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Seroepidemiological analysis of *Leptospiral* infection by MAT in stray dogs in Alborz, Iran

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Abstract:

Leptospirosis is a zoonotic disease with global importance, in which animals are the source of transmission of this disease through shedding in their urine. In order to diagnose this disease in dogs and reduce the risk of transmission to humans, the epidemiological study of leptospirosis is essential.

The aim of this study was the seroepidemiological analysis of *Leptospiral* infection by MAT in stray dogs in Alborz, Iran.

One hundred ten blood samples from stray dogs were collected to detect the antibodies against *leptospira* interrogans serovars by Microscopic Agglutination Test (MAT).

The prevalence of positive MAT tests in stray dogs were 21.84%. The following protocol confirmed that the most common titers were 1:200 (50%) and 1:400 (25%). At the end, the most prevalent *Leptospira* serovars were L.Canicola (33.33%) and the lowest belonged to L.Pomona

(4.1%). No significant difference between age and sex of dogs and their MAT titer was observed ($P > 0.05$).

The results showed that there is high prevalence of leptospirosis in stray dogs of Koohsar in Alborz province. Leptospirosis is a zoonosis disease, therefore the disease in humans and animals (especially dogs) should be studied continuously.

Key words: Dogs, *Leptospiral*, Leptospirosis, MAT, Seroepidemiology

Introduction:

Leptospirosis is a zoonosis disease that affects most mammalian species (McCallum, et al. 2019). This infection has recently been recognized as an emerging public health problem all over the world, especially in tropical countries (Miotto, et al. 2018a).

The northern provinces of Iran are more susceptible to this disease because of their humidity and high rainfall (Mansour-Ghanaei, et al. 2005). The disease is transmitted to humans by direct or indirect contact with the urine of infected animals (McCallum, et al. 2019). The epidemiology of this disease is complex and recirculation of the pathogen among wild and domestic animals is needed for the stableness of infection in an area. (Goldstein 2010).

Infection in dogs may cause variable symptoms; some dogs may have mild or no signs of infection and sometimes they may die (Miotto, et al. 2018a).

Dogs are the major cause for transmission of *leptospiral* infection to human, and *L.interrogans* serovar Canicola and Icterohaemorrhagiae are known to be the most contaminant agent of dogs around the world (Hernández Ramírez, et al. 2017). Reduction of the risk of transmission to

humans depends on the detection of the organism spread in dogs. Findings suggest that the presence of bacteria through the urine may vary without showing any clinical symptoms. Besides, asymptomatic and chronic carrier dogs can also act as sources of infection. Diagnosis of infected dogs is an essential matter to public health. Stray dogs, due to their lifestyle and the absence of vaccination, suffer from this disease more often than domestic dogs (Llewellyn, et al. 2016; Miotto, et al. 2018a).

Laboratory diagnosis of leptospirosis can be performed by several methods: direct microscopy, culture, serological, and molecular methods such as Polymerase Chain Reaction (PCR) (Khaki 2016; Miotto, et al. 2018b).

Microscopic detection of *Leptospira* is not a reliable method due to the thinness of the bacteria (Murray, et al. 2015). The culture is the most accurate method for identifying *Leptospira* spp. but take almost three months and has a relatively long doubling time and often diagnosed based on antibody detection (Miotto, et al. 2018a).

Polymerase Chain Reaction (PCR) test on specimens enables rapid and direct diagnosis, at least in the early and convalescent stages of infection. PCR is a preventive approach, but need to complete laboratory equipment and highly skilled staff (Khaki 2016).

Microscopic agglutination test (MAT) is the gold-standard serological test for leptospirosis and provides an estimate of the antibody titer present in the serum of the dog. MAT can identify the infected serovar of the bacterium, so it can help to detect the probable animal source of infection. Therefore, the most appropriate method to detect *leptospiral* antibodies is MAT (Khaki 2016).

Objectives: A few data are available on the prevalence and rate of leptospirosis in dogs especially among stray dogs living in Alborz province, Iran. This research is the first report on *leptospiral* infection in stray dogs living in this district.

Therefore, the aim of this study was the seroepidemiological analysis of *Leptospiral* infection by MAT in stray dogs in Alborz, Iran.

Materials and Methods:

Population study: The study group consisted of 110 dogs, including 48 male and 62 female dogs aged between 3 months to 5 years old, who were selected among stray dogs living in Koohsar region of Alborz province in April to July 2018. All dogs were apparently healthy without any clinical symptoms of leptospirosis.

Sampling: Five milliliters of blood were obtained by sterile venipuncture, and collected into sterile falcon tubes, and placed near the icebox. They were then quickly transferred to the National Reference Laboratory for *Leptospira*, Department of Microbiology, Razi Vaccine & Serum Research Institute of Karaj, Iran.

Sample preparation: In the laboratory, the samples were centrifuged (Sigma, Germany) at 3000×g for 10 min, and the sera were separated and stored at -20 □.

MAT test: Serum samples were subjected to 20 *leptospira* serovres to detect the presence of *Leptospira* antibodies. Twenty *Leptospira* serovars were obtained from the *leptospira* reference Laboratory, Razi Vaccine & Serum Research Institute of Karaj, Iran.

The sera were serially diluted to 1:50, 1:100, 1:200, 1:400, 1:800, and 1:1600, et, and then, serum samples were added to the live *Leptospira* cell suspensions in 96-well round-bottomed

microtiter plates at room temperature in the dark for 2 hours (Niloofa, et al. 2015). Then, an aliquot from wells was added on a slide and observed under a 20X magnification by using dark field microscopy (Nikon 80i, Japan). Every serum that gives an agglutination of at least 50% of the *Leptospires* (compared to the control antigen) is considered as positive. Titers of 1:100 or higher were considered as positive. The highest dilution observed was considered as the result for each serovar. Negative and positive sera by adding live antigen were used as the control (Organization 2003).

Data analysis Statistical analyses were performed using Statgraphics version 18-X64. The total number of seropositive dogs was calculated based on sex and age. The X^2 test was used to measure the differences in proportions between generated categories, and $P< 0.05$ were considered as significant.

Results and Discussion:

Leptospirosis is considered as an important zoonotic disease around the world (Khaki 2016). It occurs in tropical, semitropical and temperate climates, industrialized and developing countries, and urban environments, as well as rural regions worldwide (Esfandiari, et al. 2015). The *leptospiral* infection in dogs is usually attributed to the serovars L.Canicola, L.Icterohaemorrhagiae, L.Grippotyphosa, L.Pomona, and L.Bratislava and it is associated with renal failure, liver dysfunction, and other disorders (Klaasen and Adler 2015). Dogs are considered to be the reservoir host of *Leptospira* and the presence of dogs in the family increases the risk of infection in humans (Lelu, et al. 2015). Stray dogs and dogs kept under shelter conditions, due to a higher degree of environmental exposure to pathogenic *Leptospir,* are more susceptible to the infection (Scanziani, et al. 2002).

In Iran, a few seroepidemiological studies on *leptospiral* infection in dogs have been carried out. Therefore, in order to estimate the prevalence of this disease, we investigated the prevalence of leptospirosis among stray dogs living in Koohsar region of Alborz province, Iran.

In current study the samples from stray dogs were collected to detect the antibodies against *leptospira* interrogans serovars by the reference method of microscopic agglutination test (MAT). (Niloofa et al., 2015; Organization, 2003).

Based on the MAT test, a total of dogs 24 (21.84%) from the 110 studied dogs had a positive MAT titer and 86 (78.26%) had a negative MAT titer (Table 1). There were 10 positive titers (21.27 %) and 38 titers negative cases (78.72%) in the serum of male dogs. While in female dogs, 14 (22.22%) and 48 (77.77%) cases had positive and negative serum titers, respectively. Female dogs were at significantly greater risk of leptospirosis than male dogs (Risk Ratio=1.06). The mean age of male and female dogs was 21.6± 13.2 and 28.2± 15 months, respectively (Table 1). The most prevalent positive cases (No=7) (29.16%) were observed between 2-3 years old. The lowest prevalence (No=2) (8.33%) was in 4 and 8 months old (Table 1). No significant difference in seroprevalence was observed between sex and age of the dogs. ($P > 0.05$) (Table 1).

In a study conducted in Mashhad in 2003 showed that the *leptospiral* positive titer in dogs was 41.6% (Talebkhan, et al. 2003) which the titer was more than our results (21.84%). However our finding was similar to the results of other countries; for example, 21.27% prevalence in Madras in India (Venkataraman and Nedunchelliyan 1992), 25.1% in Los Rios Region, Chile, 21.4% in Paraiba, Brazil, and 21.3% in Temuco, Chile (Lelu, et al. 2015).

In our study, there was a little difference between the male and female dogs. This finding is in contrast to the results from previous studies, in which the number of infected male dogs was

more than female dogs (Ward, et al. 2004). The results of our study showed that the most infections in stray dogs were seen at the ages of 2 to 3 years old (29.16%), which is similar to a study conducted in USA (Ward, et al. 2004). But in another study conducted in Mashhad, it was found that younger dogs with a larger body were more susceptible to the infection (Talebkhan, et al. 2003).

The findings of the present study showed that the positive titers ranged from 1:100 to 1:1600, which is similar to a study conducted in Iran (Zeynali, et al. 2003).

In the present study, the most prevalent serovar was L.Canicola (33.33%), followed by L.Icterohaemorrhagia (25%), and L.Grippotyphosa (20.83%), while the serovar with lowest prevalent was L. Pomona (4.1%) (Table 2). This result is consistent with previous studies (Kikuti, et al. 2012; Lelu et al. 2015; Talebkhan, et al. 2003; Venkataraman & Nedunchelliyan. 1992; Zeynali, et al. 2003).

It seems that stray dogs in Koohsar region are exposed to increased risk of leptospirosis, which is possibly due to exposure to wildlife habitats. This may create a risk to human public health. The results provided useful information on seroprevalence of this organism and the high rate of *leptospiral* infection in dogs in Koohsar, Alborz. In Iran. Serological identification of the isolates with MAT also showed that L. Canicola and L. Icterohaemorrhagiae were the dominant serovars in this region.

Due to the fact that leptospirosis is a zoonotic disease, studies should be carried out continuously on the prevalence and monitoring of the disease, and there is also a need for further studies to better understand the epidemiology of leptospirosis in dogs in urban and rural environments.

Ethics:

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest:

The authors declare that they have no conflict of interest.

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Table1. The number and percentage of dogs evaluated

variable		All dogs n= 110(%)	Seropositive n= 24(%)	Seronegativen= 86(%)	P-Value
Sex	Male	48 (42.72)	10 (21.27)	38 (78.72)	> 0.05
	Female	62 (57.27)	14 (22.22)	48 (77.77)	
Age (months/years)	3 months	2 (1.81)	0 (0)	2 (2.32)	> 0.05
	4 months	8 (7.27)	2 (8.33)	6 (6.97)	
	6 months	4 (3.63)	0 (0)	4 (4.65)	
	8 months	10 (9.09)	2 (8.33)	8 (9.30)	
	10 months	4 (3.63)	0 (0)	4 (4.65)	
	1-2years	16 (14.54)	4 (16.66)	12 (13.95)	
	2-3 years	31 (28.18)	7 (29.16)	24 (27.90)	
	3-4 years	21 (19.09)	6 (25)	15 (17.44)	
	4-5 years	14 (12.72)	3 (12.5)	11 (12.79)	

Table 2. seropositivity for *Leptospira* serovars in stray dogs

Titer / Serovar	1:100 No. (%)	1:200 No. (%)	1:400 No. (%)	1:800 No. (%)	1:1600 No. (%)	Total No. (%)
Canicola	0	4(16.66)	2(8.33)	1(4.16)	1(4.16)	8 (33.33)
Icterohaemorrhagiae	0	3(12.5)	2(8.33)	1(4.16)	0	6 (25)
Grippotyphosa	1(4.16)	2(8.33)	2(8.33)	0	0	5 (20.83)
Serjo hardjo	1(4.16)	1(4.16)	0	0	0	2(8.33)
Atumnnalis	1(4.16)	1(4.16)	0	0	0	2 (8.33)
Pomona	0	1(4.16)	0	0	0	1 (4.16)
Total No. (%)	3 (12.5)	12 (50)	6 (25)	2 (8.33)	1 (4.16)	24 (100)