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Detection of leptospiral antibodies by microscopic agglutination test in north–east of Iran

Ehsanollah Sakhaee^{1*}, Gholam Reza Abdollah pour²¹Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran²Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Iran

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ABSTRACT

Objective: To detect leptospiral antibodies by microscopic agglutination test (MAT) in north–east of Iran. **Methods:** This study was conducted to evaluate prevalence of human leptospiral infections by MAT, using six current reference strains of *Leptospira interrogans* in north–east of Iran. A total of 285 serum samples were collected from three north–east provinces of Iran, from December, 2009 to June, 2010. **Results:** Antibodies were detected at least against one serovar of *Leptospira interrogans* in 45 sera (15.79 %) among 285 samples at a dilution 1:100 or greater. Positive titers against more than one serovar were detected in 24 sera of the positive samples. Therefore, there were 75 positive reactions against different serovar of *Leptospira interrogans*. Positive titers were recorded against serovar *icterohaemorrhagiae* (31 samples), *hardjo* (26 samples), *grippotyphosa* (7 samples), *pomona* (5 samples), *canicola* (4 samples) and *ballum* (2 sample). **Conclusions:** In present study the most prevalent (*Leptospira icterohaemorrhagiae*) and the least prevalent (*Leptospira ballum*) serovar are different from previous studies. Maybe, species and prevalence of serovars change during the time in one area and between regions.

1. Introduction

Leptospirosis is a tropical zoonosis caused by pathogenic *Leptospira* species. The “gold standard” serodiagnostic method, the microscopic agglutination test (MAT), may give an indication of the serogroup to which the infective serovar belongs but can rarely identify it [1]. However, studies conducted by Ellis *et al.* in Northern Ireland and by Mackintosh *et al.* in England indicate that active leptospiral infections often occur in the absence of detectable agglutination titers [2, 3].

A number of serological techniques are used in the diagnosis of leptospirosis, each having its own sensitivity and specificity. It is often necessary to use a number of techniques, either together or successively, to make a reliable diagnosis. The enzyme–linked immunosorbent

assay (ELISA) and the MAT is the laboratory methods generally used. The ELISA technique has been compared with the MAT for the diagnosis of leptospirosis in human, cattle and cousins which indicated that there is high correlation between the ELISA and MAT results [4–6].

In the present study, the prevalence of human leptospiral infections was determined by MAT, using six current reference strains of *Leptospira interrogans* in north–east of Iran.

2. Materials and methods

2.1. Sample collection and processing

A total of 285 serum samples were collected from three north–east province of Iran, from December, 2009 to June, 2010. Samples were collected aseptically using sterile 5 mL syringe. Serum was separated by centrifugation of blood at 3 000 g for 10 minutes at room temperature, the sera were transferred into 1.5 mL sterile micro tube (Eppendorf) and were kept at –20 °C until use. These samples were submitted to the *Leptospira* Research Laboratory (<http://leptolab.ut.ac>).

*Corresponding author: Ehsanollah Sakhaee, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran.

Tel: 00989132952830

Fax: 00983413222047

E-mail: Ehsan_Sakhaee@yahoo.com, Ehsan_Sakhaee@mail.uk.ac.ir

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ir) of Teaching and Research Hospital of the Faculty of Veterinary Medicine at the University of Tehran.

2.2. Microscopic agglutination test

MAT was performed in *Leptospira* Research Laboratory as follows: a 7–10 day culture of *Leptospira interrogans* in liquid medium (GRA–Sina) was used as antigen. The density of leptospires was assessed using a counting chamber (Petroff–Hauser USA) and adjusted to 2×10^8 leptospires/mL. Six reference strains of *Leptospira interrogans* which were used as antigen including *hardjo*, *pomona*, *icterohaemorrhagiae*, *grippotyphosa*, *canicola* and *ballum*. All serum samples were serially diluted in phosphate buffer solution (PBS) in a microtiter plate (Greiner), starting from 1 in 50 dilution, using 2–fold dilution (1 in 100, 200, 400, 800 and 1600). Then, 10 μ L of serum dilution was added to 10 μ L of appropriate antigen on a microscopic slide and was placed in a petri dish with moist paper to avoid evaporation, and incubated at 30 °C for 90 minutes. Finally the slide was examined under dark–field microscope (Olympus Bx50). One antigen control and two (positive and negative) standard serum controls were used each time. Titers 1:100 or greater were considered positive. The end–point titer was determined as the highest serum dilution showing agglutination of at least 50% of the

leptospires.

3. Results

Antibodies were detected at least against one serovar of *Leptospira interrogans* in 45 sera (15.79 %) among 285 samples at a dilution 1:100 or greater. Positive titers against more than one serovar were detected in 24 sera of the positive samples (Table 1). Therefore, there were 75 positive reactions against different serovar of *Leptospira interrogans*.

Table 1

Frequency (%) and number of positive serum samples by MAT at ad:lu:ion 1:100.

Number of serovars	Number of positive sera	Frequency (%)
One serovar	21	7.37
Two serovars	19	6.67
Three serovars	4	1.40
Four serovars	1	0.35
Total	45	15.79

As Table 2 shows, Positive titers were recorded against serovar *icterohaemorrhagiae* (31 samples), *hardjo* (26 samples), *grippotyphosa* (7 samples), *pomona* (5 samples), *canicola* (4 samples) and *ballum* (2 sample).

Table 2

Number and frequency of serum samples with positive titer against each serovar, at different dilution (n,%).

Serovar	Dilutions					Total
	1: 100	1:200	1: 400	1: 800	1:1 600	
<i>Grippot yphosa</i>	6(8.00)	1(1.33)	0(0.00)	0(0.00)	0(0.00)	7(9.33)
<i>Pomona</i>	3(4.00)	1(1.33)	1(1.33)	0(0.00)	0(0.00)	5(6.67)
<i>Icterohae morrhagiae</i>	17(22.67)	6(8.00)	6(8.00)	1(1.33)	1(1.33)	31(41.33)
<i>Canicola</i>	3(4.00)	1(1.33)	0(0.00)	0(0.00)	0(0.00)	4(5.33)
<i>Ballum</i>	2(2.67)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(2.67)
<i>Hardjo</i>	16(21.33)	8(10.67)	1(1.33)	1(1.33)	0(0.00)	26(34.67)
Total	47(62.67)	17(22.67)	8(10.67)	2(2.67)	1(1.33)	78(1.00)

4. Discussion

The earliest study (1967) on leptospirosis prevalence in Iran indicated that there are 31% serum positive titer against *Leptospira interrogans* in cattle and 17% in sheep[7]. Another study showed that the prevalence of serum positive titer against leptospiral antigen has been about 24.6% in Tehran suburb dairy farms[8]. Results of studies on leptospirosis prevalence in other regions in Iran include: between 3 to 30.7 % in Tehran suburb[9], 24.24% in Mashhad suburb[10], 32% in Shiraz suburb[11], 46.8% in Karadj suburb[12], 22% in Gilan province[13–16] and finally 53.73% in Ahwaz suburb[7].

Results of previous studies about prevalence of each serovar of *Leptospira* in Iran has shown that *Leptospira hardjo* was the most (67.7%) and *Leptospira icterohaemorrhagiae* the least (0.8%) prevalent serovars in Tehran suburb[8], *Leptospira icterohaemorrhagiae* was the most and *Leptospira pomona*

the least prevalent serovars in Tehran suburb[17], *Leptospira icterohaemorrhagiae* was the most and *Leptospira pomona* the least prevalent serovars in Mashhad suburb[10], *Leptospira pomona* was the most prevalent serovar in Neyshabour suburb[18], *Leptospira grippotyphosa* was the most prevalent serovar in Urmia[19], *Leptospira canicola* was the most (39.9%) and *Leptospira hardjo* the least (4.7%) prevalent serovars in Karadj suburb [12]. *Leptospira grippotyphosa* was the most prevalent serovar in Gilan province[13–16], *Leptospira canicola* was the most and *Leptospira grippotyphosa* the least prevalent serovars in Shiraz suburb[11], *Leptospira canicola* was the most prevalent serovar in tribal area of west central of Iran[20], and finally *Leptospira grippotyphosa* was the most and *Leptospira ballum* the least prevalent serovars in Ahvaz[7].

In present study the most prevalent (*Leptospira icterohaemorrhagiae*) and the least prevalent (*Leptospira*

ballum) serovar are different from previous studies[7–27]. Maybe, species and prevalence of serovars change during the time in one area and between regions.

Conflict of interest statement

We declare that we have no conflict of interest.

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