Serologic study on leptospiral infection in goats in Khorramabad, west Iran

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ABSTRACT-Leptospirosis is a zoonosis of worldwide distribution, caused by Leptospira interrogans. It is an acute infectious, systemic and septisemic disease which had resent outbreaks in some parts of Iran. This study was conducted on 180 goats in Khorramabad area in the West of Iran in order to seroprevalence of leptospiral infection. On the bases of age these goats were divided in 5 groups. Blood samples were collected from the goats 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, Grippotyphosa, Icterohaemorrhagiae, Canicola, 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 leptospiral infection was 11.67% in goats. 9.52% of male goats and 90.48% of female goats were positive. Also the most seropositive cases were observed in 4-6 years old goats. About 23% of infected goats were in the non-moist stables and 77% were in the moist (marshy) stables. There was significant difference between in two kind of stables, Sex and aging prevalence (P<0.05). The highest number of reactors in goats (52.17%) was due to serovar Canicola, followed in descending order by Grippotyphosa (26.09), Pomona (13.04%) and Icterohaemorrhagiae (8.7%). Two sample (9.52%) was positive for more than one serotype. 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.© 2014 Bull. Georg. Natl.Acad. Sci.

Key words: Seroprevalence, Leptospira, goats, MAT, Khorramabad.

Leptospirosis is a common global zoonotic disease of man and in all farm animals species specially in sub-tropical and tropical regions of the word (Agunloye, 2002 ; Cousing and Robertson, 1986 ; Radostits et al. 2007). It is caused by spirochetes belonging to the genus Leptospira. All the pathogenic leptospires were formerly classified as members of the species Leptospira interrogans; the genus has recently been reorganized and pathogenic leptospires are now identified in several species of Leptospira. Leptospirosis is a significant occupational hazard in the cattle and pig industries in certain areas (Ellis et al. 1994 ; Faber et al. 2000 ; Hartskeeri et al. 2004). Most leptospiral infections in sheep and goat are asymptomatic (Agunloye, 2002 ; Cousing and Robertson, 1986 ; Radostits et al. 2007). Pyelonephritis is the most frequently encountered clinical manifestation of leptospirosis in goats however, abortion and stillbirth are serious problems (Ellis et al. 1994 ; Faber et al. 2000 ; Hartskeeri et al. 2004). Affected lams and kids may manifest fever, jaundice and hemoglobinuria, which may also result in death (Agunloye, 2002 ; Cousing and Robertson, 1986 ; Radostits et al. 2007).

Milk drop syndrome in cattle at milky goats have also been reported (Quinn et al. 2002). Non-specific disease characterized by fever, jaundice, anorexia and lethargy may also occur. Leptospirosis can be readily transmitted between species including between animals and humans through infected urine, contaminated soil or water or other

body fluids (Barwick et al. 1998). Veterinarians can be infected through contact of mucous membranes or skin lesions with urine or tissues from an infected animal. Human leptospirosis can be highly variable, ranging from asymptomatic infection to sepsis and death headache, myalgia, nausea and vomiting are common complaints however, neurologic, respiratory, cardiac, ocular and gastrointestinal manifestations can occur (Ellis et al. 1994 ; Roth and Gleckman, 1985). In rare instances, leptospirosis can be fatal. Leptospirosis is classified into 2 broad categories; host-adapted and non-host-adapted. An animal infected with a host-adapted serovar of the organism is a maintenance of reservoir host. Cattle are the maintenance host for some of the serovars, thus serological surveys of cattle in the world has found that relatively high percentages of the sera had antibodies against numerous leptospiral serovars but sheep has been accepted as accidental or incidental hosts for the most leptospiral serovars (Radostits et al. 2007). However, persistent leptospiruria due to leptospira hardjo in sheep were no contact with cattle has occurred (Radostits et al. 2007) and also widespread leptospiral infection in merino rams in Australia, suggest that sheep may be a maintenance host at least for some of the serovars such as hardjo. This could complicate control of the infection in cattle and also the infected sheep are the potential zoonotic risk to abbatior, worker, sheep farmer and shearers which previously had not been considered (Ellis et al. 1994). Considering that the high leptospiral seroprevalence rates of the cattle and buffalos in previous studies in Iran (Firouzi and Vandyousefi, 2000 ; Shoaei, 1993) 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. Prevalence of leptospiral infection in goats was unknown in khorramabad. Prevention of occupational leptospirosis among veterinarians involves early identification of infected animals, reducing contact with affected animals (particularly urine and other body fluids) and the use of waterproof barrier clothing (Ellis et al. 1994). 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 media, and prolonged incubation period (Radostits et al. 2007). Therefore, recognition of leptospiral infection has been based generally on serological 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 the enzyme-linked immunosorbent assay (ELISA) (O.I.E., 2000). Previous serological surveys of leptospiral infection in Khorramabad were carried out cattle and horse. These surveys indicated that leptospiral infection is common in these animals. Because, there was no evidence of the study on leptospiral infection in goat in Khorramabad, this study was carried out to determine the prevalence of leptospiral antibodies in goat and compare with other farm animals.

Material And Methods
Blood samples were taken from 180 goats from 16 goat herds in 4 suburbs of Khorramabad, West of Iran, during the period September 2013 to May of 2014. On the basis of age, these goats were divided in 5 groups (<1, 1 - 2, 2 - 4, 4 – 6 and over 6 years). 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 four were 46, 43, 44 and 47, respectively. Ten ml of blood were collected from the jugular vein of each goat. The blood samples were allowed to clot and were centrifuged for 10 min at 3000g. 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 leptospira interrogans: Pomona, Grippotyphosa, Icterohaemorrhagiae, Canicola, Hardjo and Ballum using the Microscopic Agglutination Test (MAT) in the Leptospiros Research Laboratory of veterinary faculty of Tehran University. 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 microtitation plates were incubated at 29°C for 2 hours. The plates were examined under darkfield microscopy. The results were considered positive when 50% or more of leptospiirae at dilution of 1:100 or greater were found. The results were analysed by chi-square and Fisher’s exact test to determine the difference between two sexes and moist stable and different groups of age was significantly related to the prevalence of leptospiral antibodies.

Results
About 21 (11.67%) from 180 goats that tested were positive for at least one leptospiral antigen. Some samples were positive for two leptospiral antigens. 2 male (9.52%) goats and 19 female (90.48%) goats were positive in MAT test. There was significant difference between seropositives and sex (P<0.05) (Table 1). On the base of age, 1 goats (4.76%) in less than 1 year group, 2 goats (9.53%) in the 1 – 2 years group, 6 goats (28.57%) in the 2 - 4 years...