Original Study

Claus Thomas*, Venance Msoffe, Natalie Van Houtte, Ginethon Mhamphi, Joachim Mariën, Christopher Sabuni, Isaac Makundi, Jahashi Nzalawahe, Robert Machang'u and Herwig Leirs

Molecular detection of *Leptospira* and *Bartonella* in *Mastomys natalensis* and its ectoparasites in Morogoro, Tanzania

https://doi.org/10.1515/mammalia-2023-0031
Received March 7, 2023; accepted June 27, 2023;
published online July 25, 2023

Abstract: Rodents play an important role in the transmission of zoonotic diseases. This study investigated the prevalence of *Leptospira* spp. and *Bartonella* spp. in *Mastomys natalensis* and its ectoparasites (fleas and mites) in selected villages of Morogoro, Tanzania. *Mastomys natalensis* were captured live in fallow habitats using Sherman® traps and anesthetized using Halothane. Blood samples were obtained from the retroorbital sinus Ectoparasites were removed from the fur using a hard brush and preserved in 70 % ethanol. Real time–qPCR was used to detect Leptospira spp. and *Bartonella* spp. from *Mastomys natalensis* blood and ectoparasites respectively. The study revealed a relatively larger number of males than females captures. *Leptospira* spp. was demonstrated in one out of 100 *Mastomys natalensis*. For *Bartonella* spp., prevalence of (14 %) was recorded in mites with a higher proportion in mites from adult male *Mastomys natalensis* than females. Upon Sanger sequencing, four positive samples showed a complete sequence of the *ITS* gene. Indicating that all samples belonged to *Uncultured Bartonella*. Low prevalence of *Leptospira* spp. and a high prevalence of *Bartonella* spp. was observed in *Mastomys natalensis*. Further exploration of rodent pathogens is recommended to raise awareness of the role of commensal rodents in disease transmission via their ectoparasites.

Keywords: *Bartonella* spp.; ectoparasites; *Leptospira* spp.; *Mastomys natalensis*; mites

1 Introduction

Rodents are mammals with broad and complex ecological range; they are cosmopolitan except in Antarctica (Makundi et al. 2003). Given their high prolificacy, rodents have successfully colonized a wide variety of habitats and environments throughout the world (Cortez et al. 2018; Dahmana et al. 2020; Holt et al. 2006; Meerburg et al. 2009). *Mastomys natalensis* is an abundant and dominant species within the small mammal community of Morogoro, Tanzania, and it is known to carry a number of infectious agents of diverse diseases, including: Bartonellosis, leptospirosis, rickettsioses, lyme borreliosis (Katakweba et al. 2012; Machang'u et al. 2004; Mariën et al. 2022).

Rodent borne pathogens may enter a human host through diverse routes including ectoparasite bites, direct contact with rodent excreta or consumption of food and water contaminated with fecal materials of the small mammals (Boey et al. 2019; Machang'u et al. 2004).

Leptospirosis is a rodent borne zoonosis of worldwide distribution (Machang'u et al. 2004). The disease is caused by a spirochete of the genus *Leptospira,* which consists of 22 species (Boey et al. 2019). According to WHO at least

***Corresponding author: Claus Thomas**, Department of Wildlife Management, Sokoine University of Agriculture, Morogoro, Tanzania; Department of Microbiology, Parasitology and Biotechnology, Sokoine University of Agriculture, Morogoro, Tanzania; and Department of Microbiology and Parasitology, St. Francis University College of Health and Allied Sciences, Ifakara, Tanzania, E-mail: clausthomas2017@gmail.com. https://orcid.org/0000-0002-6916-5087
Venance Msoffe, Department of Wildlife Management, Sokoine University of Agriculture, Morogoro, Tanzania; and Mkwawa University College of Education, Iringa, Tanzania
Natalie Van Houtte, Joachim Mariën and Herwig Leirs, Evolutionary Ecology Group, Department of Biology, University of Antwerp, Antwerp, Belgium
Ginethon Mhamphi, Department of Wildlife Management, Sokoine University of Agriculture, Morogoro, Tanzania. https://orcid.org/0000-0002-8354-2301
Christopher Sabuni, Institute of Pest Management, Sokoine University of Agriculture, Morogoro, Tanzania
Isaac Makundi and Jahashi Nzalawahe, Department of Microbiology, Parasitology and Biotechnology, Sokoine University of Agriculture, Morogoro, Tanzania
Robert Machang'u, Department of Microbiology and Parasitology, St. Francis University College of Health and Allied Sciences, Ifakara, Tanzania

1.03 million cases of leptospirosis occurred annually, worldwide, with 58,900 deaths in the past decade. In the East and Central African region, leptospirosis is known to be prevalent for more than three decades (Machang'u et al. 2004). Tanzania is among the tropical countries of Sub-Saharan Africa where leptospirosis is endemic (Mgode et al. 2021).

Any mammal can be infected with one or more *Leptospira* serovars, however, rodents are the most commonly affected animals which are also the major natural reservoirs of this microorganism (Dehio 2001; Mgode et al. 2021). In animals, including humans, leptospirosis present with varieties of clinical manifestations, from mild, flue-like symptoms to severe, febrile conditions associated with, inappetance, stiffness, abdominal pain and occasionally may cause fatal septicaemic complications (Desai et al. 2009).

Bacteria of the genus *Bartonella* are small, fastidious, slow-growing gram-negative aerobic rods (Dehio 2001). To date they are 45 described species and subspecies of *Bartonella* of which 20 are found in rodents (Gutiérrez et al. 2015; Malania et al. 2016; Theonest et al. 2019).

It is reported that, rodent associated *Bartonella* are the cause of human infections particularly in areas where humans have close contact with rodents. Relatively recently, *Bartonella* spp. have been reported to cause febrile illnesses in humans in northern Tanzania (Theonest et al. 2019).

The *Bartonella* spp. cause a disease with a wide range of clinical manifestations in humans and animals, for example: Cat-scratch fever, trench fever, Carrion's disease, bacteremia with fever, bacillary angiomatosis, peliosis, endocarditis and neuroretinitis (Broecke 2021; Malania et al. 2016). *Bartonella* spp. cause damage of the endothelial cells and erythrocytes of their mammalian hosts (Malania et al. 2016).

Studies on *Leptospira* spp. and *Bartonella* spp. transmission through rodents and their ectoparasites have shown that rodent urine and rodent ectoparasites are major drivers in the transmission of leptospirosis and bartonellosis respectively (Boey et al. 2019; Dehio 2001; Mariën et al. 2022). However, studies on the genotypes of these pathogens, prevalence of the diseases caused and their modes of transmission in rodents, specifically *M. natalensis* and their ectoparasites in Morogoro needs further exploration. This study, therefore, aimed at determining the prevalence, genotypes and modes of transmission of *Leptospira* spp. and *Bartonella* spp. pathogens in Morogoro. The findings of this study will contribute to the design of effective control strategies of these diseases and their carriers.

2 Materials and methods

2.1 Study sites

This study was carried out in fallow lands at Choza and Kiroka villages, within Morogoro municipality (6°50′34.9794″S; 37°38′8.232″E) between January and March 2021. This period coincides with the breeding season of *M. natalensis* in the study areas (Broecke 2021). The study area experience a bimodal rainfall pattern characterized by short rains from November to January and long rains from March to May each year. During the period of January to March, the land is commonly dry, covered with short grasses and scattered bushes. The dry season lasts for six (6) months from May to November (Rija et al. 2014; Van Aelst and Holvoet 2018) (Figure 1).

2.2 Rodent trapping and sample collection

During the study, *M. natalensis* (n = 50) were live captured from each of the villages, using Sherman® traps (standard medium size LFA: 7.6 × 8.9 × 23 cm). Sample size was determined by considering time of the study, budget and animal ethics as described by Aplin (2003). The trapping was conducted for three consecutive nights and inspected every morning for captures over the period of 3 months (Table 2). In each study village, five transect lines were set in fallow land, each laid with 30 Sherman® traps. A 10 m space was set from line to line and from trap to trap. A mixture of peanut butter and maize bran was used as the bait. Captured *M. natalensis* were anaesthetized using halothane and blood samples were drawn from the retro-orbital sinus using capillary tubes. Collected blood was preserved in EDTA vacutainer tubes and a drop of the blood from each rodent was absorbed on a filter paper (Claus et al. 2020).

Fleas and mites were removed from the fur of the anaesthetized animals using a hard brush then collected in a clean dish and covered with a white paper, as described by Claus et al. (2020). After sample collection, the rodents were humanely killed using an overdose of halothane (Mariën et al. 2022).

Mites (n = 250) and fleas (n = 80) obtained were preserved in Eppendorf tubes containing 70 % ethanol before being transported to the Institute of Pest Management (IPMC) in Morogoro. The ectoparasites were morphologically identified using a bright–field digital microscope (Zeiss Primor Star Axiocam ERaC5C) at 0.5 objective lens and a pictorial identification guide as described in Angelakis and Raoult (2014), Desai et al. (2009), and Kim et al. (2005). The fleas and mites, identified as *Xenopsylla cheopis and Laelaptine* spp. respectively were sorted and pooled separately according to their host and then crushed in a sterile mortar. Before DNA extraction (Van Houtte et al. 2014), the *X. cheopis* pulp were divided into 20 aliquots, while *Laelaptine*s spp. were aliquoted into 80 pools.

2.3 DNA extraction

Genomic DNA of the *M. natalensis* blood, *X. cheopis* and *Laelaptine*s spp. were extracted using the NucleoSpin® Tissue (MACHEREY-NAGEL GmbH & Co. KG, Germany), following the manufacturer's instructions. The DNA extracts were then stored at −20 °C.

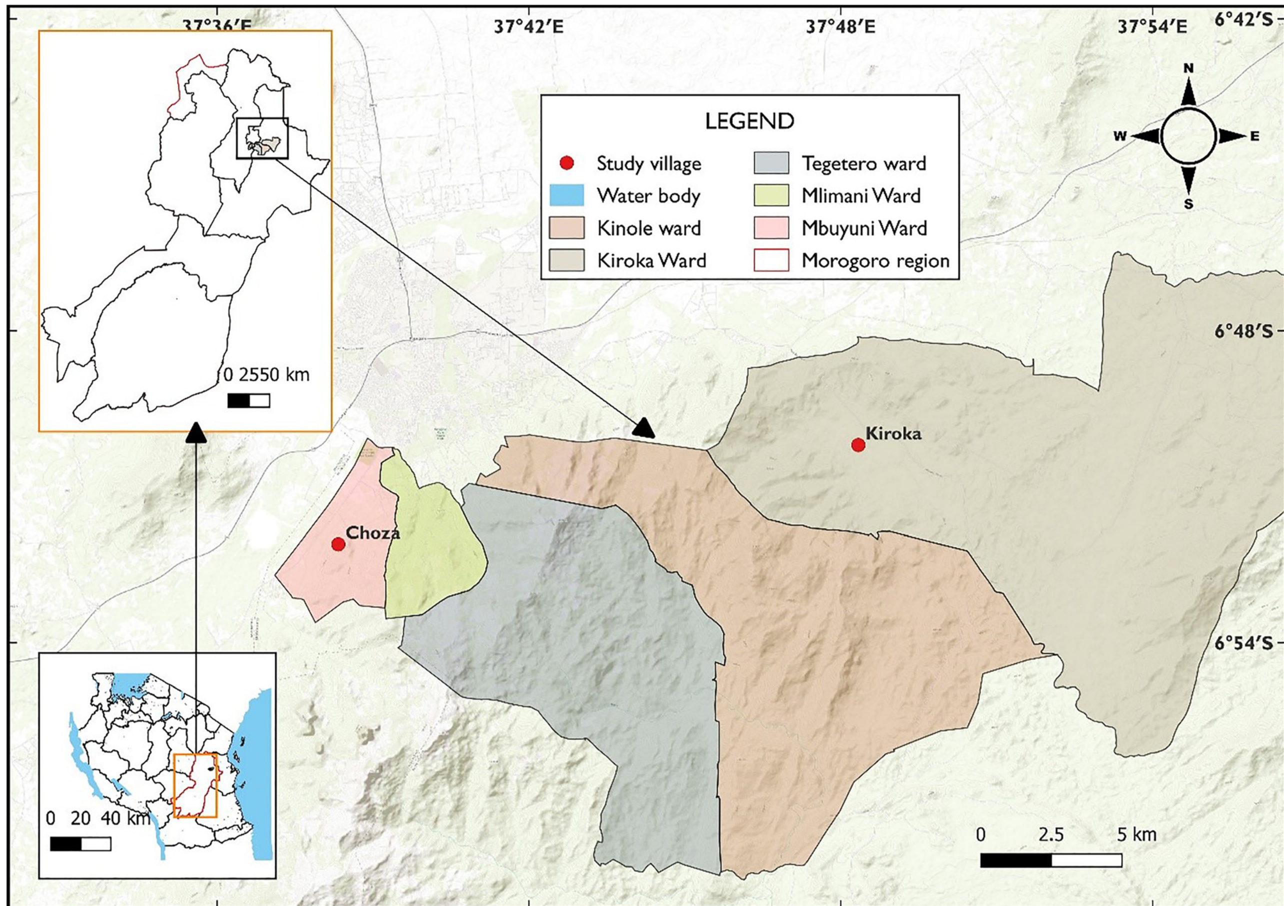

Figure 1: Map of selected study villages of Morogoro.

2.4 Molecular screening of *Bartonella* spp.

DNA extracts were screened for *Bartonella* spp. by real time-qPCR using the following pairs of primers and probes (Meerburg et al. 2009; Van Houtte et al. 2014). Barto_ITS3 F(5′GATGCCGGGGAAGGTTTTC3′), Barto_ITS3_R(5′-GCCTGGGAGGACTTGAACCT-3′) and Barto_ITS3 _Probe (6FAM-5′GCGCGCGCTTGATAAGCGTG-3′) (Invitrogen, Thermo Fisher Scientific, and Belgium).

Amplification of Internal Transcribed Spacer (ITS) gene was conducted in a final volume of 20 µL containing 10 µL of 2 × Eurogentec TakyonTM Mix (Eurogentec, Liège, Belgium), 1 µL of each primer (0.5 µM), 0.12 µL of probe, 2.5 µL of DNase-free water, and 5 µL of DNA template. The real time–qPCR was performed on the StepOneTM Real-Time PCR system (by Thermo Fisher Scientific) using the following thermal profile: an incubation step at 50 °C for 2 min for eliminating PCR amplicons, then an activation step at 95 °C for 3 min followed by 40cycles of denaturation at 95 °C for 15 s and an annealing-extension at 60 °C for 30 s. Samples were confirmed as *Bartonella* spp. if they tested positive for the first and second runs on real time–qPCR (Mariën et al. 2022) Further amplification of the ITS region (453–780 bp) (Sokhna et al. 2013)-(Böge et al. 2021) was done using conventional PCR system before sequencing (Sokhna et al. 2013). The amplification reactions were conducted in a final volume of 15 µL, containing 7.5 µL of Hot Goldstar master mix, 0.3 µL of each primer, 5.4 µL of DNA free water and 2.5 µL of DNA template. Reactions were conducted in a thermal cycler (TProfessional Basic Thermocycler by Biometra) under the following amplification conditions; 40 cycles for 30 s at 94 °C, for 30 s at 66 °C, for 50 s at 72 °C. PCR products were prepared with DNA Gel Loading Dye (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania) for gel electrophoresis in 1 % agarose. Visualization was done using UV light. Amplicons of positive samples were purified and then Sanger sequenced with forward and revers primers at Neuromics Support Facility- Vlaams Instituut voor Biotechnologie (Antwerp-Belgium). The DNA extracts from the ectoparasites were also screened for other potential rodent pathogens including; *Borrelia* spp., *Babesia* spp. and *Anaplasma* spp. by real time-qPCR.

2.5 Molecular screening of *Leptospira* spp.

The blood DNA extracts were screened for *Leptospira* spp. by real time-qPCR using the following pairs of primers and probes (Dahmana et al. 2020):

Primer F lip32B (AGCTCTTTTGTTCTGAGCGA), Primer R lip32BR (TACGAACTCCCATTTCAGCGATTA), Probe (FAM-AAAGCCAGGACAAGCG CCG-NFQ-MGB) (Invitrogen, Thermo Fisher Scientific, Belgium). Amplification of 16S-RNA gene was conducted in a final volume of 10 µL containing 5 µL of 2 × master mix, 0.5 µL of each primer forward and reverse, 0.2 µL of probe, 1.0 µL of DNase-free water, and 1 µL of DNA template. The real time–qPCR was performed on the StepOneTM

Real-Time qPCR system by (Thermo Fisher Scientific) using the following thermal profile: pre incubation step at 95 °C for 3 min by one cycle then an activation step at 95 °C for 15 s for 40 cycles, followed by 40 cycles of denaturation at 60 °C for 30 s and an annealing-extension at 60 °C for 30 s (Cortez et al. 2018).

2.6 Statistical analysis

Confidence intervals (95 % CI) for the prevalence of *Bartonella* spp. and *Leptospira* spp. in *M. natalensis* was determined at the level of alpha of 0.05 (Böge et al. 2021).

The statistical difference in *Bartonella* spp. infection of mites from males and females of *M. natalensis* was determined using a two-tailed student's *t*-test.

Prevalence (%) was calculated as number of individuals infected divided by the number of individuals examined. Statistical analysis and molecular analyses were done using *R* Statistical Software 4.1.3 and Genious software respectively.

3 Results

3.1 Molecular detection of *Leptospira and Bartonella* spp. in *M. natalensis* and their ectoparasites

One out of 100 *M. natalensis* screened for *Leptospira* spp by real time–qPCR, was positive 1(1 %), (95 %, CI: 4.7–7.9 %), indicating a relatively low prevalence of leptospirosis in the multimammate rats in the study areas.

Following real time-qPCR, *Bartonella* spp. were detected in mites, but not in fleas at a prevalence of 14 (14 %), (95 % CI: 7.08–20.9 %). The positive samples were further amplified by conventional PCR followed by sequencing. Of the 14 samples, four showed a complete sequence of the *ITS* gene (Table 1). The sequenced samples belonged to the same *Bartonella* strain (RN3BJ), predominantly *Uncultured Bartonella* (MW194941).Others were*Bartonella queenslandensis* (MZ570393), *Candidatus Bartonella thailandensis* (FJ411484,) and *Bartonella* sp. (EF190331). *Borrelia* spp., *Babesia* spp. and *Anaplasma* spp. were not detected. *Bartonella* spp. infection was shown to be higher in mites from adult male *M. natalensis* than females. However the difference was not considered significant ($p > 0.05$). Juvenile *M. natalensis* were relatively few in the study areas and no ectoparasites were found on them. The study revealed a larger population of males than females in the study areas (Table 2).

Table 1: Mites from *Mastomys natalensis* screened for *Bartonella* spp. by real time qPCR.

Study location	*M. natalensis* mites positive for *Bartonella* spp.	*Bartonella* spp.-positive males	*Bartonella* spp.-positive females	P-value
Choza	6/14(42 %)	4/6 (67 %)	2/6 (33 %)	0.06
Kiroka	8/14 (58 %)	5/8 (63 %)	3/8 (37 %)	0.07

Table 2: *Mastomys natalensis* captured in a three-month period (trap nights, sex and age).

	Choza			Kiroka		
	Jan	Feb	Mar	Jan	Feb	Mar
Trap nights	3	3	3	3	3	3
Number of traps	150	150	150	150	150	150
M. natalensis captured	10	19	21	11	19	20
Males	7	10	10	7	10	10
Females	3	9	11	4	9	10
Adults	9	19	20	11	18	20
Juveniles	1	0	1	0	1	0
Mites collected from *M. natalensis*	17	40	54	29	42	50
Fleas collected from *M. natalensis*	6	10	14	12	11	21

4 Discussion

In this study, the prevalence of *Leptospira* spp. and *Bartonella* spp. in *M. natalensis* and their ectoparasites (*Laelaptine* spp. and *X. cheopis*) in selected areas of Morogoro were determined. There was no co-infestation found of mites and fleas in the multimmamate rats. This finding is in agreement with report by (Kaminskienė et al. 2017).

Molecular analysis of the pathogens demonstrated a relatively low prevalence of *Leptospira* in *M. natalensis.* This finding contradicts with a previous report by (Katakweba et al. 2012), which states that, a dry environment is limiting the survival of *Leptospira* spp. because leptospirosis is a water borne disease. Therefore, the prevalence would be expected to be higher during the study period which was between the end of the short rain season and beginning of long rains which would be favorable for the *Leptospir*a spp. to survive in the environment. Therefore, it is plausible to believe that, the overall prevalence of leptospirosis in *M. natalensis* in the study areas is relatively low.

Other studies by Kaminskienė et al. (2017) and Mgode et al. (2021) have also reported a low prevalence of *Leptospira* spp. in *M. natalensis*, but a comparatively higher prevalence in *Rattus rattus* and *Cricetomys* spp. This relatively small proportion of *Leptospira* spp. infected *M. natalensis* may also be due to their ecology and habitats which differ from those of *R. rattus* and *Cricetomys* spp. (Massawe et al. 2005). Low prevalence of *Leptospira* spp. in

blood samples has also been reported by Cortez et al. (2018) and Bal et al. (1994), suggesting a number of factors contributing to the low prevalence, including: the species of the rodent, habitat and development of immunity with age. However, leptospirosis has a short phase in which the infectious agent is found in the circulatory system (leptospiremia) before entering the kidney tubules where it remains for a long time with intermittent discharge with urine in relatively large numbers (Bal et al. 1994).

*Laelaptine*s spp. from 14 rodents tested positive for *Bartonella* spp. by real time–qPCR, however, upon conventional PCR and sequencing, four samples demonstrated a complete *ITS* gene sequence with preponderance of *Uncultured Bartonella.* These findings agreed with previous studies done elsewhere, showing that *Bartonella* strains found in rodent ectoparasites belonged to *Uncultured Bartonella* spp. (Angelakis and Raoult 2014; Böge et al. 2021; Dehio 2001; Gundi et al. 2009). In this study, other species of *Bartonella* spp. that were detected and known to be zoonotic pathogens includes: *Bartonella queenslandensis, Candidatus Bartonella, Thailandensis Bartonella* spp. In this study, more mites were isolated from males than females *M. natalensis*, however the difference was not significant. This is in agreement with studies by Beery (2018).

This study also showed that, the overall prevalence of *Bartonella* spp. in *M. natalensis* mites was higher in males than females. This could be explained by the fact that, males have a larger home range compared to females, thus, predisposing them to more frequent contacts with the pathogens. This is in agreement with Ferrari et al. (2004) and Kataranovski et al. (2011), who suggested that males have a bigger role in driving the dynamics of transmission of infections.

Bartonella spp. infection was higher in mites from adult *M. natalensis* than in juveniles, because, adults had a higher chance of exposure to the pathogens than the juveniles. The detection of *Bartonella* spp. in mites has been reported by Alsarraf et al. (2017) which proposed that, ticks and mites be added to the list of potential reservoirs and vectors of pathogens, however, further investigations are necessary to describe their potential roles in *Bartonella* infection and transmission.

5 Conclusion and recommendation

This study has shown a relatively low prevalence of *Leptospira* spp. and higher prevalence of *Bartonella* spp. in mites from *Mastomys natelensis* in the study areas, suggesting that mites are potential reservoirs *cum* vectors of bartonellosis. Additional studies on the role of fleas as reservoirs or vectors of bartonellosis are essential.

Since *Leptospira* spp. and *Bartonella* spp. are zoonotic pathogens transmitted by rodents and their ectoparasites respectively. Surveillance for these bacteria should be considered alongside studies of other rodent borne pathogens such as, *Borrelia* spp., *Babesia* spp. and *Anaplasma* spp.

Research ethics: Risk assessment was submitted to and approved by the Ethical Committee and Decision Board of Sokoine University of Agriculture (SUA), Tanzania Wildlife Research Institute (TAWIRI) and Tanzania Commission for Science and Technology (COSTECH), permit number 2022-401-NA-2021-084.
Acknowledgments: The authors would like to thank the staff of SUA, SFUCHAS, Ifakara Health Iinstitute (IHI) (Molecular Biology Division) and the Regional Administrators of Morogoro and Sokoine University of Agriculture, Institute of Pest Management (IPM) field assistants and the Evolutionary Ecology group of the University of Antwerp for their support and cooperation during the study.
Author contributions: CT conceptualized the research idea, was involved during the data collection and manuscript write up. VM, NH, GM, JM undertook the field survey, collected data and performed the data analysis. CS and JN reviewed and edited the manuscript. IM and RM supervised the research work, reviewed and edited the manuscript. HL supervised the research work, reviewed, edited the manuscript, funded the research work and finalized the manuscript.
Research funding: This work was financially supported by the Research Foundation-Flanders (FWO) and ACE-IRPM & BTD of the Sokoine University of Agriculture.
Conflict of interest statement: The authors declare that they have no conflicts of interest regarding this article.

References

Alsarraf, M., Mohallal, E.M.E., Mierzejewska, E.J., Behnke-Borowczyk, J., Welc-Falęciak, R., Bednarska, M., Dziewit, L., Zalat, S., Gilbert, F., Behnke, et al. (2017). Description of *Candidatus Bartonella fadhilae* n. sp. and *Candidatus Bartonella sanaae* n. sp. (*Bartonellaceae*) from *Dipodillus dasyurus* and *Sekeetamys calurus* (*Gerbillinae*) from the Sinai Massif (Egypt). Vector-Borne Zoonotic Dis. 17: 483–494.

Angelakis, E. and Raoult, D. (2014). Pathogenicity and treatment of *Bartonella* infections. Int. J. Antimicrob. Agents 44: 16–25.

Aplin, K.P. (2003). *Field methods for rodent studies in Asia and the Indo-Pacific*. Australian Centre for International Agricultural Research, Canberra.

Bal, A.E., Gravekamp, C., Hartskeerl, R.A., De Meza-Brewster, J., Korver, H., and Terpstra, W.J. (1994). Detection of leptospires in urine by PCR for early diagnosis of leptospirosis. J. Clin. Microbiol. 32: 1894–1898.

Beery, A.K. (2018). Inclusion of females does not increase variability in rodent research studies. Curr. Opin. Behav. Sci. 23: 143–149.

Boey, K., Shiokawa, K., and Rajeev, S. (2019). Leptospira infection in rats: a literature review of global prevalence and distribution. PLoS Negl. Trop. Dis. 13: e0007499.

Böge, I., Pfeffer, M., Htwe, N.M., Maw, P.P., Sarathchandra, S.R., Sluydts, V., Piscitelli, A.P., Jacob, J., and Obiegala, A. (2021). First detection of *Bartonella* spp. in small mammals from rice storage and processing facilities in Myanmar and Sri Lanka. Microorganisms 9: 658.

Broecke, B.V. (2021). Linking behavior, co-infection patterns, and viral infection risk with the whole gastrointestinal helminthes community structure in Mastomys natalensis. Front. Vet. Sci. 8: 15.

Claus, A.T., Abdul, A.S., Rhodes, H.M., Robert, S., and Stella, T.K. (2020). Prevalence of Rickettsia typhi in rodent fleas from areas with and without previous history of plague in Mbulu district, Tanzania. Afr. J. Microbiol. Res. 14: 65–70.

Cortez, V., Canal, E., Dupont-Turkowsky, J.C., Quevedo, T., Albujar, C., Chang, T.-C., Salmon-Mulanovich, G., Guezala-Villavicencio, M.C., Simons, M.P., Margolis, E., et al. (2018). Identification of *Leptospira* and *Bartonella* among rodents collected across a habitat disturbance gradient along the inter-oceanic highway in the southern amazon basin of Peru. PLoS One 13: e0205068.

Dahmana, H., Granjon, L., Diagne, C., Davoust, B., Fenollar, F., and Mediannikov, O. (2020). Rodents as hosts of pathogens and related zoonotic disease risk. Pathogens 9: 202.

Dehio, C. (2001). *Bartonella* interactions with endothelial cells and erythrocytes. Trends Microbiol. 9: 279–285.

Desai, S., van Treeck, U., Lierz, M., Espelage, W., Zota, L., Sarbu, A., Czerwinski, M., Sadkowska-Todys, M., Avdicová, M., Reetz, J., et al. (2009). Resurgence of field fever in a temperate country: an epidemic of Leptospirosis among seasonal strawberry harvesters in Germany in 2007. Clin. Infect. Dis. 48: 691–697.

Ferrari, N., Cattadori, I. M., Nespereira, j., Rizzoli, A., and Hudson, P. J. (2004). The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse Apodemus flavicollis. Ecol. Lett. 7: 88–94.

Gundi, V.A.K.B., Taylor, C., Raoult, D., and La Scola, B. (2009). *Bartonella rattaustraliani* sp. nov., *Bartonella queenslandensis* sp. nov. and *Bartonella coopersplainsensis* sp. nov., identified in Australian rats. Int. J. Syst. Evol. Microbiol. 59: 2956–2961.

Gutiérrez, R., Krasnov, B., Morick, D., Gottlieb, Y., Khokhlova, I.S., and Harrus, S. (2015). *Bartonella* spp. infection in rodents and their flea ectoparasites: an overview. Vector Borne Zoonotic Dis. 15: 27–39.

Holt, J., Davis, S., and Leirs, H. (2006). A model of Leptospirosis infection in an African rodent to determine risk to humans: seasonal fluctuations and the impact of rodent control. Acta Trop. 99: 218–225.

Kaminskienė, E., Radzijevskaja, J., Balčiauskas, L., Gedminas, V., Paulauskas, A. (2017) Laelapidae mites (Acari: Mesostigmata) infesting small rodents in the Curonian Spit, Lithuania. Biologija 63, https://doi.org/10.6001/biologija.v63i2.3528.

Katakweba, A.A.S., Mulungu, L.S., Eiseb, S.J., Mahlaba, T.A., Makundi, R.H., Massawe, A.W., Borremans, B., and Belmain, S.R. (2012). Prevalence of haemoparasites, Leptospires and Coccobacilli with potential for human infection in the blood of rodents and shrews from selected localities in Tanzania, Namibia and Swaziland. Afr. Zool. 47: 119–127.

Kataranovski, M., Mirkov, I., Belij, S., Popov, A., Petrović, Z., Gačić, Z., and Kataranovski, D. (2011). Intestinal helminths infection of rats (*Ratus* [sic] *norvegicus*) in the Belgrade area (Serbia): the effect of sex, age and habitat. Parasite 18: 189–196.

Kim, C.M., Kim, J.Y., Yi, Y.H., Lee, M.J., Cho, M., Shah, D.H., Klein, T.A., Kim, H.C., Song, J.W., Chong, S.T., et al. (2005). Detection of Bartonella species from ticks, mites and small mammals in Korea. J. Vet. Sci. 6: 327–334.

Machang'u, R.S., Mgode, G.F., Assenga, J., Mhamphi, G., Weetjens, B., Cox, C., Verhagen, R., Sondij, S., Goris, M.G., and Hartskeerl, R.A. (2004). Serological and molecular characterization of leptospira serovar Kenya from captive African giant pouched rats (*Cricetomys gambianus*) from Morogoro Tanzania. FEMS Immunol. Med. Microbiol. 41: 117–121.

Makundi, R.S., Kilonzo, B.S., and Massawe, A.W. (2003). Interaction between rodent species in agro-forestry habitats in the western Usambara Mountains, north-eastern Tanzania, and its potential for plague transmission to humans. SUA IR (2003-1).

Malania, L., Bai, Y., Osikowicz, L.M., Tsertsvadze, N., Katsitadze, G., Imnadze, P., and Kosoy, M. (2016). Prevalence and diversity of Bartonella species in rodents from Georgia (Caucasus). Am. J. Trop. Med. Hyg. 95: 466–471.

Mariën, J., Vanden Broecke, B., Tafompa, P.J.J., Bernaerts, L., Ribas, A., Mnyone, L.L., Mulugu, L.S., and Leirs, H. (2022). Host related factors determine co-occurrence patterns between pathogenic bacteria, protozoa, and helminths in populations of the multimammate mouse, *Mastomys natalensis*. bioRxiv 2022-01.

Massawe, A.W., Rwamugira, W., Leirs, H., and Makundi, R.H. (2005). Influence of land preparation methods and vegetation cover on population abundance of Mastomys natalensis in Morogoro, Tanzania. SUA IR (2005-12).

Meerburg, B.G., Singleton, G.R., and Kijlstra, A. (2009). Rodent-borne diseases and their risks for public health. Crit. Rev. Microbiol. 35: 221–270.

Mgode, G.F., Mhamphi, G.G., Massawe, A.W., and Machang'u, R.S. (2021). Leptospira seropositivity in humans, livestock and wild animals in a semi-arid area of Tanzania. Pathogens 10: 696.

Rija, A.A., Bugingo, A., Said, A., and Mwamende, K.A. (2014). Wet season bird species richness and diversity along urban-rural gradient in Morogoro municipality and surrounding areas, Tanzania. Tanzania J. Forestry Nat. Conserv. 83: 1–12.

Sokhna, C., Mediannikov, O., Fenollar, F., Bassene, H., Diatta, G., Tall, A., Trape, J.F., Drancourt, M., and Raoult, D. (2013). Point-of-care laboratory of pathogen diagnosis in rural Senegal. PLoS Negl. Trop. Dis. 7: e1999.

Theonest, N.O., Carter, R.W., Amani, N., Doherty, S.L., Hugho, E., Keyyu, J.D., Mable, B.K., Shirima, G.M., Tarimo, R., Thomas, K.M., et al. (2019). Molecular detection and genetic characterization of Bartonella species from rodents and their associated ectoparasites from northern Tanzania. PLoS One 14: e0223667.

Van Aelst, K. and Holvoet, N. (2018). Climate change adaptation in the Morogoro Region of Tanzania: women's decision-making participation in small-scale farm households. Clim. Dev. 10: 495–508.

Van Houtte, N., de Bellocq, J.G., Akaibe, D., Wilschut, L., Verheyen, E., Amundala, N., Socolovschi, C., Falay, D., Raoult, D., Laudisoit, A., et al. (2014). High prevalence of *Rickettsia typhi* and *Bartonella* species in rats and fleas, Kisangani, Democratic Republic of the Congo. Am. J. Trop. Med. Hyg. 90: 463–468.