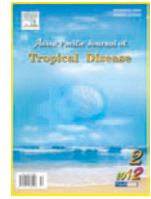




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Epidemiological patterns of *Leptospira* spp. among slaughterhouse workers in Zanjan– Iran

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ABSTRACT

Objective: To determine epidemiological patterns of *Leptospira* spp. among slaughterhouse workers in Zanjan– Iran during 2011. **Methods:** 98 human blood samples collected from slaughterhouse workers in Zanjan province, in 2011. The serum samples were analyzed with the microagglutination test (MAT) in order to detect antibodies against *Leptospira* interrogans. **Results:** The results of this study showed that from 98 Samples, 34 Samples (34.7%) were positive to different leptospiral serovars. The lowest dilution that each serum was considered positive was 1:200. The most prevalent *Leptospira* serovars was Hardjo (47.8%), Grippityphosa (15.2%), and the lowest belonged to serovar Sejro (4.3%). **Conclusions:** Leptospirosis is a work–related disease and slaughterhouses' workers face a higher risk than other individuals in danger of contracting it which, in turn, leads to transmittance of the infection into the food and protein cycle. Early diagnosis of the disease could prevent its occurrences in these groups of workers.

1. Introduction

Leptospirosis is a worldwide infectious zoonosis, caused by pathogenic spirochetes of the genus *Leptospira* spp. [1–3]. Human infection results from exposure to infected urine of carrier mammals, either directly or via contamination of soil or water. Leptospirosis is associated with occupational and recreational activities [2,4]. Leptospirosis typically has two clinical forms: anicteric and icterohaemorrhagic. Fever, chills, headache, severe myalgia, conjunctival suffusion, anorexia, nausea, vomiting, and malaise usually characterizes acute leptospirosis [2,5]. These diseases affect many groups of workers which keep direct or indirect contact with animals, mainly without adequately using protection measure [6,7]. In slaughterhouse workers transmission occurs through contact with urine, blood and organs from infected animals [3,7]. The microscopic

agglutination test (MAT), the standard reference test for the detection of leptospiral–specific antibodies. This test measures the ability of the patient's serum to agglutinate live leptospiras. In Iran, leptospirosis was first reported in 1968 [8]. The aim of this study was to test the epidemiological patterns of *Leptospira* spp. among cattle industrial slaughterhouse workers in Zanjan– Iran

2. Materials and methods

The Zanjan province is situated in the northwestern region of Iran and it has moderate climate conditions. In this research, the industrial slaughterhouse that is located in Zanjan province underwent studying.

In this study, a total of 98 samples of serum were collected from industrial slaughterhouses workers of Zanjan–Iran. The inclusion criteria were all the workers of the aforementioned slaughterhouses who were in close contact with live animals and their secretions, and with carcasses. These workers performed different functions in the slaughterhouses. In addition, each of the slaughterhouse workers involved in

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the study signed an individual informed consent.

The serum samples were obtained by venous puncture and were transferred to the National Reference Laboratory for *Leptospira*, Department of Microbiology, Razi Vaccine & Serum Research Institute, Karaj, Iran. Serum was separated by centrifugation in 3000 g for 10 mins and stored at -20°C until their analysis under laboratory conditions. The Serum samples were tested for antibody to *Leptospira* interrogans serovars: Autumnalis, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, Pomona and Sejroe, using the microscopic agglutination test (MAT). The antigens were 7 to 14-day-old live cultures grown in EMJH liquid medium. Serum samples were diluted to 1:100, 1:200, 1:400 and 1:800. For each test series, positive and negative controls were used. 10^{μ}L of antigen were mixed with the same volume of serum dilutions and incubated for 90 min at 30°C . For positive serums, the lowest titer of the test 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. The results of the MAT were analyzed, using statistical package for social sciences, version 16. Chi-square and Fisher's exact tests were used.

3. Results

The result showed the overall seroprevalence of leptospirosis among slaughterhouse workers obtained in this study was 98 (34.7%). The most prevalent *Leptospira* serovars to which antibodies were detected include Hardjo (47.8%), Grippotyphosa (15.2%), Canicola (13.0%). Seven samples (20.6%) showed serological reaction with more than one serovar. One sample (3.0%) showed serological reaction with four serovars: Canicola, Autumnalis, Hardjo and Icterohaemorrhagiae, and four samples (11.8%) showed reaction with three serovars: Canicola, Grippotyphosa and Hardjo. two samples (5.9%) showed serological reaction with two serovars: Grippotyphosa and Icterohaemorrhagiae (Table 1).

Table 1. Number and frequency of serum samples with positive titer against each serovar, at different dilution.

serovar	Dilutions(n,%)			
	1:200	1:400	1:800	Total
Harjoe	7(30.4%)	9(56.2%)	6(85.7%)	22(47.8%)
Grippotyphosa	2(8.7%)	5(31.25)	0	7(15.2%)
Canicola	6(26.1%)	0	0	6(13.0%)
Sejroe	2(8.7%)	0	0	2(4.3%)
Autumnalis	2(8.7%)	0	1(14.3%)	3(6.5%)
Pomona	0	0	0	0
Icterohaemorrhagi	4(17.4%)	2(12.5%)	0	6(13.0%)
Total	23(50.0%)	16(34.8%)	7(15.2%)	46(100.0%)

Concerning the demographic characteristics of the slaughterhouse workers, the age groups of 20–30 years and 50–60 years had a P value of 0.549. Also, the groups with 0–10 and 20–35 years as slaughterhouse workers have a risk for

the presence of antibodies against *Leptospira* spp. ($P=0.377$) (Table 2). All individuals who were in direct contact with the cattle were greatly stricken with the disease.

Table 2.

Risk factors associated with the seroprevalence of leptospirosis among slaughterhouse workers from the province of zanjaan- Iran.

Variable	Seropositive %	Seronegative %	P-value
Age group			
20–30 years old	34.7	65.3	0.007
35–60 years old	26.4	73.5	0.549
Years as slaughterhouse workers			
0–10 years old	73.5	26.4	0.377
20–35 years old	29.4	70.5	0.377

4. Discussion

Leptospirosis, is an occupational zoonosis, which affects people in close contact with infected animals or their secretions. The World Health Organization has classified it as a reemerging disease[2,9]. The results of the present study showed that out of the 98 serological samples that were obtained 34 samples (34,7%) was positive. The majority of positive serum samples reacted to leptospira serovar Hardjo (47.8%); the other commonly reacting serovars being leptospira serovars Autumnalis(6.5%) and Icterohaemorrhagiae (13%), Canicola (13%) and Grippotyphosa (15.2%) and Sejroe (4.3%). Leptospirosis often occurs in the clinical form and therefore the argument generally arises around the serological assaying and the existence of antibodies against the serovers of *Leptospira*. In this study, the individuals with seropositive exhibited positive reaction with the titer of 1:200 and in the individuals with the titer of 1:200 and higher the symptoms of the disease can be clearly diagnosed. Ebrahimi et al. reported the serological prevalence of *Leptospira* serovers at %48.5 in the central regions of Iran[10]. Similar results have been reported for other countries; i.e., prevalence of 15% in Nicaragua [11], 13.1% in Colombia [12], prevalence of 2.6% in Brazil [13] and 3.3% for Chile [14]. In Bombay of India, from every 169 individual's suspect of being stricken with *Leptospira*, 74 people (43.7%) were diagnosed positive from the serological aspect [15]. In the research that was performed at the slaughter workers of Mexico, out of the 292 serological samples that were obtained 8.22% was positive. [16] In this study, the most prevalent *Leptospira* serovars was Hardjo and the lowest belonged to serovar Sejroe. In Iran, the highest prevalence rate has been related to the serovar Hardjo (54.12%) and following down the ladder they have been for Pomona, Canicola and Icterohaemorrhagiae. [10] In Italy, the most frequent serovar was Icterohaemorrhagiae [17]. The serovars identified in Mexico have been Tarassovi, Icterohaemorrhagiae, Pyrogenes, Grippotyphosa and Panama; the abovementioned authors also reported the presence of Hardjo, Bratislava, Pomona and Canicola [1,18]. The serovar Hardjo has been diagnosed in slaughterhouse workers from Chile [19]. In a study at the slaughterhouses workers of Mexico the serovars to which antibodies were

detected include Hardjo (56.52%), Bratislava (56.52%), Pomona (13.04%), Muenchen (13.04%), Canicola Hond Utrech IV (8.7%) and Wolffi (4.35%) [16]. The serological prevalence of Leptospirosis in the slaughterhouses workers of this study were higher as compared with the one belonging to the other slaughterhouses of the country [16,20,21]. The individuals' age also is considered as an occupational risk factor for Leptospirosis and therefore the age group afflicted with the disease in this study was similar to those which were reported by the other researchers [16,20,22]. In the present study 33.3% of the positive samples belonged to those individuals who had the employment duration of longer than 10 years which meant they had been in direct contacts with the cattle. This result is consistent with previous studies [16,20–22]. In the opinion of the researchers, inappropriate slaughtering and transporting of infected carcasses cause the high epidemic of Leptospirosis among the slaughter houses' workers.

The infection rate for the individuals employed in the Slaughter house of Zanjan is increasing which, in turn, will increase the probability of food contamination by these individuals. It seems that use of gloves and masks during contacts with carcasses for their methodical placement and transportation could lower a significant level of risk for food products' contamination in the slaughter house to a minimum possible.

Conflict of interest statement

We declare that we have no conflict of interest.

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