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☞ **NEWS**

Sero-prevalence of leptospirosis and differentiation in Blood Parameter between positive and negative cases in dogs of Kathmandu Valley

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Abstract

A cross-sectional study was carried out from August 2016 to December 2016 in Kathmandu valley. A total of 70 blood samples were collected from street dogs and tested against *Leptospira* infection using Immunocomb Canine *Leptospira* Antibody Test Kit. Out of 70 blood samples tested, 8 samples (11.4%) seemed to be positive. For the gender wise sero-prevalence in the dogs, male was found to be 11.8% (2/17), whereas female was found to be 11.3% (6/53). The sero positivity rate was found to be 10%, 13% and 12% in Kathmandu, Bhaktapur and Lalitpur districts respectively. Average mean and standard deviation of total leucocyte count of positive and negative cases were 79750±12090 /cu mm and 11026.98±4316.742 /cu mm respectively. The mean and standard deviation of blood glucose level of positive and negative cases were 114±15 mg/dl and 67.73±9.89 mg/dl, blood urea level of positive and negative cases were 20.12±11.19 mg/dl and 14.85±6.33 mg/dl, Serum Glutamic Pyruvic Transaminase (SGPT) level of positive and negative cases was 112.75±24.38 U/l and 47.01±24.32 U/l, Serum Glutamic Oxaloacetic Transaminase (SGOT) level of positive and negative cases were 109.87±9.89 U/l and 81.19±23.61 U/l, protein level of positive and negative cases were 8.25±1.83 gm/dl and 8.68±1.78 gm/dl, calcium level of positive and negative cases were 14.37±2.92 mg/dl and 8.66±2.07 mg/dl, phosphorus level in Positive and negative cases were 24.375±2.065 mg/dl and 4.00±1.82 mg/dl respectively. Levels of Blood parameters (TLC, Blood Glucose, SGPT, SGOT, and Calcium & Phosphorus) were found to be increased and significant between positive and negative cases P<0.05, where blood urea and protein remained constant and non-significant

P>0.05. Detection of *Leptospira* antibody in non- vaccinated dogs indicates the significant threat to human population and demand unique strategic action plan in the spirit of one health alliance for the control and prevention of Leptospirosis in animal and human.

Key words: Leptospirosis, seroprevalence, stray dogs, Kathmandu

Introduction

Leptospirosis is an important bacterial zoonotic disease with worldwide distribution (Adler and de la Peña Moctezuma, 2009; Bharti et al., 2003 and WHO, 1999). It is most commonly found in tropical or sub-tropical countries. The disease is caused by more than 200 different serotypes of the pathogen species *Leptospira interrogans* (Sessions and Greene, 2004). The most prevalent serovars such as *Canicola*, *Icterohaemorrhagiae*, *Pomona*, *Bratislava*, and *Grippotyphosa* are associated with dogs (Greene et al., 2006 and Bolin, 1996). In recent years, epidemiological trend of this disease has been increased and it has become a reemerging disease due to changing infectious serovars (Alton et al., 2009). Rodents and domestic mammals, such as cattle, pigs and dogs, serve as major reservoir hosts and maintain *Leptospira spp* serovars in the environment (Faine et al., 1999; Yannagihara et al., 2007, Vinelz et al., 1996). Dogs are an important factor in the occurrence of human infections because they act as an epidemiological link between reservoirs from the environment and people. The stray dogs population suffer from this zoonoses more often than pet dogs do due to their lifestyle and the absence of immune prophylaxis. Stray dogs may become infected by direct or indirect contact with mammalian reservoir hosts as a result of rummaging through garbage and hunting when searching for food, via water ingestion from puddles, by sniffing other animals' urine, licking the genital tract of females, and mating. Infection in dogs may result in very variable symptomatology; while some dogs have mild or no signs of the disease, for others the illness can quickly become serious and can even cause death (Van de Maele et al., 2008).

The first report of a suspected leptospiral infection in Nepal was in a Nepali soldier in 1981 (Brown et al., 1981). Since then, a number of serological studies have been carried out in Nepal, showing the presence of antibodies against *Leptospira* in human and animals (Rai et al., 2000; Murdoch 2004; Myint et al., 2010; Nepal et al., 2013; Joshi, 2000 and Sharma et al., 2012).

In Nepal, some NGOs/INGOs have been taking measures to solve the problems of stray dogs for their placement in shelters and implementation of the catch–neuter–release program. Data regarding the health status of these dogs related to leptospirosis are very scarce. The aim of this study was to determine the sero-prevalence and differentiation of blood parameters in positive and negative cases of dogs of Kathmandu Valley. This study will give the scenario of leptospirosis in dogs and pathological changes in blood and urine parameters due to leptospirosis. It is important to be able to diagnose and treat leptospirosis in dogs, both for the health of the dogs and the health of the owners themselves in the spirit of one health approach, as leptospirosis is a zoonotic disease.

Methods

This prospective cross sectional study was conducted from August 2016 to January 2017 in Kathmandu valley. Different dog welfare centers of Kathmandu, Bhaktapur and Lalitpur districts were selected for blood sample collection. Ethical approval was taken from Nepal Veterinary Council before conducting this study.

Blood sample collection and analysis

A total of 70 unvaccinated stray dog blood samples were collected from radial vein using 21 G needle syringes and transported to National Zoonoses and Food Hygiene Research Centre (NZFHRC) Chagal, Kathmandu for serum separation and testing. Gender and age of dogs were also reported. Age was estimated by examining the teeth of dogs. Duplicate blood sample were sent to Manmohan Memorial Hospital for testing the haematological parameters. The blood samples were allowed to clot and then centrifuged at 2000 rpm for 15 minutes. The serum sample was then kept separated and stored in a cryovial at 2-8°C until tested. Serology was done by using ELISA test Kit (Biogal's Immunocomb Canine Antibody Test Kit). The Immuno Comb test which is based on the "dot – ELISA" technology is more sensitive than the Microscopic Agglutination Test (MAT), yet it does not presume to identify the specific serotype.

The ImmunoComb® Canine Leptospira test kit is suitable for the detection of rising antibody levels due to infection with any of the following serovars: *L. canicola*, *L. icterohemorrhagiae*, *L. grippityphosa* and *L. Pomona* serovars.

Statistical analysis

Data were analyzed using SPSS software (18th version) and Microsoft excel (2007 version). Descriptive and parametric tests (Z test for significance of difference means) were calculated.

Results

A total of 70 dog sera were tested by using immuno comb dot-ELISA. Out of 70 cases, 8 (11.42%) cases were found to be positive for leptospirosis infection. Among 70 dogs, 17 were male and 53 were female dogs. The sero-positivity rate was found to be 2 (11.8%) in male and 6 (11.3%) in female. The highest sero-positivity rate was found to be 4 (11.8%) in 12-24 month age groups and there was no infection in the 48-60 and 60-72 month age groups (Table No.1).

Table No.1: Age and gender wise distribution of seropositivity cases of Leptospira in dogs

Parameters	Frequency	ELISA Test	
		Positive	Negative
Gender			
Male	17	2(11.8)*	15(88.2)
Female	53	6(11.3)	47(88.7)
Age(months)			
0-12	14	1(7.14)	13
12-24	24	4(16.67)	20
24-36	14	2(14.28)	12
36-48	9	1(11.11)	8
48-60	5	0(0)	5

Table No. 4: Differentiation in blood parameters in positive and negative cases

Parameters	Units	Positive cases			Negative cases			P-value
		Mean	SD	Range	Mean	SD	Range	
Leucocyte count	/cu mm	79750	12090	79750+12090	11026.98	4316.742	11026.98+4316.742	<0.0001
Differentiate count								
Neutrophils	/cu mm	27512	17830	27512+17830	5748.54	3778.08	5748.54+3778.08	0.0006
Lymphocyte	/cu mm	42981	18544	42981+18544	4701.63	3648.71	4701.63+3648.71	<0.0001
Eosinophil	/cu mm	2210	2100	2210+2100	260.03	215.73	260.03+215.73	0.0088
Monocytes	/cu mm	1648	1153	1648+1153	352.14	447.64	352.14+447.64	0.0016
Basophils	/cu mm	1052	1369	1052+1369	159.873	169.12	159.873+169.12	0.0655
Blood Glucose	mg/dl	114	15	114+15	67.73	9.89	67.73+9.89	<0.0001
Blood Urea	mg/dl	20.12	11.19	20.12+11.19	14.85	6.33	14.85+6.33	0.1933
SGPT	U/I	112.75	24.38	112.75+24.38	47.01	24.32	47.01+24.32	<0.0001
SGOT	U/I	109.87	9.89	109.87+9.89	81.19	23.61	81.19+23.61	<0.001
Protein	gm/dl	8.25	1.83	8.25+1.83	8.68	1.78	8.68+1.78	0.5307
Calcium	mg/dl	14.37	2.92	14.37+2.92	8.66	2.07	8.66+2.07	<0.0001
Phosphorus	mg/dl	24.375	2.065	24.375+2.065	4.00	1.82	4.00+1.82	<0.0001

60-72	4	0(0)	4
Total	70	8(11.42)	62(88.58)

*parenthesis indicates percentage

Among 8 positive samples 1 sample was highly positive reaction to Leptospira, 4 samples were positive reaction to Leptospira and 3 samples showed low positive reaction to Leptospira (Table 2). For the area wise distribution of sero-prevalence, the highest seropositivity rate 2/15(13%) was recorded in Bhaktapur followed by Lalitpur 3/25(12%) and Kathmandu 3/30(10%) respectively (Table 3).

Table No. 2: Sero-prevalence of Leptospirosis in dogs

Results	Frequency
Negative	62(88.6)*
Highly positive reaction	1(1.5)
Positive reaction	4(5.7)
Low positive reaction	3(4.2)
Total	70

*Parenthesis indicates percentage

Table No.3: Areas wise distribution of sero-prevalence of Leptospirosis in dogs

Districts	Tested sera	Frequency
Kathmandu	30	3(10)*
Bhaktapur	15	2(13)
Lalitpur	25	3(12)
Total	70	8(11.4)

*Parenthesis indicates percentage

For the differentiation in blood parameters of positive and negative cases, average mean and standard deviation of total leucocyte count of positive and negative cases were 79750±12090 /cu mm and 11026.98±4316.742 /cu mm respectively. The mean and standard deviation of blood glucose level of positive and negative cases were 114±15 mg/dl and 67.73±9.89 mg/dl, blood urea level of positive and negative cases were 20.12±11.19 mg/dl and 14.85±6.33 mg/dl, Serum Glutamic Pyruvic Transaminase (SGPT) level of positive and negative cases were 112.75±24.38 U/I and 47.01±24.32 U/I, Serum Glutamic Oxaloacetic Transaminase (SGOT) level of positive and negative cases were 109.87±9.89 U/I and 81.19±23.61 U/I, protein level of positive and negative cases were 8.25±1.83 gm/dl and 8.68±1.78 gm/dl, calcium level of positive and negative cases were 14.37±2.92 mg/dl and 8.66±2.07 mg/dl, phosphorus level in Positive and negative cases were 24.375±2.065 mg/dl and 4.00±1.82 mg/dl respectively. Levels of Blood parameters (TLC, Blood Glucose, SGPT, SGOT, and Calcium & Phosphorus) were found to be increased and significant between positive and negative cases P<0.005 where blood urea and protein remained constant and non-significant P>0.05 (TableNo.4).

Discussion

The seroprevalence of Leptospirosis in Kathmandu Valley was found to be 11.4%. Previous studies demonstrate that seroprevalence of *Leptospira* among cattle and buffaloes were found to be 5.5% and 11% respectively in Nepal (Joshi and Joshi, 2000a, 2001b). Similar serological survey was done throughout Nepal by Dyson et al., (2000). They found the prevalence rate of 17% in different species of livestock, and suggested that chronic infection is very common.

Chuan-Jiang Lai et al. (2005) found 45.6% seropositivity of *Leptospira* in dogs in Northern Taiwan. However, Tongkorn M. et al. (2006) found 11% in Thailand and Gautam R. et al., (2010) found 8.1% in USA seropositivity of *Leptospira* antibodies in dogs.

In a survey conducted in Japan over a period of three years, 1.2% seroprevalence was observed in dogs (Ryu 1975). In another study conducted by Abdoel T. H. (2011), the seroprevalence was 21.3% in dogs. In a study conducted by Dayou Shi et al. (2012), the seroprevalence of Leptospirosis was 7.3%. The prevalence of *Leptospira* antibodies in dogs has varied among different countries: 21.3% in India (Venkataraman and Nedunchellian, 1992) and 6.36% in Italy (Cerri et al., 2003). Climate may be an important factor affecting the prevalence of *Leptospira* in each area. The suitable climate for *Leptospira* is the tropical climate, and the prevalence of *Leptospira* has been found to be the highest in the rainy season (Ward et al., 2002).

The highest number of leptospira positive cases belonged to the age group 12-24 months (i.e. less than 1 year of age). This might be due to the fact that the samples collected were more in number in this age group. This is similar to the study done by Ghneim et al. (2007). However, Ward et al. (2004) found dogs between 4 and 9 years of age were more likely to acquire infection than dogs less than 1 year of age.

Leptospira positivity was equal in males and female in this study. However, Harkin and Gartrell in 1996 and Miller et al. (2007) identified more infected females than males while in 1992 Rentko et al. (1992) and Zwijnenberg et al. (2008) found more infected males than females. The prevalence rate seemed to be higher in Bhaktapur (13.33%) district.

In this study, the levels of blood parameters (TLC, Blood Glucose, SGPT, SGOT, and Calcium & Phosphorus) were found to be significant between positive and negative cases $P < 0.005$ where blood urea and protein were non-significant $P > 0.05$. The Immuno Comb® ELISA kit has a mixture of antigens of four of these most common serovars in dogs. Hence, the positive results obtained in the present study are likely to indicate exposure to one or more of these common serovars. However, a lack of serovar-specific data in the present study makes it difficult to conclude on the predominant serovar. Infection with serovar Icterohaemorrhagiae has classically been associated with either acute haemorrhagic disease or liver failure and uraemia (Wohl, 1996). In contrast, patients with classic infections from serovar Canicola are likely to exhibit acute renal failure associated with less hepatic involvement (Wohl, 1996). Infections from serovars Pomona, Grippityphosa and Bratislava have recently been reported to be predominantly associated with renal involvement and less consistent hepatic involvement (Adin & Cowgill 2000; Birnbaum et al., 1998; Brown et al. 1996; Okewole & Ayoola 2009). Protein, calcium and phosphorous estimated high in positive cases which is consistent with other previous studies. Birnbaum et al. (1998) found hyperphosphatemia in 50% of the dogs. With those high numbers it is suggested to take a phosphate sample in dogs suspected or diagnosed with Leptospirosis. The development of hyperphosphatemia could be due to development of a renal failure,

but it can also be due to delayed serum separation, dietary excess, hemolysis or another cause (Ettinger & Feldman, 2010).

Conclusion

Detection of *Leptospira* antibody in non- vaccinated dogs indicate the significant threat to human population and demand unique strategic action plan in the spirit of one health alliance for the control and prevention of Leptospirosis in animal and human. Levels of blood parameters (TLC, Blood Glucose, SGPT, SGOT, and Calcium & Phosphorus) were increased and significant where blood urea and protein remained constant and non-significant.

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NEWS:

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