

# High-level seroprevalence against *Leptospira interrogans* serovars among wild foxes, jackals and stray dogs in the North Khorasan Province, Iran

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## Abstract

**Background:** Leptospirosis is an important, neglected zoonotic disease that affects people and animals in humid (sub)tropical regions. Wild canines carry the pathogen and may contaminate natural resources which may then act as a source of human infection.

**Objectives:** The study was designed to understand the seroprevalence of leptospirosis among domestic and wild canines in Bojnurd County, Northeast Iran.

**Methods:** A total of 77 serum samples, comprising 29 sera from asymptomatic wild canines [foxes ( $n = 25$ ) and jackals ( $n = 4$ )] and 48 sera from asymptomatic stray dogs, was investigated. Serovars were identified and antibody titres were measured by standard microscopic agglutination test (MAT) using serial serum dilutions.

**Results:** Among all serum samples, 44.1% reacted positively to a *Leptospira interrogans* serovars. The average percentage of positive reactions was higher in stray dogs than in wild canines although this did not reach statistical significance (55.2% and 37.5%,  $p = 0.159$ ). Positive reactions with *L. i. Pomona*, *L. i. Australis* and *L. i. Tarasovi* was detected only among jackals and foxes. Among the stray dogs, the highest number of positive sera were for *L. i. Grippityphosa* (61.1%) and *L. i. Canicola* (50%). The highest titre detected was for *L. i. canicola* (1:1600) in two stray dogs and against *L. i. Icterohaemorrhagiae* and *L. i. Pomona* (1:800) in a single jackal.

**Conclusions:** The study revealed that leptospirosis is endemic among various canine species in the North Khorasan Province of Iran. Detailed monitoring of canines is necessary for better understanding the epidemiology of infection in our and other Iranian regions.

## KEYWORDS

fox, Iran, jackal, leptospirosis, stray dogs

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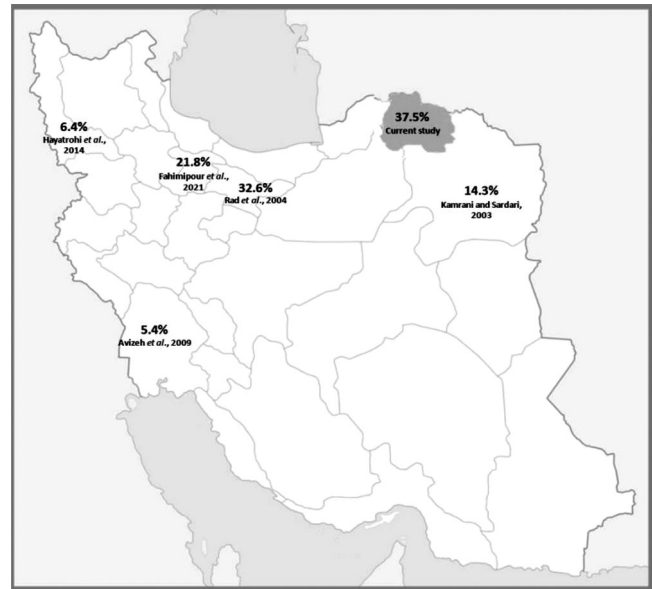
## 1 | INTRODUCTION

Leptospirosis, a globally neglected zoonotic disease causing fever for days to weeks, affects people and animals in humid (sub-)tropical regions (Ullmann & Langoni, 2011). Leptospirosis is mild in 90% of all cases but may generate severe complications in other patients. Although the main symptoms are fever, muscle pain and headaches, the disease can also lead to serious organ failure (kidneys, liver) and haemorrhaging. The disease is spread by a large variety of both wild and domestic animals which are natural reservoirs of *Leptospira* spp. (Adler & de la Peña Moctezuma, 2010). A wide variety of animals host *Leptospira interrogans* and many of these are asymptomatic *Leptospira* renal carriers. They contaminate the environment by shedding bacteria in their urine and they may develop symptoms only after long incubation periods (Adler & de la Peña Moctezuma, 2010). The precise epidemiology of leptospirosis in a specific niche is defined by the close contact between the particular *Leptospira* serovars and their specific maintenance hosts (Fratini et al., 2020). The seroprevalence of leptospirosis in humans and different animals in Iran has been studied before (Khalili et al., 2020). Leptospirosis is endemic in the North Khorasan province, as recent studies using the microscopic agglutination test (MAT) unveiled past and present infections among both rodents (Arzamani et al., 2018) and humans (Hashemi et al., 2021). Leptospirosis infection, its associated prevalence and the dominant serovars were reported as being different in canines around the world (Ab Rahman et al., 2018; Ambily et al., 2013; Aslantaş et al., 2005; Azocar-Aedo et al., 2017; Lelu et al., 2015; Samir et al., 2015; Shi et al., 2012). Similar reports on seroprevalence have emerged from different geographic regions of Iran as well (Avizeh et al., 2009; Fahimipour et al., 2021; Jamshidi et al., 2008; Rad et al., 2004; Torkan & Momtaz, 2019). Wild and feral canines carry the pathogen and contaminate the environment including soils, surface waters, streams and rivers where the bacteria can survive for weeks to months. This acts as one of the most significant sources of infection. In the current study *Leptospira* spp. seroprevalence among foxes, jackals and stray dogs in Bojnurd County, Northeast of Iran, was investigated for the first time.

## 2 | METHODS AND MATERIALS

### 2.1 | Study population and blood sample collection

This study was conducted in the North Khorasan province in the North-east of Iran (37.47 N and 57.33 E) (Figure 1). In a six months period, from April to September in 2020, stray dogs and wild canines were collected by the municipal animal control department and examined by veterinarians. All asymptomatic animals subjected to this study. A total of 77 blood samples were randomly selected from 29 asymptomatic wild canines [foxes ( $n = 25$ ) and jackals ( $n = 4$ )] and 48 also randomly selected asymptomatic stray dogs were included in the current study. Five millilitres of blood were collected aseptically from each animal and centrifuged at 3000 rpm for 10 min. Sera were kept at  $-20^{\circ}\text{C}$  in micro-tubes. The sera were transferred to the *Leptospira* Research



**FIGURE 1** Study location and illustration of the rate of leptospiral positive reactions among canines in different geographic regions of Iran

Laboratory of the Veterinary Research and Teaching Hospital at the University of Tehran for further analysis while maintaining cold chain management.

### 2.2 | Microscopic agglutination test (MAT)

All serum samples were subjected to MAT in duplicate to determine the exposure of individual animals to the various serovars of *L. interrogans* bacteria (Nilloofa et al., 2015; Sakhaee, 2011; Sakhaee et al., 2010). A seven to 10 days' culture of different serovars of *L. interrogans* in a liquid medium (GRA-Sina, Sinajen, Tehran, Iran) was used as a source of cellular antigens. The density of leptospires was checked using a counting chamber (Petroff-Hauser USA) and adjusted to  $2 \times 10^8$  cell/ml. All serum samples were serially diluted in phosphate buffer solution (PBS), starting from 1 in 50 dilutions, using twofold dilution (1 in 100, 200, 400, 800 and 1600). Then, 10  $\mu\text{l}$  of serum dilution was added to 10  $\mu\text{l}$  of the appropriate antigen in a 96-well plate and incubated at  $30^{\circ}\text{C}$  for 90 min. Upon completion of incubation, samples from individual wells were transferred to a microscopic slide and examined using a dark-field microscope (Olympus Bx50). One antigen control and two (positive and negative) standard serum controls were used for each 96 well plate (Sakhaee, 2011). Samples with 50% agglutination were considered positive. According to the OIE Terrestrial Manual, a titre of 1:100 diluted serum is interpreted as positive and serves international trade purposes (World Organization of Animal Health-OIE, 2019). All samples were tested against nine leptospiral antigens (*L. interrogans* serovar Hardjo, *L. interrogans* serovar Tarasovi, *L. interrogans* serovar Australis, *L. interrogans* serovar Icterohaemorrhagiae, *L. interrogans* serovar Pomona, *L. interrogans* serovar Grippotyphosa, *L. interrogans* serovar Autumnalis, *L. interrogans* serovar Canicola and *L. interrogans* serovar Ballum).

**TABLE 1** Distribution of positive MAT results as measured in canine serum samples

Type of canines	Positive/total cases (%)	Number	Serovar						
			Hardjo titre	Australis	Tarasovi	Canicola	Ictero.	Pomona	Grippi.
Stray dogs	18/48 (37.5)	7							100
		1					100		
		6**				100–1600			
		2				100			100
		1	100			100			200
		1	100						200
Jackal/fox	16/29 (55.2)	1*		400			800	800	
		1*		100		100			
		1*				100			
		1*			200				400
		3						100	
		2							100
		2			100				
		2					100		
		1		200					
2		100					100		
Total	48/77 (44.1)		2	5	3	11	4	6	14

\*Jackal.

\*\*Titre 200 = 3 cases, Titre 100 = 1 case and Titre 1600 = 2 cases.

Hardjo: *L. interrogans serovar Hardjo*, Australis: *L. interrogans serovar Australis*, Tarasovi: *L. interrogans serovar Tarasovi*, Canicola: *L. interrogans serovar Canicola*, Ictero: *L. interrogans serovar Icterohaemorrhagiae*, Pomona: *L. interrogans serovar Pomona*, Grippi: *L. interrogans serovar Grippotyphosa*.

## 2.3 | Statistical analysis

Fisher exact testing was conducted for comparing the leptospiral seroprevalence among the different animals using SPSS software (version 20).

## 3 | RESULTS

Among the 77 samples studied, 44.1% reacted positively to at least one serovar-specific antigen preparation of *L. interrogans*. The rate of positive reactions was higher in stray dogs versus wild canines (55.2% and 37.5%), but this was not statistically significant ( $p = 0.159$ ). The highest detected frequency was for *L. interrogans serovar Grippotyphosa* ( $n = 14$ ) and *L. interrogans serovar Canicola* ( $n = 11$ ) (Table 1).

Positive reactions with *L. interrogans serovar Pomona* ( $n = 6$ ), *L. interrogans serovar Australis* ( $n = 5$ ) and *L. interrogans serovar Tarasovi* ( $n = 3$ ) were detected only among sera collected from jackals and foxes. No positive reaction against *L. interrogans serovar Canicola* was detected among foxes. Among the stray dogs, the highest frequency of positivity was for *L. interrogans serovar Grippotyphosa* ( $n = 11/18$ , 61.1%) and *L. interrogans serovar Canicola* ( $n = 9/18$ , 50%). Antibodies against *L. interrogans serovar Hardjo* ( $n = 2$ ) were detected among stray dogs only (Table 1).

The highest detected titre was 1:1600 for *L. interrogans serovar Canicola* in two stray dogs and a titre of 1:800 against *L. interrogans serovar Icterohaemorrhagiae* and *L. interrogans serovar Pomona* in a single jackal (Table 1). Among dog samples, a positive triple reaction against *L. interrogans serovar Hardjo* (1:100), *L. interrogans serovar Canicola* (1:100) and *L. interrogans serovar Grippotyphosa* (1:200) was spotted. All tested jackal samples showed positive reactions. A single jackal sample revealed a response at 1:800 against *L. interrogans serovar Icterohaemorrhagiae* and *L. interrogans serovar Pomona* followed by 1:400 against *L. interrogans serovar Australis* (Table 1).

## 4 | DISCUSSION

Leptospirosis is one of the most significant re-emerging infectious diseases in Iran (Parhizgari et al., 2017). The present study investigated the prevalence of antibodies against serovar-specific leptospiral antigens among wild canines and stray dogs using the MAT (Niloofa et al., 2015). Clinical samples were collected from animals captured in the North Khorasan province in Iran. The location of the study is in a mountainous area with 9 months of cold weather. Conditions such as these should always be considered in our type of study but the precise impact of climate is ill defined.

**TABLE 2** Prevalence and the most dominant leptospiral serovars reported from Iran and other region of world among dogs

Study place	Reported prevalence %	Serovars	Current study
Iranian province			
North Khorasan	37.5	<i>L. interrogans Grippityphosa</i> (61.1%) and <i>L. interrogans Canicola</i> (50%)	Fahimipour et al. (2021)
Alborz	21.8	<i>L. interrogans Canicola</i> (33.3%), <i>L. interrogans Icterohaemorrhagiae</i> (25%) and <i>L. interrogans Grippityphosa</i> (20.83%)	Avizeh et al. (2009)
Khuzestan	5.4	<i>L. interrogans Hardjo</i> (44.5%), <i>L. interrogans Ballum</i> and <i>L. interrogans Icterohaemorrhagiae</i> (22.2%)	Kamrani and Sardari (2003)
Khorasan Razavi	14.3	<i>L. interrogans Canicola</i> (11.98%), <i>L. interrogans Pomona</i> (4.79%) and <i>L. interrogans Hardjo</i> (2.39%)	Hayatirohi et al. (2014)
West Azerbaijan	6.4	<i>L. interrogans Hardjo</i> (44.5%), <i>L. interrogans Icterohaemorrhagiae</i> and <i>Ballum</i> (22.2%),	Rad et al. (2004)
Tehran	32.6	<i>L. interrogans Canicola</i> (9%), <i>L. interrogans Icterohaemorrhagiae</i> (5.7%) and <i>L. interrogans Grippityphosa</i> (3.7%)	Altheimer et al. (2020)
Thailand	12.1	<i>L. interrogans Sejroe</i> (4.4%) and <i>L. interrogans Icterohaemorrhagiae</i> (3.7%),	Ambily et al. (2013)
India	71.12	<i>L. interrogans Autumnalis</i> (23.9%) and <i>L. interrogans Australis</i> (19.17%)	Aslantaş et al. (2005)
Turkey	43.96	<i>L. interrogans Bratislava</i> (66%) and <i>L. interrogans Canicola</i> (21.5%)	Roqueplo et al. (2015)
Sudan	74.2	<i>L. interrogans Autumnalis</i> (> 70%) and <i>L. interrogans Icterohaemorrhagiae</i> (> 60%)	Roqueplo et al. (2015)
Gabon	34.6	<i>L. interrogans Autumnalis</i> (> 30%) and <i>L. interrogans Icterohaemorrhagiae</i> (> 70%)	Roqueplo et al. (2015)
Ivory Coast	58.1	<i>L. interrogans Autumnalis</i> (60%) and <i>L. interrogans Grippityphosa</i> (40%)	Samir et al. (2015)
Egypt	11.3	<i>L. interrogans Icterohaemorrhagiae</i> (47.3%) and <i>L. interrogans Canicola</i> (52.6%)	Mayer-Scholl et al. (2013)
Germany	32	<i>L. interrogans Australis</i> (24%), <i>L. interrogans Grippityphosa</i> (20%) and <i>L. interrogans Pomona</i> (9%).	Major et al. (2014)
Switzerland	28.1	<i>L. interrogans Australis</i> (70.5%) and <i>L. interrogans Bratislava</i> (69.1%)	López et al. (2019)
Spain	25.8	<i>L. interrogans Icterohaemorrhagiae</i> (19.4%) and <i>L. interrogans Bratislava</i> (8.5%)	Piredda et al. (2021)
Italy	29.9	<i>L. interrogans Icterohaemorrhagiae</i> (57%) and <i>L. interrogans Bratislava</i> (22%)	Tagliabue et al. (2016)
Italy	49	<i>L. interrogans Australis</i> (39.3%) and <i>L. interrogans Icterohaemorrhagiae</i> (32.1%)	Alton et al. (2009)
Canada	8	<i>L. interrogans Autumnalis</i> (31.3%) and <i>L. interrogans Bratislava</i> (15.8%)	Leiu et al. (2015)
Chile	25.1	<i>L. interrogans Canicola</i> (51.6%)	

We report specific antibodies against various *L. interrogans* serovars found in jackals and foxes. The prevalence of specific antibodies against those leptospiral serovars detected among foxes in this study (55.2%) is higher than the prevalence reported in native foxes in Chile (7.7%) (Galarce et al., 2021), red foxes from Spain (47.1%) (Millán et al., 2009) and Croatia (33.8% and 31.25%) (Slavica et al., 2008; Slavica et al., 2011). The prevalence was slightly lower than recorded among red foxes from Croatia (57.6%) (Milas et al., 2006). In a recently conducted study on jackals, Nakonechnyi et al. (2019) found positive reactions against *Leptospira interrogans* among all nine animals that were tested.

The documented overall prevalence of 37.5% among dogs in this study is almost 2.5-fold higher than the estimated overall prevalence of 14.6% (95% CI: 3.49–25.77) for dogs in Iran (Khalili et al., 2020). The highest recorded prevalence was from Tehran (32.6%) (Rad et al., 2004), following Alborz (21.8%) (Fahimipour et al., 2021), Khorasan Razavi (14.3%) (Kamrani & Sardari, 2003), West Azerbaijan (6.4%) (Hayatrohi et al., 2014) and Khuzestan (5.4%) (Avizeh et al., 2009). The serological prevalence reported by the current study is in the mean but broad range among those reported from other regions of the world (8% to 74.2%) (Table 2). The high prevalence of leptospiral antibodies among stray dogs conceivably results from the greater risk for leptospirosis rising from spending all of their time outdoors and in urban environments as reported for large working and hunting dogs in the United States (Adin & Cowgill, 2000; Alton et al., 2009; Birnbaum et al., 1998; Ward et al., 2002).

The causative agents of leptospirosis among dogs are usually the serovars *L. interrogans serovar Canicola*, *L. interrogans serovar Icterohaemorrhagiae*, *L. interrogans serovar Grippityphosa*, *L. interrogans serovar Pomona* and *L. interrogans serovar Bratislava* (Klaasen & Adler, 2015). The high detection frequency for *L. interrogans serovar Grippityphosa* (61.1%) and *L. interrogans serovar Canicola* (50%) and the lack of positivity against *L. interrogans serovar Pomona* among dogs in the current study is similar to the results of a study conducted by Fahimipour et al. (2019) in the Alborz province. These authors reported a high prevalence of *L. interrogans serovar Canicola* (33.3%), *L. interrogans serovar Icterohaemorrhagiae* (25%) and *L. interrogans serovar Grippityphosa* (20.83%), followed by a low prevalence of *L. interrogans serovar Pomona* (4.1%) (Fahimipour et al., 2021). Studies conducted in other regions of Iran including Tehran (Rad et al., 2004), Khuzestan (Avizeh et al., 2009), West Azerbaijan (Hayatrohi et al., 2014) and a study in a nearby region of the current study in Khorasan Razavi (Kamrani & Sardari, 2003) illustrated positive reactions of sera with different serovars of *L. interrogans* (Table 2) (Figure 1). The common serovars among dogs reported here differ from those found in Asian, African, European and American countries (Table 2).

Based on serological data collected during the current study, we conclude that dogs, foxes and jackals are natural reservoirs of *Leptospira* in the area that we covered. Specific antibodies against *L. interrogans* were reported as was done among rodents in the same geographical regions before (Arzamani et al., 2018). Dogs are considered an important host for *Leptospira interrogans*, and having close contact with stray dogs may increase the risk of infection in humans (Lelu et al., 2015).

## 5 | CONCLUSION

We demonstrate that leptospirosis is a significant endemic epizootic disease in the study region. More extensive investigations on the large population of wild canines is recommended for better understanding the possible transfer of infections from wild and domesticated animals to humans. First thing to better substantiate this risk would be to perform seroprevalence studies among humans from which in the end prophylactic measures may be designed.

## CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

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## ETHICS STATEMENT

Sample collection was performed according to the rules and regulations set by the Ethical Committee of North Khorasan University of Medical Sciences (project number: IR. NKUMS. REC1391.017.).

## AUTHOR CONTRIBUTIONS

Kourosh Arzamani: Conceptualization, Methodology, Resources

Gholamreza Abdollahpour and Amir Azimian: Investigation

Hamed Ghasemzadeh-Moghaddam: Visualization, Writing – Original Draft Preparation

Alex van Belkum: Writing – Review & Editing

## DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/vms3.890>.

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