Seroprevalence of bovine leptospirosis in Garanhuns municipal district, Pernambuco State, Brazil

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ABSTRACT


The prevalence of Leptospira interrogans serovars in dairy cattle was determined by analyzing 464 serum samples from cows on 15 properties in Garanhuns municipal district, Pernambuco State, Brazil. A microscopic seroagglutination test including 12 serovars of Leptospira interrogans as antigens was used. Samples with titres ≥ 100 were considered positive. Two hundred and twenty-one (47.63 %) of the samples were positive to one or more serovars. The prevalence of the serovars was hardjo (21.98 %), bratislava (15.73 %), castellonis (11.64 %), tarassovi (10.56 %), pyrogenes (1.72 %), icterohaemorrhagiae (1.08 %), pombia (0.86 %), wolffi (0.86 %), grippotyphosa (0.86 %), djasiman (0.43 %), canicola (0.21 %), and copenhageni (0.21 %).

Keywords: Brazil, cattle, leptospirosis

INTRODUCTION

Leptospirosis is a serious zoonosis caused by Leptospira interrogans with more than 200 different serotypes, which affects mainly cattle, pigs, horses, and dogs (Amatredjo & Campbell 1975). Cattle susceptibility varies according to factors such as age, physiological state and population density (Brod & Fehberg 1992). In the Americas, the predominant serotypes are hardjo, pombia and grippotyphosa (Ellis 1984). Prevalence varies considerably between herds, regions and countries; it is highest in tropical countries with heavy rainfall and neutral or alkaline soils (Guimaraes 1982/83).

Bovine leptospirosis is endemic in Brazil and quite frequent in cattle. It causes abortions, placental retention and perinatal mortality, congenital abnormalities, milk production loss, and mastitis (Lilenbaum, Santos & Dos Santos 1995). The last two are generally associated with the hardjo serotype, which is related to the “Milk Drop Syndrome” (Higgins, Harbourne & Little 1980; Pearson, Mackie & Ellis 1980).

The aim of this study was to determine the seroprevalence of L. interrogans serovars known to affect cattle in the studied region.

MATERIALS AND METHODS

Herd description

Four hundred and sixty-four adult cows were used in this study. The animals were crossbred Holstein-Friesian, Jersey and Brown Swiss either dry or in various lactation stages. Some animals presented reproductive disturbances. They were maintained under semi-intensive management systems on 15 farms in
the municipal district of Garanhuns, Pernambuco State, northeastern Brazil, for more than a year without being vaccinated against leptospirosis. The sampling fraction was determined according to the Pan-American Center of Foot-and-Mouth Disease, considering a 10% possibility of error and 49.37% of expected prevalence (calculated based on total population) (Astudillo 1979).

Blood sampling and serological testing

A serum sample from each cow was obtained by jugular venipuncture, using disposable needles (40 x 16 mm) after disinfecting the skin with 3% iodine alcohol. Approximately 10 ml of blood was obtained from each animal. The blood samples were collected in sterile tubes that were placed at an incline to facilitate blood serum acquisition after blood clotting. The blood was then centrifuged at 804.9 g for 10 min, the serum decanted and stored in cryotubes at −18°C until testing was performed.

The test was the standardized microscopic seroagglutination, read under a dark-field microscope, according to WHO (Myers 1985) and the Ministry of Health—National Health Foundation (Brasil 1995). The batch of living antigens used was based on recommendations of FIOCRUZ/RJ (Anon. 1995) and included 12 serovars considered important for cattle (Table 1). Dilutions of the antigens used were 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200, as proposed by Myers (1985) and Anon. (1995).

Investigative questionnaire

When each batch of serum samples was collected, the farmers concerned were requested to complete a questionnaire in order to acquire information about the farms and animals. Farm management practices such as feed, semi-intensive system, water quality and the type of terrain as well as sanitary data like vaccination, presence of reproductive disturbances and the use of anthelmintics were evaluated.

Statistical analysis

The samples were analyzed using descriptive analysis. Absolute and relative frequencies of positive animals on the studied farms and the agglutination titres related to L. interrogans serovars were evaluated. The questionnaires were analyzed by the EPI-INFO and Chi-square tests to determine associations between levels of prevalence and independent variables.

RESULTS

A seropositive prevalence of 47.63% was obtained for one or more of the serovars with titres of ≥ 100, while 52.37% of the animals tested seronegative.

Seropositive animals were found on all 15 farms (Table 2), and many serotypes of L. interrogans were present. These results revealed the widespread prevalence of infection, which demonstrates a high capacity for the organism to survive in the environment, ease of transmission and possibly the contribution of many other susceptible species of animals in facilitating the maintenance of the agent in nature. In this study, samples of the serum antibodies to the following 12 serovars were detected: hardjo (22.00%), bratislava (15.73%), castellonis (11.00%), tarassovi (10.66%), pyrogenes (1.72%), ictero haemorrhagiae (1.08%), grippotyphosa (0.86%), pomona (0.86%),
TABLE 3 Distribution of the agglutination titres in cow sera, with dilutions 1:100 to 1:3200 for 12 *Leptospira interrogans* serovars

<table>
<thead>
<tr>
<th>Serovars</th>
<th>Dilutions/titres</th>
<th>1:100</th>
<th>1:200</th>
<th>1:400</th>
<th>1:800</th>
<th>1:1600</th>
<th>1:3200</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardjo</td>
<td></td>
<td>34</td>
<td>41</td>
<td>13</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>102</td>
</tr>
<tr>
<td>Bratislava</td>
<td></td>
<td>43</td>
<td>18</td>
<td>11</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>73</td>
</tr>
<tr>
<td>Castellonis</td>
<td></td>
<td>34</td>
<td>17</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>54</td>
</tr>
<tr>
<td>Tarassovi</td>
<td></td>
<td>26</td>
<td>15</td>
<td>5</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>49</td>
</tr>
<tr>
<td>Pyrogenes</td>
<td></td>
<td>5</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>8</td>
</tr>
<tr>
<td>Icterohaemorrhagiae</td>
<td></td>
<td>5</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>Pomona</td>
<td></td>
<td>3</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>Wolffi</td>
<td></td>
<td>3</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>Grippotyphosa</td>
<td></td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>Dyasiman</td>
<td></td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Canicola</td>
<td></td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Copenhageni</td>
<td></td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>157</td>
<td>96</td>
<td>32</td>
<td>2</td>
<td>16</td>
<td>3</td>
<td>1</td>
<td>307</td>
</tr>
</tbody>
</table>

wolffi (0.86%), djasiman (0.43%), copenhageni (0.21%) and canicola (0.21%).

Table 3 shows the distribution of the agglutination titres in relation to the *L. interrogans* serovar antigens used. It can be seen that a higher frequency of agglutination titres was obtained at dilutions 1:100 (157) and 1:200 (96).

The analysis of the questionnaires did not reveal any association between reproductive disturbances and the level of leptospirosis prevalence in the animals.

DISCUSSION

Results of the prevalence of seropositive animals obtained in this study are in agreement with similar studies performed in other regions of Brazil (Abuchaim & Dutra 1985; Ribeiro, Gouveia & Silva 1988; Brod, Martins, Nussbaum & Feberg 1995; Negrão, Mólnar & Mólnar 1999) and in other countries (Miller, Wilson & Beran 1991; Niang, Will & Kane 1994; Cantu-Covarrubias & Banda-Ruiz 1995), but the titres obtained are higher than those of Girio, Mathias, Silva & Franceschini (1990) and Rodrigues, Muller & Freitas (1999) and lower than those obtained by Lilenbaum *et al.* (1995) and Lilenbaum & Santos (1996). The prevalence of antibodies to *L. interrogans* varies considerably between countries, regions, areas and even farms, which might explain the different prevalence rates found in various regions or in one region.

Animal susceptibility varies according to age, physiological state, population density, presence of rodents and wildlife on the farm, access to water and feed, as well as climatic and environmental factors (Brod & Feberg 1992). In this study, however, a correlation between prevalence of infection and the variables population density, type of soil and conditions of water and feed troughs was not obtained.

The semi-intensive management system was used on all studied farms.

The prevalence of bovine leptospirosis in Brazil has been studied by many researchers, the first reference dating back to 1958 (Guida & Barros 1958) when 3.7% of animals were reported positive. Subsequent studies demonstrated a higher incidence of infection in different regions of Brazil (Abuchaim & Dutra 1985; Ribeiro *et al.* 1988; Brod *et al.* 1995; Lilenbaum *et al.* 1995; Lilenbaum & Santos, 1996; Negrão *et al.* 1999; Rodrigues *et al.* 1999).

A positive correlation between rainfall and leptospirosis in cattle has been reported, indicating that during the current study period (October to December 1998), when there was heavy rainfall, an increase in the number of seropositive animals would be seen. This, however, did not occur.

On the 15 farms a high prevalence of seropositive animals to the hardjo, bratislava and castellonis serovars was seen and a low or no prevalence to the copenhageni, djasiman and grippotyphosa serovars. This even distribution may contribute to a characterization of the disease in the studied municipal district. The wolffi, pomona, icterohaemorrhagiae, pyrogenes and tarassovi serovars showed an average level of reactors amongst the animals.

*Leptospira hardjo* was the most prevalent serovar in the studied region, which is in agreement with studies in Brazil by Abuchaim & Dutra (1985), Ribeiro *et al.* (1988), Lilenbaum & Santos (1996), Brod *et al.* (1995) and Negrão *et al.* (1999). These authors emphasized the importance of bovine as reservoirs in various regions of the country. Since the 1980's when the hardjo serovar was first included in the batch of antigens tested, it has proven to be the predominant serovar in cattle (Miller *et al.* 1991; Brod *et al.* 1995;
The prevalence of the hardjo serovar is 23.78%, the second highest, which is in agreement with the findings of Lilenbaum & Santos (1996) and Negrao et al. (1995). These authors stated that this serovar has increased in the last few years in Brazil. Rodrigues et al. (1999) found 10.24% of animals seropositive to the bratislava serovar, although it was only the seventh most prevalent in this study. Thirteen of the 15 farms had animals positive to this serovar, which may be related to incidental infections involving contact of bovine with other species, mostly swine, since the farmers adopt a semi-intensive management system and some raise cattle together with other species.

The castellonis and tarassovi serovars represented 17.59% and 15.96% respectively, of the seropositive animals, confirming the findings of Caldas, Sampaio, Tishcenko & Viegas (1977) and Negrao et al. (1999). The castellonis serovar was considered by Langoni, Marinho, Baldini & Da Silva (1995) the second most prevalent in sheep. This suggests that infection of cattle by these serovars is related to the presence of different species on seven of the farms. However, a better knowledge of the importance and ecology of these serovars and others, such as pyrogenes, djasiman and copenhageni, is needed. The last named three are generally reported as of low incidence and their significance in cattle is not well known. Prospective studies should focus on the correlation of seroconversion or high antibody titres and clinical disease associated with L. interrogans serovars.

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REFERENCES


