Seroprevalence of Leptospiral Infection in sheep in Khorramabad, west Iran

SHAHRAM MALEKI
Department of Internal Medicine, Faculty of Veterinary Medicine, Lorestan University, P. O. Box: 465, Khorramabad, Iran.

ABSTRACT
Leptospirosis is a zoonosis of global distribution, caused by infection with pathogenic of the genus leptospira. Leptospira is recognized as an important public health problem worldwide and affects virtually all mammals. This study was conducted on 200 sheeps from five suburbs of Khorramabad area in the West of Iran at Winter and Spring of 2014 in order to seroprevalence of leptospiral infection. On the bases of age these sheeps were divided in 5 groups. Blood samples were collected from the sheeps and the sera were removed and stored at -20°C until ready for tested. They were initially screened at serum dilution of 1:100 against 6 live antigens of leptospira interrogans serovar Pomona, Canicola, Grippotyphosa, Icterohaemorrhagiae, Hardjo and Ballum using the Microscopic Agglutination Test (MAT) and samples were considered positive, if 50% or more of agglutination of leptospires in a dilution of 1:100 or greater was found. Sera with positive results were titrated against reacting antigens in serial twofold dilution from 1:100 to 1:1600. The prevalence of leptosomal infection was 14% in sheeps. 85.71% of female sheeps and 14.29% of male sheeps were positive. The highest number of reactors in sheeps (48.39%) was due to serovar Canicola, followed in descending order by Pomona (25.81%), Grippotyphosa (16.13%), Icterohaemorrhagiae (6.45%) and Hardjo (3.22%) . Three sample (10.71%) was positve for more than one serotype. Also the most seropositive cases were observed in 4-6 years old sheeps but There was no significant difference among age groups (P=0.147). About 21% of infected sheeps were in the non-moist stables and 79% were in the moist (marshy) stables. There was significant difference between in two kind of stables and Sex prevalence (P<0.05). The majority of titer levels were 100 for all the serovars. These results confirmed that the majority of leptospiral infections are asymptomatic and the presence of antibodies in the absence of infection indicates exposure to the organism in these animals and also, indicate the risk of exposure of organism to other animals. However, the results of this survey indicate that leptospiral infection is common in sheep in Khorramabad and that various serovars concur in the etiology.

Keywords: Leptospirosis, Seroprevalence, MAT, Sheep, Khorramabad.
control of the infection in cattle and also the infected sheep are the potential zoonotic risk to abattoir, worker, sheep farmer and shearsers which previously had not been considered (9). 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 (7, 22). Therefore, recognition of leptospirosis 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) (20). Considering that the high leptospirosis seroprevalence rates of the cattle and buffalo in previous studies in Iran (11, 25) and with attention to the fact that sheep are usually in contact with cattle directly or indirectly in the most regions of the province (Lorestan), therefore this is predicted that sheep may be one of the important animals in epidemiology of the infection in Iran. Previous serologic surveys in Khorramabad were carried out on horse, cattle and goat. These surveys showed that leptospirosis infection is common in these animals. Because, there was no evidence of the study on leptospirosis infection in sheep in Khorramabad, this study was carried out to determine the seroprevalence of leptospirosis infection in sheep and compare with other farm animals.

1-MATERIALS AND METHODS

Blood samples were taken from 200 sheeps from 18 sheep herds in 5 suburbs of Khorramabad, West of Iran, during the period September 2013 to May of 2014. According to dental formula, these sheeps were divided into five age groups (<1, 1 - 2, 2 - 4, 4 – 6 and over 6 years old). 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. The numbers of samples from suburb one to five were 42, 40, 39, 40 and 39, respectively. Ten ml of blood were collected from the jugular vein of each sheep. The blood samples were allowed to clot and centrifuged for 10 min at 3000 g. After centrifugation, the serum was removed and stored at -20°C until ready for test. The sera were tested for antibodies to 6 live serovars of *Leptospira interrogans*: Pomona, Grippotyphosa, Icterohaemorrhagiae, Canicola, Hardjo and Ballum using the Microscopic Agglutination Test (MAT) in the Leptospira Research Laboratory, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. According to the methods of OIE (20), 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 hours. The plates were then examined by dark-field microscopy. The results were considered positive when ≥ 50% of agglutination of leptospires at the test serum dilution of ≥ 1:100 were observed (20). Sera with positive results were titrated against reacting antigens in serial two-fold dilutions from 1:100 to 1:1600. The results were analysed by chi-square and Fisher’s exact test to determine the difference between two sexes, moist stable, various suburbs and different groups of age was significantly related to the prevalence of leptospiro antibodies.

2-RESULTS

Antibodies against one or more serovars were detected in 28 (14%) sheep. Some samples were positive for two leptospirol antigens. 24 female (85.71%) goats and 4 male (14.29%) goats were positive in MAT test. There was significant difference between seropositives and sex (P<0.05) (Table 1). The highest number of reactors was for canicola (48.39%) followed by Pomona (25.81%), grippotyphosa (16.13%), icterohaemorrhagiae (6.45%) and hardjo (3.22%) (Table 2). All of sera were seronegative for other serovars (Table 2). Out of the sheeps that were seropositive for leptospirosis, three sample (10.71%) was positive for more than one serotype (Table 2). The majority of titre levels were 1:100 for all serovars and the frequency of 1:100, 1:200 and 1:400 were 70.97, 25.81 and 3.22%, respectively (Table 3). On the base of age, 1 goats (3.57%) in less than 1 year group, 3 goats (10.71%) in the 1–2 years group, 8 goats (28.57%) in the 2-4 years group, 10 goats (35.72%) in the 4-6 years group and 6 goats (21.43%) in the over 6 years group were positive for leptospiroa. There was no significant difference among age groups (P=0.147), but there was a tendency in young sheep to be more seropositive than adult sheep (Table 4). Distribution of leptospirosis infection in sheep among various suburbs was also significantly different (P<0.05) (Table 5). In central suburb, none of the examined sheep has shown antibodies against various serovars of *leptospira interrogans*. About 78.57% of infected goats were in the moist (marshy) stable and 21.43% were in the non-moist stable and there was a significant difference between the prevalence of disease in two kind of stable (P<0.05) (Table 6).