Serologic study on leptospiral infection in sheep in Ahvaz, southwestern Iran

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Summary

To investigate the seroprevalence of leptospiral infection in sheep in Ahvaz, southwestern Iran, blood samples were taken from 181 female sheep. Sera were stored at -20°C until use. They were initially screened at serum dilution of 1:100 against six live antigens of *Leptospira interrogans* serovars *pomona*, *canicola*, *hardjo*, *ballum*, *icterohaemorrhagiae*, *grippotyphosa* using the microscopic agglutination test (MAT). The samples were considered positive if ≥50% of agglutination of leptospire in a dilution test serum of ≥l:100 were observed. Sera with positive results were titrated against reacting antigens in serial two-fold dilutions from l:100 to 1:1600. Antibodies against one or more serovars were detected in 27 (14.9%) sera at dilution ≥1:100. Antibodies against more than one serovar were found in 5 (18.5%) positive sera. Among the positive sera, antibodies were most frequent to serovar *pomona* (43.8%) followed by *canicola* (21.9%), *icterohaemorrhagiae* (12.5%), *grippotyphosa* (9.4%), *ballum* and *hardjo* (each of them 6.3%). The results of this survey indicate that leptospiral infection is common in sheep in Ahvaz and that various serovars concur in the etiology.

Key words: Leptospirosis, Seroprevalence, Epidemiology, Iran, Sheep

Introduction

Leptospirosis is a common global zoonotic disease of man and in all farm animal species especially in sub-tropical and tropical regions of the world. Most leptospiral infections in sheep and goat are asymptomatic but may result in high fever, abortion, stillbirth, agalactiae and prenatal death. Affected lambs and kids may manifest fever, jaundice and haemoglobinuria which may result in death (Cousing and Robertson, 1986; Radostits *et al*., 2000).

Unfortunately, a definitive diagnosis of leptospirosis is difficult to make. Most of diagnostic laboratories do not attempt to isolate leptospires because of their fragile nature, cost and complexity of the isolation method, and long incubation period (Donahue *et al*., 1991; Radostits *et al*., 2000). Therefore, recognition of leptospiral infection has been based generally on serologic evidence. 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 (MAT) and enzyme-linked immunosorbent assay (ELISA) (OIE, 2000).

Previous serologic surveys in Ahvaz were carried out on cattle, buffalo, horse and donkey (Haji Hajikolaei *et al*., 2006, 2005a, 2005b). These surveys showed that leptospiral infection is common in these animals. Because, there was no study on leptospiral infection in sheep in Ahvaz, this study was carried out to determine the seroprevalence of leptospiral infection in

sheep.

Materials and Methods

Blood samples were taken from 181 female sheep from five suburbs of Ahvaz, southwest of Iran, between January and March, 2003. None of animals had been vaccinated against leptospires. According to dental formula, these sheep were divided into four age groups, namely 1, 2, 3 and ≥4 years old. The numbers of samples from suburb one to five were 53, 38, 34, 31 and 25, respectively. At the time of blood collection, all animals appeared healthy with no clinical signs suggestive for leptospirosis. Ten ml of blood was collected from jugular vein of each sheep. The samples were allowed to clot and centrifuged for 10 min at 2500 × g. After centrifugation, the serum was removed and stored at -20°C until use.

The sera were tested for antibodies against six live antigens of *Leptospira interrogans* (*L. interrogans* serovar *pomona*, *canicola*, *hardjo*, *ballum*, *icterohaemorrhagiae*, *grippotyphosa*) using the MAT, in Leptospiral Research Laboratory, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. According to the methods of OIE (2000), sera were initially screened at a dilution of 1:100 against these antigens. At first, a serum dilution at 1:50 was made and a volume equal to the diluted serum volume of each antigen was added to each well of micro-titration plates, making the final serum dilution of 1:100. The micro-titration plates were incubated at 29°C for two hrs. The plates were then examined by dark-field microscopy. Results were considered positive when ≥ 50% of agglutination of leptospires at the test serum dilution of ≥ 1:100 were observed (OIE, 2000). Sera with positive results were titrated against reacting antigens in serial two-fold dilutions from 1:100 to 1:1600.

Obtained results were statistically analysed using chi-square and Fisher's exact test with significance level at 0.05.

Results

Antibodies against one or more serovars were detected in 27 (14.9%) sheep. The highest number of reactors was for *pomona* (43.8%) followed by *canicola* (21.9%), *icterohaemorrhagiae* (12.5%), *grippotyphosa* (9.4%), *ballum* and *hardjo* (each of which 6.3%). The majority of titre levels were 1:100 for all serovars and the frequency of 1:100, 1:200, 1:400 and 1:800 were 56.3, 25, 12.5 and 6.3%, respectively (Table 1).

Antibody against more than one serovars were found in five (18.5%) sera, so that mixed infection of *pomona* and *canicola*, *canicola* and *grippotyphosa*, *hardjo* and *ballum* were seen in three, one and one positive sera, respectively.

There was no significant difference among age groups (P = 0.839), but there was a tendency in young sheep to be more seropositive than adult sheep (Table 2).

Distribution of leptospiral infection in sheep among various suburbs was also not significantly different (P = 0.112) (Table 3). In suburb 3, none of the examined sheep has shown antibodies against various serovars of *L. interrogans*.

Discussion

We found that the seroprevalence of leptospiral infection in sheep in Ahvaz was 14.9%. The reported results of seroprevalence of leptospiral infection in sheep are different from country to country.

Table 1: Distribution of serovar specific antileptospiral antibodies and their titration in seropositive sheep

Serovar	1:100	1:200	1:400	1:800	Total
grippotyphosa	2 (6.3%)	1 (3.1%)	0 (0%)	0 (0%)	3 (9.4%)
canicola	3 (9.4%)	2 (6.3%)	1 (3.1%)	1 (3.1%)	7 (21.9%)
pomona	6 (18.8%)	5 (15.6%)	3 (9.4%)	0 (0%)	14 (43.8%)
icterohaemorrhagiae	3 (9.4%)	0 (0%)	0 (0%)	1 (3.1%)	4 (12.5%)
hardjo	2 (6.3%)	0 (0%)	0 (0%)	0 (0%)	2 (6.3%)
ballum	2 (6.3%)	0 (0%)	0 (0%)	0 (0%)	2 (6.3%)
Total	18 (56.3%)	8 (25%)	4 (12.5%)	2 (6.3%)	32 (100%)

Table 2: Distribution of leptospiral infection in sheep stratified by age

Age (year)	No. positive	No. negative	Total
1	4 (19.1%)	17 (81.0%)	21
2	5 (16.1%)	26 (83.9%)	31
3	5 (17.9%)	23 (82.1%)	28
≥4	13 (12.9%)	88 (87.1%)	101
Total	27 (14.9%)	154 (85.1%)	181

Table 3: Distribution of leptospiral infection in sheep in various suburbs of Ahvaz

Suburb	No. positive	No. negative	Total
1	9 (17.0%)	44 (83.0%)	53
2	7 (18.4%)	31 (81.6%)	38
3	0 (0%)	34 (100%)	34
4	6 (19.4%)	25 (80.6%)	31
5	5 (20%)	20 (80%)	25
Total	27 (14.9%)	154 (85.1%)	181

These differences may be the consequence of environmental factors and control efforts. The environmental factors have been shown to have influential effects on development of leptospiral infection in animal and human beings. Long-term survival of pathogenic leptospires outside the host requires a warm, moist environment with a near neutral pH (Miller *et al*., 1991). So that the prevalence of leptospiral infection in sheep based on serologic survey has been reported to be 14.3% in Bolivia (Ciceroni *et al*., 1997), 19.7% in Argentina (Draghi *et al*., 1984), 4.2% in Egypt (Maronpot and Barsoum, 1972), 60.4% in India (Sratname *et al*., 1992), 6.1% in Italy (Ciceroni *et al*., 2000) and 16.8% in Greece (Burriel *et al*., 2002).

In contrast to the previous studies in Ahvaz, the prevalence of antibodies to one or more serovars of *L. interrogans* was 53.8, 58.7, 27.9 and 40% in cattle, buffalo, horse and donkey, respectively (Haji Hajikolaei *et al*., 2006, 2005a, 2005b). Although the significance of these differences was not defined, but it may be due to difference 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 (Maronpot and Barsoum, 1972). According to the report of Rocha (1988), the prevalence of leptospiral infection in cattle, sheep, goat, and horse in Portugal was 15.3, 3.3, 5.0 and 43.3%, respectively. In Turkey, 44.77% of cattle and 8% of sheep reacted to one or more serovar of *L. interrogans* (Ozdemir and Erol, 2002). In Malaysia 40.5, 31 and 10% of cattle, buffalo and sheep reacted to one or more serovar of *L. interrogans*, respectively (Bahaman *et al*., 1987).

Pomona was present as the predominant serovar in seropositive sheep in this study. On the other hand, in a previous study in Ahvaz, the predominant serovars were *grippotyphosa* in cattle, horse and donkey and *canicola* in buffalo (Haji Hajikolaei *et al*., 2006, 2005a, 2005b). It is probable that these serovars may be adapted to and maintained by these farm animals in Ahvaz. 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 (Ciceroni *et al*., 1997), *wollfi*, *pomona* and *ballum* in Argentina (Draghi *et al*., 1984), *hebdomadis* in the UK (Hathaway *et al*., 1981), *pomona* in India (Manickavel *et al*., 1991), *autumnalis* in Egypt (Maronpot and Barsoum, 1972), *castellonis* in Italy (Ciceroni *et al*., 2000) and *pomona* in Malaysia (Bahaman *et al*., 1987) 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 in 18.5% of seropositive sheep. In serologic tests for leptospirosis such as MAT, the results often indicate infection with more than one serovar (Hathaway *et al*., 1981; Egan and Yearsley, 1989; Firouzi and Vandyousefi, 2000; Haji Hajikolaei *et al*., 2006, 2005a, 2005b). 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 leptospiral infection in sheep in Ahvaz is endemic and occurs mostly in subclinical form.

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