Lake Victoria. (a) Schematic depiction of the Ugandan shoreline of Lake Victoria illustrating the main island groups. (b) Outline map of Ngamba and Kimi Islands. (c) Aerial photograph of Ngamba Island with the NICS camp highlighted (with white arrow). (d) Anesthetized chimpanzee undergoing annual health check in NICS veterinary laboratory in 2011; (inset top) ultrasound image of chimpanzee liver depicting pipestem fibrosis, (inset bottom) micrograph (-100) of egg of *Schistosoma mansoni* within rectal biopsy.
Box 1. Ngamba and Kimi Islands and their histories

Ngamba Island is 25 km southeast from Entebbe, accessible only by boat, and is approximately 40,000 m² in area, with second generation rainforest covering the majority of the island. The island very clearly differs from its neighbor Kimi Island, the latter hosts a busy fish landing site with an associated itinerant fishing community which lives in marginalized and very impoverished conditions (Figure 1).

After purchasing a long-term lease, the Ngamba Island Chimpanzee Sanctuary (NICS) was established in October 1998 to act as a conservation zone for the maintenance and care of orphaned and rescued chimpanzees that are unlikely to survive reintroduction to the wild. With 44 resident chimpanzees (http://ngambaisland.com/chimps/chimps.html), NICS is currently working almost at full carrying capacity. Although animals are semi-captive and return each evening to sleeping quarters, they spend most of their day foraging and roaming around the island. The wildlife conservation work of NICS was featured by Mark Carwardine and Stephen Fry in the BBC program ‘Last Chance to See’.

NICS is managed by the Chimpanzee Sanctuary and Wildlife Conservation Trust (CSWCT) (http://pasaprimates.org/) and strongly advocates conservation of chimpanzee habitats in Uganda through community education and political lobbying.

On Ngamba Island, there is a full-time veterinarian, with several animal keepers, and a well-equipped veterinary laboratory. This affords ad hoc and routine animal health checks, inclusive of more advanced procedures (e.g., X-ray and surgery). The facility also acts as a convenient base for medical outreach projects undertaken on nearby islands such as on Kimi and Koomo Islands in which NICS staff actively contribute.

Figure 1. Aerial images of the islands of (a) Ngamba and (b) Kimi very clearly showing differences in human habitation and forestation. The red cross denotes the same island from different viewpoints.

First detection of infected snails and afflicted chimpanzees

A key factor determining local transmission of *S. mansoni* is the presence of *Biomphalaria*, of which two species of snail in Lake Victoria were formally recognized, *Biomphalaria sudanica* and *Biomphalaria choanomphala* [27]. This appraisal has been deemed inaccurate for recent genetic characterization and morphological studies have now revealed that the distinctions between these two ‘species’ were due to ecophenotypy of a single entity. *B. choanomphala* var. *sudanica* is an ecomorph of marshy habitats fringing the lake, whereas *B. choanomphala* var. *choanomphala* exists as an ecomorph in the lake proper. *B. choanomphala* var. *choanomphala* was typically considered to be abundant on submerged vegetation in sheltered bay areas and not thought to be found on wave exposed shorelines and upon rocky substrates [28].

As part of a lake-wide malacological appraisal of *Biomphalaria*, snail surveys penetrating into the offshore islands of the lake took place which afforded an initial inspection of Ngamba and Kimi Islands in 2008 [22,25]. This revealed the presence of *B. choanomphala* as well as several snails shedding *S. mansoni* cercariae patent infected. This key finding then alerted NICS to the possibility of local transmission of intestinal schistosomiasis and focused the attentions of the chief veterinarian to the prospect that the chimpanzees could be at risk of infection. Schistosomiasis had not previously been considered within the diagnostic repertoire of the annual health checks. Even though this initial snail collection site was not accessible to the chimpanzees (i.e., it was adjacent to the staff camp and boat docking platform), it was thought prudent to administer a prophylactic dosing of praziquantel (40 mg/kg) to all animals within their feed. Indeed, administration of praziquantel in such a manner has become an annual standard policy within the NICS’s disease management plan. To confirm if the animals were infected, a later detailed parasitological inspection of the animals and staff was...
therefore planned, alongside a more intensive malacological inspection in an attempt to estimate the risk of infection at key points along the NICS shoreline.

In February and March 2010, the detailed parasitological inspection took place using a variety of stool, urine and serological testing methods, inclusive of PCR detection of schistosome DNA in stool as commonly used in routine medical diagnosis of this disease in The Netherlands [24]. A total of 39 chimpanzees and 37 CSWCT staff were examined for *S. mansoni* using both egg detection methods and schistosome urine antigen rapid diagnostic tests based upon circulating cathodic antigen (CCA). In addition, recovered schistosome eggs and (or) hatched miracidia were examined by DNA barcoding to establish putative epidemiological crossover between humans and chimpanzees. All diagnostic methods conclusively inferred the presence of intestinal schistosomiasis in the chimpanzees with an overall seroprevalence of antibodies against schistosome soluble egg antigen (SEA) by ELISA in excess of 90% [24]. Three animals actively excreting schistosome eggs were identified and DNA barcoding analysis of miracidia hatched from chimpanzee stools revealed the presence of three DNA barcodes commonly found in people living on the Lake Victoria shoreline, inclusive of NICS staff, as well as two novel DNA types at that time of schistosome sampling [24,26,29,30].

Although it was clear that the chimpanzees were infected with *S. mansoni*, the ability of chimpanzees to void schistosome eggs capable of hatching into viable miracidia firmly suggested that these animals were capable of currently maintaining a local, presumably zoonotic transmission of schistosomiasis independently of humans outside an originally assumed anthropozoonotic cycle [24]. This possibility was further enforced when the island’s perimeter was surveyed for *Biomphalaria* at four key sites and not only revealed the presence of large numbers of *B. choanomphala* var. *choanomphala*, even on the wave exposed shoreline, but also that up to 75% of snails were carrying pre-patent schistosome infections as ascertained by detection of *S. mansoni* DNA in PCR assays (Figure 2). During these surveys the malacological collection team was also ambushed by chimpanzees on the water margins, although without incident, showing that the chimpanzees regularly patrolled this shoreline along well-beaten

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**Figure 2. Biomphalaria on Ngamba Island.** (a) Outline map of Ngamba Island depicting four snail collecting sites in February 2010 where sampled Biomphalaria were screened for schistosome DNA by PCR. The associated pie charts represent the prevalence of pre-patent infections at each of the four sites; snails were sampled twice (in 2008 and 2010) at the lakeshore site adjacent to the camp. (b) Photograph of wave action and large pebble substrate on the exposed shoreline on a calm day, on other days the wave height can be up to 1 m with boat landing impossible. (c) Biomphalaria (indicated with black arrow) found clinging to the underside of a large pebble which was taken from the background agglomeration of rocks.
foraging tracks often in daily survey of their island territory (Figure 3a).

The PCR screening of snails for schistosome DNA very clearly revealed that snail populations were being exposed to miracidia that could have only come from two possible sources in this part of the forested area shoreline: (i) the chimpanzees themselves as they defecated near or directly into the lake while exploring these lake margins; or (ii) from local fishermen who had encroached within the 200 m no-fishing zone, presumably at night, having used human fecal material to attract fish or had relieved themselves on dry land while the chimpanzees were in their sleeping pens. Given that chimpanzees are normally fearful and timid of entering water, the second alternative was given greater credence until NICS staff began to follow and directly observe chimpanzees on the islands margins. It was then observed that certain animals regularly entered the water and in so doing probably acquired and spread their schistosome infections (Figure 3b).

Further environmental sampling and appraisals
This initial malacological appraisal was further enforced in June 2010, upon a more detailed microscope perimeter search of 20 lakeshore sites, each separated by approximately 75 m and noted by GPS [30]. Several other populations of B. choanomphala var. choanomphala at other locations along the shoreline were found, including some habitats which had hitherto been considered too wave-exposed for the presence of this snail, inclusive of other freshwater molluscan genera (Figure 2b) [30]. Intriguingly, it would appear that Biomphalaria has adapted its behavior to these wave-exposed shorelines by clinging to the underside of large pebbles and small boulders, and upon more turbulent days probably penetrates even deeper into this honeycomb structure made by these rocky agglomerations, affording them both protection and refuge from the detrimental effects of wave action.

Subsequent examination of snails collected during this and further surveys have revealed infected snails at sites accessible to chimpanzees. Most unexpectedly, one infected snail was also found to be coinfected with Schistosoma rodhaini, a rodent schistosome previously found in the Kenyan sector of the lake, although this Ngamba Island population was judged genetically distinctive [29]. This unique finding also alerts NICS researchers to the potential for this schistosome to perhaps also establish within the chimpanzee population but has yet not been encountered.

Ongoing disease surveillance
As it stands, despite prophylactic treatments with praziquantel, the infection status of chimpanzees on NICS remains of concern, mainly in part due to the putative treatment failure in several animals when given praziquantel tablets in their food and based upon the results of the parasitological surveys in the summer of 2010 [31]. Together with additional surveys in March 2011, a body of new evidence relating to the pathological effects of long-term natural S. mansoni infection is now being gathered, courtesy of the excellent veterinary expertise and facilities on NICS and continuing collaborations with local Vector Control Division, Ministry of Health teams as part of the activities within the Ugandan National Control Program for Bilharzia (Figure 1d).

As far as the authors are aware, prior to these observations, no recent information was available as to the clinical manifestations of naturally acquired intestinal schistosomiasis within chimpanzees [4]. Thus in March 2011, a pilot assessment of schistosome-associated morbidity was initiated by a team of multidisciplinary researchers by implementing portable ultrasonography during the annual
‘knock-down’ health check [31]. At these times animals were lightly anesthetized for approximately 45 min and subjected to a full veterinary investigation inclusive of ultrasound imagery and rectal snap biopsy. The findings were particularly startling, for it was clearly shown that several animals which had had a chronic history of infection, were also shown to have very clear signs of liver fibrosis upon ultrasonography consistent with image pattern D of advanced pipistem fibrosis as classified upon consideration of the World Health Organization (WHO) Niamey grading system (Figure 1d, insets) and calcified eggs were also found upon biopsies [32]. To increase the putative efficacy of praziquantel dosing, during anesthesia animals also received a stepped-up oral treatment at 60 mg/kg dosing as delivered by a gastric tube and this was well-tolerated [31]. However, the performance of this dosing and levels of putative reinfection(s) is scheduled to be assessed in June 2012 using noninvasive parasitological sampling.

Can schistosomes be stopped from monkeying-around?
In terms of general animal welfare on NICS, schistosomiasis needs to be actively controlled and hopefully eliminated but there are several local considerations that need to be kept in mind both from short- and long-term perspectives. First of all, general reductions in putative anthropozoonotic potential are clearly needed, including increased annual coverage of preventive chemotherapy in NICS staff and in neighboring fishing communities. This should help to dampen levels of schistosome egg excretion into the environment, especially upon those occasions when infected people frequent the NICS margins potentially contaminating its shoreline. As might be expected, to more rigorously enforce the no-fishing zone and stop all encroachment of fishermen onto the island is probably unobtainable. Moreover, a heavier handed policy might even become counterproductive by alienating the local human populace to the objectives of the NICS. Thus, to ensure that a positive relationship continues, CSWCT is developing strategic initiatives to promote both health and community welfare activities within nearby fishing communities whilst educating the recipients to the goals and motives of NICS (Box 1).

Further activities, specifically undertaken on NICS itself, should involve an integrated approach requiring consideration of three principal factors, notwithstanding the subtleties in adapting these to chimpanzee- and humanspecific settings: (i) good sanitation, handling and disposal of all fecal waste to reduce environmental contamination whenever possible; (ii) disease monitoring and active treatment of infected individuals; and (iii) control of water contact and aquatic exposure alongside some environmental management of local populations of Biomphalaria. Of the above options, water supply and sanitation control within the human populace on NICS is already excellent but in the parts of the island where chimpanzees roam freely, measures to contain their fecal input are impractical due to indiscriminant defecation of these animals. More effective disease surveillance is obvious but is hampered by the need for mobilization of additional funds to synergize with the annual health checks, which are already costly. Better access to praziquantel with more effective administration is immediately beneficial but it would appear that for certain chimpanzees the dosing of 40 mg/kg in food has been insufficient for cessation of egg excretion. Although stepped-up supervised dosing at 60 mg/kg, as delivered by a gastric tube, has been attempted it is not yet clear how effective this has been in the short or long term and thus further parasitological surveillance is clearly warranted.

Of the remaining alternatives, control of snail populations with chemical molluscicides, although possible, will probably not be cost-effective in this lacustrine setting and on a shoreline which shelves steeply along with a honeycomb substrate where chemicals would probably fail to mix and penetrate. Moreover, the application of chemical control agents strongly conflicts with the strict conservation ethos of the sanctuary. Thus, among all alternatives, options to reduce water contact of the chimpanzees should be preferentially explored. This could be done by reducing access in the more risky areas of the shoreline by further fencing off parts of this shoreline but in the short term this will be expensive to set up and in the long term very difficult to maintain. It must be remembered that chimpanzees, which are particularly intelligent and resourceful creatures, often interfere with and potentially destroy such fencing measures as part of their territorial enforcement and routine daily patrolling.

Concluding remarks
Despite being set in a unique and carefully controlled environment, the situation on NICS serves as a potent example for the opportunity for anthropozoonotic transmission of intestinal schistosomiasis and the pervasiveness of risk-of-infection along a local shoreline in light of a putative zoontic cycle. Veterinarians and conservation managers should be made more aware of the occurrence of schistosomiasis, especially when wildlife is to be maintained in ex situ settings and natural aquatic boundaries are utilized. Future disease management plans on NICS should recognize the difficulties of care of presently infected animals and maintaining long-term individual welfare (i.e., optimization of praziquantel treatments), alongside putting in place locally appropriate measures to mitigate other contaminating and (or) exposure enhancing behaviors. Obviously, the latter is more easily targeted to the human populace rather than the chimpanzee population. Looking to the future, reducing or eventually stopping schistosomiasis transmission on NICS will require a concerted long-term multidisciplinary effort in which careful parasitological and malacological surveillance is very clearly needed.

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