Risk Factors of Leptospirosis in Khuzestan, South West of Iran, 2012

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Background: Leptospirosis as a common zoonotic disease is the widest spread infection worldwide. Human is infected via direct contact with infected animals or through exposure or drinking contaminated water infected by animal urine.

Objectives: The aim of this study was to identify risk factors for leptospira infection in Khuzestan which is expected to be an endemic area for this infection.

Patients and Methods: As part of an investigation on rural area in Khuzestan, this comparative study was conducted in the region. Sixty five cases, which were positive for IgM anti-leptospira antibodies, and 295 controls that were negative for IgM antibodies included in the study. A questionnaire including variables related to leptospirosis exposure was administered. SPSS-16 using Chi square and Fisher exact test were used to compare data. Differences with P-values less than 0.05 were considered as significant. The 95% confidence interval (CI) for the odds ratios (OR) were calculated.

Results: Occupation in rice farm was the most important risk factors (OR: 5.32, 95% CI = 2.74-10.43, P < 0.0001). Other risk factors were as: exposure to rat/rodent in house (OR: 3.53, 95% CI = 1.98-6.29, P < 0.0001), swimming in river or brooks (OR: 4.02, 95% CI = 2.21-7.47, P < 0.0001) and keeping cattle in house (OR: 11.53, 95% CI = 3.50-37.97, P < 0.0001).

Conclusions: The main risk factors for this infection in Khuzestan are rice farming and keeping animals (such as cattle) as well as contact to rodent in houses. Another frequent risk factor was swimming in river or brooks.

Keywords: Leptospirosis; Risk Factor; Case-Control Study

1. Background

Leptospirosis as a common zoonotic disease is the most wide spread infection worldwide. Humans most commonly become infected through occupational, recreational, or animal contact with the urine of carrier animals, either directly or indirectly by contaminated water or wet soil (1). Human leptospirosis has been reported in Northern area of Iran; Guilan and Mazandaran (2, 3). Wide spread of animal leptospirosis is observed in most area of Iran (3, 4). Clinical spectrum of leptospirosis is broad ranging from subclinical to severe fatal illness, but the most frequent initial clinical presentation is fever, head ache and myalgia (5-8). Leptospirosis outbreaks have been reported following swimming in and drinking contaminated water. Although, skin barrier is the classically acknowledged route of transmission; however, previous studies have described that ingestion of contaminated water should be considered as an important risk factor for illness. Previous studies have also found that ingestion of leptospira contaminated water and food was associated with disease (5, 6).

Certain occupational groups, including veterinarians, butchers, shepherds, rice-farmers, fishermen, sugarcane workers, sewer workers, and military personnel, are considered to be at increased risk of leptospirosis (1, 9). Leptospirosis as an emerged infectious disease is considered as an important public health problem worldwide (10).

Some people of Iranian urbanities are accustomed to on weekends to travel to villages where there is a risk of exposure to infection. Although, in reported studies some risk factors for leptospirosis is described, but these factors varies in different geographic area and socioeconomic status (11-13).

Most leptospirosis cases are diagnosed by serology. The reference standard assay is the microscopic agglutination test (MAT), but in limited resource area due to diagnostic capacity of laboratories, other serological test measuring anti leptospira – IgM antibodies are used to detect recent or current leptospirosis (2, 14-16). Cross reaction IgM-antibody may be associated with other spirochetes organisms and autoimmune diseases (5).

2. Objectives

There are few and limited studies on leptospirosis, but no reports on risk factors for this infection in Iran (2, 4).
To identify potentially risks factors for *leptospirosis* in rural area in Iran, We conducted this study. The study was conducted in Khuzestan Province, a province in the southwestern region, which is suspected to have latent epidemic of *leptospirosis*. The finding of this study enables local health authorities to plan disease prevention and control.

### 3. Patients and Methods

In a cross-sectional study from December 2012 to February 2012, two hundred and eighty person in rural area of Khuzestan in South west Iran were studied. Rural inhabitants in rice farming areas in south, north and mid zone of the province; Shadegan, Baghemalek and Ahvaz, respectively were randomly selected for this study using detailed maps of the cities in Khuzestan Health Center. A standardized questionnaire that included variables related to *leptospira* exposure were administered at each participant. Required data collected by this questionnaire included socioeconomic status, sanitation system, water source, food source, animals, rats or rodents contact. In addition, demographic information, occupational and environmental exposure was included. Sample size was calculated according to statistics formula based on prevalence in previous studies.

Blood samples were collected by trained lab personnel during the interview. Collected blood specimens were frozen at -2°C until testing. The samples were tested for anti-*leptospira* IgM antibodies using an IgM anti-*leptospira* enzyme-linked immunosorbent assay. The kit used was Serion ELISA classic ESR 125M (D-97076 Wurzburg, Germany, 2012). To standardize the tests, first all associated IgM antibodies against other spirochetes as well as rheumatoid factors (RF) were removed by washing and adding buffering protein in the test field, and then anti Leptospira – IgM antibody was measured. According to the kit’s instructions a test with value of more than 20 IU/mg was considered positive and interpreted as an evidence of recent or current infection. Values 15-20 IU/mg defined as borderline as and lower than 15 were negative. Of total 288 samples, 8 samples with borderline results were excluded. Individuals with positive tests were defined as cases and those with negative results were defined as control. Data of cases and controls were statistically compared.

SPSS software system, version 16 was used to derive descriptive statistics and in subsequent multivariable analyses. Chi square and Fisher exact test were used to compare data in both groups. Differences with P-values less than 0.05 were considered as significant. The 95% confidence interval (CI) for the odds ratios (OR) were calculated.

### 4. Results

Two hundred and eighty persons including 160 male with mean age of 43.7 ± 21.2 years and 120 female with mean age of 41.9 ± 22.7 years were enrolled.

Demographic characteristics including sex and age groups among cases and controls are shown in Table 1. Gender was not significantly associated with infection (P = 0.06), although infected men were more than women. The infection rate among male was higher than males (66.2% vs. 54.3%). Age was significantly associated with infection (P = 0.006). Person aged above 35 years were more infected than those below 35 years.

Household facility such as having radio, television, refrigerator that reflects socioeconomic status, sanitary facility such as water source, household animal ownership (e.g. horse, cow, sheep) and rodent exposure (rodents/rats in house) that are *leptospiros* exposure related data are shown in Table 2. There were significant differences between case and control (P < 0.05).

Occupation in rice farm was the main risk factors (OR: 5.32, 95% CI = 2.71-10.43, P < 0.0001). Other risk factors were: exposure to rat/rodent in house (OR: 3.53, 95% CI 1.98-6.29, P < 0.0001), swimming in river or brooks (OR: 4.02, 95% CI 2.21-7.47, P < 0.0001), keeping domestic cow or horse/donkey (OR: 11.53, 95% CI 3.50-37.97, P < 0.0001). Individual activities such as taking bath, gathering water of river or brook, washing clothes and travel out of community in both groups were similar. Outdoor activities of studied persons such as occupation, swimming and walking are shown in Table 3.

### 5. Discussion

In this study, 22.5% of rural community in this region was community in the region was seropositive for *leptospirosis*. Rural inhabitants due to their life style and jobs are in direct and indirect contact by animals and rats or rodents. In the present study by comparative analysis of data of seropositive and sero-negative individuals, we found that rice-farm occupation was an important risk factor in rural area. Rice farmers due to light touch bare feet with contaminated water are at higher risk of exposure to the infectious agent. Contact with rodents, domestic animals and rat in rural houses were also shown to be an important risk factor for the farmers as well as other village inhabitants. House of Iranian villagers because of keeping animals such as cattle, sheep, and dog in addition to stocked farm crops such as rice or wheat is a good place for rodent/rat entrance, therefore peoples in rural community at home are also exposed with *leptospiroli* sources. Another risk factor was swimming in water sources such as rivers, lakes or brooks in the villages or in neighboring area. Our findings is in consistent with other studies, contact with rodents and rats, exposure to the water of rivers and streams with high probability of contamination with urine of rodents/rat or other animals and working with bare feet in farms are considered as the main risk factors for *leptospirosis* (10-12, 17-19).
Table 1. Demographic Characteristics Among Seropositive (Case) and Seronegative (Control) Persons

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases (n=65) IgM+; No. (%)</th>
<th>Controls (n=215) IgM-; No. (%)</th>
<th>OR (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>43 (66.2)</td>
<td>117 (54.3)</td>
<td>1.64 (0.92-2.92)</td>
<td>0.06</td>
</tr>
<tr>
<td>Female</td>
<td>22 (33.8)</td>
<td>98 (45.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, (y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>0 (0.0)</td>
<td>2 (0.9)</td>
<td>2.24 (1.21-4.14)</td>
<td>0.006</td>
</tr>
<tr>
<td>15-35</td>
<td>17 (26.2)</td>
<td>93 (43.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;35</td>
<td>48 (73.8)b</td>
<td>120 (55.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aAbbreviations: OR: Odds ratio (95% confidence interval), --: Not applicable
b Statistically significant (P< 0.05), OR: Odds ratio (95% confidence interval)

Table 2. Household Factors of Health Facilities, Socioeconomics, Animal Contacts and rat or Rodents in Houses Among Seropositive (case) and Seronegative (control) Persons

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases (n=65) IgM+; No. (%)</th>
<th>Controls (n=215) IgM-; No. (%)</th>
<th>OR (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health center access</td>
<td>63 (96.9)</td>
<td>213 (99.1)</td>
<td>--</td>
<td>0.23</td>
</tr>
<tr>
<td>Safe drinking water</td>
<td>49 (75.4)</td>
<td>182 (84.7)</td>
<td>0.56 (0.28-1.09)</td>
<td>0.06</td>
</tr>
<tr>
<td>Sanitary latrine</td>
<td>41 (63.1)</td>
<td>196 (91.2)</td>
<td>0.17 (0.08-0.31)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Fair socio economic</td>
<td>45 (69.2)</td>
<td>193 (89.8)</td>
<td>0.26 (0.13-0.51)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Animal contact</td>
<td>62 (95.4)</td>
<td>138 (64.2)</td>
<td>11.53 (3.50-37.97)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Rat/rodent in house</td>
<td>40 (61.5)</td>
<td>67 (31.2)</td>
<td>3.53 (1.98-6.29)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

a Statistically significant (P< 0.05)

Table 3. Outdoor Activities Among Seropositive (case) and Seronegative (control) Persons

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases (n=65) IgM+; No. (%)</th>
<th>Controls (n=215) IgM-; No. (%)</th>
<th>OR (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmers</td>
<td>52 (80)</td>
<td>92 (42.8)</td>
<td>5.32 (2.71-10.43)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Shepherds</td>
<td>3 (4.6)</td>
<td>11 (5.1)</td>
<td>0.89 (0.19-3.52)</td>
<td>0.58</td>
</tr>
<tr>
<td>Sugar cane</td>
<td>14 (21.5)</td>
<td>39 (18.1)</td>
<td>1.21 (0.56-2.44)</td>
<td>0.32</td>
</tr>
<tr>
<td>Habitual</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swimming in river</td>
<td>28 (43.1)</td>
<td>34 (15.8)</td>
<td>4.02 (2.21-7.47)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Bare foot walking</td>
<td>17 (26.1)</td>
<td>54 (25.1)</td>
<td>1.09 (0.62-1.99)</td>
<td>0.49</td>
</tr>
<tr>
<td>Cloth washing</td>
<td>20 (30.7)</td>
<td>73 (33.9)</td>
<td>0.92 (0.41-1.51)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

In the present study although, low socioeconomic and low level of sanitary latrine were more observed in infected case than in non-infected person, however, unfavorable economic situation and lack of sanitary latrine cannot be considered as independent risk factor for infection. These factors seem to be indicators of low income and life habits in farm workers. In this study gender and age were not considered as independent risk factors. Although males are infected more than females, but, this effect is not statistically significant. The mean age of both groups was not significantly different, but those older than 35 years were the most affected. It is a fact in most of villages in the region usually young people is not interested to work in villages and towns, so they prefer to stay in the big city to work. Our results are in agreement with the results of some reports from Guilan and Shahre-Kord in Iran (2, 3, 20) and Bangladesh (21), but are different with some other reports in the world (5, 10, 12, 13). The reason for these differences is attributed to difference in socioeconomic, life style, religious behavior, female involvement in occupational activities and geographical variations.

The study was limited by serological diagnosis which may mimic recent infection from previous infection. As mentioned in methodology section isolation of Leptospira by urine or blood culture because of long time duration and technical limitation on diagnostic capacity in the region was not done in this study, in addition results of serological diagnosis are acceptable for study purposes. In conclusion, the most important risk factors for this
infection are rice farming, keeping animals (such as cattle, sheep, and dog as well as contact to rodent) in houses. Other risk factors were swimming in river or brooks.

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Authors’ Contribution
All authors have participated equally in this study.

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