Prevalence of Serum Antibodies Against Six Leptospira Serovars in Sheep in Tabriz, Northwestern Iran

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Abstract: Leptospirosis is an infectious zoonosis and its prevalence in sheep is unknown in Tabriz, northwestern Iran. Rodents and wildlife are a major source of infection that discharges the bacteria from their urine. The aims of the survey were evaluation of the seroprevalence of Leptospiral infection in sheep and the relationship between seroprevalence and animal and environmental factors in Tabriz. In this study, 359 serum samples randomly collected from sheep slaughtered in Tabriz industrial slaughterhouse since December 2004 to November 2005. Sera were stored at -20°C until use. They were initially screened at serum dilution of 1:100 against 6 live antigens of Leptospira interrogans serovars pomona, canicola, hardjo, ballum, icterohaemorrhagiae, grippotyphosa using the microscopic agglutination test (MAT). The samples were considered positive if ≥ 50% of agglutination of leptospire in a dilution tests serum of ≥1: 100 were observed. Sera with positive results were titrated against reacting antigens in serial 2-fold dilutions from 1: 100 to 1:800. 66 sera (18.4%) at dilution ≥1:100 were seropositive against 1 or 2 of serovars. Grippotyphosa and canicola were detected as the most prevalent serovars with 39.7 and 30.2%, respectively. Prevalence rates of other serovars were 16.4% for pomona, 8.2% for hardjo and 5.5% for icterohaemorrhagiae. All of samples were seronegative for ballum. Statistical analysis of the results showed that the rates of the infection in the autumn (37%) and spring (21%) were significantly higher than the other seasons (0.001 < p < 0.005). The rate of the infection has been statistically increased with the aging (0.001 < p < 0.005) and the animals with 3 and 4 pair’s permanent teeth (4-5 years old) had the highest infection rates. The infection rate in the female animals group was higher than the males ones (0.01 < p < 0.05). Thus, the serological infection rate in sheep in tabriz is relatively high and consequently the preventive methods must be applied to prevention of the spread of disease and its transmission to the human and other farm animals.

Key words: Leptospirosis, seroprevalence, sheep, MAT, Tabriz

INTRODUCTION

Leptospirosis is an important infectious disease of livestock animals and humans caused by the pathogenic leptospires which are classified into one species of Leptospira interrogans containing over 212 serovars. The infection has a worldwide distribution (Radostitis et al., 2007). The distribution of isolates and prevalence of serovars varies according to geographical regions and countries and depends on environmental and host factors. The source of the infection is an infected animal which contaminates pasture, drinking water and feed by infective urine, aborted fetuses and uterine discharges (Carter and Chengappa, 1991; Quinn et al., 2002). The infection is particularly, important due to causing abortion, stillbirth, mastitis, milk drop syndrome, occurrence of the acute disease and economic losses in farm animals (Saglam et al., 2007). Considering that the infection is usually developed by the native serovars, identification of the serovars in each region is very important in epidemiology of the infection in the same region (Cerri et al., 2003). Urine is the chief source of contamination because animals, even after clinical recovery, may shed leptospirae in the urine for long periods and act as carriers (Quinn et al., 2002). Leptospirosis is classified into 2 broad categories: host-adapted and non host-adapted. An animal infected with a host-adapted serovar of the organism, is

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a maintenance or reservoir host. Cattle are the maintenance host for some of the serovars, thus serological surveys of cattle in the world have found that relatively high percentages of the sera had antibodies against numerous leptospiral serovars, but sheep has been accepted as accidental or incidental hosts for the most leptospiral serovars (Bulu et al., 1990, Radostitis et al., 2007). However persistent leptosporia due to L. hardjo in sheep where no contact with cattle has occurred (Radostitis et al., 2007) and also widespread leptospiral infection in merino rams in Australia, suggest that sheep may be a maintenance host at least for some of the serovars such as hardjo (Ellis et al., 1994). This could complicate control of the infection in cattle and also the infected sheep are a potential zoonotic risk to abattoir worker, sheep farmer and shearsers which previously had not been considered (Ellis et al., 1994).

Considering that the high leptospiral seroprevalence rates of the cattle and buffalos in previous studies in Iran and East Azerbaijan province (Firouzi and Vandyousefi, 2000; Hasanpour et al., 2007; Shouei, 1993) and with attention to the fact that sheep are usually in contact with cattle directly or indirectly in the most regions of the province, therefore this is predicted that sheep may be one of the important animals in epidemiology of the infection in Iran. Prevalence of leptospiral infection in sheep was unknown in Tabriz.

The purposes of the survey were to determine the seroprevalence rate of the leptospiral infection, to determine the titer of the antibodies against to different Leptospira serovars and evaluation of the relationship between seroprevalence and animal and environmental factors in sheep slaughtered in Tabriz, Iran.

MATERIALS AND METHODS

Animals and samples: The minimum sample size on the basis of the 95% confidence rate and the reviewed prevalence rates of other studies as well as the confounding rate equal to 5% was determined 359 sheep. Blood samples were stratified randomly taken from 365 sheep slaughtered in industrial slaughterhouse of Tabriz since December 2004 to November 2005. Ten milliliter of blood was collected from jugular vein of each sheep. None of animals had been vaccinated against leptospires. The animals were classified in to 5 age based groups, without permanent teeth and with 1, 2, 3 and 4 pair's permanent teeth. At the time of blood collection, all the animals appeared healthy with no clinical sign suggestive of leptospirosis. The samples were allowed to clot and centrifuged for 10 min at 2500 g after centrifugation; the serum was removed and stored at -20°C until use.

Serology: The sera were tested for antibodies against six live antigens of leptospira interrogans (L. interrogans serovar grippotyphosa, icterohaemorrhagiae, hardjo, pomona, canicola and ballum with regarding to the previous surveys in Iran) using Microscopic Agglutination Test (MAT) in leptospiral research laboratory, University of Tehran. EMJH base 23% solutions (PH = 7.4) used for preparation of the different dilutions of sera (1:50 to 1:800). Adequate amounts of the 7-10 days old pure and without secondary contaminated leptospira cultures were being used as antigens. Load of the organisms was evaluated by a dark field microscope. The MAT was performed according to the methods of OIE (2000): sera were initially screened at a dilution of 1:100 against these antigens. At first, a serum dilution at 1:50 was made and a volume equal to the diluted serum volume of each antigen was added to each well of micro-titration plates, making the final serum dilution of 1:100. The micro-titration plates were incubated at 29°C for 2 h. The plates were then examined by dark-field microscopy. Results were considered positive when ≥50% of agglutination of leptospires at the test serum dilution of ≥1:100 were observed (OIE, 2000). Sera with positive results were titrated against reacting antigens in serial 2-fold dilutions from 1:100 to 1:800.

Statistical analysis: Results were statistically analyzed using the software SPSS 11.5 with the aid of Chi-Square and Fishers exact tests with 95% confidence interval to assess the association between the infection rates and age, sex and season.

RESULTS

Antibody prevalence's, as determined by positive results at a 1:100 dilution or higher, against one or more studied serovars was 18.4% (66/359) (Table 1).

Results of MAT: The highest number of reactors was for serovar grippotyphosa (39.7%) followed by canicola (30.2%), pomona (16.4%), hardjo (8.2%) and icterohaemorrhagiae (5.5%). Antibody against more than one serovars were found in seven (10.6%) sera of seropositive ones, so that mixed infection of grippotyphosa and canicola, grippotyphosa and pomona were seen in 5 and 2 sera, respectively. All of the samples were seronegative for serovar ballum (Table 2).

There are 73 recognizable Antibody titers from the all of 66 seropositive samples. The majority of titre levels were 1:100 for all serovars and the frequency of 1:100, 1:200 and 1:400 dilutions were 78.2% (57/73), 19.1%(14/73) and 2.7% (2/73), respectively (Table 2).
The distribution of the infection concerning varies seasons, comparison of infection rates between the seasons and the results of the statistical analyses are described in Table 3 and 4.

Statistical analysis of the results showed that the rates of the infection in the autumn (37%) and spring (21%) were significantly higher than the other seasons (0.001 < p < 0.005).

Seropositivity for Leptospirosis in the studied animals according to age (permanent teeth pairs) and sex is shown in Table 5.

### Table 1: Seroprevalence of Leptospirosis in sheep slaughtered in Tabriz industrial Abattoir-Iran

<table>
<thead>
<tr>
<th>No. of tested</th>
<th>No. of positive (%)</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>359</td>
<td>66</td>
</tr>
<tr>
<td>a: Results of MAT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serovar</th>
<th>1:100</th>
<th>1:200</th>
<th>1:400</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grippotyphosa</td>
<td>26 (35.6%)</td>
<td>3 (4.1%)</td>
<td>0 (0%)</td>
<td>29 (39.7%)</td>
</tr>
<tr>
<td>Canicola</td>
<td>14 (19.9%)</td>
<td>6 (8.2%)</td>
<td>2 (2.7%)</td>
<td>22 (30.2%)</td>
</tr>
<tr>
<td>Pomona</td>
<td>10 (13.7%)</td>
<td>2 (2.7%)</td>
<td>0 (0%)</td>
<td>12 (16.4%)</td>
</tr>
<tr>
<td>Hardjo</td>
<td>4 (5.5%)</td>
<td>2 (2.7%)</td>
<td>0 (0%)</td>
<td>6 (8.2%)</td>
</tr>
<tr>
<td>Icterohaemorrhaginiae</td>
<td>3 (4.1%)</td>
<td>1 (1.4%)</td>
<td>0 (0%)</td>
<td>4 (5.5%)</td>
</tr>
<tr>
<td>Ballum</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>57 (78.2%)</td>
<td>14 (19.1%)</td>
<td>2 (2.7%)</td>
<td>73 (100%)</td>
</tr>
</tbody>
</table>

### Table 2: Distribution of serovar specific anti-leptospiral antibodies and their titration in seropositive sheep

<table>
<thead>
<tr>
<th>Season</th>
<th>No. of tested</th>
<th>No. of positive (%)</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>110</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Spring</td>
<td>89</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Summer</td>
<td>80</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Autumn</td>
<td>80</td>
<td>30</td>
<td>37.5</td>
</tr>
<tr>
<td>Total</td>
<td>359</td>
<td>66</td>
<td>18.4</td>
</tr>
</tbody>
</table>

Statistical analysis: 0.001 < p < 0.005

### Table 3: Seropositivity for Leptospirosis in the slaughtered sheep according to the season of sampling

### Table 4: The results of statistical analysis of Seropositivity for Leptospirosis at each season as compared with other ones

<table>
<thead>
<tr>
<th>Comparison of seasons</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter-Spring</td>
<td>0.005 &lt; p &lt; 0.01</td>
</tr>
<tr>
<td>Winter-Summer</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Winter-Summer</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Spring-Summer</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Spring-Summer</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Spring-Autumn</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Summer-Autumn</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

### Table 5: Seropositivity for Leptospirosis in the slaughtered sheep according to age and sex

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of positive (%)</th>
<th>Prevalence (%)</th>
<th>p-value</th>
</tr>
</thead>
</table>

#### DISCUSSION

Leptospirosis is an infectious zoonotic disease and infections with different serotypes of the leptospires in any geographical area are important on the epidemiology and pathogenesis of the disease in the region. Cattle are maintenance host for many serotypes of the bacteria, thus previous studies on cattle have demonstrated relatively high prevalence rates of the infection in different country and even various regions in Iran. Seroprevalences of leptospirosis infections in cattle of Tehran suburb dairy farms at 1990 and 2001 were 31.2% (Momeni, 1990) and 46.8% (Golie, 2001) and in East Azerbaijan province at 1993 and 2007 were 48.5% (Shoaei, 1993) and 24% (Hasanpour et al., 2007), respectively.

Sheep are not naturally maintenance hosts for some of the serotypes such as *Pomona* or *hardjo* and are likely to have infections of relatively short duration, producing severe pathologic effect. However, persistent leptospiuria and high seroprevalence rates of the infection in sheep where no contact with cattle have occurred suggest that sheep may be a maintenance host for some serovars. This could complicate control of the infection in cattle and sheep and infected sheep are a potential zoonotic risk to humans such as abattoir workers, sheep farmers and shearers which previously had not been considered (Radostits et al., 2007).

We found that the seroprevalence of leptospirosis infection in sheep in Tabriz was 18.4%. The reported results of seroprevalence of leptospirosis infection in sheep are different from region to region or country to country. These differences may be the consequence of environmental factors and control efforts. The environmental factors have been shown to have influential effects on development of leptospirosis infection in animal and human beings. Long-term survival of pathogenic leptospires outside the host requires a warm, moist environment with a near natural pH (Miller et al., 1991). So that seroprevalence of leptospirosis infection in sheep has been reported to be 60.4% in India (Satar et al., 1992), 19.7% in Argentina (Draghi et al., 1984), 16.8% in Greece (Burriel et al., 2003), 14.3% in Bolivia (Cicero et al., 1997), 6.1 and 12.3% in Italy (Cicero et al., 2000; Cerri et al., 2003), 42% in Australia (Ellis et al., 1994). 40% in Belize (Everard et al., 1988) and 32% in Croix (Ahl et al., 1992). The results of this study showed that the serological infection rate in sheep in Tabriz is relatively high and consequently the preventive methods must be applied to prevent the spread of disease and its transmission to the human and other farm animals.
In comparison to previous studies in Tabriz, the prevalence of antibodies to one or more serovars of *L. interrogans* was 48.5% (Shoaei, 1993) and 24% (Hasanpour et al., 2007) in cattle. Although, the significance of these differences was not defined, but it may be due to differences in susceptibility of these animals. Leptospirosis occurs in sheep and goats with less frequency than in cattle. So that the prevalence of leptospiral infection in cattle, buffalo and sheep in Egypt was 34.5, 26.1 and 4.2%, respectively (Maronpot and Barsoum, 1992). In Turkey, 44.77% of cattle and 8% of sheep reacted to one or more serovar of *L. interrogans* (Ozdemir and Erol, 2002). In Malaysia 40.5, 31 and 10% of cattle, buffalo and sheep reacted to one or more serovar of *L. interrogans*, respectively (Bahaman et al., 1987). In the present study, like some of the other ones (Sratname et al., 1992; Ahi et al., 1992; Ellis et al., 1994; Everard et al., 1988) seroprevalence rate of leptospiral infection in sheep was relatively high, which emphasize the important role of sheep on the epidemiology of the infection.

In this study *grippotyphosa* and *canicola* were detected as the most prevalent serovars with 39.7 and 30.2%, respectively. With attention to the fact that the rodents and dogs are the major maintenance hosts for *grippotyphosa* and *canicola* serovars, respectively (Radostitis et al., 2007) and considering that frequent contacts between sheep and these animals in the flock of the region, the relatively high prevalence of these serovars in this study are justified. Thus the preventive methods must be applied to control of the infection in rodent and accompanied flock dogs.

On the other hand, in previous studies in Tabriz and Ahvaz, the predominant serovars in cattle were *pomona*, *grippotyphosa* (Hasanpour et al., 2007) and *Pomona* (Haji Hajikolaei et al., 2007), respectively. It is probable that these serovars may be adapted to and maintained by these farm animals in Tabriz. There is a need for further investigation on clinical cases of leptospirosis to determine whether this serovar is the main cause of leptospirosis in this area. The predominant leptospira serovars in serological reaction varies somewhat from country to country. For example, *poi* and *pomona* in Bolivia (Ciceroni et al., 1997), *wolffi*, *pomona* and *ballum* in Argentina (Draghi et al., 1984), *hebdomadis* in the UK (Hathaway et al., 1981), *pomona* in India (Manickavel et al., 1991), *autumnalis* in Egypt (Maronpot and Barsoum, 1992), *castellonis* in Italy (Ciceroni et al., 2000), bratislava in Greece (Barriel et al., 2003), *canicola* in Portugal (Levett et al., 1996)icterohaemorrhagiae, *pomona* in south America (Saglam et al., 2007) and *pomona* in Malaysia (Bahaman et al., 1987) were the predominant serovars in sheep. In addition, one serovar may be predominant in a country but none of the animal reacted with this serovar in another country. This emphasizes the need for regional surveys for leptospirosis, since host-parasite relationship may change depending on the ecology of the region.

Antibodies against more than one serovar were found in 10.6% of seropositive sheep. In serologic tests for leptospirosis such as MAT, the results often indicate infection with more than one serovar (Hathaway et al., 1981; Firouzi and Vandyousef, 2000). This may be the result of mixed serovar infection or cross-reactivity among serovars.

The high prevalence of infection and dominant titre of 1:100 reveal that leptospiral infection in sheep in Tabriz is endemic and occurs mostly in subclinical form.

Statistical analysis of the results showed that the rates of the infection in the autumn (37.5%) and spring (21%) were significantly higher than the other seasons (0.001 <p<0.005). Large amounts of raining and transferring the sheep flocks in theses seasons can increase the infection rate of leptospirosis (Howard and Smith, 1999). Although in different studies, most prevalence rate of the infection occurs in varies seasons depend on the regions and serovars. For example, *hardjo* and *pomona* were the most common detected serovars, in highly raining and dried season or areas, respectively, in Spain (Howard and Smith, 1999). These differences may be the consequence of diversity of weather and environmental factors in various regions, so that in highly raining, warm and moist environments the infection is prevalent in all seasons (Miller et al., 1991; Rocha, 1998).

The rate of the infection has been statistically increased with the aging (0.001 <p<0.005) and the animals with three and four pair’s permanent teeth (4-5 years old) had the highest infection rates. The finding is similar to the results of the other surveys. Overall the prevalence rate is increasing with aging the animals due to enhancing probability of contact of animals with organism (Ciceroni et al., 2000; Saglam, et al., 2007).

**CONCLUSION**

In overall conclusion, it seems that the serological infection rate in sheep in Tabriz is relatively high and consequently the preventive methods must be applied to prevention of the spread of disease and its transmission to the human and other farm animals.

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