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**SEROEPIDEMIOLOGICAL STUDY ON LEPTOSPIROSIS AMONG LIVESTOCK
FARMERS IN KUHDASHT, LORESTAN PROVINCE**

**SHAHRAM MALEKI^{*1}, ZEYNAB AMRAEI², GHOLAMREZA TALEI³,
GHOLAMREZA ABDOLLAHPOUR⁴**

1: Department of Internal Medicine, Faculty of Veterinary Medicine, Lorestan University,
Khorramabad, Iran

2: Department of Biology, Borujerd Branch, Islamic Azad University, Borujerd, Iran

3: Department of Microbiology and Immunology, Lorestan University of Medical Sciences,
Khorramabad, Iran

4: Leptospira Research Laboratory, Faculty of Veterinary Medicine, University of Tehran,
Tehran, Iran

*** Corresponding Author: Shahram Maleki; Email: dr_shahram.maleki@yahoo.com;**

Phone: +98 9123063812

ABSTRACT

Leptospirosis is a worldwide zoonotic infection, which is usually severe and important disease. The aim in this study was seroepidemiological investigation on leptospirosis among livestock farmers of Kuhdasht, Lorestan province. The study was performed on 200 samples from livestock farmers of Kuhdasht in February 2014. The Microscopic Agglutination Test (MAT) method was used to determination of contamination of samples to leptospirosis. For the final dilution of leptospiral infection was performed dilute steps up to 1: 400 dilution. 48 serum samples (24%) were positive among 200 tested sera at the 1:100 dilution. Serum samples were shown positive reaction with two *L. Grippotyphosa* and *L. canicola* serovars. The prevalence of *Grippotyphosa* 66.67% and *Canicola* 33.33% were resulted by MAT. 31 samples (64.58%) were related to males and 17 cases (35.42%) females among 60 positive samples. The highest prevalence of

Leptospira serovars was founded in more than 50 years group with 16 cases. P. value based on gender was 0.01. Livestock farmers are one of the risk groups for leptospirosis disease, because of the presence of pathogenic *Leptospira* species in animals. Livestock farmers can prevent the occurrence of leptospiral infection by controlling their health and safety.

Keywords: Leptospirosis, *Leptospira*, Farmers, Kuhdasht, MAT

INTRODUCTION

Leptospira species; gram-negative and spiral-shaped are belonged to the family of leptospiraceae; and the phylum of spirochaetes (1,2). The genus *Leptospira* is divided into 20 species (9 pathogens, 5 intermediate and 6 saprophytes), based on phylogenetic analysis and DNA (3). Saprophytic *Leptospira* species do not cause any disease in humans (e.g., *L. biflexa*), and mild clinical signs are caused by intermediate species (3). Pathogenic *Leptospira* species are including *L. interrogans*, *L. borgpetersenii*, *L. santarosai*, *L. noguchi*, *L. weilii*, *L. kirschneri*, *L. alexanderi*, *L. alstonii* and *L. kmetyi*, though, the pathogenicity of two species: *L. alstonii* and *L. kmetyi* have not known yet (3,4). About 260 serovars have been identified for *Leptospira* (5). Producing antibody in human against special LPS of each serovar established human immunity (5). Some *Leptospira* serovars were isolated from Malaysian hospitals Included Pyrogenes, Autumnalis, Canicola, Hebdomadis, Icterohemorrhagiae, Pomona, Grippotyphosa,

Celledoni, and Sejroe by Tan et al in 1970 to 1986 (6). Pathogenic *Leptospira* species are being caused a zoonotic disease called leptospirosis (7). Leptospire are colonized in the renal tubules of animals (mammals sensitive), and host animals were remained infected for a long time (7). Livestocks (cattle and sheep) are the sources of leptospirosis, and they should be safe to prevent disease transmission to humans (8,9). *Leptospira* species are important issue of world public health; because of their long-term presence in the environment, transmitted to humans through damaged skin and mucosa, creations of mortality and economic losses (1,7,10). Half a million of leptospirosis cases are reported each year (45 cases per 100,000 people) around the world (11). Leptospirosis is more common in the warm seasons, tropical, and rainy areas (11,12).

The incubation for leptospirosis in humans is about 10 days and might be asymptomatic (12). Leptospirosis emerges in two forms: anicteric and icteric (13). Anicteric form is

subclinical and associated with symptoms such as fever, chills, abdominal pain, nausea, diarrhea, and muscle pain (13). Liver failure, severe jaundice, and death are caused by icteric form (13). Clinical signs of leptospirosis are mild and hard to diagnose (14). The severity of leptospirosis symptoms are depended on factors such as species or serovars causing disease, the age of patients, and the patients' immune system (14). Severe leptospirosis is caused to lung problems, Multi-Organ Dysfunction Syndrome (MODS), and acute kidney injury (15). There is less culture for diagnosis of leptospirosis because of time-consuming requirement of high expertise (16). The PCR in comparison with culture is a method which is better for diagnosis of *Leptospira* (16). Bacteremia is created by *Leptospira* during 7-10 days after symptoms of infection, and antibodies are detectable in the blood (16,17). These antibodies are being detected by laboratory methods such as ELISA, Indirect Fluorescent Antibody Technique (IFAT), and Microscopic Agglutination Test (MAT) (17). The MAT is a gold standard method for diagnosis of infection by *Leptospira* serovars with global application (17,18). Our purpose was seroepidemiological study on leptospirosis among livestock

farmers in Kuhdasht, Lorestan province by the MAT method.

MATERIALS AND METHODS

100 males and 100 females livestock farmers of Kuhdasht city were sampled in February 2014. Samples were performed from healthy individuals and those who had been experienced clinical symptoms similar to leptospirosis. For MAT and determination of sera contamination were used by WHO standard guidelines (9). 6 common antigens of *Leptospira interrogans*: *L. australis*, *L. grippityphosa*, *L. hardjo*, *L. icterohaemorrhagiae*, *L. canicola*, and *L. pomona* were used in this test. 7 days culture without contamination of *Leptospira interrogans* in broth a medium GRA-Sina was used to produce antigens. Blood samples were placed in temperature of laboratory about 60 minutes, then, they were transferred to 4° C. Samples were centrifuged at 3000 rpm for 10 minutes. Sera were separated from blood samples and were transferred to microtubes. Samples (sera) were frozen at -20° C to prevent damage for test. Samples were moved in freeze condition to the *Leptospira* research laboratory (college of veterinary) in Mrdabad, Karaj. Preparation of serum dilutions was used by PBS sterile solution. First, 20 µl of each serum sample was added to 980 µl of PBS sterile solution to produce a 1:50

dilution, then, antigen was added to samples and obtained the final dilution of 1 : 100 to perform the MAT (minimum dilution used in test was 1 : 100). After that, 10 μ l of each sample (1 : 100 dilution) was added to 10 μ l of antigen on a slide, and was placed for 1-2 hours at 30° C (by petri dish). Slides were investigated by the dark-field microscope (Olympus Bx50); 100 \times magnification to estimate the agglutination. Samples with more than 50% agglutination were reported positive and less than 50% agglutination, negative. For sure, the test was performed by control the testing were used antigen control and standard serum controls (positive and negative). 1 : 200 and 1 : 400 dilutions were prepared by 2-Fold Dilution to determine the final titer of positive sera in 1: 100 dilution. Following that, 10 μ L of antigen was added to 10 μ L of each dilution and were incubated at 30° C for 1-2 hours. Stages of determining the agglutination were performed by the dark-field microscope for each dilution (as before) to finish the test. The highest dilution that represented at least 50% agglutination included one or more serovars, was considered as a final titer of antibody in positive serum sample.

RESULTS

48 samples (24%) were positive for *Leptospira* by the MAT results tested of 200

blood samples. Of the 48 positive samples; 31 samples (64.58%) were positive for males and 17 (35.42%) for females with P. value 0.01. The most positive cases were belonged to more than 50 years, then 41-50 year groups (**Table 1**).

Serum samples were reacted positive by *L. canicola* and *L. grippityphosa* serovars among 6 serovars. The highest frequency was related to *L. grippityphosa* with 32 positive samples (**Table 2**).

It explained that the 8 cases of 48 positive samples were reacted positive by both *L. canicola* and *L. grippityphosa* serovars simultaneously. In this case, the serovar that was positive at the higher dilutions was considered as the main serovar. 16 samples in the MAT were positive for dilutions more than 1 : 100 by titration results; 15 samples for 1 : 200 and 1 sample for 1 : 400 dilutions (**Table 3**).

The MAT results for both *L. canicola* and *L. grippityphosa* Serovars among 16 positive serum samples in dilutions more than 1 : 100 are shown in **Table 4**.

DISCUSSION

Pathogenic *Leptospira* species can stay alive for a long time in good environmental conditions (humidity and heat) (19). Domestic animals (cattle, sheep, and dog) as the host of these bacteria are considered important to

the spread of leptospirosis (19). *Leptospira* is released by animals' urine in the environment (20). Human infection to Leptospirosis is caused by contact with the urine of infected animals (20).

The diagnosis of leptospirosis is difficult for many doctors, and definite diagnosis is possible by laboratory methods. The MAT has global application as a reference test for diagnosis of leptospirosis. In this study, the prevalence of leptospirosis was 24% in livestock farmers of Kuhdasht and *L. grippotyphosa* and *L. canicola* serovars had the highest and the lowest prevalence, respectively. P. value with 0.01 was significant for gender and it was shown that leptospiral infection is varied according to gender.

Studies have been conducted on the prevalence of leptospirosis around the world. The distribution of leptospirosis in humans was reported 24.6% in the hospital of university of Guilan (north of Iran) by Honarmand et al in 2005 (21). The serological study of leptospiral infection of sheep in Ahvaz was reported 14.9% of the incidence of leptospirosis by MAT (Hajikolaei et al (2007)) (22). The highest number *Leptospira* serovars was related to Pomona with 14 cases (43.8%), Canicola 7 cases (21.9%) and Icterohaemorrhagiae 4

cases (14.5%), respectively (22). This study shows that the transmission of leptospirosis is caused by sheep act as a host; therefore, livestock farmers are susceptible to leptospirosis.

A study on dairy cattle has been performed by MAT in industrial farms of Hamedan (Bahari et al) in 2011 (23). 18 samples were positive for *Leptospira* serovars among 80 blood samples (23). The highest prevalence was related to Canicola (21.25%) (23). This study is another reason for susceptibility of livestock farmers to leptospirosis.

In a study on 404 patients with fever, 155 patients with leptospirosis were founded by MAT in Sri Lanka (Agampodi, et al) (24). The prevalence of Pyrogenes (28.7%) and Hardjo (18.8%) serovars has been reported in this study (24). In a study on 142 samples of a cattle slaughtered of Nigeria was founded that 5 samples had antibodies against Hardjo serovar (2012) (25). In this study, evidence show that severe leptospirosis is caused by Icterohaemorrhagiae, Hardjo, and Grippotyphosa serovars (26).

Risk factors for leptospirosis have been examined on 567 abattoir workers by MAT in New Zealand (Dreyfus et al in 2014) (27). They have founded that 11% of workers have

antibodies in their sera against Hardjo and Pomona serovars (27).

CONCLUSION

Leptospirosis has been related by individuals' job and environment (28). Flooding and be in contact with trash are the most important factors to *Leptospira* infections (29). Leptospirosis might lead to kidney, liver and most organs failure (30). Generally, leptospirosis is expanding and must be considering to controlling the disease.

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NLR-independent signaling pathways. PLOS Negl Trop Dis 2014; 8:e2664.

Table 1: Number and frequency percentage of positive serum samples according to age groups

Age Group (Year)	1 : 100 Dilution MAT	
	No. of Positive	Frequency%
20-30	5	10.42
31-40	12	25.00
41-50	15	31.25
> 50	16	33.33
Total	48	100

Table 2- Number and frequency percentage of positive samples for 6 serovars tested

Serovar	1 : 100 Dilution MAT	
	No. of Positive	Frequency%
Grippityphosa	32	66.67
Canicola	16	33.33
Pomona	0	00.00
Hardjo	0	00.00
Icterohaemorrhagiae	0	00.00
Australis	0	00.00
Total	48	100%

Table 3: Number and frequency percentage of positive samples MAT in dilutions > 1 : 100.

Titer	Dilutions > 1 : 100 MAT	
	No. of Positive	Frequency%
1 : 200	15	31.25
1 : 400	1	2.08
Negative	32	66.67
Total	48	100

Table 4: Number and frequency percentage of *Leptospira* serovars in 16 positive samples in dilutions > 1 : 100

Serovar	Antibody Titer		Total
	1 : 200	1 : 400	
	Number of Positive (%)	Number of Positive (%)	
Grippityphosa	11 (68.75%)	1 (6.25%)	12 (75.00%)
Canicola	4 (25.00%)	0 (00.00%)	4 (25.00%)
Total	15 (93.75%)	1 (6.25%)	16 (100%)