The Journal of Infectious Diseases

BRIEF REPORT

Rodents as Natural Hosts of Zoonotic *Schistosoma* Species and Hybrids: An Epidemiological and Evolutionary Perspective From West Africa

Stefano Catalano,[1] Mariama Sène,[2] Nicolas D. Diouf,[2,3] Cheikh B. Fall,[4] Anna Borlase,[1] Elsa Léger,[1] Khalilou Bâ,[5] and Joanne P. Webster[1]

[1]Centre for Emerging, Endemic and Exotic Diseases, Department of Pathobiology and Population Sciences, The Royal Veterinary College, University of London, Hatfield, United Kingdom; [2]Unité de Formation et de Recherche des Sciences Agronomiques, d'Aquaculture et de Technologies Alimentaires, Université Gaston Berger, Saint-Louis, Senegal; [3]Institut Supérieur de Formation Agricole et Rurale, Université de Thiès, Bambey, Senegal; [4]Faculté de Médecine, de Pharmacie et d'Odontologie, Université Cheikh Anta Diop, Dakar, Senegal; [5]Centre de Biologie et de Gestion des Populations, Institut de Recherche pour le Développement, Campus ISRA-IRD Bel Air, Dakar, Senegal

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.

Keywords. Africa; One Health; *Schistosoma*; wildlife reservoir; zoonoses.

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, approximately 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 catalyzed [1].

Received 5 October 2017; editorial decision 26 December 2017; accepted 17 January 2018.
Correspondence: S. Catalano, MSC, Centre for Emerging, Endemic and Exotic Diseases (CEEED), Department of Pathobiology and Population Sciences, The Royal Veterinary College, University of London, Hatfield AL9 7TA, United Kingdom (scatalano@rvc.ac.uk).

The Journal of Infectious Diseases® 2018;XX00:1–5
© The Author(s) 2018. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com.
DOI: 10.1093/infdis/jiy029

Schistosoma haematobium, responsible for human urogenital schistosomiasis, and *Schistosoma mansoni*, an agent of human intestinal schistosomiasis, are both endemic across sub-Saharan Africa. Although *S. haematobium* is essentially specific to humans, *S. mansoni* can naturally infect rodents, nonhuman 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 agroindustrial growth as a consequence of the guaranteed fresh water supply [5]. Recently, although 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%) (our unpublished data).

Alterations of the natural landscape may have also favored the progressive loss of ecological barriers and the creation of permanent freshwater bodies (ie, 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 follows: (1) alternative definitive hosts and amplifiers of *S. haematobium* and *S. mansoni* transmission to humans; and (2) biotic hubs where

Downloaded from https://academic.oup.com/jid/advance-article-abstract/doi/10.1093/infdis/jiy029/4819098
by guest
on 02 March 2018

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 9 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. (our unpublished data), and we considered them as independent replicates given that the shortest distance between 2 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 × 10 × 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 (ie, 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 harboring 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].

Deoxyribonucleic acid (DNA) from individual adult schistosomes was extracted using the QIAGEN DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. Deoxyribonucleic acid from individual miracidia stored on Whatman FTA cards was extracted as described [10]. Deoxyribonucleic acid 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 χ^2 test, significant when $P \leq .05$. 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 2531 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 4-toed hedgehogs (*Atelerix albiventris*) and anuran amphibians. We detected *Schistosoma* spp. in the portal system and mesenteric vessels out of 7 of 172 *M. huberti* (prevalence 4.1%; intensity range 2–35; median intensity 4) and 6 out of 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 out of 21) and 1.9% (1 out of 52) in *M. huberti* from the villages of Temey and Nder, respectively, whereas prevalence was 8.7% (4 out of 46) and 2.7% (2 out of 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 an *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 analyzed specimens. In contrast, different haplotypes were found when aligning the COI sequences of *S. mansoni* (identity ≥98.2%) and *S. bovis* (identity ≥98.7%). Intraspecific nucleotide polymorphism translated in missense mutations in the amino acid sequence of COI's protein

Downloaded from https://academic.oup.com/jid/advance-article-abstract/doi/10.1093/infdis/jiy029/4819098
by guest
on 02 March 2018

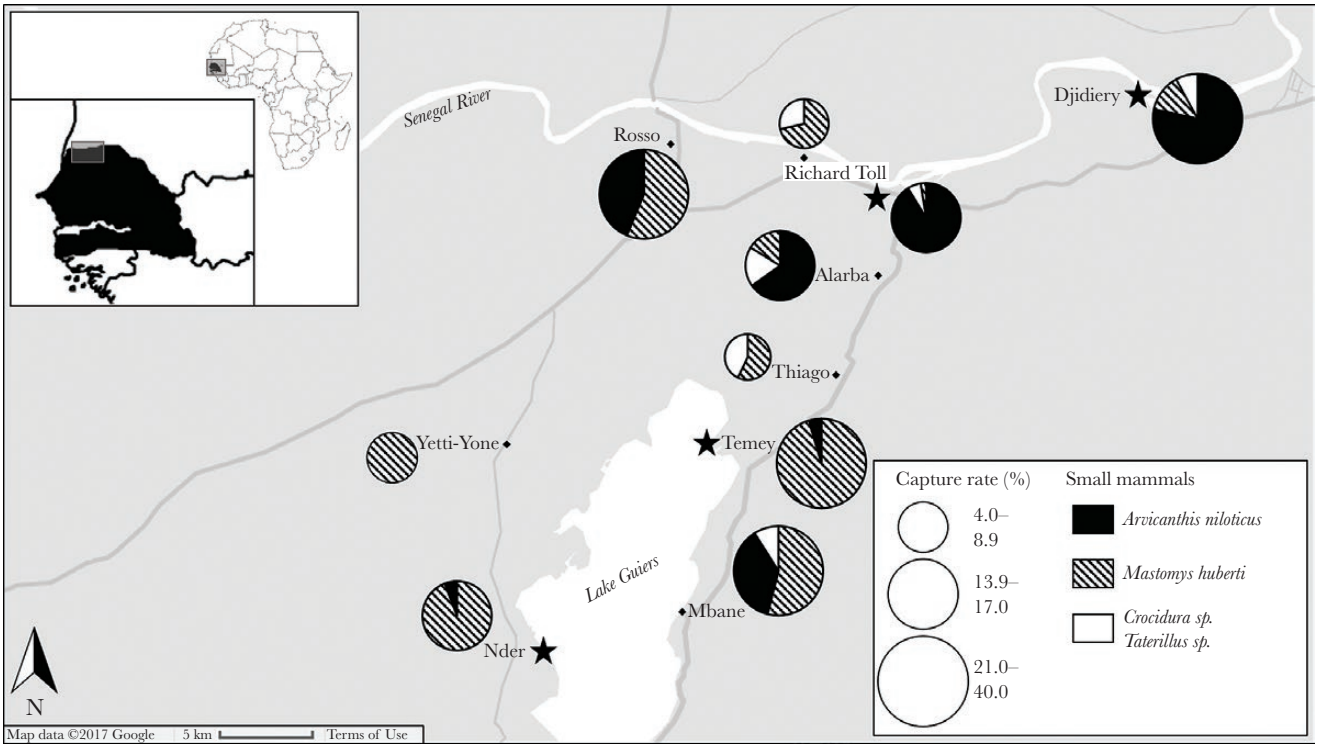

Figure 1. Geographic location of the study sites in northern Senegal and overall capture rates of wild small mammals at each locality. The star symbol indicates the sites where rodents infected with *Schistosoma* spp. were found.

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-MF919428 (COI).

The estimated *Schistosoma* spp. prevalence was significantly higher in adult rodents when compared with juveniles ($\chi^2 = 4.842$; d.f. = 1; $P = .028$) and in the village of Temey when compared with other study sites ($\chi^2 = 49.406$; d.f. = 11; $P < .001$). Schistosome prevalence did not significantly vary when tested against host species, gender, season, and habitat ($P > .05$).

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. It is interesting to note that 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]. Although 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 programs, 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*

Downloaded from https://academic.oup.com/jid/advance-article-abstract/doi/10.1093/infdis/jiy029/4819098
by guest
on 02 March 2018

Table 1. Infection Prevalence of *Schistosoma mansoni*, *Schistosoma bovis*, and *Schistosoma haematobium*/*S. bovis* hybrid in Small Mammals Captured at 12 Sites in the Areas of Richard Toll and Lake Guiers, Senegal

	Arvicanthis niloticus			*Mastomys huberti*			CRO	TAT
Location (Capture Rate)	**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
		Sb 13.8%	*Sb* 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
		Sm 2.1%	*Sm* 1.4%					
		Sb 2.1%	*Sb* 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
					Sm 40.0%	*Sm* 28.6%		
					Sh/Sb 6.7%	*Sh/Sb* 4.8%		
Nder (13.9%)	1	4	5	21	31	52	0	0
					Sm 3.2%	*Sm* 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

Abbreviations: CRO, *Crocidura* shrews; RT, Richard Toll; *Sb*, *Schistosoma bovis*; *Sh/Sb*, *Schistosoma haematobium*/*S. bovis*; *Sm*, *Schistosoma mansoni*; TAT, *Taterillus* gerbils.

Note: 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.

*Crop field as trapping habitat.

†Riparian vegetation as trapping habitat.

spp. in the Senegal River Basin may be 2-fold, 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.

CONCLUSIONS

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.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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

Downloaded from https://academic.oup.com/jid/advance-article-abstract/doi/10.1093/infdis/jiy029/4819098
by guest
on 02 March 2018

can compensate their sacrifice, we honor all the wild animals trapped during this study.

Financial support. This work was funded by the Royal Veterinary College and the Zoonoses and Emerging Livestock Systems 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. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Tchuem Tchuenté LA, Rollinson D, Stothard JR, Molyneux D. Moving from control to elimination of schistosomiasis in sub-Saharan Africa: time to change and adapt strategies. Infect Dis Poverty **2017**; 6:42.
2. Standley CJ, Dobson AP, Stothard JR. Out of animals and back again: schistosomiasis as a zoonosis in Africa. In: Rokni MB, ed. Schistosomiasis. InTech **2012**: 209–30.
3. Webster JP, Gower CM, Knowles SC, Molyneux DH, Fenton A. One health - an ecological and evolutionary framework for tackling Neglected Zoonotic Diseases. Evol Appl **2016**; 9:313–33.
4. Duplantier JM, Sène M. Rodents as reservoir hosts in the transmission of *Schistosoma mansoni* in Richard-Toll, Senegal, West Africa. J Helminthol **2000**; 74:129–35.
5. Van den Broeck F, Maes GE, Larmuseau MH, et al. Reconstructing colonization dynamics of the human parasite *schistosoma mansoni* following anthropogenic environmental changes in Northwest Senegal. PLoS Negl Trop Dis **2015**; 9:e0003998.
6. Léger E, Webster JP. Hybridizations within the genus *Schistosoma*: implications for evolution, epidemiology and control. Parasitology **2017**; 144:65–80.
7. Huyse T, Webster BL, Geldof S, et al. Bidirectional introgressive hybridization between a cattle and human schistosome species. PLoS Pathog **2009**; 5:e1000571.
8. Webster BL, Diaw OT, Seye MM, Webster JP, Rollinson D. Introgressive hybridization of *Schistosoma haematobium* group species in Senegal: species barrier break down between ruminant and human schistosomes. PLoS Negl Trop Dis **2013**; 7:e2110.
9. Granjon L, Duplantier JM. Les Rongeurs de l'Afrique Sahélo-Soudanienne. IRD éditions, Collection Faune et Flore Tropicales, **2009**: 215.
10. Gower CM, Shrivastava J, Lamberton PH, et al. Development and application of an ethically and epidemiologically advantageous assay for the multi-locus microsatellite analysis of *Schistosoma mansoni*. Parasitology **2007**; 134:523–36.
11. Liccioli S, Kutz SJ, Ruckstuhl KE, Massolo A. Spatial heterogeneity and temporal variations in *Echinococcus multilocularis* infections in wild hosts in a North American urban setting. Int J Parasitol **2014**; 44:457–65.
12. Hanelt B, Mwangi IN, Kinuthia JM, et al. Schistosomes of small mammals from the Lake Victoria Basin, Kenya: new species, familiar species, and implications for schistosomiasis control. Parasitology **2010**; 137:1109–18.
13. Rudge JW, Webster JP, Lu DB, Wang TP, Fang GR, Basáñez MG. Identifying host species driving transmission of *Schistosomiasis japonica*, a multihost parasite system, in China. Proc Natl Acad Sci U S A **2013**; 110:11457–62.
14. Riley S, Carabin H, Bélisle P, et al. Multi-host transmission dynamics of *Schistosoma japonicum* in Samar province, the Philippines. PLoS Med **2008**; 5:e18.
15. Han BA, Schmidt JP, Bowden SE, Drake JM. Rodent reservoirs of future zoonotic diseases. Proc Natl Acad Sci U S A **2015**; 112:7039–44.

Downloaded from https://academic.oup.com/jid/advance-article-abstract/doi/10.1093/infdis/jiy029/4819098
by guest
on 02 March 2018