SEROPREVALENCE OF LEPTOSPIRAL INFECTION IN BUFFALO (BUBALUS BUBALIS)

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Abstract

To investigate the prevalence of leptospiral infection in buffalo (Bubalus bubalis) in Ahvaz, blood samples were taken from 189 (98 females and 91 males) buffaloes slaughtered in abattoir. Sera were initially screened at serum dilution of 1:100 against six live antigens of Leptospira interrogans: Pomona, Canicola, Hardjo, Ballum, Icterohaemorrhagiae and Grippotyphosa using the microscopic agglutination test and sera with positive results were titrated against reacting antigens in serial twofold dilution from 1:100 to 1:1600. Antibody against one or more serovars were detected in 111 (58.73%) sera at dilution ≥1:100. Antibody against more than one serovar were found in 33 positive sera. Among the positive sera, antibodies were most frequent to serovar Canicola (24.52%) followed by those to Hardjo (19.35%), Grippotyphosa (18.06%), Ballum (16.03%), Pomona (12.26%) and Icterohaemorrhagiae (8.39%). Of 54 heifers, 44 cows and 91 males, antibodies were found in 34 (62.96%), 26 (59.09%) and 51 (56.01%), respectively. Statistical analysis showed no significant difference between age and sex groups.

Key words: buffalo, leptospirosis, Iran.

Leptospirosis, which is caused by Leptospira interrogans serovars, is an important zoonotic disease and has become a major worldwide human concern. Although most leptospiral infections are subclinical (17), some clinical syndromes such as fever, abortion, drop in milk production, icterus, and repeated bleeding have been reported in buffalo (1, 12, 23).

Recognition of leptospiral infection has been based generally on serological evidence, as isolation has been very rarely reported from naturally infected animals. For example; the leptospiral strains isolated from buffaloes are: Andamana in India (24), not typed spirochete in Pakistan (1), Borgpeterseni in Italy (23) and Guaricura in Brazil (20). Therefore, a definitive diagnosis of leptospirosis is difficult to make and most diagnostic laboratories do not attempt to isolate leptospires because of their fragile nature, the cost and complexity of the isolation media, and the prolonged incubation period (17). A wide variety of serological tests, which show varying degrees of serogroup and serovar specificity, have been described. Two tests have a role in veterinary diagnosis: the microscopic agglutination test and ELISA (16).

Despite the fact that buffalo breeding is an important source of income in the north, north-west and south area of Iran, specially in Khouzestan province, there are only two serological studies in buffaloes in Iran (7, 25), but there are many studies of leptospirosis in cattle in some areas of Iran (9). The purpose of the study reported here was to estimate the prevalence of leptospiral infection in buffalo in Ahvaz, the center of Khouzestan province of south-west Iran.

Material and Methods

Blood samples were taken from 189 (98 females, 91 males) slaughtered buffaloes (Bubalus bubalis) from the slaughterhouse in Ahvaz, during April to August 2002. Female buffaloes were divided in two age groups, which included 41 heifers and 44 cows. None of these animals had been vaccinated against leptospires and there were no history data of leptospirosis-related symptoms at the time of sampling. Ten millilitres of blood were collected from the jugular vein of each animal. The blood samples were allowed to clot and were centrifuged for 10 min at 2 500 g. After centrifugation, the serum was removed and stored at –20ºC until testing.

The serum samples were tested for antibodies to six live antigens of Leptospira interrogans: Canicola, Grippotyphosa, Hardjo, Pomona, Icterohaemorrhagiae, and Ballum, using the microscopic agglutination test (MAT). Sera were initially screened at the dilution of
1:100 against the antigens. At first, serum dilution of 1:50 was performed 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 microtitrations plates were incubated at 29°C for 2 h. The plates were examined under dark-field microscope. Results were considered positive when 50% or more of agglutinations of leptospires at the dilution of 1:100 or greater were found (16). Sera with positive results were titrated against reacting antigens in serial twofold dilutions from 1:100 to 1:1600.

Results were analysed by chi-square test with confidence level 95% to determine whether sex or age of buffaloes was significantly related to the prevalence of leptospiral infection.

**Results**

Antibodies against one or more serovars were detected in 111(58.73%) sera at dilution of ≥1:100. The prevalence of leptospiral infection in males, heifers and cows were 56.04%, 62.96%, and 59.09%, respectively (Table 1). Regardless of sex and age of the buffaloes, the highest number of reactors was due to Canicola (24.52%), followed in descending order by Hardjo (19.35%), Grippotyphosa (18.06%), Ballum (16.03%), Pomona (12.26%) and Icterohaemorrhagiae (8.39%) (Table 2).

The majority of titre levels were between 100 to 200 for all serovars and the frequency of 100, 200, 400 and 800 were 30.33%, 40.65%, 22.58%, and 6.45%, respectively. No sera reacted to these serovars in 1:1600 or higher dilution (Table 2).

Statistical analysis showed there were no relations between sex or age and infection, but there were differences between age or sex groups with reference to the predominant serovar. So in male buffaloes, heifers and cows predominant serovars were Canicola (25%), Ballum (23.92%), and Canicola (29.72%), respectively (Table 3).

Antibodies against more than one serovars were found in 29.73% of positive sera, so 78, 21, 11, and 1 of the sera had antibodies against 1, 2, 3, and 4 serovars, respectively (Table 4).

### Table 1
Prevalence of leptospiral infection in buffaloes

<table>
<thead>
<tr>
<th></th>
<th>Number of negative samples</th>
<th>Number of positive samples</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>40 (43.96%)</td>
<td>51 (56.04%)</td>
<td>91</td>
</tr>
<tr>
<td>Heifers</td>
<td>20 (37.04%)</td>
<td>34 (62.96%)</td>
<td>54</td>
</tr>
<tr>
<td>Cows</td>
<td>18 (40.91%)</td>
<td>26 (59.09%)</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>78 (41.27%)</td>
<td>111 (58.73%)</td>
<td>189</td>
</tr>
</tbody>
</table>

### Table 2
Distribution and titres of serovar specific leptospiral antibodies in infected buffaloes

<table>
<thead>
<tr>
<th>Titres (%)</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>800</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grippo...phosa</td>
<td>11(7.10)</td>
<td>12(7.74)</td>
<td>4(2.58)</td>
<td>1(0.65)</td>
<td>28(18.06)</td>
</tr>
<tr>
<td>Pomona</td>
<td>5(3.23)</td>
<td>11(7.10)</td>
<td>3(1.95)</td>
<td>0(0)</td>
<td>19(12.26)</td>
</tr>
<tr>
<td>Ictero...hagiae</td>
<td>5(3.23)</td>
<td>4(2.58)</td>
<td>4(2.58)</td>
<td>0(0)</td>
<td>13(8.39)</td>
</tr>
<tr>
<td>Canicola</td>
<td>16(10.32)</td>
<td>16(10.32)</td>
<td>4(2.58)</td>
<td>2(1.30)</td>
<td>38(24.52)</td>
</tr>
<tr>
<td>Ballum</td>
<td>5(3.23)</td>
<td>5(3.23)</td>
<td>13(8.39)</td>
<td>4(2.58)</td>
<td>27(16.03)</td>
</tr>
<tr>
<td>Hardjo</td>
<td>5(3.23)</td>
<td>15(9.68)</td>
<td>7(4.52)</td>
<td>3(1.95)</td>
<td>32(19.35)</td>
</tr>
<tr>
<td>Total</td>
<td>47(30.32)</td>
<td>63(40.65)</td>
<td>35(22.58)</td>
<td>12(6.45)</td>
<td>155(100)</td>
</tr>
</tbody>
</table>

### Table 3
Distribution of serovar specific leptospiral antibodies in infected buffaloes

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Heifers</th>
<th>Cows</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grippo...phosa</td>
<td>12(16.67%)</td>
<td>10(21.74%)</td>
<td>6(16.21%)</td>
<td>28(18.06%)</td>
</tr>
<tr>
<td>Pomona</td>
<td>7(9.72%)</td>
<td>7(15.22%)</td>
<td>5(13.51%)</td>
<td>19(12.26%)</td>
</tr>
<tr>
<td>Ictero...hagiae</td>
<td>7(9.72%)</td>
<td>3(6.52%)</td>
<td>3(8.10%)</td>
<td>13(8.39%)</td>
</tr>
<tr>
<td>Canicola</td>
<td>18(25%)</td>
<td>9(19.56%)</td>
<td>11(29.72%)</td>
<td>38(24.52%)</td>
</tr>
<tr>
<td>Ballum</td>
<td>12(16.67%)</td>
<td>11(23.92%)</td>
<td>4(10.81%)</td>
<td>27(16.03%)</td>
</tr>
<tr>
<td>Hardjo</td>
<td>16(22.23%)</td>
<td>6(13.04%)</td>
<td>8(21.61%)</td>
<td>32(19.35%)</td>
</tr>
<tr>
<td>Total</td>
<td>72(100%)</td>
<td>46(100%)</td>
<td>37(100%)</td>
<td>155(100%)</td>
</tr>
</tbody>
</table>
The prevalence of leptospiral infection in buffalo, based on serological testing, has been reported to be 1.1% - 12.22% in India (10, 18), 17% in Bulgaria (11), 5.8%-82.9% in Brazil (15, 19), 31% in Malaysia (3), 0.5% in Indonesia (4), 33.4% in Egypt (22), 11.3% in Dagestan (2), 41.93% in Sri Lanka (26), 67% in Italy (5), and 30% in west Iran (25). These reported results confirm that prevalence of leptospiral infection in buffalo is different not only between countries but also between different areas of each country. These differences may be the consequence of environmental factors. Environmental factors have been shown to influence the development of leptospiral infection in animals and human beings. Long term survival of pathogenic leptospires outside the host requires a warm and moist environment with pH that is near to neutral. Significant variation in the duration of survival of various serovars is depending on the pH of soil or water. Areas of high rainfall and subsurface water and areas near the equator have become endemic (enzootic) zones (13). In contrast to other studies on leptospiral infection in cattle and buffalo in Iran, the prevalence of leptospiral infection in buffalo and cattle in Ahvaz is higher. It may be probably due to climate condition, because the weather in Ahvaz is commonly warm and warmer than in other cities of Iran. In addition, despite very little annual rainfall, due to many river branches reaching this city, the surface water is higher than it could be expected. Miller et al. (13) showed some seasonal influence of leptospiral infection in cattle. It might be expected that more cattle become infected in warmer seasons of the year when condition are favorable for survival of leptospires in surface water and animal waste.

In contrast with leptospiral infection in cattle (53.79%), horse (27.88%), and donkey (40%), in Ahvaz, the percentage of infected buffalo (58.73%) is higher than in these animals (9, 10). Because buffaloes are mostly bred naturally, venereal transmission could have been a contributing factor to the difference in seroprevalence between buffalo and cattle or horse. In addition, buffaloes in Ahvaz are adapted to live in water and swamp and in this situation, transmission appears to be more rapid and more extensive. This could be increased chances for contact of infected water with mucous membrane of eyes, nose, and mouth. For these reasons buffaloes have greater chance of being exposed naturally to leptospires.

In this study, there was no relationship between age or sex of buffalo and infection. The findings were in agreement with the study in cattle in Ahvaz (9). Cinceroni et al. (5) showed that the prevalence of leptospiral infection in seropositive buffaloes is significantly increasing in older age, but Ellis et al. (6) reported a high prevalence of infection in young cattle in Ireland and concluded that younger animals had an important role in the epidemiology of leptospiral infection.

The predominant serovar was Canicola (24.52%). The predominant serovar giving rise of serological reaction varies somewhat between countries. For example: Pomona (45.08%) in India (18), Pomona (57%) in Bulgaria (11), Hardjo (85.4%) in Egypt (22), Hebdomadis (56.4%) in Dagestan (2), Weerasingha (30.2%) in Sri Lanka (26), and Icterohaemorrhagiae (21%) in Pakistan (4) were most common serovars in buffalo. In Leptospira infected cattle, horses, and donkeys in Ahvaz, Grippotyphosa was the predominant serovar and the prevalence was 30.07%, 33.33%, 49.51%, respectively (8, 9). Canicola was present in 24.52% of positive buffaloes in this study making it the most prevalent of all serovars for which we tested and it is probable that Canicola may be more adopted to, and maintained by the buffalo population in Ahvaz.

Antibodies against more than one serovars were found in 29.73% of positive sera. In serological tests for leptospirosis such as MAT, the results often indicate infection by more than one serovar (8, 9). 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 cannot be excluded from this interpretation.

The majority of titres were 100 and 200 for all serovars. The percentage of titre levels lower than 400 were 69.45%, 69.57% and 75.68% in positive buffalo males, heifers and cows, respectively. The high prevalence of infection and dominant titres of 100 and 200, reveal that leptospiral infection in buffalo in Ahvaz is endemic.

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References