Short Paper

Seroinvestigation of bovine leptospirosis in Shahrekord district, central Iran

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Summary

Leptospirosis is one of the most important zoonotic diseases spreading throughout the world with numerous reservoir hosts. In this study, 400 field bovine serum samples were collected from dairy farms of Shahrekord district, central Iran. Using microscopic agglutination test (MAT), 75 (18.75%) of 400 samples were found positive for different leptospiral serovars. The isolated serovars included canicola in 50.66% of samples, grippotyphosa in 21.33%, hardjo in 17.33%, icterohaemorrhagiae in 6.66% and pomona in 4.00% of samples. The percentage of positive samples was 13% in industrialized and 5.75% in unindustrialized farms.

Key words: Leptospira, Antibody, Serovar, Iran

Introduction

Leptospirosis refers to a number of disease syndromes in animals and men associated with infection by several leptospiral serovars belonging to a single species, Leptospira interrogans.

Leptospirosis affects wild rodents and domestic animals such as cattle, swine, horses, sheep, goats and dogs (Harskeel and Terpstra, 1996). The diagnosis of leptospirosis is made either by detection of antibodies in the sera or isolation of the organisms from tissue or body fluids.

Since the isolation of leptospirae is difficult and laborious, serological diagnosis is routinely used. Microscopic agglutination test (MAT) is the most commonly used diagnostic test (Balows et al., 1991).

The present study investigated anti-leptospiral antibodies in serum samples of dairy farms in Shahrekord district in central area of Iran.

Materials and Methods

The investigation was started from March, 2002 for 14 months. Blood samples were collected from 140 dairy farms (14 industrialized and 126 unindustrialized). Samples were taken only from 18 months old cows or older.

The number of blood samples from each farm varied between 1 and 30 (Mean 2.8). After separating of sera, totally 400 field serum samples were taken to the Leptospira Research Laboratory of Veterinary College of Tehran University in Mardabad, Karadj. The samples were tested for the presence of antibody against L. grippotyphosa, L. pomona, L. icterohaemorrhagiae, L. canicola
and *L. hardjo* using the MAT (Balows *et al.*, 1991).

The antigens were 4- to 14-day-old live cultures grown in Kortoff medium containing 0.1%-0.2% agar plus 5% rabbit serum. Seeds of antigens were provided by World Health Organization (WHO) based on an early requisition.

For MAT testing, serum samples were diluted at 1:50, 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200 and 1:6400 dilutions. Sera from infected and uninfected animals were used as positive and negative controls. Ten microliter of each antigen culture were mixed with same volume of serum dilutions and incubated at 29±1 °C for 90 min (National Veterinary Services Laboratories, 1987).

For positive sera, the titer of the test was expressed as the reciprocal of the highest dilution giving more than 50% agglutination. Using governorship map, the district was divided into four regions as Kiar, Lar, Taghanak and Shahrekord.

Each serum tested was categorized according to its reactions to the tests for *L. grippotyphosa*, *L. pomona*, *L. icterohaemorrhagiae*, *L. canicola*, *L. hardjo* and industrialized or unindustrialized farms. The level of statistical differences between leptospiral titers and the above-mentioned four regions was determined, using Chi-squared test.

**Results**

Of the 400 serum samples of dairy cattle, 75 (18.75%) were found positive at a titer of 1:100 or more. This study showed that 57.14% of industrialized and 17.46% of unindustrialized farms were positive for one or more serovars of leptospirae at a titer of 1:100 or more. The seropositive rates of the tested cows in these farms were 24.18% and 12.43%, respectively (Table 1).

**Table 1: Number of farms, samples and seropositive cows**

<table>
<thead>
<tr>
<th>Farm</th>
<th>No. of farms</th>
<th>No. of farms positive</th>
<th>No. of samples</th>
<th>No. of cows seropositive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Industrialized</td>
<td>14</td>
<td>8 (57.14%)</td>
<td>215</td>
<td>52 (24.18%)</td>
</tr>
<tr>
<td>Unindustrialized</td>
<td>126</td>
<td>22 (17.46%)</td>
<td>185</td>
<td>23 (12.43%)</td>
</tr>
<tr>
<td>Total</td>
<td>140</td>
<td>30 (21.42%)</td>
<td>400</td>
<td>75 (18.75%)</td>
</tr>
</tbody>
</table>

As is shown in Table 2, positive samples at titers of 1:100, 1:200, 1:400 and 1:800 were 49.33%, 33.33%, 12.00%, and 5.33%, respectively.
Table 2: Microscopic agglutination test (MAT) titers against leptospiral serovars. Percentages in brackets indicate ratio of serovar positive to total seropositives

<table>
<thead>
<tr>
<th>Serovar</th>
<th>1:100</th>
<th>1:200</th>
<th>1:400</th>
<th>1:800</th>
<th>Total (%) serovar positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. grippotyphosa</em></td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>16 (21.33%)</td>
</tr>
<tr>
<td><em>L. pomona</em></td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3 (4.00%)</td>
</tr>
<tr>
<td><em>L. icterohaemorrgiae</em></td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 (6.66%)</td>
</tr>
<tr>
<td><em>L. canicola</em></td>
<td>11</td>
<td>18</td>
<td>6</td>
<td>3</td>
<td>38 (50.66%)</td>
</tr>
<tr>
<td><em>L. hardjo</em></td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>13 (17.33%)</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>25</td>
<td>9</td>
<td>4</td>
<td>75 (100%)</td>
</tr>
<tr>
<td>Percentage</td>
<td>49.33</td>
<td>33.33</td>
<td>12.00</td>
<td>5.33</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3: Comparison of seropositive and seronegative cows in four regions of Shahrekord district at a titer of 1:100 or more

<table>
<thead>
<tr>
<th>MAT results</th>
<th>Shahrekord</th>
<th>Taghanak</th>
<th>Lar</th>
<th>Kiar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>52</td>
<td>8</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Negative</td>
<td>184</td>
<td>62</td>
<td>50</td>
<td>29</td>
</tr>
<tr>
<td>total</td>
<td>236</td>
<td>70</td>
<td>60</td>
<td>34</td>
</tr>
</tbody>
</table>

Table 3 shows number of seropositive cows in four regions of Shahrekord districts. Using Chi-square test, differences are not statistically significant at p>0.05.

Discussion

In this study a total of 400 field bovine serum samples were studied. All serum samples tested by MAT against leptospira serovars *grippotyphosa*, *pomona*, *icterohaemorrhagiae*, *canicola* and *hardjo*.

The majority of positive sera reacted to serovar of *canicola* (50.66%), with other commonly reacting serovars being *L. grippotyphosa* (21.33%), *hardjo* (17.33%), *icterohaemorrhagiae* (6.66%) and *pomona* (4.00%). In most regions of Iran, dogs are used as herd watcher, hence, a higher prevalence of serovar *canicola* (50.66%) detected in this study.

In one study 427 bovine serum samples were tested in Urmia-Iran and *Leptospirae serovars* *grippotyphosa*, *hardjo*, *canicola* and *pomona* were the most prevalent serovars, respectively (Zeinali et al., 1998).

Reports indicating high prevalence of serovar *hardjo* in bovine populations (White et al., 1982; Dhaliwal et al., 1996). However, its low prevalence in our study (17.33%) may be due to localization of this serovar in bovine genital systems (Ellis et al., 1985).

Higher seroprevalence of leptospiral serovars in industrialized farms (57.14%) as compared to unindustrialized farms (17.46%) reflects the role of intensive rearing on the epidemiology of the disease. Since the numbers of seropositive cows in the four regions of the district are not statistically different, it was concluded that there is no endemic infection in these regions.
It should be pointed out that there is a difference between infection status and seroprevalence. A seropositive diagnosis indicates that the animals has been exposed to the infective agent in the past, whereas, seronegative animals may not necessarily be free from infection (Quinn et al., 2002). The present study indicates seroprevalence, not infection status.

At any given time, on the basis of antibody titer alone, it is impossible to predict how recently an animal has been exposed to infection, because the rate of decline of antibody varies from cow to cow (Elder et al., 1985).

References