

# Confirmed Infection with Intestinal Schistosomiasis in Semi-Captive Wild-Born Chimpanzees on Ngamba Island, Uganda

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## Abstract

**Background:** Intestinal schistosomiasis, caused by *Schistosoma mansoni*, is endemic to Lake Victoria, with high prevalence of the disease observed in human lakeshore communities. However, nonhuman primates have recently been overlooked as potential hosts of the disease, despite known susceptibility.

**Methods:** Using a variety of stool, urine, and serological diagnostic methods, 39 semi-captive wild-born chimpanzees and 37 staff members at Ngamba Island Chimpanzee Sanctuary, Lake Victoria, Uganda, were examined for *S. mansoni* infection. Miracidia recovered from stool were DNA barcoded to investigate cross-over between humans and chimpanzees. The island was also surveyed for *Biomphalaria* intermediate host snails, which were examined for infection with *S. mansoni*.

**Results:** Chimpanzees were unequivocally shown to be infected with intestinal schistosomiasis with a seroprevalence in excess of 90%. Three egg-positive cases were detected, although the sensitivity of the diagnostic tests varied due to earlier prophylactic praziquantel treatment. Miracidia hatched from chimpanzee stool revealed three DNA haplotypes commonly found in humans living throughout Lake Victoria, including staff on Ngamba Island, as well as two novel haplotypes. At one site, a snail was observed shedding schistosome cercariae.

**Conclusions:** The anthrozoonotic potential of intestinal schistosomiasis on Ngamba Island is greater than previously thought. Moreover, the ability of chimpanzees to void schistosome eggs capable of hatching into viable miracidia further suggests that these nonhuman primates may be capable of maintaining a local zoonotic transmission of schistosomiasis independently of humans. The implications for management of captive and wild primate populations at risk of exposure are discussed.

**Key Words:** Conservation medicine—Molecular epidemiology—*Pan troglodytes schweinfurthii*—*Schistosoma mansoni*—Veterinary parasitology—Zoonosis.

## Introduction

ZOO NOTIC TRANSMISSION OF DISEASES is currently of interest to science, the media and policy makers. However, less commonly discussed are instances of anthrozoonoses, where the so-called human diseases are transmitted to wild

animals, either in captivity or when living in proximity to human communities that may have encroached upon previously pristine habitats. As expanding human populations come into ever-closer contact with wild animals, and as conservation measures increasingly involve maintenance of wild-life populations *ex situ*, the potential for disease transmission

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from humans requires careful monitoring (Epstein and Price 2009).

In nonhuman primates, there are several examples where anthroponoses have been clearly identified, and used to set new guidelines for conservation management. The best known of these involve the great apes, which are most at risk from anthroponoses due to their close genetic relatedness to humans. For example, human-habituated mountain gorillas in Central Africa have been shown to be at greater risk of *Giardia* and *Cryptosporidium* infection than nonhabituated gorillas; genotyping isolates of *Giardia* has confirmed its human origin (Nizeyi et al. 1999, Graczyk et al. 2002). Other outbreaks of human-like diseases in nonhuman primates have been recorded from well-known primate research sites, such as Gombe National Park in Tanzania, Bwindi Impenetrable Forest in Uganda, and Parc National des Volcans in Rwanda (Wallis and Rick Lee 1999). From a helminth perspective, captive chimpanzees in Copenhagen Zoo have recently been shown to be maintaining their own cycle of *Ascaris* infection within their enclosure, highlighting the possibility of cross-over between hosts, in a nonendemic setting (Nejsum et al. 2010).

Intestinal schistosomiasis is caused by infection with the trematode worm *Schistosoma mansoni*, with freshwater *Biomphalaria* snails as the intermediate host. In Uganda, Lake Victoria is a known hot-spot for intestinal schistosomiasis, where prevalence in human communities can reach 100% (Odogwu et al. 2006, Standley et al. 2009). Although the role of nonhuman primates, especially baboons, as reservoir hosts for the disease was investigated extensively in the 1960s–1970s (Miller 1960, Nelson 1960, Fenwick 1969), little attention has been paid to this topic since the advent of molecular tools. In 2000, *S. mansoni* eggs were found in the feces of a baboon in Gombe National Park in Tanzania (Murray et al. 2000), but chimpanzees were not found to be infected, which is in contrast to a prior study where two infected chimpanzees were identified (Nutter, 1993). In general, reports of schistosomiasis in chimpanzees are confined to experimental infections, where they have been shown to be permissive hosts of *Schistosoma*, including *S. mansoni* (Sadun et al. 1966, 1970, von Lichtenberg et al. 1971). Past surveys have exclusively relied on stool sampling and microscopy as a diagnostic, which, based on a single sample, may not accurately reflect infection status as typically seen with underdiagnosis in humans (Booth et al. 2003). Molecular and serological methods could further revise these diagnostic appraisals (Bergquist et al. 2009).

Ngamba Island in Lake Victoria is a sanctuary for rescued, orphaned, and confiscated chimpanzees. Created in 1998 by the Chimpanzee Sanctuary and Wildlife Conservation Trust (CSWCT), it is located ~23 km from Entebbe in an area of Mukono District known to be highly endemic for schistosomiasis (Standley et al. 2009). Most of the 44 chimpanzees are free to roam daily throughout the 100-acre forested island and have access to the shoreline at many points. Numerous observations of chimpanzee water-contact have been made, so it is hypothesized that these chimpanzees have had exposure to cercariae of *S. mansoni*. This appeared more plausible after a malacological survey on Ngamba Island in 2008 had confirmed the presence of infected *Biomphalaria* snails around the island (Standley et al. 2009).

On the basis of this observation of infected snails, an informal spot check for schistosomiasis involving three different

diagnostics was carried out on 10 chimpanzees resident on the island. Four individuals tested positive based on one or more diagnostic, which resulted in blanket prophylactic treatment with praziquantel for all the chimpanzees in February 2009 for immediate animal welfare improvement and initiated the detailed research investigation reported here.

In this study, we employed a range of specialized diagnostic tools, many never before used on chimpanzees, to determine the prevalence and intensity of infection with *S. mansoni*. By further surveying the island for snail hosts and the staff of the island for the disease, we attempted to create a comprehensive picture of the status and transmission potential of intestinal schistosomiasis on Ngamba Island. By genotyping parasite eggs and/or miracidia recovered from the chimpanzees and staff, we sought to ascertain whether chimpanzees were infected with the same genetic lineages of *S. mansoni* as found in people, thus indicating shared or even anthroponotic transmission. Our intention was to improve the health and welfare of the chimpanzees and staff living there, as well as provide recommendations for rehomed and wild primate conservation.

## Materials and Methods

### Sample collection

The survey was carried out in January–February 2010, when the majority of samples were collected. Blood samples had been collected during the annual health checks of the chimpanzees in September–October 2009. These had been centrifuged to isolate sera, which were then frozen at  $-20^{\circ}\text{C}$  and stored at the Entebbe Virus Research Institute, where they were later analyzed.

Urine and feces from chimpanzees were collected non-invasively by Ngamba Island staff caregivers. Urine was collected directly from individual animals, by catching it in named collection containers, attached to long poles, held through the bars of the sleeping enclosure. Stool produced by a particular individual was noted and its position recorded; it was gathered once the animals had been moved out for their daily free-roaming of the island. These samples were transported directly to the laboratory facilities for processing.

Staff working on Ngamba Island were also invited to submit stool, urine, and finger prick blood samples, which were collected by a Vector Control Division (Ministry of Health) community nurse. All participants gave written informed consent before sample collection.

### Analysis of the samples

A variety of diagnostic tests were carried out on the samples collected. Due to the opportunistic nature of stool and urine collection from the chimpanzees, not all tests could be performed for all individuals. Similarly, in cases where only small amounts of stool were obtained from an individual, not all stool-based diagnostics could be performed on the sample, leading to unavoidable gaps within the final dataset.

Sera were defrosted and used in soluble egg antigen (SEA)-enzyme-linked immunosorbent assay (ELISA) tests for *Schistosoma* (IVD) at a dilution of 1:40, as per the manufacturers' instructions. Urine samples were tested for micro-hematuria using commercially available Hemastix<sup>®</sup> (Bayer), to assess for the possibility of *Schistosoma haematobium* infec-

tions (French et al. 2007), although this parasite is not known to be endemic to this part of Lake Victoria. Fifteen microliters of urine was used in a circulating cathodic antigen (CCA) urine lateral flow test, which is a highly sensitive measure of presence of adult *S. mansoni* worms (Stothard et al. 2006, Standley et al. 2010b). Results were semiquantitative and scored as "trace," "single positive," "double positive," or "triple positive" based on band intensity, and cross-checked by a second observer.

Stool samples were used to create duplicate Kato-Katz thick smears according to published methods (Katz et al. 1972). Where indicated by positive CCA tests or Kato-Katz slides, the remainder of the stool was used for Percoll separation and examination under the microscope for *S. mansoni* and other eggs (Eberl et al. 2002) and for hatching using a Pitchford funnel (Pitchford 1959). Schistosome miracidia that hatched from eggs from the Pitchford funnel were collected in 2.5  $\mu$ L of water and placed on Whatman® FTA indicator cards for later extraction of DNA.

Approximately 0.5 g of sieved stool was suspended in 1 mL 100% ethanol and used for a quantitative polymerase chain reaction (real-time PCR) detection method for *Schistosoma*. For DNA isolation, 200  $\mu$ L of feces suspension was centrifuged and the pellet was washed twice with 1 mL of phosphate-buffered saline. After centrifugation the pellet was re-suspended in 200  $\mu$ L of 2% polyvinylpyrrolidone (Sigma) suspension and heated for 10 min at 100°C. After sodium-dodecyl sulfate-proteinase K treatment (2 h at 55°C), DNA was isolated using QIAamp DNA-easy 96-well plates (QIAGEN). In each sample, 10<sup>3</sup> PFU/mL Phocin Herpes Virus 1 (PhHV-1) was included within the isolation lysis buffer (Verweij et al. 2001, Niesters 2002).

*Schistosoma* real-time PCR including PhHV-1 as an internal control was performed using primers and probes as described previously (Obeng et al. 2008).

#### DNA sequencing of schistosome eggs and miracidia

Genomic DNA was extracted from the Whatman FTA Indicator cards according to standard protocol (Gower et al. 2007). PCR amplifications were performed on a portion of the cytochrome oxidase sub-unit 1 (*cox1*) gene using the ASMIT 1 and ASMIT 2 primers (Bowles et al. 1992). The *cox1* gene was selected as a marker due to the known ability of this fragment for species-level identification in schistosomes, as well as significant intraspecific variation, useful for analysis of geo-

graphical patterns and population dynamics. Most importantly, these primers have recently been used widely on *S. mansoni* from school-aged children from Lake Victorian shoreline communities, creating a large dataset of haplotypes against which future DNA sequences can be directly compared (Stothard et al. 2009, Standley et al. 2010a). PCR products were observed on a 2% agarose gel, stained with GelRed™ (Biotium). Positively amplified samples were sequenced using an Applied Biosystems Big Dye Kit (version 1.1) and an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems). The resulting sequences were assembled and visually edited in Sequencher v 4.8 (Gene Codes Corporation) before being compared to the existing database of *S. mansoni cox1* sequences on GenBank.

#### Malacological surveys

The shoreline of Ngamba Island was divided into 20 equally distanced, global positioning system-referenced sites, which were surveyed for presence and abundance of *Biomphalaria* snails. Sampling was semiquantitative; two collectors were deployed at each site for 10 min, sampling ~15 m of shoreline. All snails collected were shed to detect infection with schistosomes by exposing the snails to direct sunlight for 1–3 h at midday.

#### Treatment and clinical examinations

In February 2009, all chimpanzees were treated with albendazole and praziquantel and again with praziquantel in February 2010. The animals were also treated with ivermectin in September–October 2009.

All Ngamba Island staff members who volunteered samples were treated with albendazole, and those who tested positive for schistosomiasis by any diagnostic were also treated with praziquantel. Before treatment, each participant was also invited to take part in a full clinical consultation with a medical doctor (Christoffer van Tulcken) and community nurse (Aaron Atuhaire), who recorded reports of symptoms and medical and treatment histories.

#### Ethics clearance

Ethics clearance to take samples from the chimpanzees was granted by the Uganda Wildlife Authority and the Uganda National Council for Science and Technology (UNCST). Permission to survey and treat human adults was given by

TABLE 1. RESULTS OF DIFFERENT DIAGNOSTIC TESTS FOR *SCHISTOSOMA MANSONI* INFECTION IN CHIMPANZEES AND STAFF MEMBERS ON NGAMBA ISLAND

Diagnostic	Target group	Total number sampled	Number of positives	% Prevalence (95% CIs)
<i>Schistosoma</i> enzyme-linked immunosorbent assay	Chimpanzees	31	29	93.5 (78.6–99.2)
	Staff	23	14	60.9 (38.5–80.3)
Circulating cathodic antigen test	Chimpanzees	20	10	50.0 (27.2–72.8)
	Staff	34	15	44.1 (27.2–62.1)
Kato-Katz double smear	Chimpanzees	19	1	5.3 (0.1–26.0)
	Staff	27	5	18.5 (6.3–38.1)
Percoll	Chimpanzees	9	1	11.1 (0.3–48.2)
	Staff	25	3	12.0 (2.5–31.2)
Real-time polymerase chain reaction <i>Schistosoma mansoni</i>	Chimpanzees	24	13	54.2 (32.8–74.4)
	Staff	24	10	41.7 (22.1–63.3)

CI, confidence interval.

TABLE 2. NUCLEOTIDE BASE CHANGES ACROSS THE ASMIT FRAGMENT OF THE COI GENE IN THE 12 HAPLOTYPES RECOVERED FROM CHIMPANZEES

Haplotype and chimpanzee sequence	Base number					
	1696	1706	1844	1868	1908	1935
H1 S3, S5, S8, S10, S11	C	T	T	T	A	T
H16 S7, K1	C	T	C	A	A	G
H36 S1, S2	C	T	T	A	A	T
H132 <sup>a</sup> S4, S5	T	T	C	A	A	G
H133 <sup>a</sup> S9	C	C	T	A	G	T

<sup>a</sup>Corresponds to previously unknown haplotypes.

Note: "S" and "K" refer to different individual chimpanzees.

UNCST and the National Health System Local Research Ethics Committee at St. Mary's Hospital in London as part of the national control program for bilharzia and intestinal worms.

## Results

Intestinal schistosomiasis was found in the chimpanzees across all the diagnostic tests used. Of the five diagnostic techniques, the highest prevalence for *Schistosoma* was observed using the SEA-ELISA for both the chimpanzees (93.5%) and the staff (60.9%) in 2010 (Table 1). CCA urine lateral flow tests were less sensitive than the SEA-ELISA for detecting schistosomiasis, but the results followed the same pattern as the serum observations (Table 1). In terms of intensity of the CCA test reaction, seven chimpanzees had trace readings, two were single positives, and one was a double positive. The staff had five trace readings, nine single positive readings and one triple positive reading.

The microscopy-based diagnostics on stool (Kato-Katz and Percoll) detected one egg-patent chimpanzee, and two further

individuals produced eggs that were collectable via the Pitchford funnel methodology and subsequently hatched into miracidia, proving their viability. All three of these chimpanzees had positive CCA tests and two had strong positive SEA-ELISA results; serum was not available for the third. Real-time PCR detection of *S. mansoni* DNA in stool revealed similar prevalence levels to those observed with the CCA tests, in both humans and chimpanzees (41.7% and 54.2%, respectively); seven chimpanzees and five humans had high levels of DNA in the stool (Ct [cycle threshold] < 30), whereas three chimpanzees and three humans had moderate amounts of DNA (Ct 30–35) and three chimpanzees and two humans had low amounts of DNA (Ct > 35). Of the chimpanzees who were egg-positive (either by Kato-Katz or Pitchford), one had high levels of *Schistosoma* DNA as detected by real-time PCR, another had moderate levels, and the third's feces was not archived in ethanol and so was not able to be tested.

The miracidia collected from the chimpanzees were genotyped for variation in the ASMIT fragment of the mitochondrial cytochrome oxidase sub-unit one (*cox1*) gene. The sequences were compared to the database of haplotypes established in 2009 (Stothard et al. 2009) and 2010 (Standley et al. 2010a), which had included previous analysis of cercariae from field-caught snails from Ngamba Island in 2008. The 12 haplotypes recovered from chimpanzee miracidia (11 from one individual and 1 haplotype from another individual) corresponded to three previously known haplotypes: H1, H16, and H36 (GenBank acquisition numbers GQ415163.1, GQ415179.1, and GQ415211.1 respectively), as well as two novel ones, labeled H132 and H133 (GenBank acquisition numbers HM031081 and HM055377). Twenty miracidia collected from two staff members were also sequenced and corresponded to haplotypes H1 (the most common locally), H16, H17, H35, H38, and a novel haplotype, named H137 (GenBank acquisition number HM055378). The total size of the database now stands at 137 unique haplotypes, reflecting the high genetic diversity of *S. mansoni* in this region. Tables 2 and 3 show the number and position of variable positions between all haplotypes.

The malacological survey along the shoreline of Ngamba Island revealed *Biomphalaria* populations at 16 of 20 sites

TABLE 3. NUCLEOTIDE BASE CHANGES ACROSS THE ASMIT FRAGMENT OF THE COI GENE IN THE 20 HAPLOTYPES RECOVERED FROM STAFF MEMBERS

Haplotype and human sequence	Base number												
	1703	1754	1769	1844	1865	1868	1877	1890	1908	1935	1986	1995	2012
H1 G2, G8, G9, G10, E3, E4, E8, E10	T	A	T	T	A	T	G	A	A	T	T	C	A
H10 G4, G6, E5	T	A	T	C	A	T	G	A	A	T	T	C	A
H16 E1, E9	T	A	T	C	A	A	G	A	A	G	T	C	A
H17 E2	T	A	T	T	A	T	G	A	A	T	T	T	A
H35 G1, G3, G5	C	G	C	T	G	T	A	A	A	T	C	C	G
H38 G7	T	A	T	T	A	A	G	A	G	T	T	C	A
H137 <sup>a</sup> E6, E7	T	A	T	T	A	T	G	G	A	T	T	C	A

<sup>a</sup>Corresponds to a previously unknown haplotype.

Note: "G" and "E" refer to individual staff members whose names are withheld to protect identity.

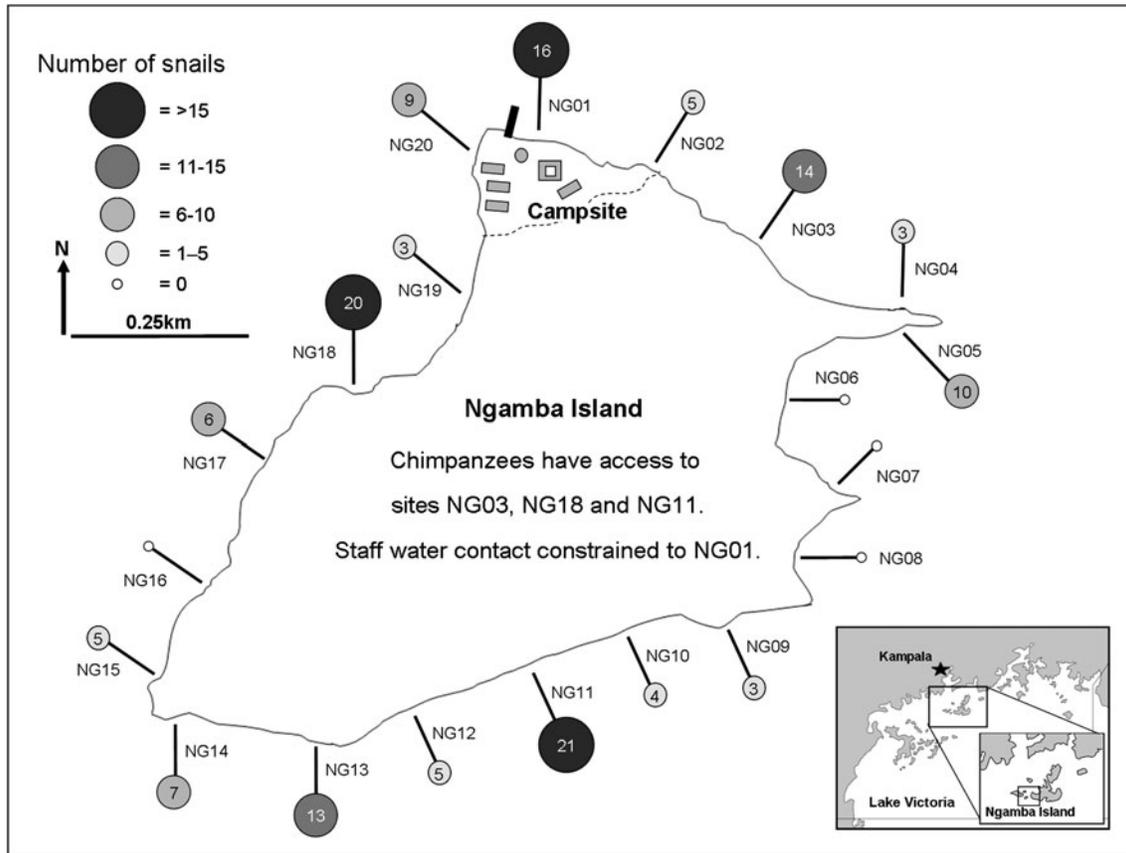


FIG. 1. Map of Ngamba Island showing snail collection sites and abundance of *Biomphalaria* observed.

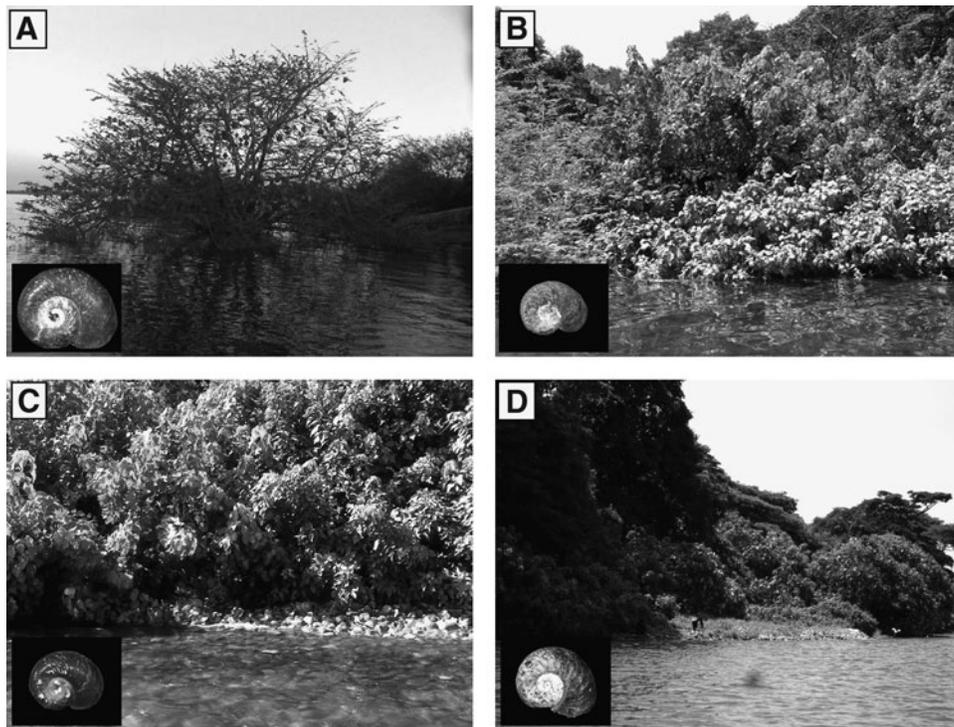


FIG. 2. Site pictures from NG01 (A), NG03 (B), NG11 (C), and NG18 (D). Insets show shell pictures of individual *Biomphalaria* collected from these sites, all displaying typical *Biomphalaria choanomphala* shell shape.

sampled, with an average of nine snails collected during the 10-min sampling period (Fig. 1). On the basis of shell characters, all snails were identified as *Biomphalaria choanomphala* (Fig. 2). Habitats varied from sheltered coves with mud/sand substrate and aquatic vegetation to rocky exposed shores, the latter not being considered a typical habitat favored by *Biomphalaria* (Fig. 2). Thirteen of the sites where snails were found were within the free-roaming area accessible to the chimpanzees. A snail from site NG01 was found to be shedding *S. mansoni* cercariae, and these were barcoded as above, and corresponded to barcodes H14 and H100 (GenBank acquisition numbers GQ415176.1 and GQ415283.1), both found in Uganda on previous surveys.

Clinical consultation with the staff revealed that only 55.6% of those surveyed had previously taken praziquantel. Eight of 27 staff members who answered questions about their symptoms reported to have suffered abdominal pain, diarrhea, or other gastrointestinal symptoms in the last month. Two of these staff members were found to be egg-patent for *S. mansoni* based on Kato-Katz double smears, and a third was CCA positive.

## Discussion

This conjoint parasitological and malacological survey has comprehensively demonstrated that chimpanzees are infected with *S. mansoni* and these animals are at risk of anthrozoönotic transmission from sympatric human populations. Although prevalence estimates varied by diagnostic test, positive cases of intestinal schistosomiasis among the surveyed animals could be found by each method.

### Parasitological findings: chimpanzees and staff members

One important finding was that the disparity in the prevalence as detected by egg excretion compared to that by SEA-ELISA was greater for the chimpanzees than for the human staff members. While this effect could be the result of the blanket prophylactic treatment with praziquantel in 2009, it could also suggest that chimpanzees are generally less permissive hosts. This could result in enhanced clinical manifestation of the disease, as a higher proportion of eggs could be trapped in tissues and organs. If surveys in the future could measure clinical signs of the disease, such as ultrasonography examinations of the liver and spleen in the chimps, it would assist in testing this hypothesis, thus providing a direct health benefit from this research. Further, in humans there is evidence that immunosuppression in patients co-infected with schistosomiasis and human immunodeficiency virus can result in reduced egg excretion, although these studies are contested (Karanja et al. 1997, Kallestrup et al. 2005). Retroviruses in chimpanzees might have a similar effect; on Ngamba Island, none of the chimpanzees surveyed are infected with simian immunodeficiency virus, but around 70% are infected with simian foamy virus, a closely related retrovirus (Mugisha et al. 2010); it is not known what effect this underlying infection might have on egg excretion patterns of schistosomes.

The staff members on Ngamba showed high seroprevalence of schistosomiasis and treatment with praziquantel was reported in over half of the participants. This

could be responsible for the reduced prevalence seen in egg-detection diagnostic tests, although the relatively high number of positive CCA tests indicates the likely persistence of adult worms.

### Molecular epidemiology of *S. mansoni* on Ngamba Island

Our study is the first to report direct genetic testing of the schistosome miracidia recovered from chimpanzees or indeed any nonhuman primate with naturally acquired infections, without resorting to laboratory passage or invasive procedures. Common haplotypes were found in both chimpanzees and staff; the same haplotypes were also found abundantly in people living across the Lake Victoria shoreline (Standley et al. 2010a). These results have important implications: chimpanzees can clearly act as hosts for forms of *S. mansoni* that are capable of infecting humans, and *vice versa*. From a conservation medicine perspective, this proves that in regions where wild chimpanzees come into close contact with human communities that carry the infection, they too are at considerable risk from contracting the disease contingent on water contact where intermediate host snails are present. Considering that other primate species have also been shown to be permissive to experimental schistosome infections (Sadun et al. 1966), these findings should be taken on board by primate conservationists, who should integrate monitoring and potentially treatment into existing disease management. Further, if the Ngamba Island chimpanzees were to be reintroduced into the wild, they should be treated with praziquantel before release, to avoid establishment of the disease in a wild setting.

### Transmission dynamics: *Biomphalaria* distributions and infection status

From the perspective of investigating the micro-scale transmission dynamics on Ngamba Island, the malacological surveys found *Biomphalaria* snails in a variety of habitat types and in a number of sites where the chimpanzees can access the lake. The paucity of patent infections observed across both surveys (in 2008 and 2010), with none in areas that chimps can access, demonstrates how traditional measures of determining infection status may greatly underestimate the risk of a particular locality, and so alternative methods, such as PCR screening, are urgently required. A follow-up survey in June 2010 did detect a shedding snail at site NG03, where chimps have been observed to access the water. When genotyped, some cercariae were found to be *S. mansoni*, but others were *Schistosoma rodhaini*, a closely related schistosome of rodents. *S. rodhaini* has been shown to be viable in primates, especially in the context of co-infection with *S. mansoni*; the two species have further been observed to hybridize (Nelson and Teesdale 1965, Morgan et al. 2003). This finding supports the suggestion that the chimpanzees on Ngamba Island are and continue to be at risk from exposure to *S. mansoni*, and further introduces the possibility of transmission of *S. rodhaini* or hybrids to humans and chimpanzees on the island.

### Implications

One direct result of this research, from an animal welfare perspective, could be to reduce the water access available to

the chimpanzees at points where snails are most abundant. For example, no *Biomphalaria* were found at four sites, which could be retained as places where the chimps could access the water. Likewise, measures can be taken to prevent staff and visitors from exposing themselves to the disease through unnecessary water contact, or else they could be encouraged to take postimmersion measures (Ramaswamy et al. 2003). A further immediate consequence of these surveys has been the addition of praziquantel to the annual regime of de-worming. This will immediately improve the health of those already suffering from the disease; a further line of research could be to investigate appropriate drug dosage for chimpanzees for maximum efficacy.

Above all, these results stress the importance of considering captive animals, whose health can be monitored more readily, as a model for understanding the dynamics of disease between humans and wildlife and molecular epidemiology more generally. Given the ever-increasing importance of research into zoonotic diseases, our study presents the benefits of also examining animals for signs of emerging infection with human pathogens, with obvious conservation and welfare implications.

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### Disclosure Statement

The authors declare that they have no competing interests.

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