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Full Length Research Paper

Seroprevalence of canine leptospirosis, in Urban and Periurban, Morogoro, Tanzania

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A cross-sectional study was carried out in the Morogoro region, Tanzania, to determine the seroprevalence of canine *Leptospira* exposure. A total of 232 sera were collected from apparently healthy dogs in Mvomero, Morogoro Urban and Morogoro Rural districts. The microscopic agglutination test (MAT) was performed following standard procedure using panel of six *Leptospira* serovars. Within the districts, positive reactions against five serovars were detected: Sokoine (4.3%); Pomona (4.3%); Lora (3.0%); Grippotyphosa (2.2%), and Kenya (0.9%). The overall seroprevalence was found to be 9.5%. Male dogs were at significantly greater risk than the female dogs ($p < 0.05$); but no significant difference in prevalence was observed with respect to age and breed ($p>0.05$). The growing urbanization, which allows high interaction between different maintenance hosts, may cause infection spill over and consequently a rising prevalence. The presence of *Leptospira* antibodies suggests that leptospirosis is common in this study area. Therefore, further serological surveys followed by isolation and identification of isolates in this study area and other areas of the country need to be undertaken to report infective serovars in canine population.

Key words: Seroprevalence, leptospirosis, serovars, epidemiology, dogs.

INTRODUCTION

Leptospirosis is one of the most important bacterial diseases in dogs (Ghasemzadeh and Namazi, 2015). Due to the climate change and shift in infective serovars, it is now regarded as re-emerging disease (Chomel, 2014; Knoepfler, 2015). Serosurveillance followed by production and usage of vaccines consisting of circulating serovars in the region are essential preventive tools in endemic regions (Senthil et al., 2013). Human leptospirosis, has received critical attention in Tanzania and animals have been suspected to be the source of the disease (Biggs et al., 2011). Although that clinical evidence of the disease exists, no isolation of the microorganism has been achieved in dogs in the country (Barnabas and Muhairwa, 2015).

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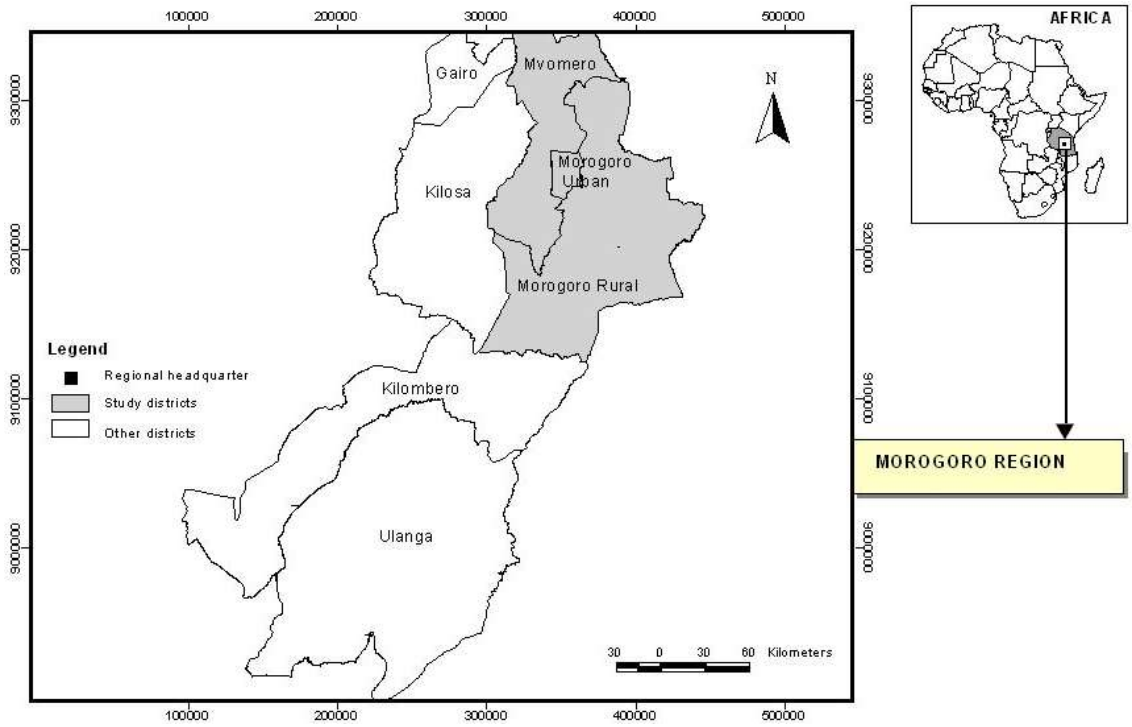

Figure 1. Map of the Morogoro region showing the study areas.

In United States and Canada, leptospirosis has been found to be more prevalent in adult male dogs (Ward et al., 2002a). In a subsequent study, Ward et al. (2002b) showed rainy tropical climate to be particularly favourable for the survival of the pathogen. Furthermore, flooding and growing urbanization are drivers for leptospirosis (Lau et al., 2010). In a study by Meeyam et al. (2006), dogs in contact with sewage-contaminated environments, or that consumed raw meat had a higher risk of contracting leptospirosis.

Canine leptospirosis has been reported in a number of African countries with varying prevalence such as Senegal 83% (Roqueplo et al., 2016), Nigeria 16.7%, Ethiopia 8.3%, South Africa (Coastal regions) 4.7%, South Africa (Gauteng Province) 50%, and Zimbabwe 15.6% (de Vries et al., 2014). In Tanzania, early studies by Machang'u et al. (1997) showed that 37% of dogs were seropositive to serogroup Icterohaemorrhagiae; this was higher than that of cattle, rodents and humans. Subsequent investigations provided further evidence of seroprevalence in African giant rats (Machang'u et al., 2004), pigs (Kessy et al., 2010) and cattle (Assenga et al., 2015). The reported seroprevalence data exhibits widespread exposure to *Leptospira* species in humans and animals in Tanzania, with dogs ranking highest, however, no clear link to the clinical disease in dogs has been established. According to Barnabas and Muhairwa (2015), records from clinic and pathology at the Sokoine University of Agriculture (SUA) Animal Hospital show the existence of seasonal morbidity and mortality suggestive of leptospirosis syndrome in adult dogs, which occur after the rainy season. Similar trends have been observed in different parts of the country such as Dar es Salaam and Dodoma, however, no research data is available to support the observations. Moreover, dogs are fed with raw condemned meats, including, kidneys from pigs and cattle, which expose them to risk of *Leptospira* infection.

There is no recent information about *Leptospira* seroprevalence in canines and no attempt has been made on identification of the circulating serogroups in Tanzania. Also, the studies carried out in dogs in the country did not determine risk factors possibly associated with the disease. The aim of this study was therefore, to determine the seroprevalence and risk factors associated with *Leptospira* infection in healthy dogs in three districts of Morogoro region, Tanzania. Knowing the *Leptospira* seroprevalence in the mentioned study areas will help to advocate a 'One Health' approach to promote multidisciplinary research efforts to improve understanding of the animal to human transmission of *Leptospira* infection in Tanzania and elsewhere.

MATERIALS AND METHODS

Study area

The study was carried out in Morogoro region, in the three districts of Mvomero, Morogoro Urban, and Morogoro Rural (Figure 1) from

November 2016 to July 2017. Morogoro region is situated between 5°58' and 10°0' S and 35°25' and 35° 30' E. The population of dogs in each district was estimated at 5645, 4624 and 10,125 in Mvomero, Morogoro Urban, and Rural districts, respectively (as per communication through District Veterinary Offices). The following wards were selected in each district; Morogoro Rural (Kingolwira and Pangawe); Mvomero (Mangae and Mzumbe); (Kihonda, Misongeni, Magadu and Mazimbu) Morogoro Urban. The study districts and wards were purposively selected based on the population of dogs.

Study animals

The study included only dogs of one year of age and above, to exclude possibility of maternal antibodies known to exist up to that age (Chappuis, 1998), currently not vaccinated against leptospirosis. Exclusion criteria included dog aggressiveness and poor health conditions not necessarily related to leptospirosis, such as emaciation and poor demeanor/obvious ill dogs.

Study design

A cross-sectional study design with random sampling of dogs was used. The sample size was estimated according to Fosgate (2009). A cross-sectional study design with random sampling of dogs was used. The wards in each district were selected purposively based on the number of dogs kept. From a list of wards that had dog population, villages were selected using a simple random technique. The list was obtained from the Village Administrative Office and was used as primary sampling unit. A total of 232 blood samples were collected. This was a convenience sampling based on the availability of both male and female dogs in the study area.

Sample collection and handling

Basic descriptive characteristics of the dog were initially recorded (that is, gender, age and breed). Apparently healthy dogs were manually restrained and blood was collected from the cephalic vein using a 25G, 2 ml syringe. Blood (2 ml) samples were then transferred to plain Vacutainer® tubes, allowed to clot overnight in a refrigerator for separation of serum. The sera were subsequently harvested and dispensed into appropriately labeled 1.5 ml cryovials, and stored at -20°C freezer at the Pest Management Centre-Leptospirosis Research Laboratory (SPMC-LRL) until subjected to MAT.

Serological analysis

Live leptospiral antigens representing six commonly found serogroups for MAT in Tanzania were used. Two of the reference serogroups Hebdomadis (*Leptospira santarosai* serovar Hebdomadis) and Pomona (*Leptospira interrogans* serovar Pomona) were initially obtained from the WHO Reference Laboratory at the Royal Tropical Institute (KIT), Amsterdam, Netherlands. The remaining were local serogroups Grippotyphosa (*Leptospira kirshneri* serovar Grippotyphosa), Icterohaemorrhagiae (*L. interrogans* serovar Sokoine), Australis (*L. interrogans* serovar Lora) and Ballum (*Leptospira borgpetersenii* serovar Kenya) provided by the Pest Management Centre, Morogoro, Tanzania. The serovars were grown in fresh Ellinghausen and McCullough medium-modified by Johnson and Harris (EMJH) (Difco-USA) for 5 to 7 days, reaching a density of 3×10^8 leptospires/ml on the MacFarland scale, according to the guidelines of WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis of the Royal Tropical Institute, Amsterdam, Netherlands.

Antibody detection

The MAT was applied to determine *Leptospira* antibodies in dog sera as described elsewhere (Cole et al., 1973). Briefly, 10 µl of the sera were mixed with 90 µl phosphate buffered saline (PBS) in 'U-shaped' microtiter plates to obtain 100 µl (1:10 dilutions). Further doubling dilutions (1:20 to 1:160) were prepared in subsequent wells and then 50 µl of the fully-grown serovars in EMJH medium was added to the sera in the microtiter plate wells and mixed gently and then incubated at 30°C for 2 h. The serum antigen mixture was then visualized by DF microscopy for the presence of agglutination and the titres recorded. A sample was considered positive if 50% or more of the microorganisms in the microtiter well agglutinated at the titre ≥1:160 (Assenga et al., 2015). This was determined by comparing 50% of spirochaetes, which remained free with a control culture diluted 1:2 with PBS (Korver, 1992). Positive samples were further diluted to titres of 1:5120 to appreciate the end point titres. Phosphate buffered saline was used as a negative control, whereby 50 µl volume of PBS was mixed with an equal volume of the different antigens.

Ethical considerations

This research was conducted in adherence with the Sokoine University of Agriculture (SUA) Code of Conduct for Research. The clearance for conducting this research was obtained from the Ethical Clearance Committee of the College of Veterinary Medicine and Biomedical Sciences, SUA, and from the District Executive Director of each of the three districts involved in this study. A verbal consent was sought from the dog.

Data analysis

Data was stored using Microsoft Office Excel® 2013. Descriptive statistics to determine prevalence was computed using EpiInfo™ software version 7.1.4.0 (2014). Statistical significance was determined at 95% CI at critical probability of ($p < 0.05$). Identification of determinant factors for *Leptospira* infection such as gender, breed and age of dogs was analyzed by Logistic Regression Model whereby all variables were entered once at a time using SPSS version 20.

RESULTS

A total of 232 dogs with no history of vaccination against leptospirosis were sampled from the three districts; Morogoro Rural (n=32), Morogoro Urban (n=122) and Mvomero district (n=78) were screened for canine leptospirosis using MAT test. The collected samples per ward are shown in Table 1. The MAT detected 14 (11.5%) positive samples from Morogoro Urban district, Morogoro Rural, 4 (12.5%) and Mvomero, 4 (5.1%). The overall *Leptospira* seroprevalence in this study was 9.5%. The serovars that showed seropositivity were: Sokoine (4.3%); Pomona (4.3%); Lora (3.0%); Grippotyphosa (2.2%); Kenya (0.9%) (Table 2). Table 3 shows that male dogs had a higher seropositivity (15.3%) compared to female dogs (2.0%). Results of the multivariable logistic regression for MAT seropositivity showed that only

Table 1. MAT test results of dogs by individual districts.

District	Ward	No. of samples	Positive samples	Seroprevalence (%)
Mvomero	Mangae	46	2	4.3
	Mzumbe	32	2	6.3
Sub total	-	78	4	5.1
Morogoro Rural	Kingolwira	12	2	16.7
	Pangawe	20	2	10
Sub total	-	32	4	12.5
Morogoro Urban	Kihonda	20	0	0
	Misongeni	46	10	21.7
	Magadu	30	4	13.3
	Mazimbu	26	0	0
Sub total	-	122	14	11.5
Total: 3 districts	8 Wards	232	22	9.5

Table 2. Prevalence of *Leptospira* serovars in canine sera collected in Morogoro Urban, Morogoro Rural and Mvomero districts.

Serovar	Number	Percentage	95% CI	
			Low	High
Hebdomadis	-	-	-	
Kenya	2	0.86	0.24	3.08
Sokoine	10	4.31	2.36	7.75
Grippotyphosa	5	2.16	0.93	4.95
Lora	7	3.02	1.47	6.1
Pomona	10	4.31	2.36	7.75

Table 3. Seroprevalence of *Leptospira* infection in dogs by sex, age and breed, in Morogoro, Tanzania.

Risk factor	Category	Seropositivity	
		Number	Percentage
Sex	Male	20	15.3
	Female	2	2.0
Age	1 year	16	11.4
	More than 1 year	6	6.5
Breed	Mongrel	17	8.7
	Mixed	5	13.5

gender difference was statistically important. Specifically, male dogs (15.3%) were more likely to have canine leptospirosis antibodies ($p < 0.004$, 95% CI = 2.1 - 44.4) compared to female dogs (Table 4).

Table 5 shows frequency of MAT titres of dog sera collected from Morogoro urban, Morogoro rural and Mvomero districts against six *Leptospira* antigens. A total of 22 sera showed relatively high titers, suggesting an active infection. Fifty-two (22.4%) of the tested animals had lower antibody levels (1:20 to 1:80) that is, below the cut-off point of 1:160 recommended (WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis of the Royal Tropical Institute, Amsterdam, The Netherlands).

Table 4. Multivariable logistic regression analysis of sex as a risk factor for leptospirosis in dogs in Morogoro, Tanzania.

Variable	p-value	OR	95% CI	
			Lower	Upper
Sex (reference female)	0.004	9.590	2.070	44.436
Age (reference more than 1 year)	0.370	1.618	0.565	4.630
Breed (reference mongrel)	0.843	0.875	0.233	3.288

Table 5. MAT titres by serovar of dog sera collected from Morogoro Urban, Morogoro Rural and Mvomero districts.

Serovar	1:20	1:40	1:80	1:160	1:320	1:640	1:2160	1:5120
Hebdomadis	3	1	1	-	-	-	-	-
Kenya	2	2	4	2	-	-	-	-
Sokoine	8	6	6	4	4	1	-	1
Grippotyphosa	4	4	0	3	2	-	-	-
Lora	4	11	5	4	3	-	-	-
Pomona	9	3	6	3	1	4	2	-

DISCUSSION

This study was carried out with the aim of establishing seroprevalence and risk factors associated with *Leptospira* infection in healthy dogs in three districts of Morogoro region, Tanzania. It was found that, 9.5% of dogs were seropositive for *Leptospira*. Age, sex and breed were the risk factors analysed, however, only sex has shown significance. Moreover, seroprevalence was higher in male as compared to female dogs. Seroprevalence among the three districts was reported as follows: Morogoro Urban district (11.5%), Morogoro Rural (12.5%), and Mvomero (5.1%).

The serology results indicate that 9.5% of dogs, with no history of vaccination had previous exposure to the *Leptospira*. These findings are consistent with those of Moch et al. (1975), who found seroprevalence of 8.3% in Ethiopia but differ from those of Mgode et al. (2015) who reported 39% prevalence in dogs. A possible explanation to the findings by Mgode et al. (2015) is that, all the reactors at titres 1: 20 were considered positive while in this study, only reactors with a titre ≥1:160 were considered positive (Cole et al., 1973; Assenga et al., 2015). According to Miller et al. (2011), there is no consensus on cut off titre that constitutes seropositivity by MAT in leptospirosis. Titres lower than 1:160 in the present study can be considered indicative of an early phase of leptospirosis or previous exposure to the organism from diverse reservoir hosts.

The current study found that dogs in the households examined have been exposed to at least five serovars, namely, Sokoine (4.3%), Pomona (4.3%), Lora (3.0%), Grippotyphosa (2.2%) and Kenya (0.9%). This study differs from that of Mgode et al. (2015), where the most predominant serovars in dogs were Sokoine (39%), Kenya (26%), Grippotyphosa (10%), Pomona (9%), Hardjo (9%), and Canicola (5%). This study agrees with studies elsewhere that, there is a high level of variation in *Leptospira* serovars in different areas. For example, in Ethiopia and Nigeria the most predominant serovar was Grippotyphosa (Moch et al., 1975; Okewole and Ayoola, 2009), while in Uganda, predominant serovars were Icterohaemorrhagiae (42.8%), Canicola (39.2%), Pyrogenes (21.4%), Tarassovi (10.7%), Grippotyphosa and Australis (7.2%) (Millan et al., 2013). Direct comparisons of seroprevalence are often difficult as the vaccination history of dogs, the MAT panel of serovars and the detection method vary among studies. Limitation of the current study was that, only six reference serovars were used in the MAT panel, living out two more serovars Hardjo and Canicola, thus might have missed the antibodies and misrepresented other unknown infecting serovar. According to Mgode et al. (2015), the requirement for leptospirosis serodiagnosis in Tanzania is to include eight serovars (Sokoine, Kenya, Grippotyphosa, Lora, Pomona, Hardjo, Hebdomadis, and Canicola). However, the panel may not be complete as other serovars could be present in the country.

Leptospirosis is therefore, a potential public health threat in the study area. This study provides information on circulating serovars in canine population and contributes to our overall knowledge to help us in designing possible preventive measures. One such measure is vaccination of dogs using the local circulating serovars to protect dog health in the study districts and elsewhere in Tanzania.

In this study, occurrence of the "pig" serovar Pomona (4.3%) in dogs, suggests that pigs in the study areas could be important maintenance hosts of this serovar. Indeed, close contact of dogs and pigs is common in the study districts due to keeping of pigs in peridomestic areas. This finding is consistent with those of United States and Canada which implicate serovar Pomona as among the main serovars causing canine leptospirosis (Prescott et al., 2002). This study has demonstrated a decline in host serogroup specificity, which according to Goldstein (2010), there are serovars pathogenic to dogs other than Icterohaemorrhagiae and Canicola. The reported seroprevalence to serovar Sokoine, which was first isolated from urine of cattle by Mgode et al (2006) and serovar Pomona which was previously reported by Kessy et al. (2010) in pigs in Morogoro suggests there is a "sharing" of *Leptospira* serovars between dogs, cattle and pigs, in Morogoro. In other countries, the serogroups of *Leptospira* found in cattle and pigs were different from those found in Morogoro. For example, in Brazilian cattle were found serogroups Sejroe, Hardjo, Tarrassovi, Bratislava, and Icterohaemorrhagiae (Guitian et a., 2001), while in pigs in Japan serogroups Australis, Icterohaemorrhagiae, and Pomona were reported (Kazami et al 2002). In Tanzania, rodents are natural carriers of *Leptospira* serovars Lora, Grippotyphosa and Kenya (Assenga, 2003; Mgode et al., 2015), which were found in this study suggesting that, rodents may be a source of *Leptospira* infection in dogs in Tanzania.

The present study observed that sera agglutinate to more than one serogroup, which implies serological cross-reactions. These reactions between serovars belonging to different serogroups can be due to mixed or two different past infections. One of the sera showed cross positivity for serogroup Icterohaemorrhagiae (*L. interrogans* serovar Sokoine) and Pomona (*L. interrogans* serovar Pomona), while two samples were positive for serogroup Grippotyphosa (*L. kirshneri* serovar Grippotyphosa) and Pomona (*L. interrogans* serovar Pomona); and one sample each for serogroup Ballum (*L. borgpetersenii* serovar Kenya), Icterohaemorrhagiae (*L. interrogans* serovar Sokoine), Grippotyphosa (*L. kirshneri* serovar Grippotyphosa), Australis (*L. interrogans* serovar Lora) and Pomona (*L. interrogans* serovar Pomona), and for serogroup Grippotyphosa (*L. kirshneri* serovar Grippotyphosa), Australis (*L. interrogans* serovar Lora) and Pomona (*L. interrogans* serovar Pomona), respectively. According to Felt et al. (2011), cross-reactions are reported in acute or early convalescent sera, whereby the host, infected previously with one serogroup, may successively become infected by another serogroup, and the recently acquired serogroup may cross-react to the previous one, leading to activation of the memory response against the subsequent serogroup.

A significantly higher seroprevalence was recorded amongst male dogs ($p < 0.004$) as compared to female dogs similar to what was previously reported by Ward et al. (2002a). This is possibly due to increased outdoor activities, sniffing habits and the licking of external genitalia by infected males and females (Luna et al., 2008; Cisneros et al., 2002). Similar trend was observed in another study conducted by Meeyam et al. (2006), who reported dogs with increased outdoor activities or that consumed raw meat to be at a higher risk of leptospirosis. However, the later was not the case in this study area, as majority of the dog owners fed their dogs with stiff porridge mixed with sardines. However, there was no significant difference in prevalence with respect to age which contradicts with Ward et al. (2002a) who reported that male dogs' age 4 to 10 years old had significantly increased *Leptospira* antibody levels. The majority of the dog sampled were the mixed breed (n = 195) and a few Mongrel breeds (n = 37). This sample was not adequate to detect differences in prevalence of positive leptospiral titres by breed in the total population of 232 dogs sampled.

This study demonstrated *Leptospira* antibodies in unvaccinated, clinically healthy dogs at titres ≥160. The findings provide further support to the assertion that dogs could serve as maintenance hosts of the spirochete to other animals. Improving hygiene and removal of rodents may also reduce the risk of infection in dogs.

Conclusion

This study has found that leptospirosis is a potential health risk to dogs in Tanzania.The significantly higher seroprevalence amongst dogs from Morogoro rural compared with those from Morogoro urban and periurban districts may be indicative of differences in area-level risk factors. Sex was identified as a risk factor for leptospirosis where male dogs are significantly at higher risk of infection as compared to female dogs. Moreover, sharing environment amongst domestic animals, that is, dogs, pigs and cattle may result into infection spill over, consequently rising prevalence and risk of acquiring leptospirosis by dog keepers.

Further serological surveys using antigen panels with more serovars is required. In addition, isolation and identification of locally circulating serovars are needed. Vaccination of dogs using local leptospiral antigens should be encouraged and done alongside thee control of distemper, hepatitis and other canine diseases. Also, disease prevention programs for pet owners need to be instituted to reduce the public health risk of pet leptospirosis. Furthermore, implementation of efficient management of rodents and infection in livestock must be emphasized.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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