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Research Article

SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH CANINE LEPTOSPIROSIS IN HARYANA, INDIA

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ABSTRACT

Leptospirosis caused by *Leptospira* spp. is a widespread zoonosis and has been recognized as a re-emerging infectious disease in humans and dogs. In Haryana, the disease is merely studied and shedding of the pathogen among dogs is poorly understood. The aim of the present study was to determine the seroprevalence of *Leptospira* infection in apparently healthy dogs and the risk factors associated with sero-positivity. Out of 239 tested serum samples, 9.62% were detected positive for leptospirosis by IgM based ELISA assay. For risk assessment of environment contamination of *Leptospira* organism, host and environmental determinants for leptospirosis occurrence were considered in the present study. Risk factors considered for risk assessment of leptospirosis were not found statistically significant. However, the study showed low risk of transmission of infection from dogs to human beings in Haryana, India.

Keywords: Enzyme linked immunosorbent assay, *Leptospira*, Risk factors, Seropositivity, Seroprevalence

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Leptospirosis is an emerging anthropozoonosis of global importance caused by bacteria of the genus *Leptospira*. The infection is contracted either through direct contact to urine of an infected animal or indirectly by exposure to *Leptospira* contaminated water. Leptospiral infection in dogs can result in an acute hepatorenal failure, or it can also lead to asymptomatic chronic carrier state. Chronic carriers may act as a source of infection and, for this reason, are of public health concern.

The diverse clinical presentations of this disease make it essential for the laboratory to play a role in diagnosis. Microscopic agglutination test (MAT) is considered as the gold standard for the diagnosis of leptospirosis. MAT has been used widely in various studies for the detection of *Leptospira* antibiodies, which is based on serology, is often used as the gold standard in the present study, seroprevalence and risk assessment for canine leptospirosis was conducted using IgM based ELISA and statistical analysis, respectively.

MATERIALS AND METHODS

Serum samples of dogs were collected from 13 districts of Haryana state, namely Panchkula (n=30), Karnal (n=1), Sirsa (n=3), Hisar (n=84), Fatehabad (n=3), Rohtak (n=39), Bhiwani (n=8), Jhajjar (n=2), Charkhi Dadri (n=1), Sonipat (n=1), Gurugram (n=14), Palwal (n=6) and Faridabad (n=31). A total of 239 blood samples were collected from the cephalic or saphenous vein of both male (n=179) and female (n=60) dogs and drawn into BD vacutainer tubes (BD Diagnostics, New Jersey, USA). The collected blood samples were left to clot for 30 min and serum was separated by centrifugation at 2000×g for 10 min. Serum samples were kept at -20ºC till further use. During transportation of serum samples to the Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences, LUVAS, Hisar, an ambient temperature of 4 ºC was maintained. All collected serum samples were tested using commercially available canine *Leptospira* IgM based ELISA kit (cat. no. LT36109EAYQ, Life technologies Pvt. Ltd. India) as per recommended procedure. Briefly, all dilution of standards were prepared by diluting a standard solution of known concentration (48 ng/L) in eppendorf tubes by following the instructions supplied with the kit. The optical density (O.D) was measured at 450 nm using a microtiter plate reader within 15 min.

The minimum detectable dose of canine *Leptospira* IgM antibodies of the ELISA kit was typically less than 0.1 ng/L in the study. A questionnaire was prepared to assess risk factors associated with leptospirosis occurrence and was filled at the time of sample collection. The association of variables derived from the questionnaire and occurrence of leptospirosis was ascertained by determining odds ratio, 95 percent confidence interval and P-values of < 0.05 was considered statistically significant. All the statistical analyses were performed using STATA ™ I.C. 15.0. The location of residents of dogs was mapped with the help of QGIS software.

RESULTS AND DISCUSSION

The optical density of standard solutions at different dilutions (concentration of IgM antibodies) i.e. at 1:2 (24 ng/L), 1:4 (12 ng/L), 1:8 (6 ng/L), 1:16 (3 ng/L), 1:32 (1.5 ng/L) was 0.9259, 0.4719, 0.2843, 0.1452 and 0.1042,

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respectively. Optical densities of positive and negative samples were in the range of 0.1097 to 0.5175 and 0.0396 to 0.0944, respectively. The ELISA titre was 1:32 i.e., as per kit guidelines, samples having IgM antibody concentration 1.5ng/L or more were taken as positive for canine *Leptospira*. Concentration of IgM antibodies in all serum samples was calculated from equation Y= 0.815X-1.119, which was derived from standard curve (Fig. 1).

Out of tested 239 serum samples, 23 (9.62%) were positive for canine *Leptospira* IgM antibodies. In a study of Kerala, out of 205 canine serum samples (healthy vaccinated-35, healthy unvaccinated-30 and rest were acute infected), 146 (71.12 %) were positive, which included 30 healthy vaccinated and acutely infected but all the healthy unvaccinated were found negative (Ambily *et al.*, 2013). In Bareilly, Utter Pradesh, out of the ten sera samples from clinically suspected dogs, only one was observed to be positive for leptospirosis (Inbaraj *et al.*, 2019).

Seroprevalence of *Leptospira* was reported from other countries i.e in Chile of South America- 3% (Moya *et al.*, 2018) followed by in Malaysia- 3.1% (Lau *et al.*, 2017) and Brazil- 8.82% (Silva *et al.*, 2017), that was found lower than the present study.

The seropositivity by serological methods was more than the present study, in studies of other countries i.e. in Switzerland- 55.7%, Colombia- 36.46% and in Malaysia-22.2% (Goh *et al.*, 2019).

In different categories of risk factors seropositivity, 95% CI, p-value and odds ratio was calculated (Table 1.). Non-sporting/others breed group dogs were showed highest seroprevalence (13.21%). In the present study, breed was not statistically associated with occurrence of infection, in conformity with the study conducted in Brazil (Kikuti *et al.*, 2012). However, other studies have reported high risk of infection in non-descriptand working breeds (Harland *et al.*, 2013; Azocar-Aedo and Monti, 2016).

In young adult age group (7 months-3 years) of dogs, a higher (10.08%) sero-prevalence was recorded. Young adult age group dogs likely to more travel with their owners to recreational areas and are at increased risk of acquiring the infection. In this study, higher number screened dogs were from young adult age group compared other age groups of dogs (Table 1). This might have contributed to a significant association between disease and young adult age group. In present study, age did not represent a risk factor, which is in agreement with other studies conducted in Brazil, Thailand and Pakistan (Kikuti *et al.*, 2012; Saleem *et al.*, 2013). Azocar-Aedo and Monti (2016) found that dogs less than 1 year old age were more sero-positve to leptospirosis than other age groups; however, statistically significant association was not reported by the researchers. In contrast, Saleem *et al.* (2013) reported higher occurrence of leptospirosis in dogs with age more than 6 months.

In the present study, association between sex and sero-prevalence of leptospirosis was found non-significant. The studies of other authors are in agreement with present study which showed higher sero-prevalence in male dogs but not found statistically significant (Kikuti *et al.*, 2012). However, Azocar-Aedo and Monte (2016) and Goh *et al.* (2019), reported statistically significant association of higher sero-prevalence leptospirosis with male dogs.

Sero-prevalence of leptospirosis was more in unneutered dogs as compared to neutered dogs which lead to the hypothesis that sexually intact animals manifest more often risky behaviours like sniffing and marking their territories. Other studies had also shown significantly higher prevalence rates in neutered dogs than unneutered ones (Major *et al.*, 2014).

In present study, unvaccinated dogs showed higher (11.39%, n=79) seroprevalence as compared to vaccinated group of dogs (Table 1). No statistically significant difference was found between the vaccinated and unvaccinated groups. It was considered that the antibodies present in unvaccinated groups of dogs were produced by infection. In Japan, a study performed by Iwamoto *et al.* (2009), out of 243 unvaccinated dogs, 41 (16.9%) were positive by MAT and ELISA which was higher as compared to present study (11.39%).

In the present study, dogs living in villages showed higher (11.43%, n=35) sero-prevalence than living in farm houses and flats/houses (p=0.86) (Table 1). This observation is in agreement with a study conducted in Switzerland, wherein no significant association was found in living environment and sero-prevalence (Delaude *et al.*, 2017). An increased sero-prevalence of leptospirosis was found in a study of dogs living in rural as compared to urban areas in Northern California. In contrast, a meta-analysis identified a higher risk in urban environments (Azocar-Aedo and Monti, 2016).

In the present study, a high sero-prevalence (12.5%, n=24) was observed in dogs kept outdoor but was not statistically significant (Table 1). Goh *et al.* (2019) found that shelter dogs were housed in large numbers in limited space, thus increasing possible dog-to-dog contact (Cutler *et al.*, 2010). It may increase risk of transmission of leptospirosis and prolonged dog handler-dog contact time increases the risk of sero-positivity, suggesting a major role of dogs.

Seroprevalence in dogs kept with other pets or farm animals was more (14.11%, n=85) than others which were not kept with other companion animals (7.14%, n=154

(Table 1). Dogs kept with buffaloes showed highest (16.1%, n=31) sero-prevalence because of their natural infection with the disease but no statistically significant difference was found. In farm animals disease can be in subclinical form and considered as a potential source of leptospirosis to dogs.

Dogs that were not used to swimming showed higher (10.1%, n=178) sero-prevalence as compared to dogs having habit of swimming but the difference was not statistically significant (Table 1). High sero-prevalence might be due to more number of samples (n=178, 74.47%) taken from this category of dogs. In contrast, in a study, dogs used to swimming were at higher risk of infection than not used to swimming (Ghneim *et al.*, 2007).

Dogs used to drink from outdoor sources of water were more (11.63%, n=86) seropositive than the dogs not used to drink from outdoor sources of water (Table 1). But association between drinking from outdoor sources of water and sero-prevalence was not statistically significant. A significant association was observed between occurrences of leptospirosis in dogs and presence of wastewater bodies near the home where dogs were kept in Argentina (Cardenasa *et al.*, 2019).

In the present study, the dogs which were provided with commercial food showed higher (Table 1) seroprevalence than other category of dogs given homemade and both homemade and commercial food (p=0.32, Table 1). This finding may be due to contamination of storage places of commercial feed with rodents and their urine and unhygienic conditions during preparation of feed. Aguiar *et al.* (2007) also reported that dogs fed commercial food were more likely to get the infection than dogs fed home diets.

In category of dogs which have no record of contact with wild animals showed more 9.79%, n=143) sero-prevalence (Table 1). However, no statistically significant difference was found in different categories. In another study conducted by Ghneim *et al.* (2007), wild animals were found as a source of infection and transmission of leptospirosis to dogs, domestic animals and human beings.

Rodents are recognized as the most significant reservoir of leptospires worldwide (Delaude *et al.*, 2017). In present study, dogs living in houses infested with rodents were showing higher (11.4%, n=114) sero-positivity than other category of dogs but associations were not statistically significant (Table 1). In a study of Malaysia (Goh *et al.*, 2019) presence of rats was shown as the significant predictor for the dogs' seropositivity for leptospirosis.

Highest seroprevalence was found in Sirsa (33.33%, n=3) and Fatehabad (33.33%, n=3) districts of Haryana but the difference (Table 2, Fig. 2) was not statistically

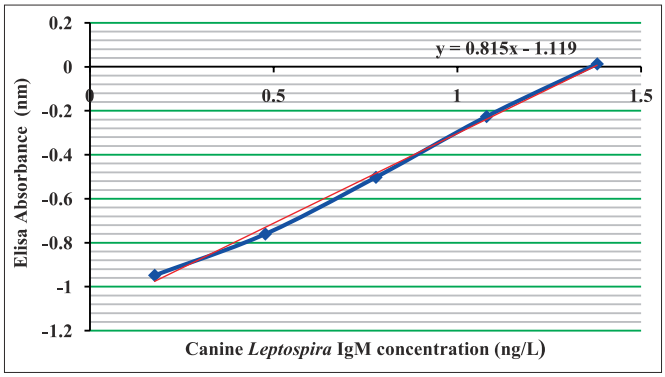

Fig. 1. Standard curve depicting concentration of Leptospira IgM antibodies determined in standard diluents

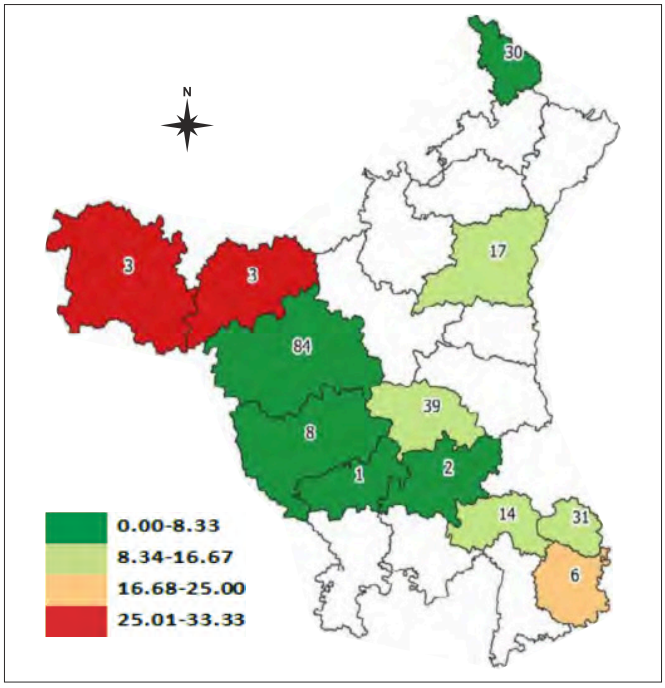

Fig. 2. Map showing seropositivity in different districts of Haryana (Hisar- 8.33%, Bhiwani- 0%, Charkhi Dadri- 0%, Faridabad - 12.9%, Fatehabad- 33.33%, Gurugram- 14.29%, Jhajjar- 0%, Karnal-11.76%, Palwal- 16.68%, Panchkula- 3.33%, Rohtak- 10.26%, Sirsa- 33.33%, Sonipat- 0%)

significant. However, in Sirsa and Fatehabad, there was little or no rainfall during the whole year. But high seroprevalence in these areas than other areas may be due to variations in number of samples collected from different areas of Haryana i.e. less number of samples were collected from Sirsa and Fatehabad (n=3). Climate may be an important factor affecting the prevalence of Leptospira in each area. Acha and Szyfres (2001) emphasized the higher occurrence of leptospirosis in tropical and subtropical regions, due to the better viability of the agent in this type of environment.

The risk factors taken in the present study did not show any statistical association with seroprevalence of leptospirosis. Questionnaires that aim to determine the risk

Table 1. Seroprevalence of leptospira in different categories of risk factors

Sr. No.	Risk Factor	No. of dogs	No. positive (%)	95% CI	p-value	Odds Ratio
1	Breed					
	i) Toy and Terrier	33	1 (3.03)	0.08-15.76		
	ii) Sporting	78	6 (7.69)	2.88-15.99	0.086	0.89
	iii) Working/Utility	75	9 (12.00)	5.64-21.56		
	iv) Non-sporting/others	53	7 (13.21)	5.48-25.34		
2	Age					
	i)Young adult (7months-3year)	119	12 (10.80)	5.32-16.95		
	ii) Adult (>3 years-6 years)	83	8 (9.64)	4.25-18.11	0.74	0.97
	iii) Old age (>6 years-9 years)	37	3 (8.11)	1.70-21.91		
3	Sex					
	i) Male	179	21 (11.73)	7.41-17.37	0.07	0.26
	ii) Female	60	2 (3.33)	0.64-17.75		
4	Neutered					
	i) Yes	25	2 (8.00)	0.98-26.03	0.77	0.8
	ii) No	214	21(9.81)	6.18-14.61		
5	Vaccination					
	i) Yes	142	14(9.86)	5.50-15.99		
	ii) Do not know	18	0(0.00)	0.00-18.53	0.51	1.06
	iii) No	79	9(11.39)	5.08-19.59		
6	Dogs kept in different places					
	i) Farm house	18	2 (11.11)	1.38-34.71		
	ii) Flat/house	186	17 (9.14)	5.41-14.23	0.86	1.08
	iii) Village	35	4 (11.43)	3.20-26.74		
7	Dogs kept at different locations					
	i) Indoor	206	9 (9.22)	5.64-14.03		
	ii) Both	9	1(11.11)	0.28-48.25	0.59	1.19
	iii) Outdoor	24	3 (12.5)	2.66-32.36		
8	Dogs kept with other pets or farm animals					
	i) Yes	85	12(14.1)	7.42-23.11	0.09	2.09
	ii) No	154	11 (7.14)	3.64-12.50		
9	Dogs kept with different type of animal					
	i) Buffalo	31	5 (16.13)	5.45-33.73		
	ii) Dog	50	7 (14.00)	5.82-26.74	0.5	0.78
	iii) Dog and buffalo	4	0 (0.00)	0.00-60.24		
	iv) Dogs not kept with any companion animal	154	11 (7.14)	3.72-12.74		
10	Drinking from outdoor sources of water					
	i) Yes	86	10 (11.63)	5.72-20.35		
	ii) Do not know	16	2 (12.50)	1.55-38.35	0.35	1.37
	iii) No	137	11 (8.03)	4.08-13.91		
11	Dogs given different type of food					
	i) Homemade food	160	13 (8.13)	4.40-13.49		
	ii) Commercial food	75	9 (12.00)	5.64-21.56	0.32	1.24
	iii) Both	4	1 (25.00)	0.63-80.59		
12	Swimming					
	i) Yes	29	2 (6.90)	0.85-22.77		
	ii) Do not know	32	3 (9.38)	1.98-25.02	0.77	0.91
	iii) No	178	18 (10.11)	6.10-15.51		
13	Contact with wild animals					
	i) Yes	10	0 (0.00)	0.00-30.85		
	ii) Do not know	86	9 (10.47)	4.90-18.94	0.91	1.02
	iii) No	143	14 (9.79)	5.46-15.88		
14	Dogs living in houses infested with rodents					
	i) Yes					
	ii) Do not know	114	13 (11.40)	6.21-18.71		
	iii) No	17	1 (5.88)	0.15-28.69	0.79	1.1
		108	9 (8.33)	3.88-15.23		

Table 2. Seroprevalence of *Leptospira* in different districts of Haryana State

District (No. of samples)	No. Positive (%)	95% CI	p-value	Odds Ratio
Hisar (84)	7 (8.33)	3.20-15.06		
Bhiwani (8)	0 (0.00)	0.00-36.94		
Charkhi Dadri (1)	0 (0.00)	0.00-97.50		
Faridabad (31)	4 (12.90)	3.42-28.87	0.89	0.99
Fatehabad (3)	1 (33.33)	0.84-90.57		
Gurugram (14)	2 (14.29)	1.78-42.81		
Jhajjar (2)	0 (0.00)	0.00-84.19		
Karnal (17)	2 (11.76)	1.46-36.44		
Palwal (6)	1 (16.68)	0.42-64.12		
Panchkula (30)	1 (3.33)	0.08-17.22		
Rohtak (39)	4 (10.26)	2.51-23.03		
Sirsa (3)	1 (33.33)	0.84-90.57		
Sonipat (1)	0 (0.00)	0.00-0.00		

factors for canine leptospirosis may not be suitable for use in preventive programs because of the limited degree of observation that owners may have in relation to their own dogs. The precise role of the dog in the epidemiology of leptospirosis is difficult to establish and remains controversial (Martins *et al.*, 2012). It is likely that marked differences exist between areas with different geographic, wildlife, social, and economic features, and that the role of the dog may vary depending on these parameters. A close contact of many humans with dogs and their full integration in the household bring the potential of high exposure, particularly in industrialized countries.

CONCLUSION

The present study concluded that low seropositivity was reported for leptospirosis in appraently healthy canine population in Haryana. Low seropositivity does not means that the infection cannot be transmitted to human beings. However, healthy dogs can act as a reservoir for transmission of infection to the human population. The risk factors taken for risk assessment of environment contamination of Leptospira were not found statistically significant. To predict the future epidemiological situation for leptospirosis among the dog population in Haryana and to improve its diagnosis, the constant monitoring of the population, contamination and carrier state in mouse-like small mammals, rats and farm animals, as well as expanding the range of diagnostic *Leptospira* strains with new pathogens among animals are necessary.

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