Original Article

The Emergence of the Leptospirosis among Paddy Farmers of Jahrom, South of Iran

Rahim Raoufi 1* MD, Naser Hatami 2 MD.

1. Infectious Department, Jahrom University of Medical Sciences, Jahrom, Iran.

2. Student Research Committee, Jahrom University of Medical Sciences, Jahrom, Iran.

*correspondence: **Rahim Raoufi,** Jahrom University of Medical Sciences, Jahrom, Iran. Email: Sarahim1513@gmail.com.

Abstract:

Introduction: Leptospirosis is a bacterial zoonotic disease that affects humans and a wide range of animals including mammals, birds, reptiles and amphibians. This disease has a profound impact on the lives of exposed people, such as farmers and ranchers. The aim of this study was to determine the seroepidemiology of Leptospirosis in paddy farmers of Jahrom.

Methods: This descriptive cross-sectional study was performed on 242 paddy farmers living in different parts of Jahrom city. Samples were randomly selected and 5 cc of blood was collected from each participant. After centrifugation and storage at 20 ° C, serums were tested for the presence of anti-Leptospira IgG. Data analysis was performed by SPSS version 21.

Findings: According to the data analysis, seropidemiology of Leptospirosis was 2.5% and there was a significant relationship between age and duration of employment in Rice Farmering with seropidemiology of the disease (P<0.05), but living are was not correlated with seroepidemiological prevalence.

Conclusion: This study showed that the prevalence of leptospirosis in paddy farmers of Jahrom city is 2.5%. Given the difference between the results of the present study and similar studies in Iran which may be due to differences in the geographical area and serotype of southern Iran, further studies with other serologic methods such as MAT are recommended.

Keywords: Leptospirosis, Jahrom, Paddy Farmers.

[DOR: 20.1001.1.23222913.2019.8.4.3.9]

Introduction:

Leptospirosis is one of the common zoonotic diseases that is caused by the gram-negative Leptospira interrogans bacteria [1]. The major reservoirs of the pathogenic Leptospira in nature are mammals [2]. Humans may be a random host and also excrete Leptospira through the urine for a short time and to a small extent after getting infected leptospirosis [3].

People who are in contact with Leptospira based on their occupation are also at risk of being infected, such as slaughterhouse workers, sewage workers, paddy farmers, farmers, and those who participate in water sports [4]. In terms of susceptibility to infection, there is no difference between the genders, but men account for a higher percentage of cases due to the possibility of contact [4].

Like other pathogenic spirochetes, Leptospora introngensus cannot pass through healthy skin, but can easily pass through skin scratches such as the nails

surrounding, mucous membranes of the eyes, nose and mouth, into the internal mucosa and then into the lymph and bloodstream and even its seen in the vitreous and CSF fluid [5]. Leptospirosis is a two-stage disease: In the first stage or septicemia, leptospirosis occurs in the blood, lymph, vitreous, or CSF. In the second stage, leptospira is found only in the bent renal and urinary tracts [6].

The clinical signs of Leptospira vary from asymptomatic infection to two clinical forms: The first common form of the disease consists of systemic self-limiting disease, which accounts for 90-95% of cases; and includes symptoms of headache, fever, myalgia, nausea, diarrhea, and Inflammation of the conjunctiva. The second form of the disease is more severe with high morbidity and mortality, comprising 5–10% of residual disease, and its manifestations may be a combination of renal failure, thrombocytopenia, bleeding, jaundice, liver inflammation and rarely severe hemoptysis and shock and collapse [7]. It is important to diagnose the epidemiologic history, especially occupational history of contact with contaminated animals or waters [8]. Isolation of Leptospira from the culture medium of human specimen is the gold standard for diagnosis [8].

The prevalence of leptospirosis in Iran and Mazandaran province in 2007 in the at risk occupations was as following: farmers 29.5%, fishermen 18%, miners 12.5%, ranchers 6% and slaughterhouse staff 4% [9]. In a meta-analysis study examining the prevalence of leptospirosis in Iran in 2016 [10], it was found that the prevalence of leptospirosis in Iran was 39% (95% confidence interval). Farmers (44%) were at high risk (95% CI 27-61) and prevalence was higher in males (69%; 95% CI 74-56)).

The worldwide prevalence of leptospirosis differs in countries. It is 12.6% In Malaysia [11], Somalia 11.2% [12], Italy (rural areas) 11.34% and urban areas 3.08% [12], France 1.1% [13]. Mexico, 14.2% [14], New Zealand 9.5% [15].

Due to the recent outbreak of this disease in southern Iran, two cases of leptospirosis were identified in Jahrom, southern Iran, and no case of leptospirosis has been reported in Fars province in recent years, and these two cases were the first identified cases in Jahrom. This study was carried out to determine the seroepidemiology of the disease in Jahrom city, following the emphasis of the epidemiological evidence indicating the presence of reservoirs of this disease in different parts of Fars province.

Methods:

In this descriptive cross-sectional study, the population under study was paddy farmers residing in different parts of Jahrom (Simkan, Khafar, Hacan). According to the statistics obtained from previous studies, the sample size was 242 paddy farmers residing in different parts of Jahrom city in Fars province, south of Iran (Figure 1), including the regions of Simkan, Khafar and Hacan. They were recruited by random cluster sampling. A questionnaire including age, sex, and duration of employment and living area were completed before blood sampling.

[DOR: 20.1001.1.23222913.2019.8.4.3.9]

5 cc of venous blood were taken from each participant. The samples were transferred to the laboratory and their serums were separated by centrifugation at 3000 rpm for 5 minutes and kept at -20 ° C until the serological tests were performed. Blood serums were evaluated to determine the presence of specific IgG against Leptospira by ELISA using DRG kit. ® (EIA-4245). The test consists of a 96-well plate that allows 96 tests to be performed concurrently. The first five holes of each plate were used to make the standard curve. In the first step, each plate was filled from cavity 6 with 91 of serum samples. This study was performed by indirect ELISA method. For this purpose, Leptospira antigen 1 was inserted into the plate cavities. After the patient's serum or plasma was added to IgG against leptospira, this IgG was getting bind to the surface antigens of bacteria. Next, peroxidase-bound antibody against human IgG was used to detect the presence of this IgG, and after washing to determine the presence of its substrate-labeled oxygen, water was added. H2O2 was converted to water and oxygen by the peroxidase enzyme. In this substrate there was a pigment called TMB which was producing a reaction when dyed. The amount of dye production was depending on the level of IgG against Leptospira and the amount of dye produced by ELISA Reader was measured at 450 LW to determine the presence of IgG in these patients. For data analysis, the results of the serological tests were entered into SPSS version 21 software. Frequency percentiles were then plotted and Chi-square was used for statistical analysis and evaluation of the relationship between variables. Significance level was considered 0.05.

Findings:

The present study was conducted on 242 paddy farmers of Jahrom city. In this study seroepidemiology of leptospirosis in paddy farmers was 2.5% (n = 6). All participants were male. Of the 242 paddy farmers studied, 6 were positive for IgG against leptospirosis, of which there was a statistically significant difference in the distribution of positive specimens across different age groups (p = 0/007). As can be seen in Table 1, the highest frequency of positive IgG was in the age group of 79-70 years.

Of the 242 paddy farmers studied, 87 lived in Simkan (35.9%), 146 lived in Khafar (60.3%) and 9 lived in Hacan (3.7%). Of these, 6 were positive for IgG against leptospirosis, of which 4 lived in the Simkhan region (4.5%) and 2 lived in the Khafar region (1.3%). And in the Hackans region, there were no positives cases; no statistically significant difference was seen in comparison of the living area sero-positivity. (P = 0.242).

Discussion:

Jahrom city is located in Fars province (south of Iran) with warm and dry climate. There have been no cases of leptospirosis in this province in recent years. Whereas in northern Iran we have seen different and sometimes high prevalence rates [16-21]. In the present study the prevalence of positive cases of Leptospirosis was 2.5% which was different from other studies in Iran. These include the following studies: Alikhani

[DOR: 20.1001.1.23222913.2019.8.4.3.9]

study in 2015 in Mazandaran province with 18% prevalence [16], Esfandiari study in Amol in people suspected of this disease with 17.8% prevalence [17], The Sultani study in Jouybar showed a prevalence of 63% in paddy fields [18] and a Rassaei study with 37.8% prevalence in suspected individuals in Gilan province [19]. Two separate studies in the Gilan province showed the prevalence of 21% and 35%, respectively [20-21]. In the southwest of the country in paddy fields, as shown in the 2012 Alavi and Khoshkho study in Ahwaz, we saw a 22.5% prevalence of the disease [24]. Worldwide studies have also shown a different incidence of the disease. Among the studies in France that showed a prevalence of 1.1% and 9% [15], 12% [11] and 13% [22], respectively, in different regions. One of the reasons for these differences in these studies is the diagnostic methods of Leptospirosis. The standard diagnostic method for this disease is culture, which is difficult and time-consuming to isolate the bacteria, so serologic methods are used to diagnose it. MAT is the standard and reference method, which is a time-consuming method and increases the risk of laboratory personnel infection; therefore useing other serological methods, including ELISA and IHA, which are highly sensitive and specific to the geographic region and serotype are recommended. These methods have a sensitivity and specificity of 50 to 90% depending on the common serotype. Therefore, one of the reasons for the studies discrepancy may be due to the geographical area and the serotype of southern Iran to the north. Therefore, considering our 6 positive cases with suspected symptoms, it is suggested that other non-serological methods should be considered in future studies. Another reason for the difference in results can be attributed to evaluated population; studies conducted in Mazandaran [16,17,9], and Gorgan and Ghaemshahr [23] were on suspected cases, and The whole population was not evaluated for seroepidemiology as we done.

In the present study, 66.6% of positive samples were in Simkan and 33.3% in Khafar. Despite this difference in prevalence, the difference was not statisticaly significant. Most studies in Iran have been carried out in rural areas that have been engaged in agricultural occupations and are in line with this study. Examples include Babam Mahmoudi's study in Mazandaran, Soltani's in Jouybar, Rahimi's in Gilan. Studies comparing the prevalence of the disease in urban and rural areas have been more prevalent in rural areas [19]. And it could be argued that agricultural jobs, mostly in rural areas, continue to be a risk factor for leptospirosis infection.

Duration of employment was another variable in this study that showed that with increasing employment time, the probability of contact with the agent increases. The highest frequency of positive serology was seen in the age group of 69-79 years which was statistically significant compared to other ages. Various studies have been conducted on the relationship between employment duration and positive serology and the results have been varied, including a faithful study in which temperament showed involvement in work experience of less than 7 years in 53% of cases [19]. . Also, the Sultan Majd study conducted in Zanjan shows a work experience of between 10-30 years in 70% of cases [20].

[DOR: 20.1001.1.23222913.2019.8.4.3.9]

The most common age group involved in this study was 69-79 years old, which was significant in comparison with other age groups. Studies with similar results have been found to be more likely to be in contact with older age groups: in the Gilan Rasheed study, the highest age group in the 50+ age group [19], as well as the in-depth study of the 30-30 year age group [20] and the study Sultani Majd in Zanjan also has the highest prevalence in this age group [18]. Lower age groups have shown the highest incidence of conflict in some studies. One study in Khoy showed that 20% to 30% of the age group and 50% to 30-40 years of age were involved. Also, the study in rivulets has the highest incidence of rice cultivars in the age group of 30-40 years.

Given that it seems likely to increase with age, the difference in results across studies should alert us to consider all age groups with high-risk occupations**.**

Conclusion:

Leptospirosis is an emerging disease around the world and some occupations are at a higher risk for infection acquisition. On the one hand, the nonspecific symptoms of the disease and the potential for severe illness and mortality, and on the other hand, being unknown to physicians, are calling for more and more attention from researchers

References:

Jiménez JIS, Marroquin JLH, Richards GA, Amin P. Leptospirosis: Report from the task force on tropical diseases by the World Federation of Societies of Intensive and Critical Care Medicine. J Crit Care. 2018 Feb;43:361-365.

[2] Weiss RA, McMichael AJ. Social and environmental risk factors in the emergence of infectious diseases. Nat Med. 2004;10: S70-6. doi:10.1038/nm1150

[3] Cachay ER, Vinetz JM. A global research agenda for leptospirosis. Journal of postgraduate medicine. 2005;51(3):174.

[4] Kamath R, Swain S, Pattanshetty S, Nair NS. Studying risk factors associated with human leptospirosis. Journal of global infectious diseases. 2014 Jan;6(1):3.

[5] Haake DA, Levett PN. Leptospirosis in humans. InLeptospira and leptospirosis 2015 (pp. 65-97). Springer, Berlin, Heidelberg.

[6] Rao RS, Gupta N, Bhalla P, Agarwal SK. Leptospirosis in India and the rest of the world. Brazilian Journal of Infectious Diseases. 2003 Jun;7(3):178-93.

7. Haake DA, Levett PN. Leptospirosis in humans. InLeptospira and leptospirosis 2015 (pp. 65-97). Springer, Berlin, Heidelberg.

8. Sykes JE, Hartmann K, Lunn KF, Moore GE, Stoddard RA, Goldstein RE. 2010 ACVIM small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention. Journal of veterinary internal medicine. 2011 Jan;25(1):1-3.

9. Babamahmoodi F, Salmani Mojaveri M, Babamahmoodi A. Seroepidemiology of leptospirosis in workers of high risk occupation in Mazandaran province – Iran 2007-2008. J Mazandaran Univ Med Sci. 2009; 19 (73) :10-15.

10. H Khayat, K Sayehmiri, F Sayehmiri, S Ghafourian, M Rezapour, A Sheikhian, L Bogdanovic, M Taherikalan. Systematic Review and Meta-Analysis of Published

[DOR: 20.1001.1.23222913.2019.8.4.3.9]

Literatures: How Much do We Know about Reliable Prevalence of Leptospirosis in Iran?. West Indian Med J. 07 Jul, 2016

. DOI: 10.7727/wimj.2016.112.

11. El IJ, Bahaman AR. A review of human leptospirosis in Malaysia. Tropical biomedicine. 2004 Dec;21(2):113-9.

12. Cacciapuoti B, Nuti M, Pinto A, Sabrie AM. Human leptospirosis in Somalia: a serological survey. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1982 Jan 1;76(2):178-82.

13. Duval G, Michault A, Baranton G, Law-Koune JD, Folio G, Bertil G, Guiserix J. Seroepidemiological study of human leptospirosis at Reunion Island. Revue d'epidemiologie et de sante publique. 1991;39(2):135-41.

14. Vado I,Cardenas M,Jimenez B etal.Clinical epidemiological study of leptospirosis in human and reservoirs in Yukatan,Mexico. Rev. Inst. Med. trop. S. Paulo Dec 2002;44(6).

15. Benschop J, Heuer C, Jaros P, Collins-Emerson J, Midwinter A, Wilson P. Sero-prevalence of leptospirosis in workers at a New Zealand slaughterhouse. The New Zealand Medical Journal (Online). 2009 Dec 11;122(1307).

16. Alikhani A, Babamahmoodi F, Alian S, Zameni F, Ghorbani A, Shojaeefar A. Sero-Epidemiological Study of Leptospirosis in Healthy People in Qaemshahr, Iran, 2015. J Mazandaran Univ Med Sci. 2016; 26 (140) :78-84

17. Esfandiari B, Yousefi M, Abu Hosseini Tabari M, et al. Epidemiologic study of Leptospirosis in patients referred to Mazandaran Province Diagnostic Center during 2004-2009, Proceedings of Second National Congress of Paddy Fever (Leptospirosis) 2012: 42-41

18. Soltani K. Epidemiological study of Leptospirosis in Jouybar city in 2010, Proceedings of the Second National Congress of Paddy Fever (Leptospirosis) 2012: 48.

19. Rassaei AS, Razvani M., Hosseini S. Mortality of Leptospirosis in Farmers of Guilan Province from 1997 to 2009, Abstracts of Second National Congress of Paddy Fever (Leptospirosis) 2012: 51.

20. Rahimi F, Vand Yousefi C, Moradi Beddandi S, Bozari M. Prevalence of Leptospirosis in Residents of Guilan Villages 2004-2005, Journal of Kermanshah University of Medical Sciences, Summer 2007; (33): 205-197.

21. Honarmand H,Eshraghi S,Khoramizadeh M.Leptospirosis in flat area Guilan province,Iran,in 2005.Infectious Disease Journal,Jul-Sep 2006:59-61.

22. El jalii M,Islam MA.Epidemiology and diagnosis of human leptospirosis in Malaysia,Thesis submitted hn fulfilmet of the requirements for the degree of doctor of philosophy in the Institute of bioscience university Putra Malaysia sep 2000.

23. Aalian Sh, Ghassemian R, Babam Mahmoodi F et al. Report of 81 Patients with Hemorrhagic Icter Leptospirosis admitted in Sari and Fayem Shahr Hospitals during 2005-2010, Abstracts of Second National Congress of Paddy Fever (Leptospirosis 19): 19 -18.

24. Alavi SM, Khoshkho MM. Seroprevalence study of leptospirosis among rice farmers in khuzestan province, South

[DOR: 20.1001.1.23222913.2019.8.4.3.9]

west iran, 2012. Jundishapur J Microbiol. 2014;7(7):e11536. doi:10.5812/jjm.11536.

Tables and Charts:

Table 1: Frequency distribution of IgG antibody against Leptospirosis in paddy farmers of Jahrom by different age groups.

Age group	n	IgG positive	
		n	%
20-29	*10*	*0*	*0*
30-39	*29*	*1*	*3.4*
40-49	*53*	*1*	*1.8*
50-59	*76*	*1*	*1.3*
60-69	*48*	*0*	*0*
70-79	*23*	*2*	*8.6*
80-89	*3*	*1*	*33.3*
total	*242*	*6*	*2.5*

Table 2: distribution of leptospirosis IgG antibody in paddy farmers of Jahrom city based on living area.

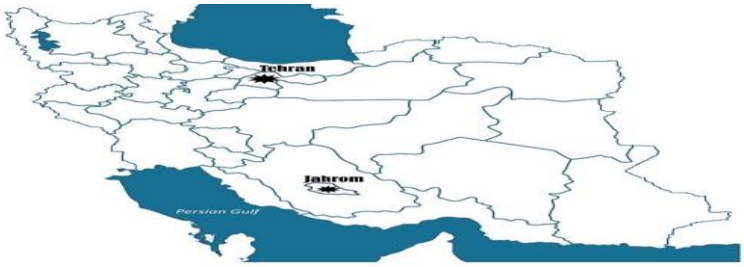
Living area	n	IgG positive	
		n	%
Simakan	*87*	*4*	*4.5*
Khafr	*146*	*2*	*1.3*
Hakan	*9*	*0*	*0*
total	*242*	*6*	*2.5*

[DOR: 20.1001.1.23222913.2019.8.4.3.9]

Table 3: Frequency distribution of IgG antibody against Leptospirosis in paddy fields of Jahrom city in based on employment duration.

Employment duration	**n**	**IgG positive**	
		n	%
1-15	*48*	*2*	*4.1*
30-15	*83*	*0*	*.*
30-45	*79*	*1*	*1.2*
+45	*32*	*3*	*9.3*
total	*242*	*6*	*2.5*

Figure 1: Geographical location of Jahrom in southern Iran.



[DOR: 20.1001.1.23222913.2019.8.4.3.9]