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†

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The prevalence of haemoparasites, leptospirosis and *Yersinia pestis* was investigated in rodents and shrews from Tanzania, Namibia and Swaziland. Blood smears originating from rodents and shrews from the three countries indicated the presence of *Trypanosoma lewisi* (72.7%; *n* =950), B*acillus* spp. (25.6%; *n* =950), *Borrelia* sp. (0.01%; *n* =950) and bipolar coccobacilli (0.01%; *n* =950). The blood smears from Namibia (*n* =26) had no haemoparasites while only 1.33% (*n* =75) of those from Swaziland showed presence of *T. lewisi*. *Leptospira interrogans* was found in rodent blood sera from Tanzania in the following serogroup proportions (*n* =350): Icterohaemorrhagiae (10.29%), Pomona (2.86%), Hardjo (1.14%), Bullum (0.86%), Grippotyphosa (1.43%) and Canicola (1.14%). Serodiagnosis of antibodies against the F1 antigen of *Y. pestis* using the enzyme linked immunosorbent assay (ELISA) was negative for all the serum samples from central Tanzania, while two samples of serum from two species of rodents, *Rhabdomys pumilio* and *Gerbilliscus leucogaster,* collected in the Kavango Region of Namibia were positive. These results suggest an enzootic plague activity in this region in Namibia. It is concluded that zoonotic agents, that are infectious to humans, are prevalent in rodents and shrews in the three countries, and that local communities should apply rodent control measures to reduce the risk of human infections.

Key words: haemoparasites, plague, leptospirosis, Africa, rodents.

INTRODUCTION

Rodents and shrews play an important role as reservoirs and hosts of many pathogens of animal and public health importance (Gratz 1994) globally. Agents of rodent-borne zoonoses include viruses, bacteria, rickettsia, protozoa and helminths (Gratz 1994, 1997). Infections with zoonotic haemoparasites are widespread in wild rodents (Korbawiak *et al.* 2005). They include borrelia, trypanosomes, bacilli, plasmodia and coccobacilli (Silayo 1992; Gratz 1997; Juha *et al.* 2003; Powelczyk *et al.* 2004). In humans, these pathogens are responsible for many rodent-borne diseases including plague, leptospirosis, toxoplasmosis, leishmaniasis and haemorrhagic fevers. Leptospirosis and plague are believed to be widespread in East and southern Africa (Machang'u *et al.* 1997; Kilonzo *et al.* 2005; Makundi *et al.* 2008; Laudisoit *et al.* 2009).

Leptospirosis is a water-borne zoonotic disease of worldwide importance, which has been largely neglected in Africa and elsewhere in the tropics (Machang'u 1992). Public health professionals, veterinarians and physicians rarely consider leptospirosis in clinical diagnosis, even in cases of pyrexia of unknown origin. The incidence of leptospirosis in human populations is not well established, and hence this disease does not feature among the priority public health concerns or in national disease prevention programmes in Africa (Machang'u 1992). Rodents and shrews are

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the major reservoirs for leptospirosis; however, maintenance hosts (animals that are capable of acting as a natural source of leptospiral infection for their own species) include various species of mammals, reptiles and amphibians. Rodents shed the microorganisms in urine, and under favourable environmental conditions (alkaline soils, mud, swamps, streams and rivers) the leptospires can survive for years. In the laboratory they can survive for eight years in Korthhof medium in glass tubes in the dark at room temperature and 10 years freeze-dried in vacuum storage in sealed ampoules as preserved cultures (Faine 1982).

Plague is a zoonotic disease for which human infection is primarily acquired through bites of infected rodent fleas with bubonic plague. The natural reservoirs of plague are wild rodents and the causative agent is a bacterium, *Yersinia pestis,* a member of the family Enterobacteriaceae (Meerburg *et al.* 2009). Natural transmission of plague to humans remains a possibility in many regions of the world, where foci exist in sylvatic rodent populations. A recent review highlights, in a historical sense, that the number of plague cases is relatively low, the disease is still widely distributed globally, that it has an innate ability to spread rapidly, and clinical symptoms can unfold quickly (Meerburg *et al.* 2009). Plague remains a disease of major public health importance globally, with several countries in the world reporting cases (WHO 2006b). It persists as a chronic disease among many species of rodents around the world, and outbreaks often occur in unpredictable patterns (Makundi *et al.* 2008). Outbreaks in Algeria and Democratic Republic of Congo (DRC) have shown that plague may re-emerge in the same areas after a long period of quiescence (WHO 2006a).

Most outbreaks of rodent-borne diseases in humans are commonly related to socio-economic deficiencies such as poor hygiene, poverty and overcrowding. However, the incidences of these diseases are grossly underestimated (Daniel *et al.* 1992; Lyla *et al.* 1995; Machang'u *et al.* 1997). In the current study, we investigated the prevalence of zoonotic disease agents in rodents and shrews captured in selected localities in rural village settings in Swaziland, Tanzania and Namibia.

MATERIALS & METHODS

Study sites

Studies were carried out in Tanzania, Namibia and Swaziland. The studies were based at Berega village (06°10′S 37°08′E), Gairo division in Central Tanzania, Kake village (18°05′S 21°29′E) in the Kavango region of Namibia and in the Lobamba village (26°46′S 31°21′E) of Swaziland.

Animal trapping

Live trapping of rodents and shrews was conducted from February 2007 to January 2008 in Tanzania, from June 2007 to May 2008 in Namibia and from March 2008 to April 2009 in Swaziland. Trapping of rodents and shrews was carried out inside houses and in peridomestic areas (fallow land surrounding rural houses using Sherman LFA Live Traps (7.5 × 9.0 × 23.0 cm; HB Sherman Traps, Inc., Tallahassee, FL) and locally made live-traps (12 × 15 × 20 cm) consisting of a wooden box with a wire mesh window on one side and snap-back door on the other. Five Sherman and five box traps were set in houses for three consecutive nights at strategic points to increase the capture rate. In peridomestic areas three box and five Sherman traps were placed in trap lines located five metres apart. Traps were inspected every morning and captured animals were identified to species level by a taxonomist. Trapping was done every month for three consecutive trap nights. Trapping was carried in the same houses during the entire study period.

Haemoparasite examination, leptospire evaluation and serological tests

Live captured animals were anaesthetized with ether and 20–25 μl of blood was drawn from the supraorbital vein using a glass capillary. A thick blood smear was prepared on a microscope slide for each animal. Once dried, the slides were immersed in 10% Giemsa stain (1:10 dilutions) for 30 min and washed under running water for 10 sec, dried and examined under a light microscope (×100 magnification with immersion oil). Serological tests of selected sera samples from rodents and shrews were conducted for antibodies using the microagglutination assay. Leptospiral antigens were evaluated for serogroups Icterohaemorrhagiae (Sokoine), Grippotyphosa (RM4), Ballum (Ballum), Canicola (Canicola), Sejroe (Hardjo) and Pomona (Pomona). Agglutination was monitored by dark-field microscopy. For isolation, kidney tissue and urine were collected from rodents and shrews. The specimens were cultured in Fletcher's media. Plague serology was conducted using the enzyme-linked immunosorbent assay (ELISA). This assay is specific for antibodies

Table 1. **a**, Prevalence of haemoparasites in blood of different species of rodents and shrews in central Tanzania.

Rodents and shrews	Blood smears tested	*Trypanosoma lewisi*	*Bacillus* spp.	*Borrelia* sp.	Bipolar coccobacilli	Total parasites	% Positive
Mastomys natalensis	317	2	44	0	0	46	14.5
Rattus rattus	467	211	16	0	0	227	48.6
Crocidura hirta	83	1	5	2	3	11	13.3
Gerbilliscus vicinus	32	1	6	0	0	7	21.9
Graphiurus cf. *murinus*	3	1	0	0	0	1	33.3
Arvicanthis neumanni	38	0	5	0	0	5	13.2
Acomys spinosissimus	3	0	0	0	0	0	0.0
Mus minutoides	3	0	0	0	0	0	0.0
Aethomys chrysophilus	2	0	0	0	0	0	0.0
Lemniscomys zebra	2					0	0.0
Total	950	216	76	2	3	297	31.3
% Positive	950	22.7	8.0	0.2	0.3	31.3	

b, Prevalence of haemoparasites in blood of different species of rodents and shrews in the Lobamba region of Swaziland.

Rodents and shrews	Blood smears tested	*Trypanosoma lewisi*	*Bacillus* spp.	*Borrelia* sp.	Bipolar coccobacilli	Total parasites	% Positive
Mastomys natalensis	72	1	0	0	0	1	1.39
Lemniscomys rosalia	1	0	0	0	0	0	0
Rattus rattus	2	0	0	0	0	0	0
Total	75	1	0	0	0	1	1.33
% Positive	75	1.33	0	0	0	1.33	

against the F1 antigen of *Yersinia pestis*. The test detects specific immunoglobulin G or immunoglobulin M antibodies to the F1 fraction due to *Yersinia pestis* infection.

Data analysis

The prevalence of haemoparasites and leptospirosis was compared between species, sex, seasons and habitat using General Linear Model of the program STATISTICA (Stat Soft Inc.)

RESULTS

Small mammal species

In central Tanzania, ten rodent and shrew species were captured (*Rattus rattus, Mastomys natalensis, Crocidura hirta, Graphiurus* cf. *murinus, Arvicanthis neumanni, Acomys spinosissimus., Mus minutoides, Aethomys chrysophilus, Gerbilliscus vicinus* and *Lemniscomys zebra).* In Namibia, eight species of rodents were captured (*Gerbilliscus leucogaster, Steatomys pratensis, Mus indutus, M. natalensis, Lemniscomys rosalia, Thallomys paedulcus, Saccostomus campestris* and *Rhabdomys pumilio*). In Swaziland, a total of three species was captured (*R. rattus, L. rosalia* and *M. natalensis).*

Screening for haemoparasites

A total of 1051 small mammal blood samples from Tanzania, Swaziland and Namibia were screened for haemoparasites. Tables 1a and 1b show the haemoparasites observed in samples from rodents and shrews in Tanzania and Swaziland (obtained in January during the wet season). The results show the presence of *Trypanosoma lewisi* in the blood of rodents and shrews, with *R. rattus* accounting for a higher percentage of all positive cases. No haemoparasites were observed in the blood samples from Namibia. There were significant seasonal variations ($F_{6, 70} = 86.13$, $P = 0.000$) in the number of haemoparasites observed between the species studied in Tanzania. *Rattus rattus* harboured more haemoparasites than the other species (Fig. 1). There were no significant seasonal effects on the number of haemoparasites in the species studied ($P \geq 0.05$) but significant ($F_{6,0} = 2.31$, $P = 0.043$) interactions between species and season were observed. Haemoparasites were

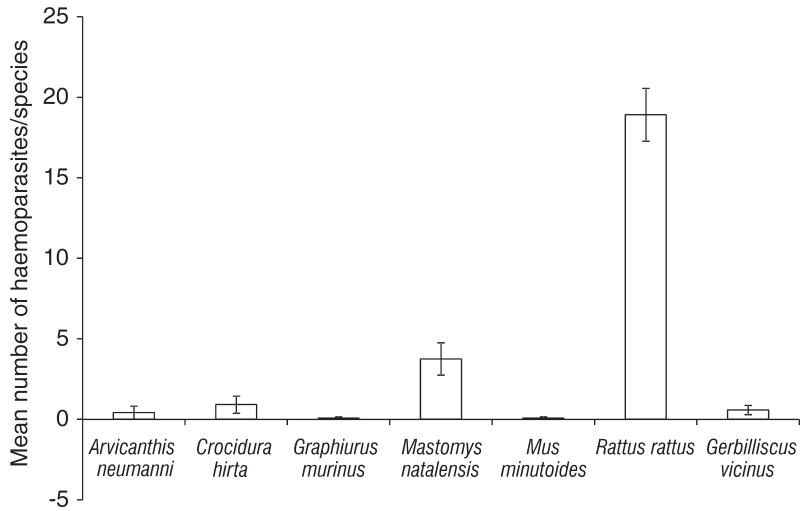

Fig. 1. Mean number of haemoparasites in different species of rodents and shrews in Central Tanzania.

more prevalent in *Rattus rattus* during the wet season than in the dry season, whereas they were more prevalent in the dry than wet season in *M. natalensis* (Fig. 2). Significant variations ($F_{1,912}$ = 78.34, P = 000) in the number of haemoparasites were observed in the two habitats studied. More haemoparasites were observed in those animals captured inside rural houses than in peridomestic areas (Figs 3 & 4).

Plague

All 452 rodent and shrew blood samples from central Tanzania were negative for antibodies against the F1 antigen of *Y. pestis* (Table 2). Two of 53 blood samples of rodents from Namibia were serologically positive for antibodies against the F1 antigen of *Y. pestis.* The two positive blood samples originated from two different rodent species, *R. pumilo* and *G. leucogaster* (Table 2). One of the samples was obtained in June (dry season) while the other was collected in March (wet season).

Leptospirosis

Table 3a shows the seroprevalence of different serogroups of *Leptospira interrogans* in rodents and shrews blood at different titres. Most of the samples tested were positive at low titres for

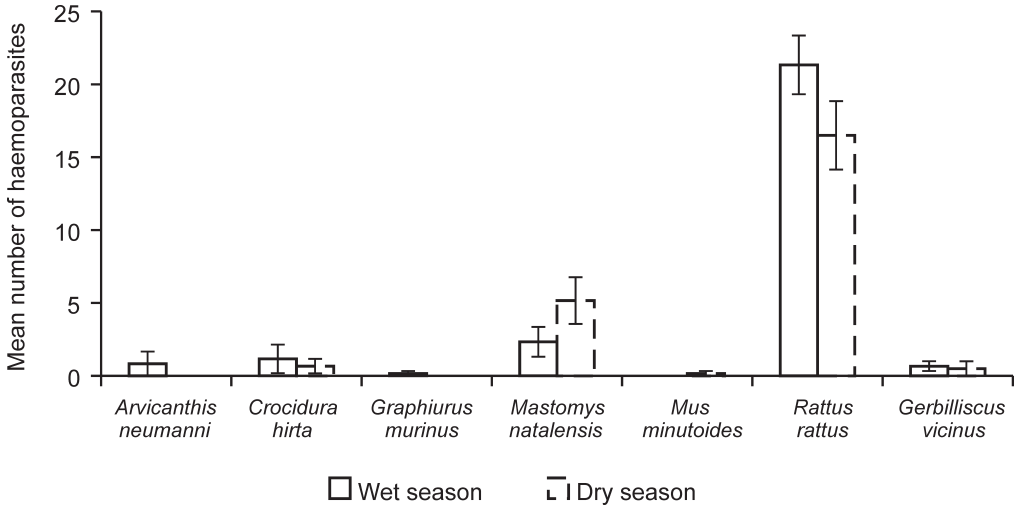

Fig. 2. Seasonal variations in the mean prevalence of haemoparasites in different species of rodents and shrews in central Tanzania.

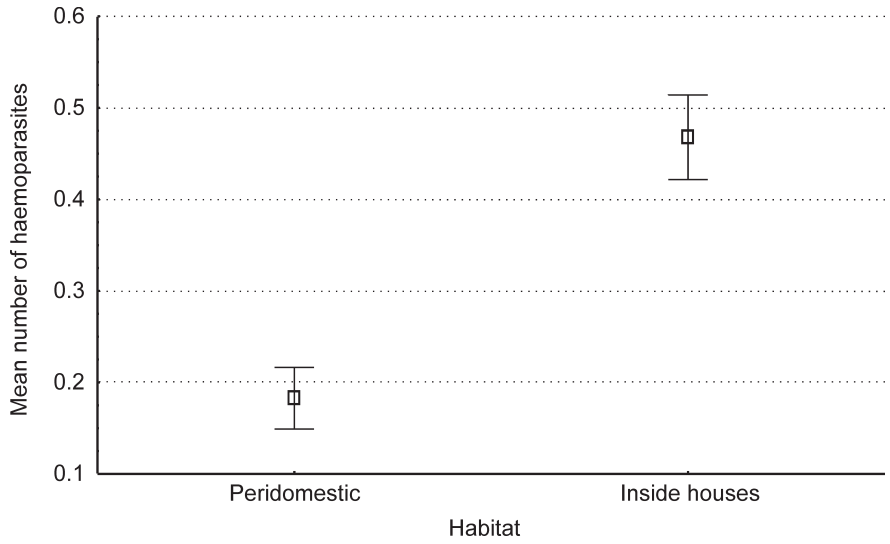

Fig. 3. Mean number of haemoparasites observed in animals captured in peridomestic areas and inside houses in central Tanzania.

leptospirosis. Blood samples from *M. minutoides* and *A. spinosissimus* were negative to all serovars of leptospirosis. *R. rattus, M. natalensis* and *C. hirta* had higher proportions of individuals which tested positive for leptospirosis than the rest of the species (Table 3b).

An analysis of the results shows that there was a significant difference ($F_{5,342}$ = 3.39, P = 0.005) in rodent and shrew species involved in the study area in Tanzania (*R. rattus* 0.147 ± 0.3; *M. natalensis* 0.168 ± 0.033; *C. hirta* 0.285 ± 0.63; *G.* cf. *murinus* 1 ± 0.26; *A. neumanni* 0.13 ± 0.07; and *G. vicinus* 0.4 ± 0.14). There were significant variations ($F_{1, 80}$ = 5.39, P = 0.02) in the prevalence of leptospirosis between two seasons. Higher prevalence of leptospirosis was observed in those animals captured during the wet seasons (0.937 ± 0.17) than in the dry season (0.35 ± 0.17). There was also a significant seasonal variations ($F_{7,80}$ = 4.81, P = 0.000) of prevalence between species (*R. rattus*

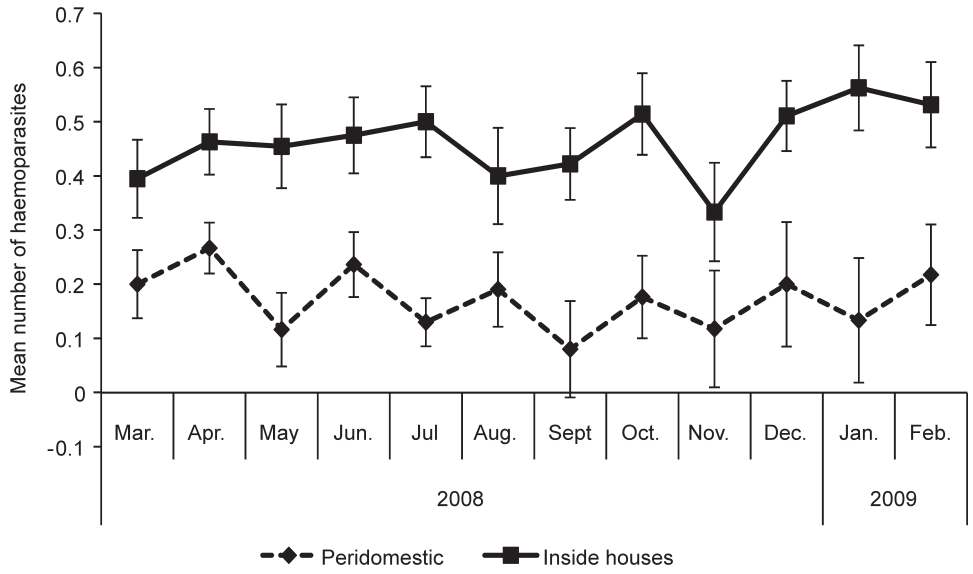

Fig. 4. Seasonal trends of haemoparasites in rodents and shrews captured in peridomestic and inside houses in central Tanzania.

Table 2. Seroprevalence of antibodies against the F1 antigen of *Yersinia pestis* in blood of rodents and shrews from central Tanzania and the Kavango region in Namibia.

Species	Tanzania*	Namibia*
Acomys spinosissimus	2 (0)	–
Arvicanthis neumanni	34 (0)	–
Crocidura hirta	43 (0)	–
Graphiurus cf. *murinus*	3 (0)	–
Mastomys natalensis	145 (0)	27 (0)
Mus minutoides	2 (0)	–
Rattus rattus	197 (0)	–
Gerbilliscus vicinus	21 (0)	–
Lemniscomys zebra	4 (0)	2 (0)
Aethomys chrysophilus	1 (0)	–
Mus indutus	–	3 (0)
Rhabdomys pumilio	–	2 (1)
Saccostomus campestris	–	3 (0)
Steatomys pratensis	–	8 (0)
Gerbilliscus leucogaster	–	6 (1)
Thallomys paedulcus	–	2 (0)
Total	452 (0)	53 (2)

*Numbers in brackets indicate positive samples.

1.92 ± 0.35; *M. natalensis* 1.75 ± 0.35; *C. hirta* 0.83 ± 0.35; *G.* cf. *murinus* 0.16 ± 0.35; *A. neumanni* 0.25 ± 0.35*; A. spinosissimus* 0.00 ± 0.35; *M. minutoides* 0.00 ± 0.35 and *G. vicinus* 0.25 ± 0.35). No variation in the prevalence of leptospirosis was observed between the two habitats ($P \geq 0.05$), but significant variations ($F_{1,344} = 4.78$, $P = 0.029$) between male and female were observed. Prevalence of leptospirosis was higher in females (0.217 ± 0.02, $n = 198$) than in males (0.126 ± 0.03, $n = 150$).

DISCUSSION

The presence of *T. lewisi* in the blood of a large proportion of individuals of *R. rattus* raises a public health concern because of the commensal nature of this species. *Rattus rattus* is thought to be a potential reservoir and vector of human or animal pathogenic trypanosomes, including *T. rhodesiense, T. gambiense, T. brucei, T. congolense, T. vivax* and *T. suis* (Silayo 1992; Juha 2003; Powelczyk *et al.* 2004). Although it has been reported that *T. lewisi* is a common blood parasite of rodents, the pathogenicity of this protozoan has not been established (Silayo 1992).

The presence of spirochetes supports previous reports on the potential role of rodents and shrews as reservoirs of *Borrelia* spp. and *Leptospira* spp. (Gratz 1977; Machang'u *et al.* 2003). Spirochetes have been detected in ticks (*Ixodes persulcatus)* and in wild rodent hosts in Russia (Sato *et al.* 1996). The presence of bacilli in rodent and shrew blood smears was not entirely unexpected since rodents are known to be carriers of various bacterial organisms (Gratz 1994). Although coccobacilli were observed in the blood smears of rodents and shrews trapped in central Tanzania, serological tests on blood sera from the same animals by ELISA were negative for antibodies against the F1 antigen of *Y. pestis.* This indicates that Gairo division in Central Tanzania was potentially plague-free. Other localities in northern and northeastern Tanzania have been reported to be plague endemic (Davis *et al.* 2006; Kilonzo *et al.* 2005; Makundi *et al.* 2008) The presence of antibodies against the F1 antigen of Y. pe*stis* in the blood of two rodent species, *R. pumilio* and *G. leucogaster,* in the Kavango region, Namibia, suggests that this is an enzootic plague area. The Kavango region is outside the known plague endemic foci, which are centred in the Ohangwema and Oshikoto regions of Namibia where human cases were previously reported (Groepe 1993; Shangula 1998). The current findings reinforce the need for regular plague surveillance in rodents in the Kavango region of Namibia in order to reduce the risks of human infections particularly because plague is now regarded as a globally re-emerging disease, with Africa reporting more cases than the rest of the world (WHO 2006b).

Leptospirosis is widely prevalent in rodents, shrews, humans and livestock in some parts of Tanzania (Machang'u 1992; Machang'u *et al.* 1997; Mgode *et al.* 2006). In central Tanzania, leptospirosis was most prevalent in species captured in houses and peridomestic areas where they interact with humans thus raising the potential of human infection. *Mastomys natalensis* and *C. hirta* were also trapped in fallow land, maize field, vegetable gardens and sugar cane plantations. These sites are associated with human activities in the rural settlements. Some farming activities, particularly for crops with high water needs such as rice, sugarcane and vegetables, predispose humans to leptospirosis infection through rodent urine-contaminated environments (Faine 1982; Faine *et al.* 1999). Leptospirosis is a major health hazard in rural communities in developing countries, particularly among the rural poor who are at greatest risk of exposure to infection emanating from long hours in the field and interactions with rodents. In addition they lack early medical intervention when they become infected (Meerburg *et al.* 2009). Recently,

Table 3. **a**, Seroprevalence of different serogroups of *Leptospira interrogans* in blood of rodents and shrews from central Tanzania.

Titres	Serogroups tested					
	Ictero. (*n* = 350)	Pomona (*n* = 350)	Hardjo (*n* = 350)	Ballum (*n* = 350)	Grippo. (*n* = 350)	Canicola (*n* = 350)
1:20	15	3	2	0	1	1
1:40	9	5	2	2	1	2
1:80	8	1	0	1	3	1
1:160	4	0	0	0		
1:320	0	1	0	0		
Total	36	10	4	3	5	4
% Positive	10.29	2.86	1.14	0.89	1.43	1.14

b, Species-specific seroprevalence of different serogroups of *Leptospira interrogans* in blood of rodents and shrews from central Tanzania.

Species	No. of sera tested	Ictero.	Pomona	Hardjo	Ballum	Grippo.	Canicola	Total positive
Rattus rattus	157	15	2	1	1	0	2	21
Mastomys natalensis	124	12	6	2	1	2	0	23
Arvicanthis neumanni	23	0	0		1	1	1	3
Gerbilliscus vicinus	7	2	0	0	0	0	1	3
Graphiurus cf. *murinus*	2	1	1	0	0	0	0	2
Crocidura hirta	35	6	1	1	0	2	0	10
Acomys spinosissimus	1	0	0	0	0	0	0	0
Mus minutoides	1	0	0	0	0	0	0	0
Total	350	36	10	4	3	5	4	62

Taylor *et al.* (2008) reported seropositive rodents for leptospirosis and toxoplasmosis in an urban environment in Durban, South Africa. Our observations in the current study are further evidence that rodent-borne zoonoses are widespread in rodents, which could predispose rural communities to infection.

Generally, there is under-reporting of rodent-borne zoonoses and insufficient attention is paid to the diagnosis of these important diseases in sub-Saharan Africa. According to Taylor *et al.* (2008) the prevalence of zoonotic agents in rodents was higher in informal settlements without improved sewage systems and housing. In rural areas in sub-Saharan Africa, communities live in settlements surrounded by habitats that are suitable for harbouring large numbers of rodents in some seasons, which increases the likelihood of intense human–rodent interactions. With increasing human population and encroachment into natural habitats, which harbour high diversity of rodents, frequent rodent–human interactions could increase the potential for contracting some zoonotic diseases, thus posing serious public health problems in Africa.

In Africa, these rodent-borne zoonoses either cause diseases, which go undiagnosed or are misdiagnosed due to lack of information on the prevalence of the causative agents (Begon 2003). Several other zoonoses that were not the subject of the current investigation include arenaviruses and hantaviruses, which cause haemorrhagic fevers in humans. These have recently been found in rodents in Tanzania (Mills 1999; Borremans *et al.* 2011; Goüy de Bellocq *et al.* 2010; Günther *et al.* 2010).

Reducing the risks to infection by rodent-borne zoonoses

It is very important to reduce the hazards of rodent- and shrew-borne diseases in areas where humans, domestic animals and rodents are living close to each other (Meerburg *et al.* 2009). Taylor *et al.* (2008) reported some of the principles that contributed to the success of the Boston Model to control rodents and their diseases at Cato Crest in

Durban, South Africa. The conditions in Cato Crest in Durban bear similarities to rural settings in Tanzania, Swaziland and Namibia, which suggests that the principles applied for reducing the risks of rodent-borne zoonotic infections could be adopted by these countries, but based on a better understanding of the local rodent species, kind of zoonoses harboured and the ecology of these species. Involving the local communities in management of rodents has been shown to be effective in reducing commensal rodent infestations (Belmain *et al.* 2008), and therefore it provides an opportunity for reducing the risks of zoonotic infections in rural communities in these countries.

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