COMPARISON OF LEPTOSPIRAL INFECTION IN THE HORSE AND DONKEY

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Abstract

In order to compare the prevalence of leptospiral infection in horses and donkeys in Ahvaz, blood samples were taken from 61 horses and 90 donkeys. 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. The prevalence of leptospiral infection was 27.88% in horses and 40% in donkeys. Both the animal species had the highest titres to serovar Grippotyphosa (33.33% and 46.51%, respectively), followed in descending order by Ballom (23.81%), Pomona (14.28%), Canicula (14.28%), Icterohaemorrhagiae (9.52%), and Hardjo (4.76%) in horses and Icterohaemorrhagiae (23.25%), Ballom (13.96%), Pomona (9.30%), Hardjo (4.65%) and Canicula (2.33%) in donkeys. Statistical analysis showed significant difference between donkeys and horses (P<0.05). In horses there was relationship between infection and sex, so that females were infected more frequently than males but in donkeys this relationship was not seen. The prevalence of infection resulting from this survey is similar to those reported from other countries but there is difference in the predominant serotype. In addition, these results confirm that the majority of leptospiral infections is asymptomatic.

Key words: horse, donkey, Leptospira, leptospirosis, Iran.

Leptospirosis which is caused by Leptospira interrogans serovars is an important zoonotic disease and has become a major worldwide human concern (7). Leptospiral infections cause both acute and chronic disease and the severity of infections is related to the virulence of the organism, susceptibility of the host, and the affected host species. Although most infections are asymptomatic, some clinical syndromes such as uveitis, abortion, stillbirth, pyrexia, icterus and periodic ophthalmia have been reported in horses (3, 4, 6, 8-10).

Unfortunately, a definitive diagnosis of leptospirosis is difficult to make. Most diagnostic laboratories do not attempt to isolate leptospires because of their fragile nature, cost and complexity of the isolation media, and prolonged incubation period (2, 8). Therefore, recognition of leptospiral infection has been based generally on serological evidence, as very few isolations have been reported from naturally infected animals (4, 8). 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 (5). A number of serological studies have indicated wide-spread evidence of leptospiral infection in horses in several countries, but there is only one study dealing with the infection in donkeys (1, 4, 6, 7, 9, 11).

The study attempted to determine the prevalence of L. interrogans antibodies in horses and donkeys from Iran. This is the first report of leptospiral infection in these animals in the country.

Material and Methods

Blood samples were taken from 61 Arabian horses (41 females and 20 males) from 3 race clubs and 90 donkeys (72 females and 18 males) from 4 suburbs of Ahvaz, South-west of Iran, during January to April of 2001. None of these animals had been vaccinated against leptospires and there was no history of leptospirosis-related symptoms or signs of the disease at the time of sampling. Ten millilitres of blood were collected from the jugular vein of each horse and donkey. The blood samples were allowed to clot and were centrifuged for 10 min at 2 500g. 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, Grippothyphosa, Hardjo, Pomona, Icterohaemorrhagiae and Ballum using the microscopic agglutination test (MAT). 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 29°C for 2 h. The plates were examined under dark-field microscopy. The results were considered positive when 50% or more of agglutination of leptospires at dilution of 1:100 or greater were found (6).

The results were analysed by chi-square test to determine whether sex of the horses and donkeys was significantly related to the prevalence of leptosprial antibodies or the difference between horse and donkey was significant.

### Results

Out of 61 horses and 90 donkeys tested, 17 (27.86%) and 36 (40%), respectively, were positive for at least one leptospiral antigen. Significant difference (P<0.05) between the horse and donkey as reactors to leptospires were found. In donkeys, there was no difference between males and females, but in horses this difference, as seen in Table 1, was highly significant (P<0.01).

The highest number of reactors in horses (33.33%) and donkeys (46.51%) was due to serovar Grippothyphosa, followed in descending order by Ballum (23.81%), Pomona (14.28%), Canicola (14.28%), Icterohaemorrhagiae (9.52%), and Hardjo (4.76%) in horses and Icterohaemorrhagiae (23.25%), Ballum (13.96%), Pomona (9.30%), Hardjo (4.65%), and Canicola (2.33%) in donkeys (Table 2).

As shown in Table 2, the majority of titre levels were between 100 and 200 for all the serovars and at least 71.43% and 76.74% were below 400 in horses and donkeys, respectively. The titres for each serovar in horses and donkeys were shown in Table 3.

Out of the horses and donkeys that were seropositive for leptospirosis, 23.53% and 16.67%, respectively, were positive for more than one serotype.

### Discussion

From this study, it is evident that leptospiral infection may exist in the horse and donkey population in Ahvaz. Whether the presence of the infection or merely persistent antibodies in the absence of infection, exposure to the organism must be acknowledged.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Horse</th>
<th>Donkey</th>
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<tbody>
<tr>
<td>Tested</td>
<td>Positive</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>41</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>17</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>G</th>
<th>D</th>
<th>I</th>
<th>C</th>
<th>H</th>
<th>B</th>
<th>Total</th>
<th>100</th>
<th>200</th>
<th>:400</th>
<th>800</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse</td>
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<td>3</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>21</td>
<td>5</td>
<td>10</td>
<td>4</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>33.33</td>
<td>14.28</td>
<td>9.52</td>
<td>14.28</td>
<td>4.76</td>
<td>23.81</td>
<td>100%</td>
<td>23.81</td>
<td>47.62</td>
<td>19.04</td>
<td>9.52</td>
<td>100%</td>
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<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Donkey</td>
<td>20</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>43</td>
<td>14</td>
<td>19</td>
<td>7</td>
<td>3</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>46.51</td>
<td>9.30</td>
<td>23.25</td>
<td>2.33</td>
<td>4.65</td>
<td>13.96</td>
<td>100%</td>
<td>32.56</td>
<td>44.18</td>
<td>16.28</td>
<td>6.9%</td>
<td>100%</td>
</tr>
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</tbody>
</table>

G - Grippothyphosa , P - Pomona, I - Icterohaemorrhagiae , C - Canicola, H - Hardjo, B - Ballum
Table 3  
Maximum of titre levels for each serotype in horses and donkeys

<table>
<thead>
<tr>
<th></th>
<th>Grippothyphosa</th>
<th>Pomona</th>
<th>Icterohaemorrhagiae</th>
<th>Canicola</th>
<th>Ballum</th>
<th>Hardjo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse</td>
<td>400</td>
<td>400</td>
<td>200</td>
<td>200</td>
<td>800</td>
<td>400</td>
</tr>
<tr>
<td>Donkey</td>
<td>400</td>
<td>400</td>
<td>400</td>
<td>100</td>
<td>800</td>
<td>200</td>
</tr>
</tbody>
</table>

There was a higher prevalence of leptospiral antibody rate in donkeys than horses. Anticipation that infection took place is highly possible due to the fact that donkeys have a greater chance of being exposed naturally to leptospires. This is because the tested horses have spent the majority of time in stable but donkeys were kept in pasture and were in contact with other animals, such as sheep, goat, and cattle being the reservoir of leptospires (8).

In seropositive donkeys, there was no difference between males and females, but in horses this difference was highly significant which was in agreement with the reports by Park et al. (6) in horses in Ohio. This may not be true for horses in general, since the number of animals used for this study were too small.

The prevalence of leptospiral infection based on serological testing has been reported to be 20.6-33.6% in USA, and 13.5% in India horse population (6, 7, 9). This was in agreement with this study in which 27.86% of horses were seropositive to one or more serovars.

The predominant leptospira serovars giving rise of serological reaction varies somewhat between countries. For example: Pomona (30.5%) in Queensland, Pomona (12.47%) in California, Bratislava (16.2%, 16.6%, 53.3%, and 22.3%), respectively, in Ohio, England, Northern Ireland, and USA, Bratislava, Copehageni, and Pyogenes (21.3%) in the Republic of Ireland, and Pomona (48.7%) in India were the most common serovars in the horse (1, 6, 7, 9, 11). Barsoum et al. (quoted after 9) reported that the horses and donkeys hospitalized in Egypt had very high titre to Pomona, Grippothyphosa, and Icterohaemorrhagiae serovars. Serovar grippothyphosa was present in 33.3% of positive horses and in 49.51% of positive donkeys in this study making it the most prevalent of all serovars for which we tested and it is probable that this serovar may be adapted to and maintained by the horse and donkey population in Ahvaz.

The majority of titre levels were between 100 to 200 for all serovars. Above 47% of positive horses and 44% of positive donkeys for each serovar had the titres of 200 and at least 70% and 76%, respectively, were below 400. Most researchers found that titres ranged between 100 and 200 and this agreed with the titres found in our study (7).

In serological tests for leptospirosis such as MAT, the results often indicate infection with more than one serovar (1, 4, 7, 9). Percentage of seropositive horses and donkeys for more than one serovar was 23.5 and 16.7, respectively. This may be the result of mixed serovar infection but the existence of cross reactivity in the MAT between the serovars is well known and can be excluded from this interpretation.

Serological testing is the laboratory procedure most frequently used to confirm the clinical diagnosis, to determine herd prevalence, and to conduct epidemiological studies. 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 (5). Therefore, the demonstration of leptospires in the genital tract and or urine only must be interpreted with full consideration of the serological results, as these findings may merely indicate that the animals were carriers.

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References