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Short Communication

Seroepidemiological Analysis of *Leptospiral* infection using MAT in Stray Dogs in Alborz, Iran

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Abstract

Leptospirosis is a zoonotic disease with global importance, and the animals are the source of transmission of this disease through shedding in their urine. Accordingly, it is essential to conduct epidemiological studies of leptospirosis in order to diagnose this disease in dogs and reduce the risk of transmission to humans. This study aimed to perform a seroepidemiological analysis of *Leptospiral* infection in stray dogs in Alborz, Iran, using the Microscopic Agglutination Test (MAT). In total, 110 blood samples were collected from stray dogs to detect the antibodies against *leptospira* interrogans serovars by the MAT. The prevalence rate of positive MAT tests in stray dogs was estimated at 21.84%. The following protocol confirmed that the most common titers were 1:200 (50%) and 1:400 (25%). In addition, the most prevalent *Leptospira* serovars were L. Canicola (33.33%), and the lowest belonged to L. Pomona (4.1%). Moreover, no significant difference was observed between the age and gender of the dogs regarding their MAT titer (P>0.05). The results also showed a high prevalence of leptospirosis in stray dogs of Koohsar in Alborz province, Iran. Since Leptospirosis is a zoonosis disease, it should be studied continuously in humans and animals, especially dogs.

Keywords: Dogs, *Leptospiral*, Leptospirosis, MAT, Seroepidemiology

Analyse Séroépidémiologique de l'Infection *leptospirale* à l'Aide de MAT Chez les Chiens Errants à Alborz, Iran

Résumé: La leptospirose est une maladie zoonotique d'importance mondiale, et les animaux sont la source de transmission de cette maladie par excrétion dans leurs urines. Par conséquent, il est essentiel de mener des études épidémiologiques sur la leptospirose afin de diagnostiquer cette maladie chez les chiens et réduire le risque de transmission aux humains. Cette étude visait à effectuer une analyse séroépidémiologique de l'infection leptospirale chez des chiens errants à Alborz, en Iran, à l'aide du test microscopique d'agglutination (MAT). Au total, 110 échantillons de sang ont été prélevés sur des chiens errants pour détecter les anticorps contre les sérotypes de *Leptospira interrogans* par le MAT. Le taux de prévalence des tests MAT positifs chez les chiens errants a été estimé à 21.84%. Le protocole suivant a confirmé que les titres les plus courants étaient 1:200 (50%) et 1:400 (25%). De plus, le *leptospire* le plus répandu les sérotypes était *L. Canicola* (33,33%) et les plus faibles appartenaient à *L. Pomona* (4,1%). De plus, aucune différence significative n'a été observée entre l'âge et le sexe des chiens concernant leur titre MAT (P>0.05). Les résultats ont également montré une forte prévalence de la *leptospirose* chez les chiens errants de Koohsar dans la province d'Alborz, en Iran. La leptospirose étant une zoonose, elle doit être étudiée en permanence chez l'homme et l'animal, en particulier le chien.

Mots-clés: Chiens, *Leptospirale*, *Leptospirose*, MAT, Séroépidémiologie

1. 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 the recirculation of the pathogen among wild and domestic animals is required for the stableness of infection in an area (Goldstein, 2010). Infection in dogs may cause variable symptoms; moreover, 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 humans; in addition, *L. interrogans* serovar Canicola and Icterohaemorrhagiae are known to be the most contaminant agents 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. Additionally, 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, including direct microscopy and culture, as well as 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.; however, it takes almost three months, has a relatively long doubling time, and is often diagnosed based on antibody detection (Miotto et al., 2018a). The PCR test on specimens enables rapid and direct diagnosis, at least in the early and convalescent stages of infection. Furthermore, it is a preventive approach; however, it requires complete laboratory equipment and highly skilled staff (Khaki, 2016).

The microscopic agglutination test (MAT) is the gold-standard serological test for leptospirosis that provides an estimate of the antibody titer present in the serum of the dog. The MAT can identify the infected serovar of the bacterium; therefore, it can help to detect the probable animal source of infection. Accordingly, the most appropriate method to detect *Leptospiral* antibodies is MAT (Khaki, 2016).

1.1. Objectives

There is lack of evidence about the prevalence and rate of leptospirosis in dogs, especially among stray dogs living in Alborz province, Iran. This study is the first report on *Leptospiral* infection in stray dogs living in this district. Moreover, this study aimed to conduct a seroepidemiological analysis of *Leptospiral* infection by MAT in stray dogs in Alborz, Iran.

2. Material and Methods

2.1. Study Population

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

2.2. Sampling

In total, five milliliters of blood were obtained by sterile venipuncture, 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 and Serum Research Institute of Karaj, Iran.

2.3. 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 -20ºC.

2.4. MAT Test

Serum samples were subjected to 20 *Leptospira* serovars to detect the presence of *Leptospira* antibodies. Totally, 20 *Leptospira* serovars were obtained from the *Leptospira* reference Laboratory, Razi Vaccine and 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. Following that, the 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). Subsequently, an aliquot from wells was added on a slide and observed under a 20X magnification 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 positive. Titers of 1:100 or higher were also considered positive. The highest dilution observed was regarded as the result for each serovar. Negative and positive sera by adding live antigens were used as the control (WHO, 2003).

2.5. Data Analysis

Statistical analyses were performed using Statgraphics (version 18-X64). The total number of seropositive dogs was calculated based on gender and age. Furthermore, the χ^2 test was used to measure the differences in proportions between generated categories, and a p-value less than 0.05 was considered statistically significant.

3. Results and Discussion

Leptospirosis is considered an important zoonotic disease across the world (Khaki, 2016). Moreover, it occurs in tropical, semitropical, and temperate climates; industrialized and developing countries; urban environments; as well as rural regions worldwide (Esfandiari et al., 2015). The *Leptospiral* infection in dogs is usually attributed to the serovars, such as L. Canicola, L. Icterohaemorrhagiae, L. Grippotyphosa, L. Pomona, and L. Bratislava; in addition, 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 those that are kept under shelter conditions, due to a higher degree of environmental exposure to pathogenic *Leptospira,* are more susceptible to the infection (Scanziani et al., 2002). In Iran, a few seroepidemiological studies have been carried out on *Leptospiral* infection in dogs. Therefore, in order to estimate the prevalence of this disease, this study investigated the incidence of leptospirosis among stray dogs living in the Koohsar region of Alborz province, Iran. In the current study, the samples were collected from stray dogs to detect the antibodies against *Leptospira* interrogans serovars by the reference method of MAT (WHO, 2003; Niloofa et al., 2015).

Based on the MAT test results, out of 110 studied dogs, a total of 24 (21.84%) and 86 (78.26%) dogs had a positive and negative MAT titer, respectively (Table 1). Furthermore, there were 10 (21.27 %) positive titers and 38 (78.72%) negative titers in the serum of male dogs. However, 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, compared to the male dogs (Risk Ratio=1.06). The mean±SD age was 21.6±13.2 and 28.2±15 months for male and female dogs, respectively (Table 1). The most prevalent positive cases (n=7; 29.16%) were observed between 2 and 3 years. On the other hand, the lowest prevalence rate (n=2; 8.33%) was noted in dogs aged between 4 and 8 months (Table 1). No significant difference was observed between the gender and age of the dogs regarding the seroprevalence (P>0.05) (Table 1).

Table 1. Number and percentage of dogs evaluated

	Variables	All dogs n=110(%)	Seropositive n=24(%)	Seronegative n=86(%)	P-Value
Gender	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)	
	1-2 years	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)	

In a study conducted in city of Mashhad during 2003, the *Leptospiral* positive titer in dogs was estimated at 41.6% (Talebkhan et al., 2003), which was more than the rates in our study (21.84%). However, this prevalence rate obtained from our findings was consistent with those in the results of the studies performed in other countries (21.27% in Madras, 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 were few differences between the male and female dogs. However, 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). Furthermore, the results of our study showed that the most infections in stray dogs were noted at the ages of 2-3 years (29.16%), which was similar to a study conducted in the USA (Ward et al., 2004). Nonetheless, 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 was 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 the lowest prevalence rate was L. Pomona (4.1%) (Table 2). This result is consistent with the findings of the previous studies (Venkataraman and Nedunchelliyan, 1992; Talebkhan et al., 2003; Zeynali et al., 2003; Kikuti et al., 2012; Lelu et al., 2015).

It seems that stray dogs in Koohsar region are exposed to an 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 the seroprevalence of this organism and the high rate of *Leptospiral* infection in dogs in Koohsar, Alborz, 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. Furthermore, there is a need for further studies to better understand the epidemiology of leptospirosis among dogs in urban and rural environments.

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)

Authors' Contribution

Study concept and design: P. Kh.

Acquisition of data: A. F. and P. Kh.

Analysis and interpretation of data: A. F., P. Kh. And S. M. B.

Drafting of the manuscript: A. F. and P. Kh.

Critical revision of the manuscript for important intellectual content: A. F., P. Kh. And S. M. B.

Statistical analysis: A. F., P. Kh. And S. M. B.

Administrative, technical, and material support: A. F., P. Kh. And S. M. B.

Ethics

The authors declare that all ethical standards have been respected in the preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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