

Title. Rodents as natural hosts of zoonotic *Schistosoma* species and hybrids: an epidemiological and evolutionary perspective from West Africa

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Running title. Zoonotic *Schistosoma* in African rodents

ABSTRACT

The complex multi-host disease dynamics of schistosomiasis and *Schistosoma* spp., including the emergence of zoonotic parasite hybrids, remain largely unexplored in West Africa. We elucidated the role of wild small mammals as reservoir for zoonotic *Schistosoma* species and hybrids in endemic areas of Senegal. We identified *Schistosoma mansoni*, *Schistosoma bovis*, and a *Schistosoma haematobium*/*S. bovis* hybrid, with local prevalence in wild rodents ranging from 1.9% to 28.6%. Our findings indicate that rodents may be an important local reservoir for zoonotic schistosomiasis in endemic areas of West Africa, amplifying transmission to humans and acting as natural definitive hosts of schistosome hybrids.

Key words. Africa; hybridization; One Health; *Schistosoma*; schistosomiasis; wildlife reservoir; zoonoses.

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Schistosomiasis is a neglected tropical disease caused by dioecious trematodes of the genus *Schistosoma*. The spatial distribution of schistosomiasis is highly focal, with transmission restricted to freshwater bodies in which specific snails act as intermediate hosts. Globally, about 250 million people are infected, with the highest burden in sub-Saharan Africa. Schistosomiasis is intimately associated with poor and rural communities where access to clean water is limited. Guided by the World Health Assembly resolution 65.21, initiatives to control and eliminate schistosomiasis as a public health issue have been recently catalysed [1].

Schistosoma haematobium, responsible for human urogenital schistosomiasis, and *Schistosoma mansoni*, an agent of human intestinal schistosomiasis, are both endemic across sub-Saharan Africa. While *S. haematobium* is essentially specific to humans, *S. mansoni* can naturally infect rodents, non-human primates and other animal hosts [2, 3]. In West Africa, small mammals as potential sources of zoonotic schistosomiasis have been investigated only once during a major human epidemic of *S. mansoni* across the Senegal River Basin in the early 1990s. During that period, Nile rats (*Arvicanthis niloticus*) and Hubert's multimammate mice (*Mastomys huberti*) were identified as suitable alternative definitive hosts of the circulating *S. mansoni* [4]. This outbreak of intestinal schistosomiasis followed dramatic anthropogenic land-use changes driven by the completion of the Diama dam in 1985, which generated a rapid agro-industrial growth as a consequence of the guaranteed fresh water supply [5]. Recently, while human intestinal schistosomiasis has remained endemic in the Senegal River Basin (baseline prevalence 1.8-68.9%), the foci of urogenital schistosomiasis have expanded throughout the region (baseline prevalence 58.3-100%) (authors' unpublished data).

Alterations of the natural landscape may have also favoured the progressive loss of ecological barriers and the creation of permanent freshwater bodies (i.e., perennial sources of

schistosomiasis transmission), supporting the co-occurrence and interspecific interactions between *S. haematobium*, *S. mansoni* and other *Schistosoma* spp. of veterinary importance [3, 6]. Advances in the application of molecular methods have revealed hybridization and introgression (see [3] for definitions) between *S. haematobium* and animal schistosomes in Senegal and other endemic countries. To date, humans are reported as the only definitive hosts of hybrids between *S. haematobium* with other *Schistosoma* spp. [6]. In the Senegal River Basin, the open and permanent water bodies and irrigation canals promote the interplay between human and animal schistosomes and the transmission of parasite hybrids in the area [7, 8]. The outcome is an entangled epidemiology in which agents of human schistosomiasis can exploit a variety of animals as reservoirs and, at the same time, hybridize with animal *Schistosoma* spp., causing spillover from zoonotic transmission.

Our aim was to elucidate the role of wild small mammals as reservoir hosts of zoonotic schistosome species and/or hybrids in a West African setting subject to dramatic anthropogenic change. We applied a multi-locus molecular approach to test the hypotheses of small mammal populations as: (1) alternative definitive hosts and amplifiers of *S. haematobium* and *S. mansoni* transmission to humans; and (2) biotic hubs where hybridization between human and animal *Schistosoma* spp. occurs, generating zoonotic hybrid parasites.

METHODS

Study Area and Sampling of Small Mammals

Between May 2016 and April 2017, we sampled nine localities in the Senegal River Basin, before and after the rainy season (June-September). The study sites were previously identified as transmission foci for *Schistosoma* spp. (authors' unpublished data), and we considered them as independent replicates given that the shortest distance between two sites

was > 3 km, above the average home range recorded for local rodent species [9]. Small mammals were captured using locally made wire-mesh live traps (26 x 10 x 10 cm) baited with peanut butter and placed adjacent to water bodies in crop fields near human dwellings and/or riparian habitat primarily represented by thick reeds (*Typha* sp.). Each evening, lines of 10-20 traps at regular intervals (5-10 m, depending on the shape and size of the site) were set over 2-3 nights per site. Each morning, the traps were inspected and the number of traps active throughout the night was recorded, considering as misfire any trap found sprung, missing, or not triggered. By-catch (i.e., the capture of non-target species) was recorded then immediately released at the point of capture.

Laboratory Analyses

The trapped small mammals were returned live to the laboratory and humanely euthanized (see Supplementary data). At post-mortem, we recorded species, gender, sexual maturity, body weight and length of each individual. Age classification of rodents as juveniles or adults was based on anatomical and sexual traits [9]. Organs from each small mammal were examined and the isolated *Schistosoma* spp. were stored in separate vials containing 95% ethanol at -20 °C. For hosts harbouring adult schistosomes, liver and large intestine were dissected and macerated by passing through a 300 µm metal sieve in water for the hatching of miracidia larval stage and their collection onto Whatman FTA[®] indicator cards [10].

DNA from individual adult schistosomes was extracted using the QIAGEN DNeasy[®] Blood & Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. DNA from individual miracidia stored on Whatman FTA[®] cards was extracted as described [10]. DNA extracts were amplified and sequenced for the complete internal transcribed spacer (ITS) region of the nuclear ribosomal DNA using the primers ETTS1 and ETTS2, and for a segment of the cytochrome *c* oxidase subunit 1 (COI) gene of the mitochondrial DNA

using the primers Cox1_Schist_5' and Cox1_Schist_3' (see Supplementary data). The ITS and COI sequences were compared by alignment with data available in the National Center for Biotechnology Information (NCBI) GenBank database (Supplementary Table 1).

Statistical Analyses

The relative abundance of small mammals was assumed to be reflected in their different capture rates, calculated for each trapping session and site as the proportion between captures and active traps set overnight [11]. Differences in the occurrence of *Schistosoma* spp. among host species (*A. niloticus* and *M. huberti*), host traits (gender and age), seasons (pre- and post-rainy season), trapping sites and habitats (riparian vegetation and crop field) were assessed using Pearson's chi-squared (χ^2) test, significant when $P \leq 0.05$. Statistical analyses were performed using EpiTools (<http://epitools.ausvet.com.au>).

Ethics Statement

Trapping activities were initiated after explicit consent from local authorities and land owners. Approval for live trapping and euthanasia of small mammals was obtained from the Clinical Research Ethical Review Board of the Royal Veterinary College, University of London (reference number: 2016 1505).

RESULTS

A total of 509 animals were captured over 2,531 trap-nights (overall capture rate 20.1%). We trapped 215 *A. niloticus* rats, 172 *M. huberti* mice, 27 *Crocidura* sp. shrews, and 6 *Taterillus* sp. gerbils. By-catch (n = 89) was mainly represented by four-toed hedgehogs (*Atelerix albiventris*) and anuran amphibians. We detected *Schistosoma* spp. in the portal system and mesenteric vessels of 7/172 *M. huberti* (prevalence 4.1%; intensity range 2-35; median

intensity 4) and 6/215 *A. niloticus* (prevalence 2.8%; intensity range 1-44; median intensity 5). No schistosomes were isolated during the inspection of the urogenital system. Among study sites, *Schistosoma* spp. prevalence was estimated to be 28.6% (6/21) and 1.9% (1/52) in *M. huberti* from the villages of Temey and Nder, respectively, while prevalence was 8.7% (4/46) and 2.7% (2/73) in *A. niloticus* from Djidiery and Richard Toll, respectively (Figure 1).

We identified *S. mansoni* in 7 *M. huberti* and 1 *A. niloticus*, and *Schistosoma bovis* in 5 *A. niloticus* based on the ITS (903-911 base pairs) and COI (861-880 base pairs) DNA regions of each adult worm. One schistosome pair parasitizing a *M. huberti* from Temey was composed of a *S. mansoni* male with a hybrid female presenting the profile of *S. haematobium* for ITS and *S. bovis* for COI (Table 1). Miracidia collected from 2 *A. niloticus* infected with *S. bovis*, 1 *M. huberti* infected with *S. mansoni*, and 1 *M. huberti* infected with both *S. mansoni* and *S. haematobium/S. bovis* were molecularly identified as either *S. mansoni* or *S. bovis*. Alignment of the ITS sequences did not detect any intraspecific variation among the analysed specimens. In contrast, different haplotypes were found when aligning the COI sequences of *S. mansoni* (identity \geq 98.2%) and *S. bovis* (identity \geq 98.7%). Intraspecific nucleotide polymorphism translated in missense mutations in the amino acid sequence of COI's protein product for *S. mansoni* from Temey (Supplementary Table 2). Sequence data from individual schistosomes were deposited in the NCBI GenBank database under the accession numbers MF776585-MF776597 (ITS) and MF919405-919428 (COI).

The estimated *Schistosoma* spp. prevalence was significantly higher in adult rodents when compared to juveniles ($\chi^2 = 4.842$; d.f. = 1; $P = 0.028$), and in the village of Temey when compared to other study sites ($\chi^2 = 49.406$; d.f. = 11; $P < 0.001$). Schistosome prevalence did not significantly vary when tested against host species, gender, season and habitat ($P > 0.05$).

DISCUSSION

A quarter of a century after the finding of *S. mansoni* in rodents of northern Senegal [4], we show that *M. huberti* and *A. niloticus* continue to serve as alternative definitive hosts of *S. mansoni*. Furthermore, our data demonstrate that *A. niloticus* can act as a suitable definitive host of *S. bovis*, a parasite of ruminants previously identified in small mammals, using molecular tools, only in *Mastomys natalensis* mice of Kenya [12]. In addition, to our knowledge, we provide the first report of *S. haematobium*/*S. bovis* hybrids in any non-human vertebrate outside of a laboratory setting, suggesting that both humans and rodents could act as natural definitive hosts of zoonotic *S. haematobium* hybrids in sub-Saharan Africa. These results, combined with the absence of pure *S. haematobium* in the examined rodents, highlight the increased host spectrum and adaptive plasticity that hybrid vigour can confer to parasites [3, 8]. Our data indicate the ability by *Schistosoma* species and hybrids to be true multi-host pathogens on a local scale, capable of crossing species barriers and maintaining transmission via zoonotic routes [3].

The molecular identification of miracidia confirms that both *S. mansoni* and *S. bovis* are able to produce viable eggs in the infected rodents. Interestingly, the substitutions characterizing the COI nucleotide sequences and amino acid translations of many *S. mansoni* specimens may indicate potential introgression of *S. haematobium* and/or *S. bovis* DNA [7]. Whilst the described multi-locus molecular analysis remains an invaluable tool for epidemiological studies of *Schistosoma* spp. and the detection of early hybridization events, the application of innovative genomic technologies would bring added value to research on multi-host zoonotic pathogens within a One Health framework. This integrated approach would provide fine-scale insight into the evolutionary pressures driving mechanisms of

spillover, host switching, hybridization and introgression between animal and human parasites [3].

In China and the Philippines, transmission maintenance and spillover to humans from rodents and other zoonotic reservoirs of *Schistosoma japonicum* continue to jeopardize control programmes, hindering public health efforts to eliminate this disease [13, 14]. In West Africa, wild rodents and other wildlife could be of paramount importance as zoonotic reservoirs of *Schistosoma* species and/or hybrids [2]. Our findings emphasize that the role of rodents as local reservoirs of *Schistosoma* spp. in the Senegal River Basin may be twofold, locally serving as either spillover hosts for the circulating schistosomes, or as key hosts in the transmission maintenance of schistosomiasis (see [3] for definitions). With rodents projected to become the dominant wildlife in human-driven environments and the main reservoir of zoonotic diseases in tropical zones [15], further data on the ecology of small mammals and schistosome transmission dynamics are necessary. A pressing question is how ongoing changes in landscape and biodiversity in West Africa will affect the future spread of synanthropic rodents and the subsequent risk of zoonotic transmission of schistosomiasis.

In conclusion, our study suggests that the role of wild rodents as potential local reservoirs of zoonotic *Schistosoma* species and hybrids in endemic areas of West Africa cannot be overlooked. The breakdown of ecological barriers in the absence of any mitigation measures may support the life cycle and interspecific interactions between human and animal schistosomes [3, 6]. Therefore, a One Health, multi-host framework would better tailor local, setting-specific schistosomiasis control strategies, enhancing public health interventions in many countries where the disease is endemic.

NOTES

Acknowledgments. We are grateful to Aidan Emery, Bonnie Webster and David Rollinson for their valuable support and hospitality at the Natural History Museum, London (UK). A very special acknowledgement to Boubacar Bâ, Alassane Ndiaye, Mapaté Gaye and Cheikh Thiam and his family for their outstanding contribution during fieldwork and for the incredible hospitality. Finally, we thank the communities of the Senegalese villages we worked with for the friendly welcome and collaboration and, although no acknowledgement can compensate their sacrifice, we honour all the wild animals trapped during this study.

Financial support. This work was supported by the Royal Veterinary College and the Zoonoses and Emerging Livestock Systems (ZELS) research initiative including the Department for International Development, Biotechnology and Biological Sciences Research Council, Economic and Social Sciences Research Council, Medical Research Council, Natural Environment Research Council, and Defence Science and Technology Laboratory (grant numbers BB/L018985/1 and BB/N503563/1).

Potential conflicts of interest. All authors report no potential conflicts of interest.

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Figure 1. Geographic location of the study sites in northern Senegal and overall capture rates of wild small mammals at each locality. The star symbol indicates the sites where rodents infected with *Schistosoma* spp. were found.

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Table 1. Infection prevalence of *Schistosoma mansoni* (*Sm*), *Schistosoma bovis* (*Sb*) and *Schistosoma haematobium*/*S. bovis* hybrid (*Sh/Sb*) in small mammals captured at 12 sites in the areas of Richard Toll and Lake Guiers, Senegal. For each trapping site we reported the capture rate (number of animals caught divided by the number of active traps) expressed as a percentage in parentheses. *Crocidura* sp. shrews and *Taterillus* sp. gerbils have been abbreviated as CRO and TAT, respectively.

Location (capture rate)	<i>Arvicanthis niloticus</i>			<i>Mastomys huberti</i>			CRO	TAT
	Juveniles (n=70)	Adults (n=145)	Total (n=215)	Juveniles (n=53)	Adults (n=119)	Total (n=172)	Total (n=27)	Total (n=6)
Richard Toll (RT)*								
Djidiery (31.6%)	17	29	46	0	2	2	2	3
		<i>Sb</i> 13.8%	<i>Sb</i> 8.7%					
Alarba (17.1%)	7	21	28	1	6	7	5	3
RT canal (14.4%)	25	48	73	0	2	2	5	0
		<i>Sm</i> 2.1%	<i>Sm</i> 1.4%					
		<i>Sb</i> 2.1%	<i>Sb</i> 1.4%					
Thiago (8.7%)	0	0	0	3	1	4	3	0
Richard Toll (RT)†								
Rosso (40.0%)	4	2	6	6	4	10	0	0
Djidiery (13.5%)	9	14	23	5	5	10	2	0
RT river (4.0%)	0	0	0	4	4	8	2	0

Lake Guiers†								
Mbane (23.7%)	0	3	3	3	39	42	7	0
Temey (22.9%)	0	1	1	6	15‡	21‡	0	0
					<i>Sm</i> 40.0%	<i>Sm</i> 28.6%		
					<i>Sh/Sb</i> 6.7%	<i>Sh/Sb</i> 4.8%		
Nder (13.9%)	1	4	5	21	31	52	0	0
					<i>Sm</i> 3.2%	<i>Sm</i> 1.9%		
Yetti-Yone (8.9%)	0	0	0	3	5	8	0	0

Lake Guiers*

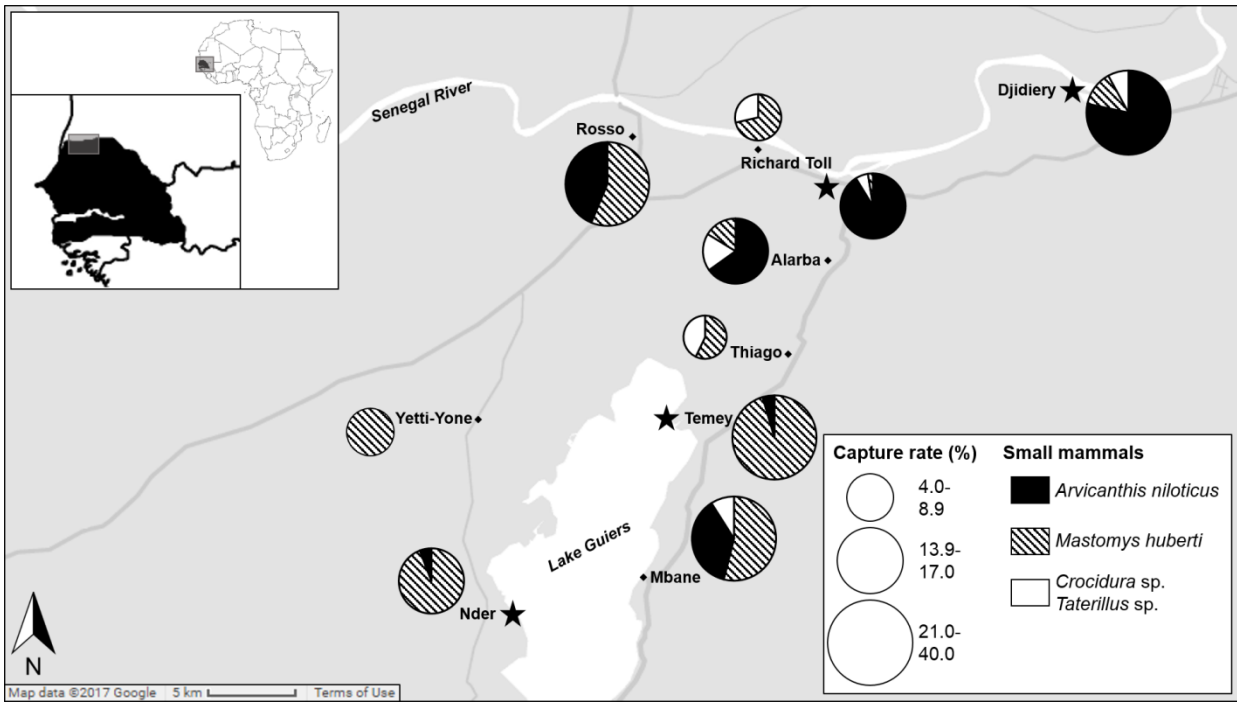
Mbane (30.6%)	7	23	30	1	5	6	1	0
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*Crop field as trapping habitat.

†Riparian vegetation as trapping habitat.

‡One host was infected with nine *Schistosoma* spp. pairs; eight pairs were composed by *S. mansoni* trematodes while a pair by male *S. mansoni* and female *S. haematobium/S. bovis* hybrid.

Figure 1.



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