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Host competence of the African rodents *Arvicanthis neumanni, A. niloticus* and *Mastomys natalensis* for *Leishmania donovani* from Ethiopia and *L.* (*Mundinia*) sp. from Ghana

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ABSTRACT

Visceral leishmaniasis caused by *Leishmania donovani* is regarded as mostly anthroponotic, but a role for animal reservoir hosts in transmission has been suggested in East Africa. Field studies in this region have shown the presence of this parasite in several mammalian species, including rodents of the genera *Arvicanthis* and *Mastomys*. Further, the natural reservoirs of *Leishmania* (*Mundinia)* sp. causing human cutaneous disease in Ghana, West Africa, are unknown. This study assessed the potential role of the Sub-Saharan rodents *Arvicanthis neumanni*, *A. niloticus* and *Mastomys natalensis* as hosts of *L. donovani* and *L. sp*. from Ghana, based on experimental infections of animals and xenodiagnoses. The distribution and load of parasites were determined *post mortem* using qPCR from the blood, skin and viscera samples. The attractiveness of *Arvicanthis* and *Mastomys* to *Phlebotomus orientalis* was tested by pair-wise comparisons. None of the animals inoculated with *L. donovani* were infectious to *P. orientalis* females, although, in some animals, parasites were detected by PCR even 30 weeks post infection. Skin infections were characterized by low numbers of parasites while high parasite burdens were present in spleen, liver and lymph nodes only. Therefore, wild *Arvicanthis* and *Mastomys* found infected with *L. donovani,* should be considered parasite sinks rather than parasite reservoirs. This is indirectly supported also by results of host choice experiments with *P. orientalis* in which females preferred humans over both *Arvicanthis* and *Mastomys*, and their feeding rates on rodents ranged from 1.4 to 5.8% only. Therefore, the involvement of these rodents in transmission of *L. donovani* by *P. orientalis* is very unlikely. Similarly, poor survival of *Leishmania* parasites in the studied rodents and negative results of xenodiagnostic experiments do not support the involvement of *Arvicanthis* and *Mastomys* spp. in the transmission cycle of *L. sp.* from Ghana.

1. Introduction

Leishmania (Kinetoplastida: Trypanosomatidae) are parasites alternating between blood feeding sand flies (Diptera: Psychodidae) and vertebrate hosts, including humans and various other mammals. Visceral leishmaniasis (VL) is a severe disease caused by parasites of the *L. donovani* complex; specifically by *L. donovani* in Asia and East Africa and by *L. infantum* in Asia, the Middle East, Europe and Latin America. VL caused by *L. infantum* is typically a zoonosis, where domestic dogs serve as principal reservoirs, although high prevalence of infection has been reported in some other mammalian species (reviewed by Quinnell and Courtenay, 2009). In contrast, VL caused by *L. donovani* is regarded as mostly anthroponotic; however, in East Africa a role for animal reservoir hosts has been suggested for many years and various findings suggest that reservoir animals may contribute to the transmission of *L. donovani* during initial outbreaks (reviewed by Ashford, 2000). In Sudan, high levels of seroprevalence and confirmed infections with *L. donovani* were found in dogs (Dereure et al., 2003), and serious risk of infection in some uninhabited areas indicates a strong probability that *L. donovani* can be zoonotic (Elnaiem et al., 1998). Indeed, previous field studies on wild mammals in East Africa have shown the presence of *L. donovani* in small carnivores (Elnaiem et al., 2001; Hoogstraal and Heyneman, 1969) and several rodent species: *Arvicanthis niloticus* (Hoogstraal and Heyneman, 1969; Elnaiem et al., 2001; El-Hassan et al., 1993; Chance et al., 1978), *Mastomys natalensis* (Elnaiem et al., 2001), *Acomys* sp. (Hoogstraal and Heyneman, 1969; Chance et al.,

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1978) and *Rattus rattus* (Hoogstraal and Heyneman, 1969; Chance et al., 1978). Recently, our team identified *L. donovani* in southern Ethiopia, using PCR and DNA sequencing of the ITS1 gene, in *Arvicanthis* sp., *Mastomys erythroleucus* and *Gerbilliscus nigricaudus* (Kassahun et al., 2015).

A *Leishmania* parasite causing human cutaneous disease in Ghana was identified as a member of the *L. enriettii* species complex in 2015 (Kwakye-Nuako et al., 2015). The species complex was classified as a new subgenus *Mundinia* one year later (Espinosa et al., 2016). The five known members of this subgenus (*L. enriettii*, *L. macropodum*, *L. martiniquensis*, *L. orientalis* and *L.* sp. originating from Ghana) are geographically widely dispersed and vary substantially in their potential to cause human disease. Although medically important, the biology of these *Leishmania* species is poorly understood and information on their natural reservoir hosts as well as vector species is scarce. Identification of reservoir hosts and vector species of Ghanaian *Leishmania* species is, therefore, a significant research challenge.

Dense populations of the rodents of the genera *Arvicanthis* and *Mastomys* live in close vicinity of humans in Sub-Saharan Africa. We recently studied the susceptibility of *Arvicanthis neumanni* (Neumann's Grass Rat), *A. niloticus* (Nile Grass Rat) and *Mastomys natalensis* (Natal Multimammate Mouse) to *Leishmania major* and demonstrated that the latter may serve as a reservoir host for this parasite (Sadlova et al., 2019). This study is a follow-up analysis of the host competence of these three rodent species for *L. donovani* and *L.* (*M.*) sp. from Ghana. *Arvicanthis niloticus* and *M. natalensis* are widespread in almost all Sub-Saharan Africa (Granjon and Ducroz, 2013; Leirs, 2013) while *Arvicanthis neumanni* with a range restricted to East Africa (absent in Ghana) was tested only for *L. donovani*. The response of these rodents to infection and their ability to infect sand flies were evaluated by experimental infections and xenodiagnosis. In addition, feeding rates of *P. orientalis* on these rodents were tested by host-choice experiments.

2. Materials and methods

2.1. Sand flies, parasites and rodents

The colonies of *P. orientalis* (originating from Ethiopia) and *P. duboscqi* (originating from Senegal) were maintained in the insectaries of the Department of Parasitology, Charles University in Prague, under standard conditions as described previously (Volf and Volfova, 2011). Two Sub-Saharan *Leishmania* strains were used: *L. donovani* strain MHOM/ET/2010/GR374 (a human isolate from Ethiopia) and *L.* (*M.*) sp. strain MHOM/GH/2012/GH5; LV757 (a human isolate from Ghana; Kwakye-Nuako et al., 2015). Promastigotes were cultured in M199 medium (Sigma) containing 10% heat-inactivated fetal bovine serum (FBS, Gibco) supplemented with 1% BME (Basal Medium Eagle, Sigma) vitamins, 2% sterile human urine and 250 μg/ml amikacin (Amikin, Bristol-Myers Squibb). To obtain higher densities of *L. donovani* metacyclic forms in the stationary-phase culture for rodent infections, a mixture of salts (sodium urate 10 mg/ml, uric acid 10 mg/ml and cysteic acid 50 mg/ml, all Sigma) was added to the medium according to Howard et al. (1987). Breeding colonies of *A. neumanni*, *A. niloticus* and *M. natalensis* were established and maintained at the animal facility of the Department of Parasitology as described previously (Sadlova et al., 2019).

2.2. Rodent infections and xenodiagnosis

Two methods were used for infecting rodents with *L. donovani* – infections initiated with sand fly-derived *Leishmania* according to Sadlova et al. (2015) and infections initiated with culture-derived promastigotes. For the first method, *P. orientalis* females, experimentally infected with *L. donovani* as described previously (Sadlova et al., 2017), were dissected on day 8 post bloodmeal (PBM), when mature infections with accumulation of metacyclic forms in the thoracic midguts (TMG) were observed. Pools of 100 freshly dissected TMG with a good density of parasites were homogenized in 50 μl of saline.

The natural vector of *L.* sp. from Ghana is not known; therefore, only culture-derived promastigotes were used for rodent infections with this *Leishmania* species. For this purpose, stationary-phase promastigotes (day 7 post inoculation) were washed twice in saline and counted using a Burker apparatus. Pools of 10^8 promastigotes were resuspended in 50 μl of saline.

Dissected salivary glands (SG) of *P. orientalis* (for *L. donovani* infections) and *P. duboscqi* females (for infections with *L.* sp. from Ghana) were pooled in sterile saline (10 glands per 10 μl of saline) and stored at −20 °C. Prior to mice inoculation, SG were disintegrated by 3 successive immersions into liquid nitrogen and added to both types (sand fly - and culture-derived) of promastigote suspensions.

Rodents were infected as described previously (Sadlova et al., 2019). Briefly, 5.5 μl of the suspension of parasites and SG were injected intradermally into the ear pinnae. The inoculum of culture-derived promastigotes comprised 10^7 parasites with 73% of metacyclic forms in *L. donovani* and 33–42% of metacyclic forms in *L.* sp. from Ghana. The inoculum of sand fly-derived *L. donovani* was 3–6 × 10^4 with 65–90% of metacyclic forms; the proportions of metacyclic forms were identified on Giemsa stained smears based on morphological criteria described previously (Sadlova et al., 2010). Animals were checked weekly for external signs of the disease until week 20–30 post infection (p.i.) when they were sacrificed.

Five to seven-day-old *P. orientalis* females (natural vectors of *L. donovani*) were allowed to feed on the site of *L. donovani* inoculation (ear pinnae) of anaesthetized rodents at 5 weeks-intervals as described previously for *L. major* infections (Sadlova et al., 2019). Fed sand fly females were separated and maintained at 26 °C on 50% sucrose until day 10–12 PBM, when females were dissected and their guts examined under the light microscope for presence and quality of *Leishmania* infections. As natural vectors for xenodiagnoses of *L.* sp. from Ghana are not available, animals infected with this *Leishmania* species were exposed to the unnatural vector *P. duboscqi* which supports the infection only until defecation of blood remnants on day 4–5 PBM (JS and TB, unpublished data). Therefore, parasite presence was determined on day 3 PBM, allowing multiplication of parasites before loss of infections, using PCR in pools of 5 whole female bodies.

2.3. Tissue sampling and quantitative PCR

Rodents were euthanized by cervical dislocation under anesthesia. Samples from both ears (inoculated and contralateral), both ear-draining lymph nodes, spleen, liver, paws and tail were stored at −20 °C for qPCR. Extraction of total DNA from rodent tissues (on equal weight samples) and sand flies was performed using a DNA tissue isolation kit (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions. Quantitative PCR (Q-PCR) for detection and quantification of *Leishmania* parasites was performed in a Bio-Rad iCycler & iQ Real-Time PCR Systems using the SYBR Green detection method (SsoAdvanced™ Universal SYBR®, Bio-Rad, Hercules, CA). Infectious loads in rodent tissues were scored using a scoring table considering the number of parasites detected: < 1000 was evaluated as low parasite loads; 1000–10000 as medium parasite loads; > 10000 as high parasite loads.

2.4. Host choice experiments and assessment of mortality and fecundity of sand flies fed on different hosts

Pair-wise comparisons between two types of hosts were performed using the same equipment as described previously (Sadlova et al., 2019). Briefly, 200 *P. orientalis* females were placed into the central cage from a row of three connected small cages and left for habituation for 20 min. Then, anaesthetized animals were placed or the forearm of a human volunteer positioned in each of the lateral cages for 1 h, after which sand flies were collected. Hosts alternated between lateral cages in each repeat and each pair of hosts was tested four times. Experiments were conducted in darkness at 24–26 °C. Fed females were maintained under the same conditions as the colony and their mortality was recorded for 4 days post-feeding. Then, females were introduced individually into small glass vials lined with wet filter papers and covered with fine mesh. A small ball of cotton wool soaked with 50% sugar solution was placed on the mesh. The numbers of eggs oviposited were compared to determine the effect of the host blood source host on *P. orientalis* fecundity (Killick-Kendrick and Killick-Kendrick, 1991).

2.5. Statistical analysis

Statistical analyses were carried out using R software (http://cran.r-project.org/). The differences in feeding preferences, mortality and fecundity of *P. orientalis* females fed on different host species were analysed by the Chi-square test. The differences in numbers of eggs laid by females fed on different hosts were tested by the nonparametric Mann Whitney U test. The relationships between weight and groups (infected and non-infected animals) and time were tested by fitting multilevel linear regression models (package "nlme"), taking into account the correlation between repeated measures of the same animal over time. The model used included interaction term between groups (categorical variable) and time (continuous independent variable).

2.6. Animal experimentation guidelines

Animals were maintained and handled in the animal facility of Charles University in Prague in accordance with institutional guidelines and Czech legislation (Act No. 246/1992 and 359/2012 coll. on Protection of Animals against Cruelty in present statutes at large), which complies with all relevant European Union and international guidelines for experimental animals. All experiments were approved by the Committee on the Ethics of Laboratory Experiments of the Charles University in Prague and were performed under permit no. MSMT-10270/2015–5 of the Ministry of the Education, Youth and Sports of the Czech Republic. Investigators were certified for experimentation with animals by the Ministry of Agriculture of the Czech Republic.

3. Results

3.1. Experimental infections with L. donovani

In total, 8 *M. natalensis*, 7 *A. neumanni* and 10 *A. niloticus* were infected by *L. donovani;* all *M. natalensis*, all *A. neumanni* and five *A. niloticus* with sand fly-derived *Leishmania*, while five *A. niloticus* were infected with culture-derived *Leishmania.* Two animals of each species served as controls inoculated with sterile saline.

None of the inoculated animals developed lesions or other external signs of the disease throughout the entire experiment. The weight of animals did not differ significantly between infected animals and uninfected controls (P = 0.126, P = 0.446 and P = 0.382 in *A. niloticus*, *A. neumanni* and *M. natalensis*, respectively) (S1 Table). All 100, 194 and 251 *P. orientalis* females used at different times p.i. for xenodiagnoses on *M. natalensis*, *A. neumanni* and *A. niloticus*, respectively, were *Leishmania*-negative.

Nevertheless, PCR performed at the end of the experiment, on week 30 p.i., revealed presence of *L. donovani* in different tissues and organs of infected rodents; the quantities of parasites were subsequently determined using qPCR. High parasite loads were found in the lymph node draining the inoculated ear of one *A. neumanni* and low parasite loads in forepaws and the inoculated ear of three *A. niloticus* (Table 1). The parasite loads in *M. natalensis* were the highest among tested rodent species. Half of the animals maintained *L. donovani* until week 30 p.i., with parasites localized mostly in liver, spleen and paws. An inoculated ear was positive in one specimen and draining lymph nodes in two animals. High parasite burdens were detected only in the spleen and liver. No parasites were detected in the right ear, tail or blood (Table 1).

3.2. Experimental infections with L. sp. from Ghana

Six *A. niloticus* (3 males and 3 females) and six female *M. natalensis* were inoculated with culture-derived promastigotes, but one *M. natalensis* died before the end of the experiment. Six animals of each species were used as controls inoculated with sterile saline. No external signs of infection were apparent on infected animals throughout the experiment. The weight of infected *A. niloticus* did not differ significantly from control animals (P = 0.98) but in *M. natalensis*, infected animals gained significantly less weight than controls (P = 0.01) (S2 Table).

Xenodiagnoses with *P. duboscqi* did not reveal *Leishmania* presence in sand flies tested by PCR – all 95 and 87 pools of sand flies used for xenodiagnoses on inoculated ears of *A. niloticus* and *M. natalensis,* respectively, on different week p.i., were negative. By the end of the experiment, on week 25 p.i., PCR failed to detect *Leishmania* DNA in any *A. niloticus*. In *M. natalensis*, the presence of *Leishmania* was confirmed in a single animal on week 20 p.i., with localization in the inoculated ear and its draining lymph nodes, the contralateral ear and forepaws. Based on qPCR, all these tissues possessed only low parasite loads.

3.3. Host choice experiments with P. orientalis

Two potential rodent hosts were offered to *P. orientalis* females in each pair-wise comparison, and each host combination was tested four times with hosts alternating between lateral cages. *Phlebotomus orientalis* females showed very low feeding rate on all tested rodents: 1.4–5.8% of females took bloodmeals during experiments (Table 2). No difference was observed in attractiveness of *A. niloticus* (the bigger species) and *A. neumanni* (the smaller species), or between *Mastomys* and *Arvicanthis (*represented by *A. neumanni)*. On the other hand, significant preferences were observed when human forearm was offered as an alternative to rodents: sand flies preferred human forearms to both *Arvicanthis* and *Mastomys* (Table 2). Engorged females that took blood meals on different hosts were further followed for comparison of mortality and fecundity. Mortality (until day 4 PBM) ranged between 15.2% and 54.5%, but was not significantly influenced by host types (Table 2). Four days PBM, females were allowed to oviposit in small glass vials where they were kept individually. Blood source did not influence significantly either the proportion of females laying eggs or the numbers of eggs laid by individual females. Median numbers of eggs per female were 36–53, depending on the experiment (Table 2).

4. Discussion

Zoonotic transmission of *L. donovani* has long been suggested in

Table 1
Presence, amount and location of *L. donovani* DNA determined by qPCR in various tissue of individual animals. IE, inoculated ear; CE, contralateral ear; DN-IE, draining lymph nodes of the inoculated ear; DN-CE, draining lymph nodes of the contralateral ear; FP, forepaws; HP, hindpaws; T, tail; L, liver; S, spleen; B, blood; -, negative results, +, < 1000 parasites; + +, 1000–10000 parasites; + + +, > 10000 parasites.

Rodent species	Individual marks of animals	IE	CE	DN-IE	DN-CE	FP	HP	T	L	S	B
A. niloticus	A1	–	–	–	–	–	–	–	–	–	–
	A2	–	–	–	–	+	–	–	–	–	–
	A3	–	–	–	–	–	–	–	–	–	–
	A4	–	–	–	–	–	–	–	–	–	–
	A5	–	–	–	–	–	–	–	–	–	–
	A6	–	–	–	–	–	–	–	–	–	–
	A7	+	–	–	–	+	–	–	–	–	–
	A8	–	–	–	–	+	–	–	–	–	–
	A9	–	–	–	–	–	–	–	–	–	–
	A10	–	–	–	–	–	–	–	–	–	–
M. natalensis	M1	–	–	–	–	–	–	–	–	–	–
	M2	–	–	–	–	+	+ +	–	+ +	+ + +	–
	M3	–	–	+ +	–	+	+	–	+ +	+ + +	–
	M4	–	–	–	–	–	–	–	–	–	–
	M5	–	–	+ +	+	+	–	–	+ + +	+ + +	–
	M6	+	–	–	–	–	–	–	–	–	–
	M7	–	–	–	–	–	–	–	–	–	–
	M8	–	–	–	–	–	–	–	–	–	–

Sudan, based on repeated outbreaks of the disease in people who had visited uninhabited areas and findings of the parasite in wild animals. However, despite a considerable effort over many years no reservoir host has been proven (reviewed by Ashford, 2000; Ashford, 1996). Here we undertook testing of the reservoir role of rodents in the genera *Arvicanthis* and *Mastomys*, as *L. donovani* parasites were repeatedly found in these rodent species during several field studies performed in East Africa (El-Hassan et al., 1993; Elnaiem et al., 2001), since studies in the 1960s when the highest prevalence was found in *A. niloticus* in the southern part of Sudan (Hoogstraal and Heyneman, 1969). Recently, the presence of *L. donovani* was reported in *Arvicanthis* and *Mastomys* in south-western Ethiopia (Kassahun et al., 2015).

The results of our laboratory study are in accordance with the above cited field studies, since we confirmed that *L. donovani* parasites survive in some individuals of *Arvicanthis* and *Mastomys* for several months. Parasites were localized not only in the viscera, but in some animals also in external organs - ears and paws, therefore, theoretically available for transmission by biting sand flies. However, all xenodiagnoses performed on ears of animals were negative. This is in contrast with results of our previous study performed with the same Ethiopian *L. donovani* strain on BALB/c mice (Sadlova et al., 2015). Mice were infected using the same method – intradermal inoculation of sand fly derived parasites in the ear pinnae. Mice did not show any signs of disease in our previous study, but 19% of the *P. orientalis* females that fed at the site of inoculation, became infected (Sadlova et al., 2015).

Parasites of the *Mundinia* subgenus from Ghana still lack their scientific name and there is no current information on their natural reservoir hosts and vector species. Our experimental infections of *A. niloticus* and *M. natalensis*, rodents present in endemic localities, did not confirm their host competence for this parasite. *Leishmania* were mostly completely lost and survived only in a single *M. natalensis* till week 20 p.i. In this animal, *Leishmania* disseminated to different tissues (draining lymph nodes of the inoculated ear, the contralateral ear and forepaws), but were present only in low parasite loads.

Generally, a failure of infectivity to sand flies can be explained by absence or low numbers of parasites in peripheral blood or in the skin. In dogs infected with *L. infantum*, high parasite numbers in skin (and blood) have been shown to be the best markers of infectiousness to sand fly vectors (Courtenay et al., 2014; Borja et al., 2016). In our study, high parasite loads were present only in the spleen and liver of *M. natalensis* and lymph nodes of *A. neumanni* infected with *L. donovani,* these being visceral organs where the parasites were not accessible to sand fly borne transmission. Skin samples derived from whole ears and paws revealed 10–700 amastigotes, compared with 14–80 thousand present in lymph nodes and viscera. A similar phenomenon was observed in the study of host competence of *Mastomys* and *Arvicanthis* for *L. major* – the *L. major* strains which produced poor skin infections in animals were not infectious to sand flies (Sadlova et al., 2019).

The main vector of *L. donovani* in Sudan and Ethiopia is *Phlebotomus* (*Larroussius*) *orientalis* (Ashford, 2000). Host choice experiments reported here showed that this sand fly species was not strongly attracted to rodents. Females clearly preferred humans as a source of blood meals. Preference of *P. orientalis* for humans and large domestic animals was also observed during field studies with animal baited traps and blood meal analyses in Ethiopia (Gebresilassie et al., 2015a, 2015b; Yared et al., 2019). Similarly, the very low engorgement rates of *P. orientalis* on rodents in our experiments (1.4–5.8%) are in accordance with the results of the study of Gebresilassie et al. (2015b), where only 1.08% of females took blood meals on small wild animals compared to 30.53% feeding on larger domestic animals. The likelihood of *L. donovani* being maintained in rodents in areas where *P. orientalis* acts as the main vector is, therefore, very low.

Taken together, the results of this study suggest that rodents infected with *L. donovani* in East Africa most probably represent parasite sinks on which the infected sand fly occasionally feed but which do not contribute to vector infection and transmission to the next host (Chaves et al., 2007). True animal reservoirs of *L. donovani* in East Africa must be confirmed with further studies, and good candidates may be small carnivores like the Egyptian mongooses, 14% of which were found infected in Dinder National Park (Elnaiem et al., 2001), or Senegal genet (*Genetta G. senegalensis*), Sudanese serval (*Felis serval phillipsi*) or White-tailed mongooses (*Ichneumia albicauda*) reported to be infected with *L. donovani* in southern Sudan and Ethiopia, respectively (Hoogstraal and Heyneman, 1969; Kassahun et al., 2015). For the *Mundinia* parasites causing cutaneous leishmaniasis in Ghana we do not have any indication from the field for identification of the reservoir host; therefore, any such findings would be highly valuable to enable further experimental research.

Table 2
Feeding preferences, mortality and fecundity of *P. orientalis* females fed on different host species. The between-species differences were tested by the Chi-squared test. The differences in numbers of eggs laid were tested by the nonparametric Mann Whitney U test.

Host combination	Host	N (%) of fed sand flies	Significance of between-species differences	Mortality post feeding: N dying/N (%)	Significance of between-species differences	Fecundity N eggs-lying/N (%)	Significance of between-species differences	Number of eggs Median (Min, Max)	Significance of between-species differences
A. neumanni vs. *A. niloticus*	*A. neumanni*	22 (1.4%)	$\chi2 = 1.231$	12/22 (54.5%)	$\chi2 = 0.746$	10/10 (100%)	$\chi2 = 0.405$	36 (5, 60)	P = 1.000
	A. niloticus	30 (2.0%)	P = 0.267	15/30 (50.0%)	P = 0.483	14/15 (93.3%)	P = 0.600	38 (2, 70)	
A. neumanni vs. *M. natalensis*	*A. neumanni*	46 (2.3%)	$\chi2 = 0.095$	7/46 (15.2%)	$\chi2 = 0.259$	37/39 (94.9%)	$\chi2 = 0.115$	49 (1, 67)	P = 0.966
	M. natalensis	49 (2.4%)	P = 0.758	12/49 (24.5%)	P = 0.192	31/37 (83.8%)	P = 0.115	48 (1, 75)	
A. neumanni vs. man	*A. neumanni*	17 (3,4%)	$\chi2 = 14.0625$	7/17 (41.2%)	$\chi2 = 0.922$	10/10 (100%)	$\chi2 = 0.376$	50 (26, 64)	P = 0.552
	man	47 (9.4%)	P = 0.0002	20/47 (42.6%)	P = 0.577	25/27 (92.3%)	P = 0.527	53 (2, 78)	
M. natalensis vs. man	*M. natalensis*	29 (5.8)	$\chi2 = 5.582$	7/29 (24.1%)	$\chi2 = 0.989$	19/22 (86.4%)	$\chi2 = 0.718$	52 (15, 66)	P = 0.918
	man	50 (10.0)	P = 0.0182	12/50 (24.0%)	P = 0.597	34/38 (89.5%)	P = 0.510	51 (5, 76)	

In conclusion, the results of this laboratory study do not support the involvement of *Arvicanthis* and *Mastomys* spp. in the transmission cycle of *L. donovani* in East Africa nor *L. sp.* in Ghana. In contrast, these rodent species most probably do comprise important reservoir hosts of *L. major* in this region (Sadlova et al., 2019).

Declaration of competing interest

None.

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Appendix A. Supplementary data

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