

## Seroprevalence of *Leptospira* spp. in Slaughtered Cattle in Zanjan, Iran

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Leptospirosis is a world-widely spread zoonotic disease with numerous sources which is caused through the infection produced by the pathogenic spirochetes of the genus *Leptospira*. This research was conducted in order to determine the infection rate of the *Leptospira* epidemic in the cattle of Zanjan Province industrial slaughter house, southwest of Iran in 2011. In this study, a total of 135 random serum samples were collected from cows. All serum samples were serologically tested by microscopic agglutination test (MAT). The serum samples were tested for antibodies against eight live antigens of *Leptospira interrogans* serovars: Australis, Icterohamorrhagiae, Hardjo, Grippotyphosa, Canicola, Autumnalis, Pomona, and Sejroe. The results of this study showed that 60 (%44.4) animals had a positive reaction against one or more serovars. The most prevalent *Leptospira* serovars was Hardjo and the least one belonged to serovar Pomona. The lowest dilution that each serum was considered positive was 1:200. Based on the results of this study and by considering higher abundance of prevalence of leptospira infection and dominant titer of 1:200 reveal that leptospiral infection among the cattle of the Zanjan Province Slaughter House is explanatory of the *Leptospira* infection for which the required hygienic measures towards preventing the Leptospirosis disease ought to be taken.

**Key words:** Iran, Zanjan, cattle, leptospirosis, Microscopic agglutination test.

Leptospirosis is a zoonotic disease caused by pathogenic spirochetes of the genus *Leptospira* that are transmitted directly or indirectly from animals to humans. The World Health Organization has classified it as a reemerging disease<sup>1,2</sup>. In cattle, this disease causes agalactia, abortion, infertility, stillbirths, birth of weak calves, and retention of fetal membranes and can culminate in the death of the infected animal<sup>3</sup>. Diagnosis of leptospirosis is based on laboratory confirmation because its clinical signs are nonspecific and may

be mistaken with other febrile diseases<sup>4,6</sup>. The microscopic agglutination test (MAT), the standard reference test for the detection of leptospiral-specific antibodies. Direct observation of *Leptospira*'s clinical samples under the dark-field microscope for the purpose of their detection is an extremely arduous task and due to the reasons for these organisms being hard to grow and high probability of contamination of their lab cultures, isolating them from the clinical samples through the cultivation method is tremendously difficult and time-consuming, and is not frequently accompanied with success<sup>5,6,7</sup>. The earliest recognized report of leptospirosis in Iran is published by Rafyi and Magami (1968). Since then

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the most prevalent *Leptospira* serovars reported in Iran includes: Hardjo, Pomona, Grippotyphosa, Canicola and Icterohaemorrhagiae<sup>8,9</sup>. Based on the sero-epidemiological studies and also the bacteriological studies conducted by the other researchers, this disease is spreading throughout our nation which is threatening the general public's hygiene and the cattle production. The detection of *Leptospira* especially in the other areas of the country which have not been subjected to thorough studies is of great significance<sup>8,9,10</sup>. As the detection of indigenous and prevalent species of pathogenic *Leptospira* in every area is an important factor in identifying the disease sources. The aim of this study was to investigate the seroprevalence of leptospirosis in cattle population in Zanjan Province industrial slaughter house, using Microscopic Agglutination Test (MAT).

### Methodology

#### Study population

In this study, a total of 135 samples were randomly collected from non-vaccinated cattle industrial slaughter house in Zanjan, northwest of Iran, in January, 2011 until June, 2011.

#### Serum samples from cattle

Bovine blood samples were collected from the jugular vein with a sterile needle under aseptic condition and were transferred to the National Reference Laboratory for *Leptospira*, Department of Microbiology, Razi Vaccine & Serum Research Institute, Karaj, Iran, in 10 ml evacuated glass tubes. Serum was separated by centrifugation in 15000×g for 20 mins and stored in 2 ml cryotubes at -20°C.

#### Microscopic agglutination test

The microscopic agglutination test (MAT) was carried out according to the methods of WHO<sup>11</sup>. The following serovars were used in this study: *Leptospira interrogans* serovars: Autumnalis, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, Pomona and Sejroe which were grown in liquid Ellinghausen McCullough Johnson Harris (EMJH) medium for 7 to 14 days. In this study, serial dilution of test serum were prepared ranging from 1:200 to 1:3200 and 10 µl of diluted test sera added to an equal volume of antigen suspension on microscope slide. Following incubation at 28-30°C for 1.5h, the slide was examined under a dark-field microscope. Agglutination was noted by observing clumps of

leptospire. The lowest dilution that each serum was considered significant was 1:100. The end point titration was the highest titration in which 50% agglutination occurred, so that the lowest titration that was considered as positive was 1:200. Needful to mention that for the purpose of controlling the confirmation of the aforementioned performed test, total of two controls were placed beside the samples of which one was a positive standard serum and the second one was negative standard serum.

### Data analysis

The results of the MAT were analyzed, using Statistical Package for Social Sciences, version 16. Chi-square and Fisher's exact tests were used. A p value  $\leq 0.05$  was considered statistically significant.

## RESULTS

In this study, the results showed that antibodies were detected at least against one serovar of *Leptospira interrogans* in 60 sera (44.4%) among 135 samples at a dilution 1:200 or greater. Positive titers against more than one serovar were detected in 30 sera of the positive samples (Table 1).

In the overall number of samples, the total of 36 Hardjo samples (%29.27), 22 Canicola samples (%17.89) 20 Autumnalis samples (%16.26), 17 Icterohaemorrhagiae (%13.82), 15 Sejroe samples (%12.20), 8 Grippotyphosa samples (%6.50), and 5 Pomona samples (%4.07) showed positive reactions. The most prevalent *Leptospira* serovars were Sejro Hardjo (% 29.27), Canicola (% 17.89) and the least prevalent *Leptospira* serovars were Pomona (%4.07). In positive sera the antibody titers of 1:200 (%54.67), 1:400(%27.64), 1:800(%10.57), 1:600(%3.25) and 1:3200 (%4.07) were detected. The highest titer was 1:200 (Table 2).

**Table 1.** Frequency (%) and number of positive serum samples by MAT at dilution 1:200

Number of serovars	Number of positive sera	Frequency (%)
One serovar	30	63.1
Two servers	22	26.2
Three serovars	8	9.5
Total	60	

**Table 2.** Number and frequency of serum samples with positive titer against each serovar, at different dilution (n, %)

Serovar	Dilutions					Total
	1:200	1:400	1:800	1:600	1:3200	
Harjoe	15(12.2)	14(11.38)	5(4.7)	0(0.00)	2(1.63)	36(29.27)
Canicola	19(15.45)	0(0.00)	1(0.81)	1(0.81)	1(0.81)	22(17.89)
Autumnalis	8(6.5)	7(5.69)	3(2.44)	1(0.81)	1(0.81)	20(16.26)
Icterohaemorrhagiae	10(8.13)	6(4.88)	0(0.00)	0(0.00)	1(0.81)	17(13.82)
Sejroe	6(4.88)	3(2.44)	4(3.25)	2(1.63)	0(0.00)	15(12.20)
Grippotyphosa	7(5.69)	1(0.81)	0(0.00)	0(0.00)	0(0.00)	8(6.50)
Pomona	2(1.63)	3(2.44)	0(0.00)	0(0.00)	0(0.00)	5(4.7)
Total	67(54.47)	34(27.64)	13(10.57)	4(3.25)	5(4.7)	123(100)

## DISCUSSION

Leptospirosis is regarded as one of the most significant and prevalent zoonotic diseases in the world and it has a higher degree of prevalence in the areas with moderate and humid climates such as the northern part of Iran<sup>[8, 10]</sup>. A chief portion of the cattle from the Zanjan Province of Iran is produced from the country's north and consequently there are favorable conditions for *Leptospira*'s growth and infection of Leptospirosis. The present study for the first time by Razi's Reference Laboratory for Vaccines and Serum Production in Karaj, Iran. The results of this study indicated that prevalence of Leptospirosis in the cattle from the Industrial Slaughter House of the Zanjan Province (northwest of Iran) is at %44.4. The earliest study on leptospirosis prevalence in Iran indicated that there are 31% serum positive titer against *Leptospira interrogans* in cattle and 17% in sheep. The similar studies that have been done by the other researchers in other parts of Iran were indicative of the relatively high rate infection among the cows. In the research that was performed at the Industrial Slaughter of Rasht (north of Iran), out of the 98 serological samples that were obtained %37.8 was positive<sup>[8,10,12]</sup>. The level of contamination of the cows in the Province of South Azerbaijan (northwest of Iran) has been as high as %48.5. In Ahvaz (southeast of Iran) the level of contamination of the cows was equal to %53.75<sup>[8, 10]</sup>. In different countries of the world, numerous studies with respect to the Leptospirosis disease were done, most of which exhibited high percentage of infection among the cattle. The reported results of seroprevalence of

leptospirosis infection in cattle are different from country to country<sup>13-15</sup>. In Portugal, 15.3% of cattle reacted to one or more serovar of *L. interrogans*<sup>16</sup>. In Malaysia, 40.5% of cattle reacted to one or more serovar of *L. interrogans*<sup>17</sup>. In Turkey, 25.42% of cattle reacted to one or more serovar of *L. interrogans*<sup>18</sup>.

In contrast to other studies in Iran, the percentage of prevalence of leptospira infection in Zanjan, Northwest of Iran, (%44.40) was higher after the city of Ahvaz in the southwest of Iran, (%53.79), high prevalence of infection and dominant titer of 1:200 reveal that leptospiral infection in Zanjan is endemic and occurs mostly in subclinical form<sup>8,10,12</sup>.

Considering its climate the province of Zanjan has the suitable conditions for growth, survivability, and transmission of the mentioned bacteria. The findings of this study display that the prevailing serovars in the area include Hardjo at %29.27, Canicola at %17.89, and Autumnalis at %16.26 out of which exist numerous reports of Hardjo serotype epidemic among the bovine population of Iran and the world<sup>19,20,21</sup>. 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, *Leptospira* Icterohaemorrhagiae was the most and *Leptospira* Pomona the least prevalent serovars in Mashhad suburb, *Leptospira* Canicola was the most (39.9%) and *Leptospira* Hardjo the least (4.7%) prevalent serovars in Karadj suburb<sup>[8,10,12]</sup>. The most prevalent serovars at the Industrial Slaughter House of Rasht (North of Iran) was related to

Pomona (%49)<sup>[12]</sup>. Through similar studies that took place at the cattle barns of Ahvaz (Southwest of Iran), after a research on the cows was done, the most prevalent serovar was introduced as Grippotyphosa (%30.70) and serovar of Hardjo (%14.35), Pomona (%18.33), Canicola %53.15, Icterohaemorrhagiae %11.5 and Ballum %10.16 were placed in a consecutive positions. The predominant serovar in sheep in Ahvaz was Pomona<sup>[9,11,12]</sup>. There are a great number of reports in regard to the abundance of these serovars and the other ones from various regions of the world<sup>[21]</sup>. The comparison of the aforementioned findings against the present study illustrates that in every area not only there exist the abundance of contamination but also an abundance of the different serovars is observed and this is indicative of transition by the diverse serovars of *Leptospira*. Importantly, not only the level for an abundance of contamination and the kind of serovar vary amongst different parts but they may also be different throughout different years, thus by depending on this, there could be a need for a new study in every area once in every few years<sup>[10]</sup>.

In this study, 60 samples (%44.4) had positive reaction. Also through another study of the researchers, similar findings corresponding to the above results have been reported<sup>[8,12]</sup>. The percentage of prevalence of leptospira infection in the Zanjan Province with comparison to the similar studies in the other cities of Iran has the second highest abundance after Ahvaz (Southwest of Iran). Based on the results of this study and by considering higher abundance of prevalence of leptospira infection and dominant titer of 1:200 reveal that leptospiral infection among the cattle of the Zanjan Province Slaughter House is explanatory of the *Leptospira* infection for which the required hygienic measures towards preventing the Leptospirosis disease ought to be taken.

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