Small Mammals in Fenced Houses as Source of Leptospirosis to Livestock Pets animals and Humans in Morogoro Municipality, Tanzania

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SUMMARY

The goal of this study was to determine the prevalence of leptospirosis in small mammals in Morogoro municipality specifically in fenced houses keeping livestock and pets. The mammals were trapped using Sherman, box and Havaharts traps. The captured mammals were anaesthetized by diethyl ether, blood was drawn either from the heart puncture by using 2ml syringe or supraorbital veins by using blood capillaries and transferred into Eppendorf tubes then centrifuged at 4000rpm for 5minutes to obtain sera. Microscopic agglutination test (MAT) was performed using Serovars namely Sokoine, Kenya and Lora on sera samples, in which U-shaped wells micro titre plates were filled with 50µl Phosphate saline (PBS) while in the first well was filled with 90µl, followed by 10µl of sera. They were serially diluted to the fourth well, then 50µl of live *Leptospira* antigen was added in each well and shaken gently. The mixture was incubated at 30ºC for two hours. Agglutination test results were examined under dark field microscope. Seventy small mammals were trapped, only two species namely *Rattus rattus* and *Mastomys natalens* is were identified. From the MAT test, 16 (10 *R. rattus* and 6 *M. natalensis)* sera samples showed positive results with respect to a particular serovar. The results indicated *R. rattus* to have high prevalence than *M. natalensis*. The overall prevalence was 22.9% whereby serovars Sokoine had 11.4%, Kenya 5.7% and Lora 5.7%. Small mammals normally shed leptospires to the environment and feed containers of livestock and pet animals through their urine. In turn leptospires get access to livestock, pet animals and humans either by being ingested or penetrated the intact skin and finally develop the disease. Control of small mammals that are reservoirs of leptopires is very important and this control will reduce the burden of leptospirosis in humans, livestock and pets found sharing the same environment

Key words: Small mammals, livestock, pets, fenced houses, humans, lepstospires

INTRODUCTION

Leptospirosis is a zoonotic bacterial disease with a worldwide distribution, and is an emerging infectious disease in humans, livestock and pet animals (Sykes *et al*., 2011). Leptospirosis is widespread throughout the world, and livestock and pets serve as both incidental hosts for various leptospiral serovar strains and maintenance hosts for the serovars related to their species (Sykes *et al*., 2011). Callte are primary reservoir of hadjo, pigs for Pomona and dogs for the serovar Canicola.They may also be infected with serovars such as Icterohaemorrhagiae, Grippotyphosa, Ballum and Pomona (Mgode *et al.,* 2015). The Leptosopra bacteria are thin, motile spirochetes with a hook-shaped end. Both saprophytic and pathogenic species exist in nature. The pathogenic spirochaetes are currently classified as a single species, *Leptospira interrogans*, and further subdivided into several serogroups and serovars based on antigenic differences (Picardeau, 2013). In theory, any parasitic *Leptospira* may infect any animal species. Fortunately, only a small number of serovars will be endemic in any particular region or country. Furthermore, leptospirosis is a disease that shows a natural nidality, and each serovars tends to be maintained in specific maintenance hosts (Birnbaum *et al*., 1998; Perret *et al.,* 2005; Sykes *et al*., 2011). In any region, domestic animal species will be infected by serovars maintained by species or by serovars maintained by other animal species present in the area or environment. The relative importance of these incidental infections is determined by the opportunity prevailing social, management, and environmental factors that provide contact and transmission of leptospires from other species (Picardeau, 2013).

Wild and domestic small animals especially rats are reservoirs of pathogenic *Leptospira* (Faine *et al*., 1999; Levett, 2001) of which they maintain the leptospires in their proximal renal tubules in the kidney and shed the organism in the urine. Rodents are generally regarded as one of the most important transmission sources of leptospirosis (Faine *et al.,* 1999). *Rattus norvegicus*, a predominantly urban dwelling rat found in close proximity to humans, is regarded as one of the main reservoirs for server Copenhageni worldwide (Faine *et al.,* 1999), and has largely replaced *R. rattus* as the dominant rat in urban settings due to its more aggressive behaviour. Several studies were conducted in different parts of the world, particularly in Brazil, the prevalence of leptospirosis in rodents by culture was 80.3% and 68.1% by MAT (Faria *et al.,* 2008). The other study conducted in Colombia showed the prevalence of leptospirosis in rodent to be 25% by MAT and 23% by culture (Agudelo *et al.,* 2009). In Tanzania the overall prevalence of leptospirosis in rodents determined using six *Leptospira* serovars was 25.8%. *Leptospira* serovar Sokoine was more prevalent than other serovers (Mgode *et al*., 2105).

Besides the significant impact on public health, leptospirosis is also an animal health problem that causes economic losses in the livestock industries, due to reproductive failure, decreased milk and meat production (Pearson *et al.,* 1980), reduced growth for example in non-vaccinated deer (Subharat *et al.,* 2012), and clinical illness, example sudden death and haemoglobinuria (Cordes *et al*., 1982).

Factors associated with an increase in the incidence of leptospirosis as well as the magnitude of outbreaks include the global warming that leads to extreme weather events such as cyclones and floods, increased rainfall, and increased world population and urbanization (Lau *et al.,* 2010; Hartskeerl *et al*., 2011). The disease prevails in urban environments of industrialized and developing countries, as well as in rural regions all over the world, and is more common in the tropics where conditions for its transmission are particularly favorable (Bharti *et al.,* 2003); however, survival is very poor in dry or cold environments (Adler and Moctezuma, 2010).

Leptospirosis is a cosmopolitan disease affecting various animal species and is considered as zoonosis. It can be transmitted directly or indirectly, mainly through contact with the carrier's urine and entering the body through mucous membranes or skin (Bharti *et al*., 2003). Wild and domestic animals are reservoirs of pathogenic *Leptospira*; they maintain the leptospires in their proximal renal tubules in the kidney and shed the organism in the urine to the environment, human foods and drinking water (Faine *et al.,* 1999). In Morogoro, little studies have been done to determine the prevalence of small mammals found in the areas where livestock and pet animals are kept. Interaction between pet animals and small mammals and pet animals feeding on small mammals, the data isscarce in thecountry thus it is less considered, despite the fact that the urban livestock and pet animals have a higher risk of infection than rural animals due to higher densities of rodents that increase exposure risk among susceptible animals (Abela-Ridder *et al.,* 2010).A research conducted in Morogoro revealed that a total of 52 *Leptospira* isolates were obtained from fresh urine and kidney homogenates, collected between 1996 and 2006 from small mammals, cattle and pigs (Katakweba *et al*., 2012; Mgode *et al*., 2015). Therefore, in order to assess, monitor, and mitigate the risk of leptospirosis in small mammals, livestock and pet animals as well as humans in Morogoro municipality it is necessary to study leptospirosis in small mammals in fenced in house.

In addition, infected animals can develop chronic renal infection and excrete the organisms in urine, thereby disseminating leptospires to other animals and constituting a potential zoonotic threat to those engaged in animal production and related industries (Cousins *et al.,* 1985; Magajevski *et al.,* 2005). Therefore, the urgent efforts for gathering sufficient data on leptospirosis for promoting awareness are highly needed. This study is aimed at establishing the existence of small mammals (rodents) in fenced houseswhere livestock and pet animals are kept since they share the environment thus creating the risk of leptospirosis being transmitted to livestock and pet animals via their excretions (urine and feces) and also their organs when consumed by pet animals.

MATERIALS AND METHODS

A cross-sectional study was carried to measure the level of exposure and carrier status before and after long rain season, namely in December and in May.The study was conducted in Morogoro municipal located at 6° 49′ 0″ S, 37° 40′ 0″ E, and sampling will was done in selected urban and peri-urban areas in fenced houses.Five claster were involved in the trapping process namely Mazimbu, Kihonda, Nanenane, Bigwa Folkland.

Sample collections

Animals were captured using Sherman, box and Havahartslive traps baited with peanut butter mixed with maize bran.The captured animals were transported to Sokoine University Pest Management Centre (SPMC) laboratory where they were anaesthetized with diethyl ether and blood samples collected. Blood was drawn from the supra-orbital vein using capillary tubes then transferred to Eppendorf tubes. Another source of blood was heart puncture performed using syringe and needle and then transferred to Eppendorf tubes. Blood was allowed to clot in Eppendorf tubesthen centrifuged at 4000rpm for 5minutes to obtain sera. The serawas separated and stored at -20°C waiting for serology test.

Serological test

The MAT test was performed using live pure leptospires culture (antigens) of three serovars Sokoine, Lora, and Kenya (local isolates from Tanzania) grown in Ellighausen-McCoullough/Johnson-Harris (EMJH) at a density of 3 x10^8 / ml on the MacFarland scale was used. Briefly the sera of 10 µl was diluted with 90 µl of phosphate buffered saline (PBS) in 'U' microtitre plates to obtain an initial dilution of 1:10. The rest of the wells were filled with 50 µl PBS. The first wells with sera were then mixed thorough and 50 µl were drawn and shifted to second well with PBS only. In the last well after thorough mixing the 50 µl were drawn and discarded. Finally, 50 µl of the full-grown antigens was added to all microtiter plate wells and mixed thoroughly of which the final dilution was 1:160. The microtitre plates was then incubated at 30°C for two hours. The serum antigen mixture was visualized under dark field microscope for agglutination or clearance, and the titers were determined. A sample was considered positive if 50% or more of the microorganisms in the microtiter well was agglutinated with antibodies in the serum. This was determined by comparing 50% of leptospirosis which remained free with a control culture diluted 1:20 in phosphate-buffered saline. The samples that agglutinated during the screening were recorded, and the sera were further diluted to determine the end point titer for each sample. The agglutinating sera was tested again at dilutions of 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560, 1:5120, 1:10240 and 1:20480 to obtain end titer. A positive control available for the tested serovars was included and for the negative control, PBS was used.

Data analysis

Data was recorded in the Microsoft excel spread sheet. Microsoft excel was used to analyze the data in which a Chi- square ($\chi 2$) was used to calculate the prevalence.

RESULTS

Total number of small mammals captured.

The total of seventy (70) small mammals were captured in the houses. On examination of all animals it was found that they were all rodents. Two species of rodents were identified namely *Mastomys natalensis* and *Rattus rattus*. *Rattus rattus* were more prevalent than the other specie as shown in Table 1.

Table 1: Total number of small mammals captured

Species	No. of animals	Prevalence (%)
M. natalensis	20	28.6
R. rattus	50	71.4
Total	70	100

Prevalence of Leptosporisis

Among 70 rodents, 16 (22.9%) were positive for leptospira. Regarding the serovars stested, 8 (11.4%) was serovar Sokoine, 4 (5.7%) was serovar Kenya and 4 (5.7%) was covered by serovar Lora. Comparing two rodent species identified in 16 rodents that were positive for lepstospirosis, 62.5% of the positives were found to be *Rattus rattus* and 37.5% of the positives were *Mastomys natalensis*. When

considering the total captures of small mammals then the prevalence within species is as indicated in Table 2.

Table 2: Prevalence of leptospirosis per rodent specie captured

Category	Tested	Positives	Prevalence (%)
M. natalensis	20	6	8.6
R. rattus	50	10	14.3
Total	70	16	22.9

Prevalence of leptospirosis in rodents in selected clusters

High prevalence of leptospirosis was observed in serovav Sokoine followed by Kenya and last one Lora. Also within clusters there was also difference in prevalence of leptospirosis in the sevoras tested and Lora was not found totally in other clusters as indicated in Table 3.

Table 3: Prevalence of leptospirosis in rodents in selected clusters

Area	Sample (n)	Serovars		
		Sokoine % infected	Kenya % infected	Lora % infected
Mazimbu	17	17.6	5.9	0.0
Kihonda	12	0.0	8.3	0.0
NaneNane	11	9.1	0.0	0.0
Bigwa	14	7.1	7.1	21.4
Falkland	16	14.3	6.25	6.25

Titers of the Serovars Tested

High titres were observed in serova Sokoineas well as high frequencies in all titres indicated in Table 4. With exception of one sample in serova sokoine with 1:160 other samples had lower titres.

Table 4: Titers of the Serovars tested (%)

Titers	Sokoine	Kenya	Lora	Total
1:40	2 (12.5)	1 (6.3)	1 (6.3)	4 (25)
1:80	5 (31.3)	3 (18.8)	3 (18.8)	11 (68.8)
1:160	1 (6.3)	0 (0)	0 (0)	1 (6.3)
Total	8 (50)	4 (25)	4 (25)	16 (100)

DISCUSSION

The purpose of this project was to determine the prevalence of leptospirosis in the small mammals captured from fenced houses in Morogoro Municipal through blood samples. Small mammals captured were only rodents and two species were identified. It was not surprising to find higher number of *Ruttus rattus* over the *Mastomys natalensis* the former specie is predominant in the houses compared to the latter which is predominant in fallow land. The latter is mainly found in houses when there is scarcity of food in the fallow lands. Studies by Katakweba et al. (2012), and Katakweba *et al*. (2013) reported the same trend as most of *Rattus rattus* were captured in and per-domestic houses

The result of the study revealed the presence of serum antibodies for the tested Leptospiral serovars. Some studies in Morogoro have documented the presence of leptospiral antibodies in rodents, livestock, pet animals, aquatic organisms and wildlife.The MAT results of this study revealed a great reaction to serovar Sokoine. This was followed by serovar Kenya, and Lora which was predominant in rodents in Tanzania (Machang'u *et al*., 2004; Mgode *et al*., 2015).Their study was generalizing and was not specific to fenced housesin this study.

Leptospirosis is widely prevalent in rodents, shrews, humans and livestock in some parts of Tanzania (Machang'u *et al*., 1997). 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 (Katakweba *et al*., 2012). These areas are associated with human activities in the rural settlement. Some farming activities particularly for crops with high water needs including rice, sugarcane and vegetables predispose leptospirosis infection to humans through rodent urine contaminated environments (Faine, 1982; Faine *et al*., 1999). From this study it is possible for livestock, pet animals and humans to contract the disease without participating in other activities as they have been pointed out.

The overall prevalence of the disease was 22.9% of which serovar Sokoine had 11.4% and this might be due the fact that serovar Sokoine is prevalent in Morogoro and is a local isolate in the study area other two serovars had 5.7% each. The results of this study support results

from previous studies that leptospirosis is a disease that still occurs in rodents in Morogoro (Katakweba *et al*., 2012). It has been observed from this study that the distribution of serovars vary from one cluster to another. Within five clusters that were involved there was a very big difference in the prevalence and distribution within the serovars tested. This finding has epidemiological implication on this disease as different factors are involved in the maintenance of different serovars in the environment

Due to less number of captured small mammals this can be based upon to give a conclusion that *Rattus rattus* (10/50) had a high number of positive cases compared to *Mastomys natalensis* (6/20). Mathematically it can be seen that despite of few *Mastomys* spp compared to *Rattus* spp more positive could be seen in Mastomys spp. This has been also observed in the study by Katakweba *et al*. (2012), where *Mastomys* spp had high prevalence than Rattus spp. This could be explained by the fact that *Mastomys* spp are interacting with other rodent species in the field and when come into the houses they spread the disease to house rodents the *Rattus rattus* and finally both species spread the disease to livestock, pets and humans. Trapping of *Mastomys natalences* in the houses has an implication of high public health implication to fenced houses.

In this study only Sokoine, Kenya and Lora were studied and found to be prevalent in fenced houses. In the previous studies (Katakweba *et al*., 2012), *Leptospira interrogans* was found in rodent blood sera from Tanzania in the following serogroup; Icterohaemorrhagiea, Pomona, Hardjo, Ballum, Grippotyphosa and Canicola in *Rattus rattus* (21/157) and *Mastomys natalensis* (23/124). These findings further confirm that small mammals especially rodents are reservoir of lepsotospiral serovars and they are of public health threats to livestock, pets and humans. Therefore, measures should be taken in order to prevent further transmission to mammals like dogs, cats, cattle and other domesticated animals which may increase the risk of human being to be infected by that particular disease. Hence vaccination should be provided to the pet animals and sanitation should be maintained to avoid any contamination from the rodents to the human that may result into the infection.

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