



Seroprevalence of leptospiral Antibodies in Humans and Domestic Animals in Iran

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ABSTRACT

Background: Leptospirosis is an important re-emerging zoonotic disease in tropical and subtropical areas and acute febrile infection and a conveyable bacterial disease of animals and humans caused by pathogenic spirochetes of the genus *Leptospira*.

Methods: Five hundred and ninety seven serum samples (159 cattle, 142 sheep, 147 goats and 149 humans) were collected from center, southeast and northeast of Iran. MAT was performed mainly as described by Turner with some modification in *Leptospira* Research Laboratory.

Results: Antibodies were detected at least against one serovar of *Leptospira interrogans* in 97 sera (17.24%) among 597 samples at a dilution 1:100 or greater.

Conclusion: The most prevalent serovar was *icterohaemorrhagiae* and the least prevalent was *canicula*.

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Introduction

Leptospirosis is an important re-emerging zoonotic disease in tropical and subtropical areas and acute febrile infection and a conveyable bacterial disease of animals and humans caused by pathogenic spirochaetes of the genus *Leptospira*. The genus *Leptospira* has currently, thirteen pathogenic and six non-pathogenic species. The species are divided in more than 250 serovars, distributed according to some in 23 serogroups (1). Typically, the disease occurs through bacterial exposure to mucous membranes and generally results in occult form or relatively mild acute clinical signs. In cattle this infection causes reproductive losses, abortion, barrenness, stillbirths or weak calves, decrease in milk production, and even death (2). Most mammalian species are natural carriers of pathogenic leptospires. These include feral, semi-domestic and farm and pet animals as important infection sources. The risk of acquiring leptospirosis is associated with contact with animals. Therefore, leptospirosis is an important occupational disease, especially affecting farmers, slaughterhouse workers, pet traders, veterinarians, rodent catchers and sewer workers (3, 4). Therefore, the objective of this paper was to update information regarding leptospirosis and its impact on public health significance.

Materials and Methods

Sample collection and processing

Five hundred and ninety seven serum samples (159 cattle, 142 sheep, 147 goats and 149 clinically healthy men and women from rural areas) were collected from center, southeast and northeast of Iran from June 2013 to April 2014. Samples were collected aseptically using sterile anticoagulant free vacutainers. Serum was separated by

centrifugation of blood at 3000 g for 10 minutes at room temperature; the sera were transferred into 1.5 µl sterile micro tube (Eppendorf) and were kept at -20°C until required. The samples were submitted to the Leptospira Research Laboratory of Teaching and Research Hospital of the Faculty of Veterinary Medicine at the University of Tehran.

Microscopic Agglutination test (MAT)

MAT was performed mainly as described by Turner (1968) with some modification in Leptospira Research Laboratory as follows: a 7–10–day–old culture of *Leptospira interrogans* in liquid medium was used as antigen (5). The density of Leptospires was assessed using a counting chamber (Petroff-Hausser USA) and adjusted to 2×10^8 leptospires/ml. Five reference strains of *Leptospira interrogans* which were used as antigen includes: *hardjo*, *pomona*, *icterohaemorrhagiae*, *grippityphosa* and *canicola*. 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 and 800). 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°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.

Results

Antibodies were detected at least against one serovar of *Leptospira interrogans* in 97 sera (17.24%) among 597 samples at a dilution 1:100 or greater (Table 6). According to table 6, positive titers were recorded against serovar *icterohaemorrhagiae* (22 samples), *hardjo* (14 samples), *grippotyphosa* (10 samples), *pomona* (44 samples) and *canicola* (7 samples). The table, present number and frequency (%) of serum samples with positive titer against each serovar, at each dilution. Number and frequency (%) of positive, negative and total sera in cattle, sheep, goats and humans have been presented in tables 1, 2, 3 and 4, respectively. Table 5 present number and frequency (%) of each serovar in cattle, sheep, goats and human. Figure 1 and 2 show frequency (%) of female and male positive sera in different area, respectively.

Discussion

Infectious diseases are transmitted globally through animal and human movements due to eco-tourism, wildlife research, reintroduction, rehabilitation, hunting, pet trade, laboratory and food industry demands and farming. These movements and activities are major contributing factors for the transfer of leptospirosis to animals and humans and the spread of the disease to new areas (6, 7). Workers employed in agriculture belong to professional groups mostly threatened by leptospirosis. Individuals working directly with animals (farmers, cowherds, veterinarians, abattoir workers, etc.) can acquire the infection by contact with contaminated urine or working in pens contaminated by infected urine, during milking, after animal bites, after contact with aborted fetuses or parts of placenta, and infected carcasses (also during the carving of slaughtered animals in abattoirs) (8, 9, 10, 11, 12, 13). In present study humans samples were taken from rural areas where most people working directly with animals. A serological survey described by

Spanish investigators of 197 persons employed in agriculture indicated positive results in 21% (15). The highest percentages of positive results were noted in the subgroups of cray-fishers, rice-workers and butchers. Furthermore, the professions connected with the possibility of exposure to direct or indirect contact with rodents, mainly with rats, are threatened by infection. This group includes, among others, individuals working in sewer systems, miners, hunters, foresters, soldiers, rodent control workers, people working in fish farms, storehouses and harbours, in piggeries, cowsheds, etc. (14, 16, 17, 18, 19, 20, 21, and 22). Results of present study show positive titer in 17.24% of cases and all of the people who had positive titer (9 cases) were working directly with animals. Many reports provide information concerning the prevalence of *Leptospira* infections among various species of domesticated animals, e.g. cattle (23, 24, 25), swine (26, 27), sheep (28, 29), horses (30, 31), etc. The majority of these animals are based on the results of serological findings. Although some of the positive serological results can be evidence of contact only with *Leptospira* (there is no proof of current infection), the results of the above-mentioned investigations among workers employed in agriculture (17, 18, 19, 21, 22) can confirm a high risk connected with animals being potential sources of infection. Worldwide there have been more than 184 distinct serovars of *L. interrogans* belonging to 20 serogroups identified. In Southeast Asia, the most common serovars associated with disease of domestic animals and humans are *icterohaemorrhagiae*, *autumnalis*, *canicola*, *pomona*, *patoc*, *grippotyphosa*, *australis* and *poi*. The first three are the most important serovars with respect to veterinary and public health perspectives (32, 33, 34). The earliest study (1967) on Leptospirosis prevalence in Iran presented that there are 31% serum positive titer against *Leptospira interrogans* in cattle and 17% in sheep (35). Another study shows that the prevalence of serum positive titer against

leptospiral antigen has been about 24.6% in Tehran suburb dairy farms (36). Results of the same studies on Leptospirosis prevalence in other regions in Iran include: between 3 to 30.7% in Tehran suburb (37), 24.24% in Mashhad suburb (38), 32% in Shiraz suburb (39), 46.8% in Karadj suburb (40), 22% in Gilan province (41) and finally 53.73% in Ahvaz suburb (42). Results of previous studies about prevalence of each serovar in Iran show that *L. hardjo* was the most (67.7%) and *L. icterohaemorrhagiae* the least (0.8%) prevalent serovars in Tehran suburb (37), *L. icterohaemorrhagiae* was the most and *L. pomona* the least prevalent serovars in Mashhad suburb (38), *L. grippityphosa* was the most prevalent serovar in Urmia (43), *L. canicola* was the most (39.9%) and *L. hardjo* the least (4.7%) prevalent serovars in Karadj suburb (40), *L. grippityphosa* was the most prevalent serovar in Gilan province (41), *L. canicola* was the most and *L. grippityphosa* the least prevalent serovars in Shiraz suburb (39), *L. canicola* was the most prevalent serovar in tribal area of west central of Iran (44), *L. grippityphosa* was the most and *L. ballum* the least prevalent serovars in Ahvaz (42). and finally *L. grippityphosa* was the most prevalent serovars in Kerman (45). In the present study, the most prevalent (*L. icterohaemorrhagiae*) and the least prevalent (*L. canicula*) serovars were different from those of previous studies. It seems that the type and prevalence of serovars change over the time in one area and from region to region.

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Conflict of interest

None declared conflicts of interest.

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