

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 recent 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 leptospire 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 world (Agunloye, 2002 ; Cousing and Robertson, 1986 ; Radostits et al. 2007). It is caused by spirochetes belonging to the genus *Leptospira*. All the pathogenic leptospire were formerly classified as members of the species *Leptospira interrogans*; the genus has recently been reorganized and pathogenic leptospire 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 lambs 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 leptospiuria 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 ; Shoaie, 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 leptospire 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 leptospire 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, Grippityphosa, Icterohaemorrhagiae, Canicola, Hardjo and Ballum using the Microscopic Agglutination Test (MAT) in the Leptospira 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 microtitration 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 leptospirae 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

group, 11 goats (52.38%) in the 4- 6 years group and 1 goats (4.76%) in the over 6 years group were positive for leptospira. There was significant relationship between aging of the goats ($P<0.05$) and increased of seropositive animals that they were observed in 4-6years old (Table 2). The highest number of reactors in goats (52.17%) was due to serovar Canicola, followed in descending order by Grippothyphosa (26.09%), Pomona (13.04%) and Icterohaemorrhagiae (8.7%) (Table 3). All of sera were seronegative for other serovars (Table 3). Out of the goats that were seropositive for leptopirosis, two sample (9.52%) was positive for more than one serotype (Table 3). About 76.2% of infected goats were in the moist (marshy) stable and 23.8% 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 4). The majority of titer levels were 100 for all the serovars (Table 5).

Discussion

Leptospirosis is an infectious zoonotic disease and infections with different serotypes of the leptospire in any geographical area are important on the epidemiology and pathogenesis of the disease in the region. Cattle are maintenance host for many serotypes of the bacteria. Thus, previous studies on cattle have demonstrated relatively high prevalence rates of the infection in different country and even various regions in Iran. Seroprevalences of *leptospiral infections* of cattle of Tehran suburb dairy farms at 1990 and 2013 were 31.2% (Moharamie, 1990), 46.8% (Goli, 2001), 14.47% (Sakhaie et al. 2005) and 40.57% (Maleki et al. 2013) and in East Azarbaijan province at 1993 and 2007 were 48.5% (Shoaei, 1993) and 24% and in ahvaz at 2006 was 53.73% (Hajikolaei et al. 2005), respectively. Sheep are not naturally maintenance hosts for some of the serotypes such as Pomona or hardjo and are likely to have infections of relatively short duration, producing severe pathologic effect. However, persistent leptospiruria and high seroprevalence rates of the infections in sheep where no contact with cattle have occurred suggest that sheep may be a maintenance host for some serovars. This could complicate control of the infection in cattle and sheep and infected sheep are a potential zoonotic risk to humans such as abbatior workers, sheep farmers and shearers which previously had not been considered (Radostits et al. 2007). We found that the seroprevalence of *leptospiral infection* in goats in Khorramabad was 11.67%. The prevalence of *leptospiral infection* in goats from another countries based on serological survey has been reported to be 1.2%, 12.3%, 13.1%, 14.3%, 16.8%, 32%, 40%, 42%, 42.1%, 55.2% and 70% in France, Italy, Nigeria, Bolivia, Greece, Croix, Belize, Australia, Egypt, India and New Zealand, respectively (Agunloye, 2002 ; Ciceroni et al. 1997 ; Flint et al. 1988 ; Maronpot and Barsoum, 1972 ; Sratnam, 1992 ; Trap and Gaumont, 1983). There are three documented report of *leptospiral infection* in goats from Iran. These studies were carried out on goats from Uremia, Ahwaz and Khoys that reported 27.5%, 10.46% and 13.3% of goats from this regions had leptospiral antibodies, respectively (Hajikolaei et al. 2007 ; Hassanpour et al. 2012 ; Zainali et al. 1997). These reported results confirm that prevalence of *leptospiral infection* in goats is different from region to region or country to country. These differences may be the consequence of environmental factors and control efforts. It seems the prevalence of *leptospiral infection* in Khorramabad is lower than of some countries specially in contrast with Uremia. Since, at present, there is no specific control strategy against leptospirosis in Khorramabad, this cannot therefore be attributed to control efforts. On the other hand, environmental factors have been shown in influence of development of *leptospiral infection* in animal and human beings. Long-term survival of pathogenic leptospire outside the host requires a warm, moist environment with a near natural pH (Miller et al. 1991). According to climate situations in Khorramabad it has been expected that *leptospiral infection* in goat was nearly similar to a previous reports in cattle in Khorramabad. Because, in previous serological surveys the prevalence of *leptospiral infection* was 20% in cattle. It may be due to the behaviors of the goat, which is differing with other animals. This animal habitually tries to use the head branches of grass and stay in muddy and swampy grounds less than others that it results to less expose to leptospire. Leptospirosis occurs in sheep and goats with less frequency than in cattle. In Turkey, 44.77% of cattle and 8% of sheep reacted to one or more serovar of *Leptospira interrogans* (Ozdemir and Erol, 2002). Although, the significance of these differences is not defined but it may be fdue to differences in susceptibility of these animals. The results of this study showed that the serological infection rate in goats in Khorram abad is relatively high and consequently the preventive methods must be applied to prevention of the spread of disease and its transmission to the human and other farm animals and emphasize the important role of goat on the epidemiology of the infection. The predominant leptospire serovars in serological reaction varies somewhat between countries. For example: pomona in India (Manickavel et al. 1991), icterohaemorrhagiae and pomona in South America (Saglana et al. 2007), poi and pomona in Bolivia (Ciceroni et al. 1997), bratislava in Greece (Burriel et al. 2003), hebdomadis in the UK (Hataway et al. 1981), castellonis in Italy (Ciceroni et al. 2000), automnalis in Egypt (Maronpot and Barsoum, 1972) and icterohaemorrhagiae in France 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.

In this study, canicola, grippotyphosa and pomona were detected as the most prevalent serovars with 52.17%, 26.09% and 13.04%, respectively. With attention to the fact that the dogs and rodents are the major maintenance hosts for pomona, canicola and grippotyphosa serovars, respectively (Radostits et al. 2007) and considering that frequent contacts between goat and these animals in the flocks of the region, the relatively high prevalence of these serovars in this study justified. Thus, the preventive methods must be applied to control of the infection in rodent and accompanied flocks dogs. In previous studies in Tehran, Tabriz, Ahvaz and Khorramabad, the predominant serovars in cattle were Pomona, Pomona, grippotyphosa and Pomona, canicola and grippotyphosa (Hajikolaie et al. 2007 ; Hassanpour et al. 2012 ; Maleki et al. 2013), respectively. It is probable that this serovar may be adapted to and maintained by these farm animals in Khorramabad. 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 region.

Percentage of seropositive for more than one serovar was 9.52% in seropositive goats. In serological tests for leptospirosis such as MAT, the results often indicate infection with more than one serovar (Egan and Yearley, 1989 ; Hajikolaie et al. 2005 ; Hataway et al. 1981). 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.

The high prevalence of infection and dominant titer of 1:100 reveal that *leptospiral infection* in goats in Khorramabad is endemic and occurs mostly in subclinical form. There was significant relationship between aging of the goats that they were observed in 4-6 years old ($p < 0.05$). In previous studies in Tabriz and Khoy, hassanpour et al. (2008) and (2012) showed the highest number of positive samples were in 2-4 years old and 4-6 years old, respectively (16). About 23.8% of infected goats were in the dry grounded stables and 76.2% were in the moist (marshy) stables and there was a significant difference of seroprevalence of disease between them ($p < 0.05$).

Laboratory procedures used in the diagnosis of leptospirosis. Leptospiral antibodies appear within a few days of infection and persist for weeks or months and in some cases, years. Unfortunately, antibody titers 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 (Roth and Gleckman, 1985). Therefore, the demonstration of leptospire in genital tract or urine only must be interpreted with full consideration of the serological results and culture or detection of leptospire in blood or body fluids as these findings may indicate that the animals were carriers. These results confirm that *leptospiral infection* may exist in the goat population in Khorramabad area and the presence of antibodies in the absence of infection indicates exposure to the organism and must be acknowledged. In addition, these results confirm that the majority of *leptospiral infections* is asymptomatic. because of the importance of *leptospira interrogans* as an abortifacient agent in goat, it will be considered as one of the possible cause of abortion in goat in Khorramabad.

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Table 1: Sex distribution in leptospiral seropositive goats.

Sex	Tested	Positive	Percent (%)
male	60	2	9.52
female	120	19	90.48
Total	180	21	11.67

Table 2: Age distribution in leptospiral seropositive goats.

Age groups (year)	Tested	Positive	Percent (%)
<1	30	1	4.76
1-2	35	2	9.53
2-4	45	6	28.57
4-6	45	11	52.38
>6	25	1	4.76
Total	180	21	11.67

Table 3: Prevalence of different leptospiral serovars in goats.

Serovar	Numbers	Percent (%)
Pomona	3	13.04
Grippityphosa	6	26.09
Icterohaemorrhagiae	2	8.7
Canicola	12	52.17
Hardjo	0	0
Ballum	0	0
Total	23	100

Table 4: Moist or non-moist stable distribution in leptospiral seropositive goats.

Stable	Tested	Positive	Percent (%)
Moist (marshy)	110	16	76.2
Non moist	70	5	23.8
Total	180	21	11.67

Table 5: Prevalence of leptospiral antibody titers to different antigens in goats.

Titer	Numbers	Percent (%)
100	16	69.57
200	7	30.43
Total	23	100