Seroprevalence of Leptospiral Infection in Ewes in Khoy - Iran

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ABSTRACT

This study was conducted on 130 ewes in Khoy area in the north of Iran in order to seroprevalence of leptospiral infection. Sera were initially screened at dilution of 1:100 against 6 live serovars of Leptospira interrogans: Pomona, Canicola, Hardjo, Ballom, Icterohaemorrhagiae and Grippotyphosa using the microscopic agglutination test (MAT). The prevalence of leptospiral infection was 28.46% in ewes. 59.45% of infected ewes were in the moist stable and 40.54% were in the non moist stable and there was significant difference between prevalence them (P<0.05). There was no significant relationship between aging of the ewes. The highest number of reactors in ewes (56.25%) was due to serovar Canicola, followed in descending order by Grippothyphosa (20.83%), pomona (12.50%), Hardjo (6.25%) and Icterohaemorrhagiae (4.16%). The majority of titer levels were between 100 and 200 for all the serovars. These results confirm that the majority of leptospiral infections are asymptomatic and the presence of antibodies in the absence of infection indicates exposure to the organism in these animals.

Key words: ewes, seroprevalence, Leptospira, Iran, MAT.

Introduction

Leptospirosis is a widely spread zoonosis of global concern [5,25]. It is caused by spirochetes belonging to the genus Leptospira. All the pathogenic leptospires were formerly classified as members of the species Leptospira interrogans; the genus has recently been reorganised and pathogenic leptospires are now identified in several species of Leptospira. Leptospirosis is a significant occupational hazard in the cattle and pig industries in certain areas. Pyelonephritis is the most frequently encountered clinical manifestation of leptospiriosis in ewes; however, abortion and stillbirth are serious problems [4,13,17,22,26,39]. Milk drop syndrome in cattle, at milky ewes have also been reported [35]. Non-specific disease characterized by fever, jaundice, anorexia, and lethargy may also occur. Leptospirosis can be readily transmitted between species, including between animals and humans through infected urine, contaminated soil or water, or other body fluids [3, 25]. Veterinarians can be infected through contact of mucous membranes or skin lesions with urine or tissues from an infected animal. Human leptospirosis can be highly variable, ranging from asymptomatic infection to sepsis and death [14,37]. Headache, myalgia, nausea, and vomiting are common complaints; however, neurologic, respiratory, cardiac, ocular, and gastrointestinal manifestations can occur [14, 37]. In rare instances, leptospirosis can be fatal. 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 a maintenance of reservoir host. Cattle are the
maintenance host for some of the serovars, thus serological surveys of cattle in the world has 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 [6,36]. However persistent leptospiuria due to L. hardjo in sheep were no contact with cattle has occurred [36] 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 [14]. This could complicate control of the infection in cattle and also the infected sheep are the potential zoonotic risk to abbatior, worker, sheep farmer and shearers which previously had not been considered[14]. Considering that the high leptospiral seroprevalence rates of the cattle and buffalos in previous studies in Iran and East Azerbaijan province [18,23,41] 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 khoy. Prevention of occupational leptospirosis among veterinarians involves early identification of infected animals, reducing contact with affected animals (particularly urine and other body fluids) and the use of waterproof barrier clothing [14]).

Diagnosis of leptospirosis can be difficult and may involve antigen detection (PCR), serological evaluation, histological examination, culture, and/or dark field microscopy [14]. A wide variety of serological tests, which show varying degrees of serogroups and serovar specificity, have been described. Two tests have a role in veterinary diagnosis: the microscopic agglutination test and ELISA [27]. A number of serological studies have indicated wide-spread evidence of leptospiral infection in ewes in several countries [10,12,24,33, 34,39,44]. The study attempted to determine the prevalence of L. interrogans antibodies in ewes in khoy area in Iran. This is the first report of leptospiral infection in these animals in the area.

Material and methods

Blood samples were taken from 130 ewes (from 10 sheep herds of khoy, North-west of Iran, during April to August of 2009. On the bases of age these ewes were divided in 4 groups ( 0-2 years,2-4 years, 4-6 years and over 6 yaers).None of these animals had been vaccinated against leptospirosis and there was no history of leptospirosis-related symptoms or signs of the disease at the time of sampling. 5-7 millilitres of blood were collected from the jugular vein of each ewe. The blood samples were allowed to clot and were centrifuged for 10 min at 3000g. After centrifugation, the serum was removed and stored at −20°C until ready for test. The serum samples were tested for antibodies to 6 live serovars of L. interrogans: Canicola, Grippo, Pomona, Icterohaemorrhagiae and Ballum using the microscopic agglutination test (MAT) in the Leptospira Research Laboratory of veterinary faculty of Tehran University. The sera were initially screened at dilution of 1:100. At first, serum dilution of 1:50 was prepared and a volume of each antigen, equal to the diluted serum volume, was added to each well, making the final serum dilution 1:100. The microtitration plates were incubated at 29oC for 2 hours. The plates were examined under darkfield microscopy. The results were considered positive when 50% or more of agglutination of leptospires at dilution of 1:100 or greater were found [33,36].

The results were analysed by chi-square test to determine the difference between different groups of age and moist stable was significantly related to the prevalence of leptosprial antibodies.

Results and discussion

37 (28.46%) from 130 ewes that tested were positive for at least one leptospiral antigen. Some samples were positive for two leptospiral antigens. On the base of age, 0 ewes (0%) in the 0-2 years group, 19 ewes (51.35%) in the 2-4 years group, 15 ewes (40.54%) in the 4-6 years group and 3 ewes (8.1%) in the over 6 years group were positive. There was no significant relationship between aging and the incidence of leptospiral infection (table 1). 59.45% of infected ewes were in the moist stable and 40.54% were in the non moist stable and there was significant difference between prevalence them (P<0.05) (table 2). The highest number of reactors in ewes (56.25%) was due to serovar Canicola, followed in ascending order by Grippo (20.83%), pomona (12.50%), Hardjo (6.25%) and Icterohaemorrhagiae (4.16%)(table 3). As shown in Table 4, the majority of titre levels were between 100 and 200 for all the serovars (52.08% and 45.83%, respectively). Out of the ewes that were seropositive for leptospirosis, 10 samples (26.39%) were positive for more than one serotype.

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 hosts 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 leptospiral infections of cattle of Tehran suburb dairy farms at 1990 and 2001 were 31.2% [31] and 46.8% [19] and in East Azarbaijan province at 1993 and 2007 were 48.5% [41] and 24% [23], 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 leptospiruria and high seroprevalence rates of the infections 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 shearsers which previously had not been considered [36].

We found that the seroprevalence of leptosomal infection in ewes in Khoy was 28.46%. The reported results of seroprevalence of leptospiral 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 efforts on development of leptospiral infection in animal and human beings. Long-term survival of pathogenic leptospiries outside the host requires a warm, moist environment with a near natural PH [30]. So that seroprevalence of leptospiral infection in sheep has been reported to be 60.4% in India [42], 19.7% in Argentina [11], 16.8% in Greece [7], 14.3% in Bolivia [8], 6.1 and 12.3% in Italy [9], 42% in Australia (14), 40% in Belize [16] and 32% in Croix [1]. The results of this study showed that the serological infection rate in sheep in Khoy 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.

In previous studies in Tabriz, The prevalence of antibodies to one or more serovars of L.interrogans was 48.5% [41] and 24% [23] in cattle and 18.4% in sheep [43]. 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 [29]. In Turkey, 44.77% of cattle and 8% of sheep reacted to one or more serovar of L.interrogans [32]. In Malaysia 40.5, 31 and 10% of cattle, buffalo and sheep reacted to one or more serovar of L.interrogans, respectively [2]. In the present study, like some of the other ones [43,1,14,16] 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 canicola and grippotyphosa were detected as the most prevalent serovars with 56.25% and 20.83%, respectively. With attention to the fact that the rodents and dogs are the major maintenance hosts for grippotyphosa and canicola serovars, respectively [36] and considering that frequent contacts between sheep and these animals in the flocks of the region, the relatively high prevalence of these serovars in this study justified. Thus the preventive methods must be applied to control of the infection in rodent and accompanied flocks dogs.

On the other hand, in previous studies in Tabriz and Alvaz, the predominant serovars in cattle were Pomona, grippotyphosa [23] and Pomona [21], respectively. In other previous studies in Tabriz, the prevalent serovar in sheep was grippotyphosa [43]. It is probable that these serovars may be adapted to and maintained by these farm animals in Khoy. 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 [8], wofli, Pomona and ballum in Argentina [11], hebdomadis in the UK [24], Pomona in India [28], autumnalis in Egypt [29], castellonis in Italy [9], Bratislava in Greece [7], canicola in Portugal [25], icterohaemorrhagiae, Pomona in south America [38] and Pomona in Malaysia [2] 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 26.39% of seropositive ewes. In serologic tests for leptospirosis such as MAT, the results often indicate infection with more than one serovar [24,18]. 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 leptosporial infection in sheep in Khoy is endemic and occurs mostly in subclinical form.

59.45% of infected ewes were in the moist stable and 40.54% were in the non moist stable and there was significant difference between prevalence them (P<0.05) that showed importance of moist stable in epidemiology of leptospira in this area.

Laboratory procedures used in the diagnosis of leptospirosis. Leptospiral antibodies appear within a few days of infection and persist for weeks or months and, in some cases, years. Unfortunately, antibody titres may fall to undetectable levels while animals remain chronically infected. To overcome
this problem, sensitive methods are needed to detect the organism in urine or the genital tract of chronic carriers [27]. Therefore, the demonstration of leptospires in the genital tract and or urine only must be interpreted with full consideration of the serological results and culture or detection of leptospires in blood or body fluids, as these findings may indicate that the animals were carriers.

These results confirm that leptospiral infection may exist in the sheep population in Khoy area and the presence of antibodies in the absence of infection indicates exposure to the organism and must be acknowledged. In addition, these results confirm that the majority of leptospiral infections is asymptomatic.

### Table 1: Age distribution in leptospiral seropositive ewes

<table>
<thead>
<tr>
<th>Age group tested</th>
<th>positive</th>
<th>percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2 years</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-4 years</td>
<td>19</td>
<td>51.35</td>
</tr>
<tr>
<td>4-6 years</td>
<td>15</td>
<td>40.54</td>
</tr>
<tr>
<td>Over 6 years</td>
<td>3</td>
<td>8.1</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>28.46</td>
</tr>
</tbody>
</table>

### Table 2: Moist or non-moist stable distribution in leptospiral seropositive ewes

<table>
<thead>
<tr>
<th>Stable</th>
<th>Tested</th>
<th>positive</th>
<th>percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moist</td>
<td>37</td>
<td>22</td>
<td>59.45</td>
</tr>
<tr>
<td>Non-moist</td>
<td>93</td>
<td>15</td>
<td>40.54</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
<td>37</td>
<td>28.46</td>
</tr>
</tbody>
</table>

### Table 3: Prevalence of different leptospiral serovars in ewes

<table>
<thead>
<tr>
<th>CGP</th>
<th>Numbers</th>
<th>Percent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>10</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>56.25</td>
<td>20.83</td>
<td>12.5</td>
<td>6.25</td>
</tr>
<tr>
<td>G - Gryppothyphosa, P - Pomona, I - Icterohaemorrhagiae, C - Canicola, H - Hardjo, B - Ballum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Some samples were positive for two leptospiral antigens</td>
<td></td>
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</tbody>
</table>

### Table 4: Prevalence of leptospiral antibody titres to different antigens in ewes

<table>
<thead>
<tr>
<th>Titre</th>
<th>Numbers</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>25</td>
<td>52.08</td>
</tr>
<tr>
<td>200</td>
<td>22</td>
<td>45.83</td>
</tr>
<tr>
<td>400</td>
<td>1</td>
<td>2.08</td>
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</table>

### Reference


