Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.com

Document heading doi:10.12980/APJTB.4.2014C1206

Seroprevalence of bovine leptospiral antibodies by microscopic agglutination test in Southeast of Iran

Mohammad Khalili[1,2], Ehsanollah Sakhaee[3], Mohammad Reza Aflatoonian[1], Gholamreza Abdollahpour[4], Saeed Sattari Tabrizi[4], Elham Mohammadi Damaneh[2], Sajad Hossini-nasab[2]

[1]*Research Center of Tropical and Infectious Diseases, Kerman University of Medical Sciences, Kerman, Iran*

[2]*Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran*

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

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

PEER REVIEW

Peer reviewer

Yuki Eshita, Associate Professor, Department of Infectious Disease Control, Faculty of Medicine, Oita University, 1-1 Idaigaoka, Hasama-machi, Yufu-shi, Oita 879-5593, Japan.
Tel: +81-97-586-5701
Fax: +81-97-586-5701
E-mail: yeshita@oita-u.ac.jp

Comments

This is a good epidemiological study in which the authors proved firstly the existence of leptospirosis in Southeast Iran by using MAT.
Details on Page 357

ABSTRACT

Objective: To evaluate serological findings of bovine leptospirosis which is a zoonotic disease with worldwide distribution caused by *Leptospira interrogans*.

Methods: One hundred and sixty seven sera were collected from 9 commercial dairy herds in jiroft suburbs, from July to October 2011. Microscopic agglutination test (MAT) was used to evaluates serological findings of bovine leptospirosis in Jiroft suburb dairy farms, Kerman province, Iran.

Results: Antibodies were found by MAT at least against one serovar of *Leptospira interrogans* in 29 samples (17.36%) among 167 sera at a dilution 1:100 or higher, and *Leptospira pomona* was the most prevalent serovar. Positive titers against more than one serovar were detected in 6 sera of the positive samples.

Conclusion: This study is the first report of leptospirosis in Southeast Iran and showed that *Leptospira pomona* was the most and *Leptospira icterohaemorrhagiae* the least prevalent serovars in Southeast Iran.

KEYWORDS
Bovine leptospirosis, MAT, Serology, Iran

1. Introduction

Leptospirosis is the most common bacterial zoonosis worldwide, caused by spirochetes of the genus *Leptospira*. There are 20 species of leptospires, consisting of over 200 serovars, circulating in a wide range of animal reservoir hosts including rats, other rodents, livestock and domestic pets[1]. *Leptospira interrogans* (*L. interrogans*) constitutes the major pathogenic leptospiral species that is responsible for human infection. *L. interrogans* can readily penetrate abraded skin and mucous membrane barriers to establish a systemic infection via haematogenous dissemination and subsequently colonizes multiple organs, particularly the kidneys and liver. While wild rodents serve as natural reservoirs, humans and a few other domesticated animals are accidental hosts in the transmission cycle of leptospirosis[2,3]. In rural areas, transmission is usually associated with farming and livestock, with increased risk during the warm and rainy months. In urban areas, infection is associated with overcrowding, poor hygiene standards, inadequate sanitation and poverty, all of which typically occur in urban slums in developing countries.

*Corresponding author: Mohammad Khalili, D.V.M, Ph.D., Associate Prof. of Microbiology, Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, P.O. Box: 76169 133, Iran.
Tel: +98 341 3202909
Fax: +98 341 3222047
E-mail: mdkhalili1@yahoo.com; mdkhalily@uk.ac.ir

Foundation Project: Supported by Research Center for Tropical and Infectious Diseases, Kerman University of Medical Sciences (Grant No.: 91/43).

Article history:
Received 22 Feb 2013
Received in revised form 27 Feb, 2nd revised form 3 Mar, 3rd revised form 10 Mar 2014
Accepted 24 Mar 2014
Available online 28 May 2014

In developed countries, infection is now increasingly being associated with outdoor recreational exposure and international travel[4].

Suitability of the environment for the survival of leptospires appears to be a critical factor in maintaining the infection and transmission to humans. Leptospires have good affinity to areas where heavy rainfall results in water logging of the land. Human populations residing in such environment are at higher risk of acquiring leptospiral infection[5].

A basic knowledge of serovars and their maintenance hosts is required to understand the epidemiology of leptospirosis in a region. Though distinct variations in maintenance hosts and the serovars they carry can occur throughout the world. The general pattern is for serogroups Hardjo bovis, Pomona, and Grippotyphosa to be recoverable from cattle[6].

The diagnosis of leptospirosis is based on two principles which include the actual isolation of the leptospiral organisms and the detection of anti-leptospiral antibodies. Isolation by culture is very time-consuming and depends on the presence of live leptospira and their ability to grow on media provided, thus serological testing is a more widely used method. The detection of anti-leptospiral antibodies can be done using tests such as the microscopic agglutination test (MAT) and enzyme-linked immunosorbent assay[7]. The MAT is the gold standard test for diagnosing leptospirosis and is the most widely used method for detecting both leptospira IgG and IgM antibodies in animal sera. The sensitivity and specificity of the MAT reported in a recent study were 91.94% and 73.77%, respectively[8]. This test can be used qualitatively and quantitatively to detect the infecting serovar and titer (World Health Organization), but it requires the propagation of live leptospiral strains to be used as antigens for a hazardous and time-consuming process in which the interpretation of the results can be subjective[9].

To the authors' knowledge, there is no report of leptospirosis in Southeast Iran; therefore, the aim of this study was to investigate the presence of anti-leptospira antibodies among dairy cattle farms by MAT, using five current reference strains of *L. interrogans* in Jiroft suburb dairy farms, Kerman province, Southeast Iran.

2. Materials and methods

2.1. Sample taking

A total of 167 sera were collected from 9 commercial dairy herds in jiroft suburbs, from July to October 2011. Sera were separated after centrifugation at 3 000 g for 10 min at room temperature and kept at −20 °C until required. These samples were submitted to the Leptospira Research Laboratory of Teaching and Research Hospital of the Faculty of Veterinary Medicine at the University of Tehran, Iran.

2.2. Microscopic agglutination test (MAT)

MAT was carried out as described by Turner (1968) with some modification in Leptospira Research Laboratory as follows: Five reference strains of *L. interrogans* which were used as antigen includes: *Leptospira hardjo* (*L. hardjo*), *Leptospira pomona* (*L. pomona*), *Leptospira icterohaemorrhagiae* (*L. icterohaemorrhagiae*), *Leptospira grippotyphosa* (*L. grippotyphosa*) and *Leptospira canicola* (*L. canicola*). All sera 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 and 400). Then, 10 μL of serum dilution was added to 10 μL of appropriate antigen on a microscopic slide and incubated at 30 °C for 90 min. 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 found at least against one serovar of *L. interrogans* in 29 samples (17.36%) among 167 sera at a dilution 1:100 or higher. Positive titers against more than one serovar were detected in 6 samples (20.68%) of the 29 positive sera (Table 1).

Table 1

Frequency (%) and number of positive serum samples by MAT at a dilution 1:100 or higher, among 167 samples.

Number of serovars	Number of positive sera	Frequency (%)
One serovar	23	13.77
Two serovars	6	3.59
Total	29	17.36

According to Table 2, the highest prevalence of positive sera by MAT was found in farm 3 (4.79%), followed by farm 5 and 9 (3.59%), farm 4 and 7 (1.80%), farm 6 (1.20%) and farm 1 (0.6%), while no positive serum was find in farm 2 and 8.

Table 2

Number and frequency (%) of total and positive sera in each farm by MAT.

	Total serum samples		Positive sera	
	Frequency (%)	Number	Frequency (%)	Number
Farm 1	4	2.39	1	0.60
Farm 2	11	6.59	0	0.00
Farm 3	33	19.76	8	4.79
Farm 4	16	9.58	3	1.80
Farm 5	34	20.36	6	3.59
Farm 6	8	4.79	2	1.20
Farm 7	17	10.18	3	1.80
Farm 8	4	2.39	0	0.00
Farm 9	40	23.95	6	3.59
Total	167	100.00	29	17.36

Positive titers were detected against serovar *L. pomona* (16