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Research Article

Determining Factors for Acquiring Human Leptospirosis from Dogs along Morogoro Municipality, Tanzania

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Abstract

Introduction: Leptospirosis is a global zoonotic disease of which natural reservoirs are rodents. However, diverse mammals, including pets, serve as transient maintenance hosts and often pass the disease to humans. Due to their mutualistic association with humans, infected dogs may serve as important vectors of leptospirosis. Human get infected after coming in contact with an environment contaminated with the urine of infected dogs. A serological study was conducted in free roaming and confined dogs in Morogoro, Tanzania, to determine the prevalence of leptospirosis in the canines.

Methodology: Blood samples were collected from 155 and 110 free roaming and confined dogs, respectively. Serum was separated by centrifugation at 3500 rpm for five minutes in the laboratory at the Institute of Pest Management and examined using the Microscopic Agglutination Test (MAT). Tested *Leptospira* spp antigens included serovars Sokoine, Pomona, Hebdomadis, Grippotyphosa and Kenya.

Results: The overall seroprevalence of leptospirosis in the dogs was 163 (61.5%), of which 88 (54.0%) and 75 (46.0%) had single and multiple leptospira serovars respectively. Furthermore, seroprevalence of 67.3% and 35.78% for free roaming and confined dogs respectively were recorded. The serovars in infected dogs were: Sokoine (54.6%), Pomona (41.1%), Grippotyphosa (23.9%), Hebdomadis (22.7%), and Kenya (19.6%). The seroprevalence for free roaming dogs was; serovar Sokoine (45.2%), Pomona (22.6%), Hebdomadis (18.7%), Grippotyphosa (16.8%) and Kenya (15.5%). In the confined dogs the serovar prevalence were Sokoine (14.7%), Grippotyphosa (7.3%), Kenya (3.7%), Pomona (22.0%) and Hebdomadis (4.6%). Of the positive dogs, 82 were males and 81 were females of which 133 and 30 were adults and puppies, respectively.

Conclusion: Higher prevalence of leptospirosis was found in free roaming than in confined dogs and serovars Pomona and Sokoine were the most frequent in the two groups of animals. Free roaming dogs have a bigger chance to be exposed to *Leptospira* spp due to broader interactions in diverse environments. Confinement and vaccination of dogs should be encouraged in Morogoro to reduce leptospirosis transmission in the animals and incidentally humans.

Keywords: Leptospirosis; Confined; Free Roaming; Dogs; Serovars; Morogoro

Introduction

Leptospirosis is a zoonotic water borne bacterial disease with a worldwide distribution, and is an emerging infectious disease in humans and dogs [1-4]. The global warming that leads to extreme weather events such as cyclones and floods, increased rainfall, and increased world population and urbanization are the factors that lead to increased disease incidences [4-7]. The disease prevails in urban and rural settings in industrialized and developing countries, especially in the tropics [8,9] compared to the dry or cold climates [10].

The disease has been neglected as a public health problem due to lack of awareness thus leading to under/misdiagnosis and reporting. Leptospirosis is ubiquitous in distribution and is both an occupational hazard and anthropo-zoonotic; also, it has been recognized as a re-emerging global public health problem due to the increased incidence in both developing and developed countries [7,11].

Until recently, the genus *Leptospira* comprised of two species: *Leptospira interrogans* that are pathogenic causing Leptospirosis and *Leptospira biflexa*, with saprophytic strains. The pathogenic spirochetes are currently classified under not less than 10 species, further subdivided into several serogroups and serovars based on antigenic differences [12,13]. There are more than 200 leptospira serovariants that are identified as pathogenic and more than 60 as non-pathogenic [14].

In dogs, leptospirosis is caused by pathogenic serovars of the Leptospira capable of causing acute fatal disease [15,16]. Wild animals, especially rats, and diverse domestic animals are reservoirs of pathogenic Leptospira [17-19]; of which they maintain in the proximal renal tubules of the kidney and shed the organisms in the urine. Leptospirosis has often been serologically identified in stray dogs [20-22]. Canine leptospirosis is worldwide distributed and, canines serve as incidental maintenance hosts for various leptospira serovar strains, primarily serovar Canicola [1], but can also be infected with other serovars, such as Icterohaemorrhagiae, Grippotyphosa and Pomona [16].

Leptospirosis is a disease that shows a natural nidality, where each serovars tends to be maintained more or less in specific maintenance hosts [1]. In any region, domestic animals can be infected by serovars maintained within or outside species. The relative importance of these incidental infections can be determined by prevailing social, management, and environmental factors which provide contact and transmission of leptospires from other species [12].

Transmission generally occurs after a susceptible animal gets in contact with infected urine, contaminated water, mud or moist soil [23]. Shedding animals pose a public health risk to humans who get in contact with urine and contaminated environments, particularly water [1]. The disease is characterized by septicaemia, renal and hepatic diseases, coagulopathies, abortions and other abnormalities, with a case fatality rate of 10% to 20% in dogs [24,25]. Infected dogs may present with renal failure or be carriers of leptospiral organisms in their proximal convoluted renal tubules. This subclinical form may take 1 to 2 years with the leptospires being persistently shed in urine [18].

In Morogoro, limited studies have been done to determine the prevalence of canine leptospirosis, therefore there is paucity of data with regard to this disease. The objective of this study was, therefore, to determine the prevalence and associated risk factors of leptospirosis in confined and free roaming dogs in Morogoro municipality.

Materials and Methods

Study areas and sample size

The study was conducted in urban and peri urban areas of the municipality of Morogoro, Tanzania and included confined and free roaming dogs The samples were obtained by convenience for the free roaming dogs from 15 administrative wards of Morogoro municipality. For confined canines, households keeping dogs were randomly selected from seven administrative wards.

Study design

This was a cross-sectional study to determine the seroprevalence of canine leptospirosis in Morogoro municipality. In addition to the screening of canine sera, questionnaires were administered to dog owners with respect to locality, whether other animals were kept in their residential areas, along with dogs, the vaccination status, age, sex and breed of the dogs.

Sample collection and processing

Blood was collected from the cephalic vein, using 25G, 5ml syringes, transferred into plain vacutainer tubes and stored in a cool box with ice packs and transported to SUA Institute of Pest Management Laboratory. The blood was then centrifuged at 3500 rpm for 5min. and the serum aspirated and transferred to sterile micro centrifuge tubes and stored at -20oC in the refrigerator until serological testing [26].

Serological test

The microscopic agglutination test (MAT) was performed on all sera by the standard method described elsewhere [27, 28]. Different Serogroups (serovars in brackets) included in MAT were: Icterohaemorrhagiae (Sokoine), Grippotyphosa (RM4), Kenya, Pomona (Pomona) and Hebdomadis. 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 approximately 3 × 108 leptospires/ml on the MacFarland scale, according to the guidelines of WHO/FAO/WOAH Collaborating Centre for Reference and Research on Leptospirosis [26]. The test involved mixing appropriate dilutions of serum with live leptospires of the serovars mentioned above and the presence of homologous antibodies was indicated by the agglutination of the leptospires, with the reported titers being the highest dilution of serum which resulted into 50% agglutination [26]. In our case, a sample was considered positive if the agglutination occurred at a titer ≥1:160 [29] compared to the negative control in which serum was replaced with phosphate-buffered saline (PBS) [30].

Statistical analysis

Results were expressed as actual numbers and proportions (%). To explore the relationship between the categorical variables in this study, Chi-square test was used. Both univariable and multivariable logistic regression analyses were used to determine the association between the dependent (Test positive compared to test negative to Leptospirosis) and independent variables. The best model was chosen based on small Akaike Information Criterion (AIC) value. P value less than 0.05 was considered significant. All the statistical analyses were performed by R statistical software version 1.4.1106.

Results

In this study, 265 blood samples from dogs were screened for leptospirosis. It was found that 163 (61.5%) of the dogs tested positive, among which 88 (54.0%) and 75 (46.0%) had single and multiple leptospira serovars respectively, whereas 102 (38.5%) dogs tested negative. The seroprevalence by the two groups were 67.3% and 35.78% in the free roaming and confined dogs respectively. The overall distribution of the serovars in infected dogs was as follows: 98 (54.6%) serovar Sokoine, 67 (41.1%) Pomona, 39 (23.9%) Grippotyphosa, 37 (22.7%) Hebdomadis, and 32 (19.6%) for Kenya. In free roaming dogs, the percentage prevalence by serovar was as follows; serovar Sokoine (45%), Pomona (23.08%), Hebdomadis (19.2%), Grippotyphosa (16.67%) and Kenya (16.03%). In the confined dogs the percentage prevalence by serovar was as follows; Pomona (22%), Sokoine (14.7%), Grippotyphosa (7.3%), Hebdomadis (4.6%) and Kenya (3.7%). The serovar Sokoine was prevalent in most of the dogs that tested positive regardless of the management system. However, for confined dogs, serovar Pomona was found in every dog positive for the disease. Furthermore, among the positive tested dogs, 82 were males and 81 were females and of these, 133 and 30 were adults and puppies respectively.

Risk factor		Groups		P value
		Infected	Non infected	
		163	102	
Sex	Male	82	56	
	Female	81	46	0.547
Breed	Mongrel	137	83	
	Cross	26	19	0.6917
Age	Adult	133	70	
	Puppy	30	32	0.0227
Management	Free range	107	49	
	Confined	56	53	0.0068
Vaccination status	Vaccinated	54	35	
	Not vaccinated	109	67	0.9481
Other livestock	Yes	67	31	
	No	96	71	0.1038

Table 1: Risk factors contribution to the Leptospirosis.

Risk factor		Odds ratio	P value
Sex	Female	1(Ref)	0.002
	Male	0.057	0.324
Breed	Cross	1(Ref)	0.208
	Mongrel	0.056	0.398
Age	Adult	1	0.000
	Puppy	0.034	0.016
Vaccination	No	1(Ref)	0.002
	Yes	0.065	0.281
Origin	Free range	02.07	0.005
	(Intercept)	1(Ref)	0.706

Table 2: Association between Infected dogs and other variables using univariate logistic regression analysis.

The univariate logistic regression analysis indicated that there were significant differences in age and management system between infected and non-infected dogs. By multivariate logistic regression analysis, puppies (OR = 0.5) and management system (OR = 1.9) were considered as independent predictive variables for testing positives for leptospirosis. There were no statistically significant differences in other variables between infected and non-infected dogs.

Serovar	Status	N (%)	P value
Sokoine	Negative	167 (63.02)	<.0001
	Positive	98 (36.98)	
Grippotyphosa	Negative	226 (85.28)	<.0001
	Positive	39 (14.72)	
Kenya	Negative	233 (87.92)	<.0001
	Positive	32 (12.08)	
Pomona	Negative	198 (74.72)	<.0001
	Positive	67 (25.28)	
Hebdomadis	Negative	228 (86.04)	<.0001
	Positive	37 (13.96)	

Table 3: Association of serovars in infected dogs.

Titres	SOK		GRYP		KEN		POM		HEBD		TOTAL	
	CND	FRD	CND	FRD	CND	FRD	CND	FRD	CND	FRD	CND	FRD
A	4	10	3	4	0	13	4	18	0	16	11	61
B	2	15	1	10	2	9	7	7	1	1	13	42
C	2	21	3	7	1	1	4	6	1	5	11	40
D	2	9	1	4	1	1	3	3	2	1	9	18
E	1	4	0	1	0	0	3	0	0	4	4	9
F	1	3	0	0	0	0	1	1	1	0	3	4
G	2	2	0	0	0	0	2	0	0	2	4	4
H	1	3	0	0	0	0	0	0	0	0	1	3
I	1	2	0	0	0	0	0	0	0	0	1	2
J	0	1	0	0	0	0	0	0	0	0	0	1
Total	16	70	8	26	4	24	24	35	5	29	57	184

Table 4: Antibody titers of the serovars tested

Key:

1. A – 1:20, B = 1:40; C = 1:80, D = 1:160; E = 1:320; F = 1:640; G = 1:1280; H = 1:2560; I = 1:5120 and J = 1:20480

2. SOK: Sokoine; GRYP: Grippotyphosa; KEN: Kenya; POM: Pomona; HEBD: Hebdomadis; CND: Confined Dogs; FRD: Free Roaming Dogs

Discussion

This study shows that there is a high prevalence of canine leptospirosis in Morogoro municipality. The results showed that free roaming dogs had a higher disease prevalence than confined dogs, which could be explained by the fact that free roaming dogs are more likely to be exposed to different contaminated environments including, abattoir areas, carelessly disposed carcasses, or during scavenging for rodents for food. The findings of this study concur with the study conducted in Morogoro by Katakweba., *et al*. [31] who identified a total of 52 Leptospira isolates from fresh urine and kidney homogenates, collected between 1996 and 2006 from small mammals, cattle, and pigs.

Apart from being exposed to different environmental contaminants, the feeding regime of free roaming dogs is not controlled. These dogs feed on any type of food available including garbage and rodents thus increasing the chance of catching leptospirosis. Confined dogs, however, are more likely to receive hygienic food and veterinary care (including vaccinations and treatment.

The local Leptospira serovars Sokoine and Kenya are the predominant serovars found in many vertebrate species, including rodents, cattle, pigs, fish, and humans in Tanzania [32-34]. It was, however, interesting that in this study, serovar Kenya was the least prevalent in both groups of the studied canines. Serovar Sokoine has been isolated from cattle, rodents (*Mastomys natalensis* and *Cricetomys ansojei*), and shrews (*Crocidura* spp.) [33, 35]. They are also the predominant serovars in tilapia and catfish species [34]. This indicates a sharing of Leptospira pathogens between terrestrial mammals such as rodents, cattle, dogs, and aquatic animals. The high prevalence of serovar Sokoine and Kenya in dogs was recently revealed [26] which agrees with the study by Mgode., *et al*. (34). Serovar Sokoine belongs to serogroup Icterohaemorrhagiae often reported in humans [17].

Serovar Pomona also showed relatively high prevalence of up to 22% and this might be due to pigs (the reservoir host for this serovar) being kept in peridomestic areas of residences keeping dogs as well [26] thus allowing the contact of the canines with excreta (urine) from pigs, rodents or cattle in the domestic environment. The antibodies to serovar Canicola which could be of interest in this study could not be detected due to the absence of its antigen in stock during the study time.

There was a significant difference between puppies and adult dogs with regard to Leptospira infection rate. Puppies were less affected than adults, possibly due to limited exposure to risk factors and possibly passive maternal immunity. Also, adult mature dogs are more likely to roam, especially when bitches are on heat. The number of puppies sampled in this study was low compared to the adults and this could have led to underestimation of the disease seroprevalence. There were no significant differences among the other risk factors (sex, breed, vaccination status and whether or not other livestock were kept in the households with the sampled dogs). The previously reported diversity of reservoir hosts of leptospirosis in Tanzania, namely rodents, bats, domestic animals [32,35-37] and this study report of leptospirosis detection in dogs call for enhancement of public awareness of this emerging zoonotic disease in Morogoro and elsewhere the country.

Conclusion

This study has shown that Leptospiral infection is prevalent in confined and free roaming dogs in Morogoro. This situation could be indicative of a wider prevalence of the disease in other susceptible animals and humans and hence a potential animal and public health significance. It is highly recommended that all domestic dogs should be vaccinated against the disease and free roaming dogs be restricted. In addition, further studies over a wider geographical coverage of Tanzania should be undertaken to map leptospirosis in the country.

Conflict of Interest

The authors declare no conflict of interests.

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