Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(11)60032-4

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

Ehsanollah Sakhaee[1*], Gholam Reza Abdollah pour[2]

[1]*Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran*

[2]*Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Iran*

ARTICLE INFO

Article history:
Received 25 December 2010
Received in revised form 3 February 2011
Accepted 8 March 2011
Available online 30 March 2011

Keywords:
Leptospirosis
Microscopic agglutination test
Human
Iran
Prevalence
Leptospiral infection
Serovar

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 ℃ 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
Foundation Project: Supported by a grant form Research Council of Shahid Bahonar University of Kerman, Iran

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 μL of serum dilution was added to 10 μL of appropriate antigen on a microscopic slide and was placed in a petri dish with moist paper to avoid evaporation, and incubated at 30 ℃ for 90 minutes. Finally the slide was examined under dark–field microscope (Olympus B×50). 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:lution 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	
Grippot yphosa	6(8.00)	1(1.33)	0(0.00)	0(0.00)	0(0.00)	7(9.33)
Pomona	3(4.00)	1(1.33)	1(1.33)	0(0.00)	0(0.00)	5(6.67)
Icterohae morrhagiae	17(22.67)	6(8.00)	6(8.00)	1(1.33)	1(1.33)	31(41.33)
Canicola	3(4.00)	1(1.33)	0(0.00)	0(0.00)	0(0.00)	4(5.33)
Ballum	2(2.67)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(2.67)
Hardjo	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.

Acknowledgments

This research was financially supported by research council of Shahid Bahonar University of Kerman.

References

[1] Angela P, Brandao D, Camargo V, Marcos V. Macroscopic agglutination test for rapid diagnosis of human leptospirosis. *J Clin Microbiol* 1998; **36**(11): 3138-3142.

[2] Ellis WA, O'brien JJ, Neill SD, Ferguson HW, Hanna J. Bovine leptospirosis: microbiological and serological findings in aborted fetuses. *Vet Rec* 1982; **110**: 147-150.

[3] Mackintosh CG, Marshall RB, Broughton ES. Serological survey for antibodies to bovine leptospiral infections in England. *NZ Vet J* 1980; **28**: 174-176.

[4] Adler B, Murphy AM, Locarnini SA, Faine S. Detection of specific anti leptospiral immunoglobulins M and G in human serum by solid-phase enzyme-linked immunosorbent assay. *J Clin Microbiol* 1980; **11**: 452-457.

[5] Adler B, Faine S, Gordon LM. The enzyme-linked immunosorbent assay (ELISA) as a serological test for detecting antibodies against *Leptospira interrogans* serovar *hardjo* in sheep. *Aust Vet J* 1981; **57**: 414-417.

[6] Cousins DV, Robertson GM, Hustas L. The use of the enzyme-linked immunosorbent assay (ELISA) to detect the IgM and IgG antibody response to *Leptospira interrogans* serovars *hardjo*, *pomona* and tarassovi in cattle. *Vet Microbiol* 1985;**10**: 439-450.

[7] Haji hajikolaei MR, Ghorbanpour najafabadi M, Abdollahpour GR. Serological study of leptospirosis in cattle in Ahvaz. *J Fac Vet Med* 2005; **60**(1): 7-14.

[8] Maghami GR. Study of role of leptospirosis in abortion in Tehran suburb dairy farms. *Vet Organization Public (Iran)* 1980; **20**: 50-66.

[9] Moharrami M, Bazargani TT, Rad P, Bokaei S. *Seroepidemiologic study of leptospirosis in Tehran suburb dairy farms*. (DVM thesis) Veterinary Faculty of Veterinary Medicine, University of Tehran; 1992.

[10] Talebkhan M, Ghadakchi H, Vand e yousefi J, Novrouzian I. *Seroepidemiologic survey of leptospiral infection on Mashhad suburb herds. Proceedings of the 3rd National Congress of Zoonosis in Iran*; 1996, p. 35-36.

[11] Firouzi R, Vand e yousefi J. Serological survey of leptospirosis in Shiraz suburb dairy herds. *Iranian J Vet Res* 2000;**1**(2): 118-123.

[12] Goli GA. *Seroepidemiologic survey of leptospirosis in Karadj herds*. Proceedings of the 23rd World Buiatrics Congress; 2002, p. 301.

[13] Asadpour Y. *Serological survey of leptospirosis in cattle in Shaft and Fuman in Gilan province*. Proceedings of the 3rd Congress of Veterinary Clinicians in Iran; 1996, p. 27-29.

[14] Eshraghi S, Honarmand H, Khoramizadeh M, Hartskeerl RA. *Detection of Leptospires serogroups which are common cause of human acute Leptospirosis in Guilan province, Iran*. 4th Scientific Meeting of the International Leptospirosis Society; 2005, p. 241.

[15] Honarmand H, Hartskreel RA, Eshraghi S, Khoramizadeh M, Ghanaei FM. *Study on the prevalence of Leptospirosis in Guilan Province, Iran*. 4th Scientific Meeting of the International Leptospirosis Society; 2005, p. 177.

[16] Yousefi-Mashhour M, Abdollahpour G. *Epidemiologic assessment of Leptospira serotypes in North Iran, Caspian Littoral, Spring and Summer 2005*. 4th Scientific Meeting of the International Leptospirosis Society; 2005, p. 52.

[17] Sakhaee E, Abdollahpour GR, Bolourchi M, Tabatabayi AM, Sattari S. *Serological evidence of bovine; leptospiral infection in aborted cows in Tehran suburb dairy farm*. Proceedings of the 4th Scientific Meeting of the International Leptospirosis Society; 2005, p. 331.

[18] Sakhaie E, Abdollahpour GR, Sattari S, Taali AR, Sharghi ARS. *Comparative seroepidemiological study of human and bovine leptospirosis in Neyshabour (Iran)*. Proceedings of the 23rd World buiatrics congress; 2002, p. 16.

[19] Jaafari M, Maham A, Azarvandi A. *Study of leptospirosis in Urmia cattle*. Proceedings of the 3rd National Congress of Zoonosis in Iran; 1996, p. 37.

[20] Abdollahpour GR. Serological survey of human leptospirosis in tribal area of west central Iran. *Iranian J Med Sci* 2003; **28**(2): 93-95.

[21] Durham PJK, Paine GD. Serological survey for antibodies to infective agents in beef cattle in north South Australia. *Aust Vet J* 1997; **75** (2): 134-140.

[22] Ellis WA, O'Brien JJ, Neill SD, Ferguson HW, Hanna J. Bovine leptospirosis: microbiological and serological findings in aborted fetuses. *Vet Rec* 1982; **110**: 147-150.

[23] Mackintosh CG, Marshall RB, Broughton ES. Serological survey for antibodies to bovine leptospiral infections in England. *New Zealand Vet J* 1980; **28**: 174.

[24] Gerritsen MJ, Olyhoek T, Smits MA, Bokhout BA. Sample preparation method for polymerase chain reaction-based semi quantitative detection of *Leptospira interroganns* serovar hardjo subtype hardjobovis in bovine urine. *J Clin Microbial* 1991; **29**: 2805-2808.

[25] Abdollahpour GR. Isolation of *Leptospira interrogans* serovar grippotyphosa from a heifer in NewSouth Wales's. *Aust Vet J* 1995; **73**(3): 109-110.

[26] Adler B, Faine S, Gordon LM. The enzyme-linked immunosorbent assay (ELISA) as a serological test for detecting antibodies against *Leptospira interrogans* serovar hardjo in sheep. *Aust Vet J* 1981; **57**: 414-417.

[27] Adler B, Cousins DV, Faine S, Robertson GM. Bovine IgM and IgG response to *Leptospira interrogans* serovar hardjo as measured by enzyme immunoassay. *Vet Microbiol* 1982; **7**: 577-585.