

Seroepidemiologic Study of Goats Leptospirosis in Khoy-Iran

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Abstract: This study was conducted on 150 goats (up to 1 to >6 years) in Khoy area in the North of Iran at Spring of 2010 in order to seroprevalence of leptospiral infection. Blood samples were collected from the animals and sera were initially screened at dilution of 1:100 against 6 live serovars of *Leptospira interrogans*: Pomona, Canicola, Hardjo, Ballum, Icterohaemorrhagiae and Grippityphosa using the Microscopic Agglutination Test (MAT). The serum positive goats were in 4-6 years and the seroprevalence of leptospiral infection was 13.3% in them. About 20% of infected goats were in the moist stables and 80% were in the moist (marshy) stables. There was a significant difference between the seroprevalence of disease in two kind of stables ($p < 0.05$). Also statistically, a significant relationship observed between the increased age of examined animals and the seroprevalence of disease ($p < 0.05$). The highest number of reactors in goats was 80% due to serovar grippityphosa and 20% due to serovar Pomona. The majority of titer levels were 100 for all the serovars. These results confirm that the majority of leptospiral infections are asymptomatic and indicate the risk of exposure of organism to other animals.

Key words: Goats, seroprevalence, leptospira, MAT, animals, Iran

INTRODUCTION

Leptospirosis is a widely spread zoonosis of global concern (Bharti *et al.*, 2003). 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 reorganised 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. Pyelonephritis is the most frequently encountered clinical manifestation of leptospirosis in goats however, abortion and stillbirth are serious problems (Bernard *et al.*, 1993; Ellis *et al.*, 1994; Faber *et al.*, 2000; Hartskeerl *et al.*, 2004; Matthews *et al.*, 1987; Rao *et al.*, 1985).

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 *L. 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 and East Azarbaijan province (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, 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 Khoy. 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).

Diagnosis of leptospirosis can be difficult and may involve antigen detection (PCR), serological evaluation, histological examination, culture and/or dark field microscopy (Ellis *et al.*, 1994). 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 and ELISA (27). A number of serological studies have indicated wide-spread evidence of leptospiral infection in ewes in several countries (Donahue *et al.*, 1991; Hathaway *et al.*, 1981; Park *et al.*, 1992; Rao *et al.*, 1985; Verma *et al.*, 1977). The study attempted to determine the prevalence of *L. interrogans* antibodies in goats in Khoy area in Iran.

MATERIALS AND METHODS

Blood samples were taken from 150 goats (from 12 goat herds of Khoy, North-West of Iran in Spring of 2010. On the bases of age, these goats were divided in 4 groups (0-2, 2-4, 4-6 and >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. About 4-6 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 3000 g.

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 *L. interrogans*: Canicola, Grippothyphosa, Hardjo, Pomona, Icterohaemorrhagiae 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 h. The plates were examined under darkfield microscopy. The results were considered positive when 50% or more of agglutination of leptospire at dilution of 1:100 or greater were found (Park *et al.*, 1992; Radostits *et al.*, 2007).

The results were analysed by Chi-square (χ^2) test to determine the difference between different groups of age and moist stable was significantly related to the prevalence of leptosprial antibodies.

RESULTS AND DISCUSSION

About 20 (13.30%) from 150 goats that tested were positive for at least one leptospiral antigen. On the base of age, 2 goats (10%) in the 0-2 years group, 6 goats (30%) in the 2-4 years group, 10 goats (50%) in the 4-6 years group and 2 goats (10%) in the >6 years group were positive. There was a significant relationship between aging of the goats and increased of seropositive animals ($p < 0.05$) (Table 1) that they were observed in 4-6 years old. About 20% of infected goats were in the non-moist stable and 80% were in the moist (marshy) stable and there was a significant difference between the prevalence of disease in two kind of stables ($p < 0.05$) (Table 2). Grippothyphosa was detected as the most-prevalent serovar with 80% other of sera were Pomona (20%). All of sera were seronegative for other serovars (Table 3). The majority of titre levels were 100 for all the serovars. About 2 male (20%) goats and 18 (80%) female goats were positive in MAT test. There was a significant difference between seropositives and sex ($p < 0.05$) (Table 4).

Table 1: Age distribution in leptospiral seropositive goats

Age groups (years)	Tested	Positive	Percentage
0-2	34	2	10.00
2-4	36	6	30.00
4-6	56	10	50.00
>6	24	2	10.00
Total	150	20	13.30

Table 2: Moist or non-moist stable distribution in leptospiral seropositive goats

Stable	Tested	Positive	Percentage
Moist	28	4	20.00
Non-moist	122	16	80.00
Total	150	20	13.30

Table 3: Prevalence of different leptospiral serovars in goats

Prevalence	C	G	P	H	I	B	Total
Numbers	0	16	4	0	0	0	20
Percent	0	80	20	0	0	0	100

G: Grippothyphosa; P: Pomona; I: Icterohaemorrhagiae; C: Canicola, H: Hardjo; B: Ballum

Table 4: Sex distribution in leptospiral seropositive goats

Sex	Tested	Positive	Percentage
Male	25	2	20.00
Female	125	18	80.00
Total	150	20	13.30

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 2001 were 31.2% (Moharamie, 1990) and 46.8% (Golie, 2001) and in East Azarbaijan province at 1993 and 2007 were 48.5% (Shoaei, 1993) and 24%, 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 abattoir 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 Khoy was 13.30%. The reported results of seroprevalence of leptospiral infection in sheep and goats are different from region to region or country to country. 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 leptospiras outside the host requires a warm, moist environment with a near natural pH (Miller *et al.*, 1991).

So that seroprevalence of leptospiral infection in animals has been reported to be 16.8% in Greece (Burriel *et al.*, 2003), 14.3% in Bolivia (Ciceroni *et al.*, 1997), 6.1 and 12.3% in Italy (Ciceroni *et al.*, 2000), 42% in Australia (Ellis *et al.*, 1994), 40% in Belize (Everard *et al.*, 1988) and 32% in Croix (Ahl *et al.*, 1992). The results of this study showed that the serological infection rate in goats in Khoy 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.

In previous studies in Tabriz-Iran, the prevalence of antibodies of one or more serovars of *L. interrogans* was 48.5% (Shoaei, 1993) and 24% in cattle and 18.4% in sheep (Hasanpor *et al.*, 2008). Although, the significance of these differences was not defined but it may be due to differences in susceptibility of these animals. 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 *L. interrogans* (Ozdemir and Erol, 2002). In the present study, like some of the other ones (Ahl *et al.*, 1992; Ellis *et al.*, 1994; Everard *et al.*, 1988; Hasanpor *et al.*, 2008) seroprevalence rate of leptospiral infection in goats was relatively high which emphasize the important role of goat on the epidemiology of the infection.

In this study, grippotyphosa and pomona were detected as the most prevalent serovars with 80 and 20%, respectively. With attention to the fact that the rodents and dogs are the major maintenance hosts for grippotyphosa and pomona 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.

On the other hand in previous studies in Tabriz and Ahvaz, the predominant serovars in cattle were Pomona, grippotyphosa and Pomona (Hajikolaie *et al.*, 2007), respectively. In other previous studies in Tabriz, the prevalent serovar in sheep was grippotyphosa (Hasanpor *et al.*, 2008). It is probable that these serovars may be adapted to and maintained by these farm animals in Khoy.

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), hebdomadis in the UK (Hathaway *et al.*, 1981), Pomona in India (Manickavel *et al.*, 1991), castellonis in Italy (Ciceroni *et al.*, 2000), Bratislava in Greece (Burriel *et al.*, 2003), Icterohaemorrhagiae, Pomona in South America (Saglana *et al.*, 2007) 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.

The high prevalence of infection and dominant titre of 1:100 reveal that leptospiral infection in goats in Khoy

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 other study in Tabriz, Hasanpor *et al.* (2008) showed the highest number of positive samples were in 2-4 years old. About 20% of infected goats were in the dry grounded stables and 80% 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 titres 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 (27). Therefore, the demonstration of leptospirae in genital tract or urine only must be interpreted with full consideration of the serological results and culture or detection of leptospiras in blood or body fluids as these findings may indicate that the animals were carriers.

CONCLUSION

These results confirm that leptospiral infection may exist in the goat population in Khoy 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.

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