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Research article

Seroprevalence of *Leptospira* infection in occupational risk groups in North Khorasan province, Iran

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ABSTRACT

Leptospirosis is an important zoonotic bacterial disease caused by *Leptospira* spp. Earlier studies from North Khorasan province (Iran) reported the presence of *Leptospira* in wild canines and rodents. To date, there is no data on the seroprevalence of leptospirosis among humans in this province. This study was performed to determine the prevalence of human leptospiral infection among people with different occupations. The study was conducted in urban and rural areas of the province. Among the serum samples collected from 278 subjects, 3 (1.1%) showed positive reaction with titer of 1:100 by the microscopic agglutination test (MAT). Positive reactions were detected against *Leptospira interrogans* Canicola and *L. interrogans* icterohemorrhagic and all these samples were from livestock farmers (n = 3/106, 2.7%). The current study revealed that, though *Leptospira* infection is low in North Khorasan province, regular monitoring of the livestock and the farmers are important.

1. Introduction

Leptospirosis is a zoonotic bacterial disease caused by different serovars of *Leptospira* spp and it is one of the most important zoonotic diseases with a worldwide distribution (Ullmann and Langoni, 2011). The incidence of infection among humans is higher in tropical regions as compared with people in temperate regions. Disease transmission occurs in both developed and developing countries and human infections can result from direct contact with infected animal or from exposure to urine-contaminated water or soil (Abdollahpour et al., 2016; Rafiei et al., 2012; Ullmann and Langoni, 2011). Due to nonspecific signs and clinical symptoms, leptospirosis often remains unrecognized. Zoonotic infections including leishmaniasis and leptospirosis were reported among rodents and wild canines (fox, jackal, and wolf) in North Khorasan province, Iran (Ab Rahman et al., 2018; Arzamani et al., 2018).

To the best of our knowledge, there has not yet been an extensive study on the sero-epidemiology of leptospirosis among humans in the North Khorasan Province of Iran. This study was performed to determine the prevalence of leptospiral infection among people with different occupations to better understand the epidemiology of the disease in the province, especially when there is report on the presence of *Leptospira* among rodents and wild canines in the region of study.

2. Materials and methods

2.1. Study area

This cross-sectional study was conducted from January to December 2018 in urban and rural areas of the North Khorasan Province which has a temperate climate and a cold winter.

2.2. Inclusion and exclusion criteria

Adult healthy workers (aged ≥18 years) with the risk of contact with animals' fresh biological exudates (urine, feces, fetus) and environments contaminated by those sources included in the study. Informed consent

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was obtained when the workers were not pregnant, had any diseases, and had no hospitalization records in the past six months.

2.3. Study population and blood sample collection

The current study was conducted among different occupational groups, including those who have close contact with animals and contaminated environment. The study subjects comprised of livestock farmers, abattoir staff, butchers, veterinarians, rice-field workers, miners, and sewage system workers. Since the occupational group who may have high risk for leptospirosis were selected for the study, no further risk factors were investigated in the present study. According to the occupation and number of people living in each region, samples were collected from 3-10 healthy individuals for each category.

A total of 278 blood samples were collected. Socio-demography such as age, gender, and occupation of each subject was also obtained. Five ml of blood was collected aseptically from each subject, centrifuged at 3000 g for 10 min, corresponding serum samples were stored at -20 °C in a micro-tube. The collected sera were transferred to the *Leptospira* Research Laboratory of Veterinary Research and Teaching Hospital, University of Tehran for further analysis under cold chain management.

2.4. Microscopic agglutination test (MAT)

All serum samples were subjected to MAT to determine the leptospira exposure through antibody titers (Niloofa et al., 2015; Sakhaee et al., 2010). The standard MAT in a duplicated format was carried out as described before by Sakhaee and Abdollah pour (2011). A seven to ten days culture of different serogroups of *Leptospira interrogans* in liquid medium (GRA-Sina) was used as antigen. All serum samples were serially diluted in phosphate buffer solution (PBS), starting from 1 in 50 dilution, using 2-fold dilution (1 in 100, 200, 400, 800 and 1600). Then, 10 μL of serum dilution was added to 10 μL of appropriate antigen in 96 well plate and incubated at 30 °C for 90 min. Upon completion of incubation, the sample from each well was transferred to a microscopic slide and examined under dark-field microscope (Olympus B×50). One antigen control and two (positive and negative) standard serum controls were used for each 96 well plate (Sakhaee and Abdollah pour, 2011). Samples with 50% agglutination were considered positive. All samples were tested against nine leptospiral antigens (*L. interrogans Hardjo, L. interrogans Tarasovi, L. interrogans Australis, L. interrogans Icterohaemorrhagiae, L. interrogans Pomona, L. interrogans Grippotyphosa, L. interrogans Autumnalis, L. interrogans Canicola, and L. interrogans Ballum).*

Table 1. Occupation of participants and their geographic distribution in North Khorasan province.

County	Occupation								Total No. (%)
	Livestock farmers	Abattoir staff	Butcher	Rice worker	Veterinarian	Further cases	Miner	Sewage worker	
Bojnurd	15	10	4	11	11	0	5	3	59(21.2)
Mane & Samalqan	15	7	3	26	1	0	0	0	52 (18.7)
Raz & Jargalan	29	1	7	0	6	7	0	0	50 (18.0)
Shirvan	7	20	14	0	8	1	0	0	50 (18.0)
Jajarm	31	3	4	0	0	1	0	0	39 (14.0)
Farouj	12	2	6	0	7	1	0	0	28 (10.1)
Total (%)	109 (39.2)	43 (15.4)	38 (13.7)	37(13.3)	33 (11.9)	10 (3.6)	5 (1.8)	3 (1.1)	278 (100)

Table 2. Leptospiral serovar distribution among the age groups.

Age groups	Negative	Serovars		Total
		Icterohaemorrhagiae	Canicola	
15–29	31	0	0	31
30–44	125	1	1	127
45–59	79	0	0	79
60–74	34	1	0	35
more than 75	6	0	0	6
	275	2	1	278

Table 3. Occupational distribution of leptospiral infection.

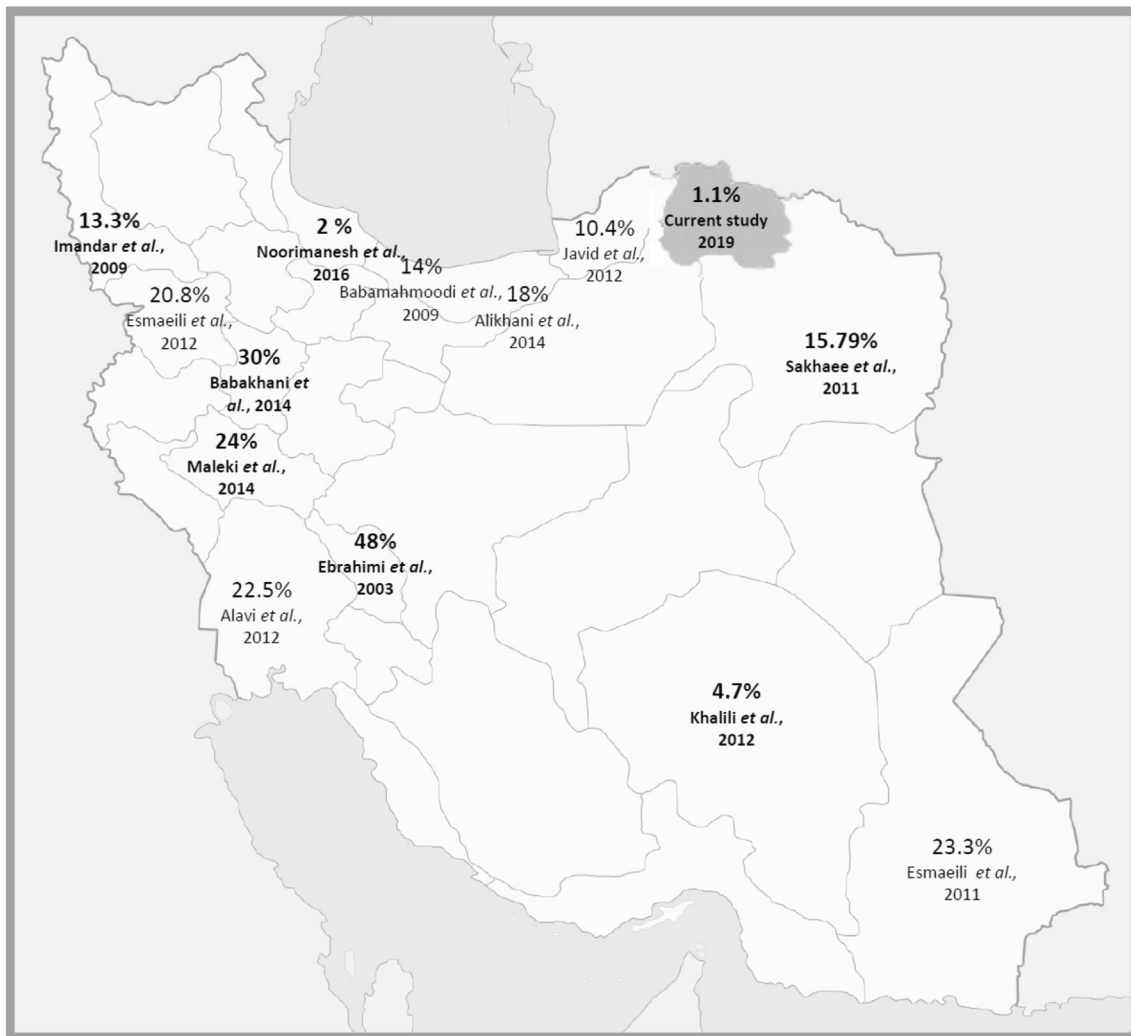| Occupation	MAT		Total
	Negative	Positive	
Livestock farmers	106	3 (2.7%)	109
Abattoir staff	43	0	43
Butcher	38	0	38
Veterinarian	33	0	33
Rice worker	37	0	37
Further cases	10	0	10
Miner	5	0	5
Sewage worker	3	0	3
Total	275	3 (1.1%)	278

Figure 1. Illustration of the rate of leptospiral positive reactions among humans using MAT (bold font) and ELISA (normal font) in different geographic regions of Iran.

2.5. Statistical analysis

The SPSS software, v. 16 was used for statistical analysis. Differences per group were tested for significance with chi-square test with Yates correction based on the result of a normality test. p-Values of <0.05 were considered to be significant.

2.6. Ethical clearance

Sample collection was performed according to the rules and regulations set by the Ethical Committee of North Khorasan University of Medical Sciences (IR.NKUMS.REC.1395.426.).

3. Results

Table 1 shows data for the samples that were collected from six different counties. The mean age of the study population was 44 ± 13 years (16–80 yrs) (Table 2). Among the investigated people 84.9% (n = 236/278) were male and 15.1% (42/278) were female.

Serum samples reacted positively to *L. interrogans Canicola*, *L. interrogans Icterohaemorrhagiae* and the highest frequency were detected for *L. interrogans icterohemorrhagic* (Table 2). In three out of 278 (1.1%) samples, a positive serological reaction was detected at a titer of 1:100 (Table 2). Two positive reactions were among female livestock

farmers (n = 2/42, 4.7%) and one was in male (n = 1/236, 0.4%). No significant difference was observed between male and female for acquiring the infection when chi-square statistic with Yates correction was used (*P* = 0.89). Positive reactions to *L. interrogans Canicola* and *L. interrogans Icterohaemorrhagiae* was seen in 30–44 and 60–74 years old livestock farmers (Tables 2 and 3). The above 60 years old livestock farmer with positive reaction was female.

4. Discussion

In the current study, the serological prevalence of specific antibodies against leptospiral antigens among different occupational risk groups was investigated by using the reference serological method MAT (Niloofa et al., 2015). Using one technique may be a drawback for study when the enzyme-linked immunosorbent assay (ELISA) increases the chance of identification of acute infections (Niloofa et al., 2015). By the way ELISA and MAT already illustrated the high correlation for detection of leptospiral antibodies (Niloofa et al., 2015; Sakhaee et al., 2010). Prevalence of infection (1.1%) was found to be low in comparison to other parts of Iran where an average rate of 19.7% and 27.8% among people have been reported in different studies by using MAT and ELISA, respectively (Khalili et al., 2020). Among the Iranian different counties, Chahar Mahaal and Bakhtiari province in the west, had the highest number of seropositive cases (48 %) (Ebrahimi et al., 2003) while the lowest

prevalence was documented in Gilan province (2%) (Noorimanesh et al., 2016). The rate of positive reactions from the geographical regions close to those included in the current study, by using MAT, was 15.79% in Khorasan Razavi, in the North east (Sakhaee E and Abdollah pour, 2011) (Figure 1). The rate of positive reactions from other places were 4.7% (Khalili et al., 2014), 13.3% (Imandar et al., 2011), 24% (Maleki, 2014), 30% (Babakhani et al., 2016), when the same technique was used (Figure 1).

When ELISA was used, the rate of positive reactions from the geographical regions close to those mentioned in the current study was 14%–18% (Babamahmoodi et al., 2009) (Alikhani et al., 2016) in Mazandaran province, followed by 10.4% in Golestan province (Javid et al., 2012). A rate of up to 23.3% seropositivity was reported from other parts of Iran by using ELISA (Alavi and Khoshkho, 2014), (Mobarez et al., 2017) and (Esmaeili et al., 2016) (Figure 1).

The serological prevalence reported in the present study is much lower when compared to several other tropical countries for example 38.5% among Indian mine workers (Parveen et al., 2016), 33.6% among wet market workers from Kuantan, Malaysia (Ab Rahman et al., 2018), 34.8% among municipal service workers in Selangor, Malaysia (Samsudin et al., 2015), 15.8% among fishing communities in Tanzania (Mgode et al., 2019). However, in the United states of America, only 2.5% of veterinarians were reported to be serologically positive (Whitney et al., 2009).

Low positive reaction here in comparison to other geographical region can be described by climate factors. The current study region has the long period of cold temperature and low amount of rainfall especially during autumn. Climatic factors play important role in acquiring leptospiral infection as human and rodent activity is affected by low temperature (Bi et al., 2002). Rodent activities are known to increase during by rainy season, hot and autumnal periods (Joshi et al., 2017).

Among the *Leptospira* serovars tested in the current study, positive reaction was observed for *L. interrogans Canicola* and *L. interrogans Icterohaemorrhagiae* which is in accordance with the results reported from the neighboring provinces Mazandran and Korasan Razavi (Babamahmoodi et al., 2009) (Sakhaee and Abdollah pour, 2011).

In another investigation conducted to determine the existence of *Leptospira* species in rodents in North Khorasan Province showed 33% positive reaction against one or two serovars, with most prevalent serovar as *L. Pomona* and the least as *Icterohemorrhagic* (Darvish et al., 2016).

Farm workers, fishermen, sewage workers, miners and veterans were found to be the highest-risk groups for leptospirosis from earlier studies (Ab Rahman et al., 2018; Joshi et al., 2017; Mgode et al., 2019; Parveen et al., 2016; Samsudin et al., 2015; Whitney et al., 2009), while we found livestock farmers (2.7%) as the most leptospiral sero-prevalent group.

5. Conclusion

The current study revealed the presence of leptospiral infection among livestock farmers in the North Khorasan province of Iran. Although less sero prevalent, leptospirosis is an endemic disease in North Khorasan province. Hence, regular monitoring, improvement in the public awareness and observance of hygienic standards by high-risk group are vital in reducing the future rate of infection in the region.

Declarations

Author contribution statement

S. Hashemi: Conceived and designed the experiments.

K. Arzamani, N. Beheshti, and A. Azimian: Performed the experiments.

G. Abdollahpour: Contributed reagents, materials, analysis tools or data.

H. Ghasemzadeh-Moghaddam: Analyzed and interpreted the data; Wrote the paper.

M. Alavinia, N. Vasanthakumari and A. van Belkum: Analyzed and interpreted the data.

Data availability statement

The authors do not have permission to share data.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Ab Rahman, M.H.A., Hairon, S.M., Hamat, R.A., Jamaluddin, T.Z.M.T., Shafei, M.N., Idris, N., Osman, M., Sukeri, S., Wahab, Z.A., Mohammad, W.M.Z.W., Idris, Z., Daud, A., 2018. Seroprevalence and distribution of leptospirosis serovars among wet market workers in northeastern, Malaysia: a cross sectional study. BMC Infect. Dis. 18, 569–673.

Abdollahpour, G., Shirzadi, M., Darvish, J., Mohammadi, Z., Yazdani, M., Mousazadeh, A., 2016. Serological survey of leptospirosis in rodent of North khorasan province, northeast of Iran. Journal of North Khorasan University of Medical Sciences 7, 725–733.

Alavi, S.M., Khoshkho, M.M., 2014. Seroprevalence study of leptospirosis among rice farmers in khuzestan province, south west Iran, 2012. Jundishapur J. Microbiol. 7, e11536.

Alikhani, A., Babamahmoodi, F., Alian, S., Zameni, F., Ghorbani, A., Shojaeefar, A., 2016. Sero-epidemiological study of leptospirosis in healthy people in qaemshahr, Iran, 2015. Journal of Mazandaran University of Medical Sciences 26, 78–84.

Arzamani, K., Mohammadi, Z., Shirzadi, M.R., Alavinia, S.M., Jafari, B., Darvish, J., 2018. Faunistic study of the rodents of north Khorasan province, north east of Iran, 2011–2013. Journal of arthropod-borne diseases 12, 127–134.

Babakhani, S., Maleki, S., Baharvand, M., Abdollahpour, G.R., 2016. Serological investigation on the incidence of Leptospira serovars among rice farmers in Veysian, Lorestan province. yafte 18, 29–37.

Babamahmoodi, F., Salmani Mojaveri, M., Babamahmoodi, A., 2009. Seroepidemiology of leptospirosis in workers of high risk occupation in Mazandaran province–Iran 2007-2008. Journal of Mazandaran University of Medical Sciences 19, 10–15.

Bi, Peng, Tong, Shilu, Donald, Ken, Parton, Kevin A., Ni, Ni, 2002. Climatic, reservoir and occupational variables and the transmission of haemorrhagic fever with renal syndrome in China. Int. J. Epidemiol. 13 (1), 189–193. In press.

Darvish, J., Arzamani, K., Abdolahpoor, G., Shirzadi, M., 2016. Rodent leptospirosis in North khorasan province, northeast of Iran. Int. J. Infect. Dis. 45, 465–470.

Ebrahimi, A., Alijani, L., Abdollahpour, G., 2003. Serological survey of human leptospirosis in tribal areas of west centarl Iran. Iran. J. Med. Sci. 28, 93–95.

Esmaeili, S., Naddaf, S.R., Pourhossein, B., Shahraki, A.H., Amiri, F.B., Gouya, M.M., Mostafavi, E., 2016. Seroprevalence of brucellosis, leptospirosis, and Q fever among butchers and slaughterhouse workers in south-eastern Iran. PloS One 11, e0144953.

Imandar, M., Hassanpour, A., Asgarlou, S., Abdollahpour, G., Sadeghi Zali, M.H., Khakpoor, M., 2011. Seroprevalence of leptospirosis in industrial livestock slaughterhouse workers in Khoy City. Scientific Journal of Kurdistan University of Medical Sciences 16, 77–85.

Javid, N., Dadgar, T., Khodabakhshi, B., Bazour, M., Sedaghat, M., Bakhshandeh-nosrat, S., Rahimi, S., Ghaemi, E.A., 2012. Seroepidemiology of anti-leptospira antibody in Golestan province, north of Iran. International Journal of Molecular and Clinical Microbiology 2, 124–127.

Joshi, Y.P., Kim, E.-H., Cheong, H.-K., 2017. The influence of climatic factors on the development of hemorrhagic fever with renal syndrome and leptospirosis during the peak season in Korea: an ecologic study. BMC Infect. Dis. 17, 1–11.

Khalili, M., Askari, N., Sakhaei, E., Ghezeljeh, A., Abdollahpour, G., 2014. Serological survey of human leptospirosis in southeast Iran. Online J. Vet. Res. 18, 29–34.

Khalili, M., Sakhaee, E., Amiri, F.B., Safat, A.A., Afshar, D., Esmaeili, S., 2020. Serological evidence of leptospirosis in Iran; A systematic review and meta-analysis. Microb. Pathog. 138, e103833.

Maleki, S., 2014. Serologic study on leptospiral infection in goats in Khorramabad, west Iran. Bull. Georgian Nat. Acad. ScI. 8, 553–558.

Mgode, G.F., Japhary, M.M., Mhamphi, G.G., Kiwelu, I., Athaide, I., Machang'u, R.S., 2019. Leptospirosis in sugarcane plantation and fishing communities in Kagera northwestern Tanzania. PLoS Neglected Trop. Dis. 13, e0007225.

Mobarez, A.M., Amiri, F.B., Esmaeili, S., 2017. Seroprevalence of Q fever among human and animal in Iran; A systematic review and meta-analysis. PLoS Neglected Trop. Dis. 11, e0005521.

Niloofa, R., Fernando, N., de Silva, N.L., Karunanayake, L., Wickramasinghe, H., Dikmadugoda, N., Premawansa, G., Wickramasinghe, R., de Silva, H.J., Premawansa, S., 2015. Diagnosis of leptospirosis: comparison between microscopic agglutination test, IgM-ELISA and IgM rapid immunochromatography test. PloS One 10, e0129236.

Noorimanesh, S., Shafighi, T., Abdollahpour, G., 2016. A survey on leptospirosis in persons referred to shahid beheshti hospital in astara. SSU_Journals 24, 366–374.

Parveen, S.M.A., Suganyaa, B., Sathya, M.S., Margreat, A.A.P., Sivasankari, K., Shanmughapriya, S., Hoffman, N.E., Natarajaseenivasan, K., 2016. Leptospirosis seroprevalence among blue metal mine workers of Tamil Nadu, India. Am. J. Trop. Med. Hyg. 95, 38–42.

Rafiei, A., Zadeh-Omran, A.H., Babamahmoodi, F., Navaei, R.A., Valadan, R., S, I., 2012. Review of leptospirosis in Iran. Journal of Mazandaran University of Medical Sciences 22, 102–110.

Sakhaee, E., Abdollah pour, G.R., 2011. Detection of Leptospiral antibodies by microscopic agglutination test in north–east of Iran. Asian Pacific journal of tropical biomedicine 1, 227–229.

Sakhaee, E., Abdollahpour, G., Bolourchi, M., Tabrizi, S.S., 2010. Comparison between microscopic agglutination test (MAT) and enzyme-linked immunosorbent assay (ELISA) for detection of leptospiral antibodies in cattle. Comp. Clin. Pathol. 19, 5–9.

Samsudin, S., Masri, S.N., Tengku Jamaluddin, T.Z.M., Saudi, S.N.S., Md Ariffin, U.K., Amran, F., Osman, M., 2015. Seroprevalence of leptospiral antibodies among healthy municipal service workers in Selangor. Advances in Public Health, e208145, 2015.

Ullmann, L., Langoni, H., 2011. Interactions between environment, wild animals and human leptospirosis. J. Venom. Anim. Toxins Incl. Trop. Dis. 17, 119–129.

Whitney, E.A.S., Ailes, E., Myers, L.M., Saliki, J.T., Berkelman, R.L., 2009. Prevalence of and risk factors for serum antibodies against Leptospira serovars in US veterinarians. J. Am. Vet. Med. Assoc. 234, 938–944.