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Research Note

Schistosoma bovis in western Uganda

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Abstract

During routine parasitological surveillance and monitoring activities within a National Control Programme for control of human schistosomiasis in Uganda, it was noted that cattle grazing in a water meadow immediately adjacent to Tonya primary school, where the prevalence of intestinal schistosomiasis in children was in excess of 90%, were unusually emaciated. To test the hypothesis that there may have been an anthropozoonotic focus of *Schistosoma mansoni* within the local herd, a young female heifer, clearly emaciated and *c.* 8 months old, was slaughtered from which schistosome worms were later recovered by dissection. As female worms inspected by microscopy were not gravid, morphological identification proved inconclusive but analysis of cytochome oxidase subunit I (COI) and small subunit (SSU) ribosomal DNA sequences from these worms identified them as *Schistosoma bovis* Sonsino, 1876. This is the first substantiated report of *S. bovis* from Lake Albert, western Uganda. Further epidemiological surveys are needed to clarify the extent of bovine schistosomiasis within this region, particularly so since this lakeside plain has been earmarked as a future game reserve.

Human schistosomiasis is a debilitating parasitic disease found throughout much of sub-Saharan Africa. In Uganda both intestinal and urinary schistosomiasis occur but the latter has a restricted distribution while the former is widespread (Doumenge *et al.*, 1987). Along the shores of Lake Victoria and Lake Albert (fig. 1a), *Schistosoma mansoni* Sambon, 1907 can be considered to be hyper-endemic and the prevalence of infection within school-age children can be nearly universal (Kabatereine *et al.*, 1996). Following support from the Schistosomiasis Control Initiative which is funded by the Bill and Melinda Gates Foundation, a National Control Programme for control of schistosomiasis was officially launched in March 2003 at Pakwach (fig. 1a), a locality on the Victoria Nile with a long association with this disease (Nelson, 1958; Ongom & Bradley, 1972; Kabatereine *et al.*, 1992).

Many of the communities along Lake Albert in the district of Hoima are extremely isolated since the escarpment descends often precipitously into the lake. With no roads and access mainly by foot, movement and trading between communities along the lake is achieved using boats. During a surveillance and monitoring visit to Tonya primary school in April 2003 (fig. 1b), where *S. mansoni* prevalence was in excess of 90%, it was noted that despite grazing on good water pasture meadow immediately adjacent to the school, all local cattle looked exceedingly emaciated. An *ad hoc* malacological survey identified numerous *Biomphalaria sudanica* (Martens, 1870), the intermediate host of *S. mansoni*, in marshy pools within this water meadow as well as smaller numbers of *Lymnaea natalensis* Krauss, 1848 and *Bulinus* inclusive of *Bulinus forskalii* (Ehrenberg, 1831) and

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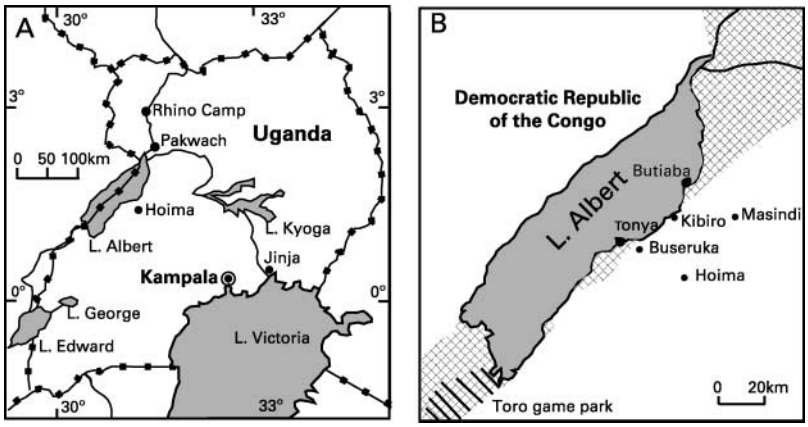

Fig. 1. A. Map of Uganda with the five major lakes; transmission of human intestinal schistosomiasis is considered to be particularly intense around the shores of Lakes Victoria and Albert while it is milder along the shores of Lakes George and Edward. Both intestinal and urinary schistosomiasis have been recorded around Lake Kyoga. Bovine schistosomiasis has been recorded around Lake Victoria region as well as in northern regions bordering Sudan. B. Sketch map of Hoima district adjacent to Lake Albert. The crosshatched regions denote natural enclosures within the Rift valley and the lake some 1500 m below the escarpment. Buseruka is the nearest health outpost station to Tonya. The Lake side plain of Tonya has been earmarked as a game reserve similar to that of Toro in the south (single hatched); Murchison game park begins some 20 km north of Butiaba. Owing to the steeply descending escarpment, there are no roads to Tonya and access is best achieved by boat from either Kibiro or Butiaba.

(?)*Bulinus ugandae* Mandahl-Barth, 1954 and (?)*Bulinus truncatus* (Audouin, 1827) in papyrus marshes fringing the lake. While *Lymnaea* and *Bulinus* are known to transmit bovine fascioliasis and schistosomiasis respectively, neither disease has been explicitly recorded in western Uganda (Dinnik & Dinnik, 1965; Torgerson & Claxton, 1999).

Given the high, local transmission of *S. mansoni* and close proximity of livestock, it was plausible that the cattle at Tonya might present an anthropozoonotic focus of *S. mansoni* since reports exist of infections in cattle (Rollinson & Southgate, 1987). Therefore to test this hypothesis, a young female heifer, *c.* 8 months old and confirmed to have been born locally, was slaughtered for examination and the internal organs were inspected for evidence of trematode infection. No worms were seen within the stomach, liver or intestinal lumen, however, a total of 15 paired schistosome worms were recovered by dissection from the mesenteric veins around the large intestine. Two worm pairs were immediately inspected under the compound microscope for intrauterine eggs, and the remaining worms were preserved in absolute ethanol for DNA analysis. The two female worms were not gravid and, as schistosome eggs were not recovered from repeated examination of faecal material, an immediate schistosome species group determination could not be made. Further inspection of faecal material from other local cattle in the field was not possible owing to immediate time constraints. Examination of the tubercules on the tegument of male worms at high magnification (× 400) showed that these worms were without spines, a characteristic of *S. bovis* Sonsino, 1876.

To identify recovered schistosomes from Tonya, the total DNA was extracted using a DNeasy DNA extraction kit (Qiagen) according to the manufacturer's instructions and fragments of small ribosomal subunit (SSU) rDNA and mitochondrial cytochrome oxidase I (COI) were amplified using polymerase chain reaction with the primers and conditions described in Littlewood *et al.* (1999) for the SSU and Lockyer *et al.* (2003) for the COI. Fragments were purified with Qiaquick columns (Qiagen) and directly cycle sequenced using ABI Big-Dye chemistry, ethanol precipitated and run on an ABI prism 377 automated sequencer. Internal sequencing primers used were as described in Littlewood *et al.* (1999) and Lockyer *et al.* (2003). Sequences were assembled and edited using Sequencher ver 3.1.1 (GeneCodes Corp.) and have been deposited in GenBank [COI – AY318827 and SSU – AY318828]. The two sequences were aligned to the SSU and COI of 13 other African and Indian *Schistosoma* sequences (Lockyer *et al.*, 2003) with the aid of ClustalW using default parameters (Thompson *et al.*, 1994).The alignment was truncated to remove portions that were not present in all species, leaving a 503 bp alignment for the COI and 1807 bp for the SSU. A Kimura-2-parameter DNA distance matrix was calculated between all sequences (table 1).

Both SSU and COI sequences for schistosomes from Tonya were identical to those previously sequenced for *S. bovis*. The occurrence of an authenticated report of bovine schistosomiasis at this locality is important for two reasons. Firstly, whilst *S. bovis* has been reported within Uganda and there are confirmed reports of natural transmission around Lake Victoria (Moné *et al.*, 2000), it is not known from the Lake Albert region and observations of *S. bovis* found in slaughterhouses in the north-west of Uganda have been attributed to imported infections from cattle raised within Sudan (Dinnik & Dinnik, 1965). The evidence that this schistosome infection was acquired locally at Tonya is persuasive; the age of the heifer and non-gravid nature of the worms (which normally start to become gravid after 32 days post-infection (see Southgate

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Table 1. Nucleotide distance (Kimura-2-parameter) matrix between worms collected from Tonya and selected schistosome species. The sequences between the Tonya schistosomes and *Schistosoma bovis* were identical. The upper diagonal portion of the matrix contains the DNA distances for SSU while the lower diagonal portion contains DNA distances for COI.

	Tonya isolate	*S. bovis*	*S. curassoni*	*S. intercalatum*	*S. haematobium*	*S. mattheei*	*S. leiperi*	*S. margrebowiei*	*S. indicum*	*S. spindale*	*S. nasale*	*S. rodhaini*	*S. mansoni*	*S. incognitum*
Tonya isolate	–	0.000	0.002	0.003	0.003	0.001	0.003	0.006	0.007	0.007	0.012	0.009	0.010	0.010
S. bovis	0.000	–	0.002	0.003	0.003	0.001	0.003	0.006	0.007	0.007	0.012	0.009	0.010	0.010
S. curassoni	0.049	0.049	–	0.001	0.001	0.001	0.002	0.004	0.005	0.006	0.012	0.008	0.009	0.010
S. intercalatum	0.064	0.064	0.058	–	0.002	0.002	0.002	0.004	0.006	0.007	0.012	0.008	0.009	0.010
S. haematobium	0.103	0.103	0.110	0.114	–	0.002	0.003	0.005	0.006	0.007	0.013	0.010	0.010	0.010
S. mattheei	0.143	0.143	0.147	0.152	0.180	–	0.002	0.004	0.006	0.006	0.012	0.008	0.008	0.009
S. leiperi	0.087	0.087	0.082	0.080	0.105	0.137	–	0.004	0.007	0.007	0.012	0.009	0.010	0.010
S. margrebowiei	0.143	0.143	0.147	0.150	0.159	0.163	0.142	–	0.003	0.004	0.009	0.006	0.006	0.006
S. indicum	0.210	0.210	0.205	0.211	0.219	0.217	0.212	0.227	–	0.002	0.007	0.005	0.006	0.007
S. spindale	0.219	0.219	0.211	0.219	0.202	0.210	0.203	0.207	0.184	–	0.007	0.004	0.005	0.006
S. nasale	0.209	0.209	0.213	0.220	0.188	0.203	0.198	0.216	0.231	0.199	–	0.010	0.010	0.010
S. rodhaini	0.240	0.240	0.246	0.264	0.226	0.254	0.241	0.273	0.263	0.240	0.240	–	0.001	0.006
S. mansoni	0.254	0.254	0.256	0.260	0.228	0.266	0.261	0.294	0.270	0.256	0.233	0.131	–	0.006
S. incognitum	0.286	0.286	0.269	0.278	0.281	0.296	0.268	0.270	0.270	0.286	0.288	0.338	0.318	–

& Knowles, 1975a)) point towards a recent infection and, even if the maternal cow carried an *S. bovis* infection acquired elsewhere, congenital transmission of *S. bovis* is unlikely (Johansen *et al.*, 2002). As *S. bovis* can utilize a wide range of species of *Bulinus* as intermediate hosts (Southgate & Knowles, 1975a,b), malacological surveillance of schistosome infections within local populations of *Bulinus* will help elucidate the precise patterns of transmission. It is perhaps worthy of note that populations of *B. forskalii* from Rhino Camp (fig. 1a) have been found to be shedding schistosome cercariae. Secondly, owing to the natural escarpment boundaries surrounding the Tonyan plain, this area has been earmarked as a future game reserve in a similar manner to the Toro game park further south (fig. 1a). Given the broad definitive host range of *S. bovis* (Rollinson & Southgate, 1987), it would be advisable to monitor the health of any such imported animals for bovine schistosomiasis after introduction; further epidemiological investigations of schistosome infections within local livestock are needed to determine the present prevalence and intensity of bovine schistosomiasis along Lake Albert.

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