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Brief Report

Serological Survey of Human Leptospirosis in tribal Areas of West Central Iran

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Abstract
Leptospirosis is one of the zoonotic diseases caused by different serovars of the species *Leptospira interrogans*. The source of infection in humans is usually either direct or indirect contact with the urine of infected animals. In this study, 400 human blood samples collected from tribal areas of Farsan and Koohrang cities in West Centeral Iran. The serum samples were analyzed employing Microscopic Agglutination Test (MAT). From 400 Samples, 194 Samples (48 .5%) were positive to different leptospiral sreovars at a minimum titre of 1/100. The highest seroprevalence was to serovar *hardjo* (54 .1% of seropositives) and the least was to serovar *grippotyphosa* (1%). Seroprevalency of women were higher than men
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Keywords • Leptospirosis • microscopic agglutination test • tribal regions

Introduction

Leptospirosis is a zoonotic disease caused by many leptospiral serovars belonging to a single species, *Leptospira interrogans*, which is pathogenic for many wild and domestic animals, as well as humans.[1] Rodents, dogs and farm animals are the most common sources of human disease. *L. interrogans* can cause subclinical infection, a mild infiuenza-like febrile illness or severe systemic disease (Weil's disease). Most human infections result from recreaional exposure to contaminated water or occupational exposure to infected animals.[2] The reference method for all serological tests is the microscopic agglutination test (MAT). This test measures the ability of the patient's serum to agglutinate live leptospiras. In this test, serial dilutions of the patient's serum are mixed with the test antigens and then examined microscopically for agglutination. Infected patients have a titre of at least 1/100 or higher.[2] This investigation was conducted to see if there is antileptospiral antibodies in human serum samples in tribal areas of Farsan and Koohrang cities in Chahar Mahal province of Iran.

Materials and Methods

In this study, 400 blood serum samples (199 men, 201 women) from 8 tribal areas of Farsan and Koohrang cities (In Chahar-Mahal province) were collected from apparently healthy adult people. Collection of samples took 4 months from July - October 2002. The MAT was carried out at the Leptospira Research Laboratory of the Faculty of Veterinary Medicine of Tehran University in Mardabad - Karaj.

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Table 1: seroprevalenc of leptospiral serovars at serum titre of 1:100

Serovar	Seropositive women		Seropositive men		Total	
	Number	Percent	Number	Percent	Number	Percent
L. grppotyphosa	1	0.51	1	0.51	2	1.03
L. pomona	24	12.24	16	8.24	40	20.61
L. hardjo	76	39.17	29	14.94	105	54.12
L. icterohaemorragiae	27	13.91	12	6.18	39	20.10
L. canicola	15	7.73	8	4.12	23	11.85

The number of samples in each tribal area was determined based on its population and blood donors were urban or farm living individuals that served as random donors. The Serum samples were tested for antibody to leptospira serovars *grippotyphosa, pomona, icterohaemorrhagiae*, *canicola* and *hardjo*, using the microscopic agglutination test.[2] The antigens were 7 to 14-day -old live cultures grown in EMJH liquid medium. Serum samples were diluted to 1:50, 1:100, 1:200, 1:400, 1:800 and 1:1600. For each test series, positive and negative controls were used. Ten microliters of antigen were mixed with the same volume of serum dilutions and incubated for 90 min at 29˚ $\pm$ 1 centigrade.[3] For positive serums, the titre of the test was expressed as the reciprocal of the highest dilutions giving more than 50% agglutination. Each serum tested was classified according to reactions to the test for *L.grippotyphosa, L. pomona, L. Icterohaemorrhagiae, L. Canicola , L. hardjo* and tribal area as well as the gender of the subject. The level of statistical differences between leptospiral titres, tribal areas, men and women was determined using standardized Z test.

Results

The results of MAT on 400 serum samples (201 women and 199 men) from 11 tribal areas demonstrated 194 (48:50%) positive samples at serum titres of 1:100 or more. In this study, 109 samples (56.18% of seropositives) belonged to women and 85 (43.81%) belonged to men which is statistically significant (Z>1.96). The most seroprevalent serovar in these areas was serovar *hardjo* (54.12%) and the least was serovar *grippotyphosa* (1.00%). Serum samples of two males were positive for serovar *Icterohaemorrhagiae* at a titre of 1:200; in higher titres there was no reaction to any serovar. Table 1 indicates seroprevalency of five leptospiral serovars in men and women serum samples.

Disscussion

This serosurvey was carried out in tribal areas of West Central Iran to determine the prevalence of leptospirosis in tribal people. Sera from both men (n=199) and women (n=201) were screened for antibodies by microscopic agglutination test (MAT). Totally 194 serum samples (48.50%) reacted to one or more leptospira serovar at a serum titre of 1:100. The majority of positive serum samples reacted to leptospira serovar *hardjo* (54.12%); the other commonly reacting serovars being leptospira serovars *pomona* and *icterohaemorrhagiae* (20.61% and 20.10%), *canicola* (11.85%) and *grippotyphosa* (1.00%). Compared to other seroinves-

Table 2: Numbers of serum samples and seropositives in 11 tribal areas

Tribal area	Number of samples			Number of seropositives			significancy at Z >1.96
	Men	women	total	Men	Women	Total	
Bazouft	86	90	176	38	71	109	s
Samsamy	15	15	30	9	3	12	s
Shahriari	15	15	30	8	13	21	s
Chelgerd	40	40	80	27	17	44	s
Philabad	10	10	20	2	3	5	ns
Deh-cheshmeh	5	5	10	1	2	3	ns
Jouneghan	5	5	10	-	-	-	-
Dashte zaree	5	5	10	-	-	-	-
Baba heidar	5	5	10	-	-	-	-
Beergan	8	7	15	-	-	-	-
Saragha-seyed	5	4	9	-	-	-	-
Total	199	201	400	85	109		s

S: significant
NS: not significant

tigations[4, 5] this study indicates higher seroprevalence of human leptospirosis that is probably due to geographical factors[6] or traditional life systems. Likewise in urban areas the seroprevalency seems to be higher.[2] Higher seroprevalency in women (56.18%) compared to men (43.81%) could be due to close contact of women and animals in tribal life. Differences in the numbers of seropositive people in some tribal areas (Table 2) are statistically significant (Z>1.96) and may indicate an endemic infection in these areas. The number of seropositive women to leptospira serovar *icterohaemorhagia* (13.91%) was significantly higher than men (6.18%), considering that the main host reservior of this serovar are rodents. It is concluded that in tribal life, women are more exposed to materials contaminated by urine of infected rodents.[7] For seropositives reacting to serovar *hardjo* also difference between women (39.17%) and men (14.94%) is statisically significant (Z>1.96). Cows are the most common host reservior for this serovar, since tribal women are more involved in cow milking and calving practices, thus explaining higher ratio of reactors to serovar hardjo .[8] It must be pointed out that seropositive diagnosis indicates past exposure to leptospira serovars and some seronegative individuals may have experienced infection in the past but since antibody titre declined, were not detected in this study .

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