Seroprevalance of Leptospirosis in Sheep in Maku, Northwest of Iran

Rezaie, H.[1]; Hassanpour, A[2*].; Abdollahpour, Gh.[2]

1- *Graduated of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran*

2- *Department of Clinical Sciences, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran*

3- *Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[*]***Corresponding author:*** Hassanpour, A

Email: alihassanpour53@gmail.com , a_hasanpour@iaut.ac.ir

Abstract

This study was conducted on 210 sheep in Maku area in Iran in order to determine seroprevalence of leptospiral infection. Sera were initially screened at dilution of 1:100 against 8 live serovars of *Leptospira interrogans*: Pomona, Canicola, Hardjo, Ballom, Icterohaemorrhagiae, Automenalis,Australis and Grippotyphosa using the microscopic agglutination test. The prevalence of leptospiral infection (At titers 100 and 200) was 15.23% in sheep. There was a significant relationship between aging and the incidence of leptospiral infection (P<0.05); however no significant relationship between breed of the shepp and the incidence of leptospiral infection was found. The highest number of reactors in sheep (62%) was due to serovar Canicola, followed in descending order by Icterohaemorrhagiae (32%) and Hardjo(6%). All of the sera were seronegatives for Pomona, Ballum, Grippotyphosa and Autominalise. The majority of titre levels were between 100 and 200 for all the serovars. These results confirm that the majority of leptospiral infections is asymptomatic and the presence of antibodies in the absence of infection indicates exposure to the organism in these animals.

Key words: Sheep, Seroprevalence, Leptospira, Iran.

Introduction

Leptospirosis is the most prevalent worldwide zoonosis, affecting a wide range of mammals including rominants, equines, rodents, and human. The disease is caused by pathogenic *Leptospira interrogans* species,and occurs from a subclinical infection to a severe syndrome with high mortality rate. Leptospirosis involves public health risk, as well as economic losses in the livestoke production industry due to decreased milk yield, abortion, stillbirth, weak calves, weight loss, reproductive complication and occasionally death (Bharti, *et al.* 2003; Radostits, *et al.* 2007). Uveitis is the most frequently encountered clinical manifestation of leptospirosis in horses; however, abortion and stillbirth are serious problems (Bernard, *et al.* 1993; Ellis, *et al.* 1983, Faber, *et al.* 2000; Harskeeri, *et al.* 2004; Hogg, 1974 and Seshagiri *et al.* 1985). Although a number of nonspecific symptoms such as fever, jaundice, abortion, pink stained milk, hemogulubinuria in cows, and stillbirth and agalactia in sheep may be considered to be the clinical signs of the disease, definitive diagnosis relies on the detection

of anti-leptospiral antibodies in serum samples (Radostits, *et al.* 2007). In other words, the efficacy of leptospira control programs in farm animals relies mainly on the direct identification of carriers (Denardi, *et al.* 2010; Schonman and Swai, 2010). ELISA (Rajeev, *et al.* 2010), PCR (Lilenbaum, *et al.* 2009), and Microscopic Agglutination Test (MAT) (Denardi, *et al.* 2010) are the main current serological methods, but MAT still being the "gold standard" is particularly recommended to differentiate the infective serovars from each other (Angela, *et al.* 1998). Leptospirosis can be readily transmitted between species, including animals and humans through infected urine, contaminated soil or water, or other body fluids (Hathaway, *et al.* 1998; Barwick, *et al.* 1998). Veterinarians may be infected through contact of mucous membranes or skin lesions with urine or tissues from an infected animal. The threat of zoonotic transmission of leptospirosis from horses is not considered great; however, it would be prudent to take basic precautions, particularly when evaluating abortions or stillbirths. 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, 1998).

Human leptospirosis is prevalent only in the northern provinces of Iran, but ruminants such as cows (Schonman and Swai, 2010), buffaloes (Denardi, *et al.* 2010) and sheep (Tooloei, *et al.* 2008) are encountered in many parts of traditional style husbandries (Nasr, 2004). Data from several indoor studies by MAT in cows (56.6%) and sheep (17.3%) suggest that the disease is prevalent in the livestock population in many regions (Hajikolaei, *et al.* 2007), A number of serological studies have indicated wide-spread evidence of leptospiral infection in horses in several countries, but there is only one study dealing with the infection in donkeys (Donahue, *et al.* 1991; Egan and Yearsley, 1989; Hassanpour and Safarmashaei, 2012; Park *et al.* 1992; Roth and GLECKMAN, 1985; Sheoran, *et al.* 2001).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 (Radostits, *et al.* 2007; Levett, 2001). The present study attempted to determine the prevalence of *L. interrogans* antibodies in sheep in Maku area in Iran.

Material and Methods

Blood samples were taken from 210 sheep (160 ewes, 50 dams) from herds of Maku, North-west of Iran, during November to Desember of 2013. On the basis of age these sheep were divided into 1-4 groups (1-2 years, 2-3 years, 3-4 years, 4-5 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. Ten millilitres of blood were collected from the jugular vein of each sheep. The blood samples were allowed to clot and centrifuged for10 min at 3000g. After centrifugation, the serum was transferred into 1.5 mL Eppendorf tubes, and stored at – 20^{o}C until it was ready to be used. Sera samples were tested for antibodies to 8 live serovars of *L. interrogans:* Canicola, Grippothyphosa, Hardjo,

Pomona, Icterohaemorrhagiae,Australis, Automenalis 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. The results were considered positive when 50% or more of agglutination of leptospires at dilution of 1:100 or greater were obtained (Park, *et al.* 1992; Piligrim and Threifall, 1999).

The results were analyzed by chi-square test to determine the difference between different groups of age of sheep which was significantly related to the prevalence of leptosprial antibodies.

Results

Thirty-two (15.23%) out of 210 tested sheep were positive for at least one leptospiral antigen. None of the samples was positive for two leptospiral antigens. Regarding the age, 2 sheep (6.25%) in the 1-2 years group, 14 sheep (43.75%) in the 2-3 years group, 8 sheep (25%) in the 3-4 years group, and 8 sheep (25%) in the 4-5 years group were positive. There was a significant relationship between aging and the incidence of leptospiral infection (table 1). The highest number of reactors in sheep (62%) was due to serovar Cacicola, followed in descending order by, Icterohaemorrhagiae(32%) however, Hardjo (6%), Ballum, Pomona, Grippothyphosaan, Automenalis, Australis were not detected among reactors (Table 2). As shown in Table 3, the presence of leptospiral antibodies at 81% and 19% was obtained at titer levels 100 and 200 for all the serovars, respectively.

Table 1: Age distribution in leptospiral seropositive sheep

Age group	tested	positive	Percent
1-2 years	25	2	6.25
2-3 years	70	14	43.75
3-4 years	66	8	25
4-5 years	49	8	25
Total	210	32	15.23

Table 2: Prevalence of different leptospiral serovars in sheep

	G	P	I	C	H	B	A	A	Total
Numbers	0	0	10	20	2	0	0	0	32*
Percent	0	0	32	62	6	0	0	0	100

G - Gryppothyphosa , P - Pomona, I - Icterohaemorrhagiae , C - Canicola, H - Hardjo, B - Ballum, A - Automenalis,A-Australis ***** Some samples were positive for two leptospiral antigens**.**

Table 3: Prevalence of leptospiral antibody titres to different antigens in sheep

Titre	100	200	400
Numbers	26	6	0
Percent	81	19	0

Discussion

In the present study, the seroprevalence survey was based on the MAT, the test usually used in serodiagnosis of leptospirosis. From this study, it was evident that leptospiral infection may exist in the sheep population in Maku. It must be acknowledged whether the infection or merely persistent antibodies in the absence of infection were evident exposure to the organism.

Out of 210 tested sheep, 15.23% were positive for leptospiral antibodies at titers 100 and 200. Leptospirosis occurs in sheep and goats with less frequency than in cattle. In Turkey, 44.77% of the cattle and 8% of sheep react to one or more serovar of *L. interrogans*. In Urmia area in Iran, 36% of cows and 19.3% sheep react to one serovar of *L.interrogans* (Ramin and Azizzadeh, 2013).

The rate of infection was 17.3% (Hajikolaei, *et al.* 2007; Tooloei, *et al.* 2008; Zakeri, *et al.* 2010) in Iran, 14.8% (Savalia and Mahendra, 2008) in India, 30.3% (Schonman and Swai, 2010) in Tanzania, 17.7% (Angela, *et al.* 1998) in Nigeria, 59.1% (Kingscote, 1985) in Canada, and 46.9% (Lilenbaum, *et al.* 2009) in Brazil. This information shows the widespread infection in Iran and the world, with the highest infection in cows and horses and the lowest in sheep (Haji Hajikolahi, *et al.* 2005; Hasanpour, *et al.* 2009; Talebkhan Garoussi, *et al.* 2003). Therefore, this should be taken into account in disease control programs.

In this study, there was a significant relationship between aging and the incidence of leptospiral infection and the incidence of leptospiral infection (Hassanpour, *et al.* 2011).

The highest number of reactors in sheep (62%) was due to serovar Canicola. It is probable that this serovar may be adapted to and maintained by the sheep in Maku area. The predominant leptospiral serovars in serological reaction varies somewhat among countries. For example, Pomona (30.5%) in Queensland, Pomona (12.47%) in California, Bratislava (16.2%, 16.6%, 53.3%, and 22.3%), respectively, in Ohio, England, Northern Ireland, and USA, Bratislava, Copehageni, and Pyogenes (21.3%) in the Republic of Ireland, and Pomona (48.7%) in India were the most common serovars in the horse (Egan and Yearsley, 1989; Park, *et al.* 1992; Roth and Gleckman, 1985; Sheoran, *et al.* 2001). In Ireland, serovar Bratislava was identified as a cause of about 25% of leptospiral abortions (Egan and Yearsley, 1989). In khoy area, a city in the Northwest of Iran, about 56.25% of the examined animal was infected by canicola as a predominant serovar in ewes (Hassanpour, *et al.* 2011). 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 server pathologic effect. However, persistent leptpspiruria and high seroprevalance rates of the infection in sheep where no contact with cattle has occurred suggest that sheep may be a maintenance host for some serovars. This could complicate the control of the infection in cattle and sheep and therfore 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).

In this study, no samples were positive for more than one serotype. In serological tests for leptospirosis such as MAT, the results often indicate infection with more than one serovar (Egan and Yearsley, 1989; Park, *et al.* 1992; Roth and Gleckman, 1985). 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.

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 (Levett, 2001). To overcome this problem, sensitive methods are needed to detect the organism in urine or the genital tract of chronic carriers (Levett, 2001; Hasanpour, *et al.* 2009). Therefore, the demonstration of leptospirae in the genital tract and or urine only must be interpreted with full consideration of the serological results and culture or detection of leptospirae in blood or body fluids, as these findings may indicate that the animals were carriers.

These results confirmed that leptospiral infection may exist in the sheep population in Maku area and the presence of antibodies in the absence of infection indicates exposure to the organism. In addition, these results confirm that the majority of leptospiral infections is asymptomatic.

References

Angela, P., Brandao, D., Camargo, D., Marcos, V. and Rui, V. (1998). Macroscopic agglutination test for rapid diagnosis of human leptospirosis. J. Clin. Microbiol. 36, 3138-42.

Barwick, R.S., Mohammed, H.O. and McDonough, P.L. (1998). Epidemiological features of equine Leptospira interrogans of human significance. Prev. Vet. Med. 36, 153-165.

Bernard, W.V., Bolin, C. and Riddle, T. (1993). Leptospiral abortion and leptospiruria in horses from the same farm. J. Am. Vet. Med. Assoc. 202, 1285-1286.

Bharti, A.R., Nally, J.E., Ricaldi, J.N., Matthias, M.A., Diaz, M.M., Lovett, J.M., Levett, P.N., Gilman, R.H. Willing, M.R., Gotuzzo, E. and Vinetz, J.M. (2003). Peru-United States Leptospirosis Consortium: Leptospirosis: a zoonotic disease of global importance. The Lancet Infectious Diseases, 3, 757-771.

De Nardi Júnior, G., Genovez, ME., Ribeiro, MG., Castro, V., Jorge, AM. (2010). An in vitro growth inhibition test for measuring the potency of Leptospira spp. Sejroe group vaccine in buffaloes. Biology, 38, 474-8.

Donahue, J.M., Smith, B.J. Redmon, K.J. and Donahue, J.K. (1991). Diagnosis and prevalence of leptospira infection in aborted and stillbirth horses. J. Vet. Diagn. Invest. 3, 148-151.

Egan, J. and D Yearsley, A. (1989). Serological survey of Leptospiral infection in horses in the Republic of Ireland. Vet. Rec. 119, 306.

Ellis, W.A., Bryson, D.G. and O'Brien, J.J. (1983). Leptospiral infection in horses in aborted equine foetuses. Equine. Vet. J. 14, 321-324.

Ellis, W.A. (1998). Leptospirosis. In: Zoonoses: biology, clinical practice and public health control. Eds., Palmer, S.R., Soulsby, L. and Simpson, D.I.H. New York: Oxford University Press, pp:115-126.

Faber, N.A., Crawford, M. and LeFebvre, R.B. (2000). Detection of Leptospira spp in the aqueous humor of horses with naturally acquired recurrent uveitis. J. Clin. Microbiol. 38, 2731-2733.

Haji Hajikolahi, M.R., Gorbanpour, M., Heidari, M. and Abdollahpor, G.R. (2005). Comparison of leptospiral infection in the horse and donkey, Bull. Vet. Inst. Pulawy. 49, 175-178.

Hajikolaei, M.R.H., Ghorbanpour, M., Gharibi, D. Abdollapour, G.R. (2007). Serological study on leptospiral infection in sheep in Ahvaz, Southwestern Iran. Iranian. J. Vet. Res. 8, 333-6.

Hasanpour, A., Monfared, N., Abdollahpour, G.R. and Satari, S. (2009). Seroprevalence of leptospiral infection in horses in Tabriz – Iran. J. Bacteriol. Res. 1(8), 97-100.

Hassanpour, A., Imandar, M., Abdollahpour. (2011). Seroprevalence of leptospiral infection in Ewes in Khoy-Iran. Adv. Environ. Biol. 5. 8. 2033-2038

Hasanpour, A. and Safarmashaei, S. (2012). Seroprevalence of leptospiral infection in horses, donkeys and mules in East Azerbaijan province. Afr. J. Microbiol. Res. 6(20): 4384-4387.

Hathaway, S.C., Little, T.W.A., Finch, S.M. and Stevens, A.E. (1981). Leptospiral infection in horses in England: A Serological study. Vet. Rec. 2, 396-398.

Hartskeeri, R.A., Goris, M.G., Brem, S., Meyer, P., Kopp, H. and Wollanke, B. (2004). Classification of Leptospira from the eye of horses suffering from recurrent uveitis. J. Vet. Med. B. 51, 110.

Hogg, G.G. (1974). The isolation of Leptospira Pomona from a sick foal. Aust. Vet. J. 50, 326.

Kingscote, B. (1985).Leptospirosis in Sheep in Western Canada. Canadian. Vet. J. 26, 164, 165-8.

Levett, P.N. (2001). Leptospirosis. Clin. Microbiol. Rev. 14, 296-326.

Lilenbaum, W., Varges, R., Ristow, P., Cortez, A., Souza, SO., Richtzenhain, LJ. et al, (2009). Identification of Leptospira spp. carriers among seroreactive goats and sheep by polymerase chain reaction. Res. Vet. Sci. 87, 16-9.

Nasr Esphehani, Z. (2004). Investigation of the leptospira antibody titer in cattle of Shahrkord, J. Vet. Med. Tehran. Univ. 58, 132-7.

Park, Y.G., Gordon, J.C. Bech-Nielsen, S. and Slemons, R.D. (1992). Factors for seropositivity to leptospirosis in horses. Prev. Vet. Med. 13, 121-127.

Piligrim, S. and Threifall, W.R.A. (1999). Serologic study of leptospirosis in mares. Equine. Pract. 21, 20.

Radostits, O.M., Gay, C.C. Hinchcliff, K.W. and Constable, P.D. (2007). Veterinary Medicine. 10 ed., Bailliere Tindall, London, pp: 1094-1123.

Rajeev, S., Berghaus, R.D., Overton, M.W., Pence, M.E., Baldwin, C.A. (2010). Comparison of FA and MAT for leptospira in pregnant and non-pregnant cows. J. Vet. Diag. Inv. 22, 51-4.

Ramin, A.G., Azizzadeh, F. (2013). Seroepidemiological detection of antibodies aginst leptospira spp using microscopic Aggutination Test in Urmia cows and sheep. Acta. Veterinaria (Beogard). 63,1, 53-61

Roth, R.M. and Gleckman, R.A. (1985). Human infections derived from dogs. Postgrad Med. 77, 169-180.

Savalia, C.V., Mahendra, P. (2008). Studies on the reservoir status of leptospirosis in Gujara. Indian. J. Field. Veterinarians. 4, 7-9

Schonman, L., Swai, E.S. (2010). Herd and animal level risk factors for bovine leptospirosis in Tanga region of Tanzania. Brazilian. J. Microbiol. 32, 298-300.

Seshagiri Rao, A., Krishna Rao, P. Ramakrishna, K. and Dhananjaya Reddy, B. (1985). Serological and clinical evidence of leptospiral infection in horses. Indian Vet. J. 62, 273-277.

Sheoran, A.S., Nally, J.E. Donahue, J.M. Smith, B.J. and Timoney, J.F. (2001). Antibody isotypes in sera of equine fetuses aborted due to Leptospira interrogans serovar pomona–Type kennewicki infection. Vet. Immunol. Immunopath. 77, 301-309.

Talebkhan Garoussi, M., Vandeussefi, J., Familghadakchi, H., Nowrouzian, I. (2003). A seroepidemiological survey of leptospiral infection in dairy cattle herds and their employees in Mashhad suburb of Iran. J. Vet. Med. Tehran. Univ. 58, 89-94.

Tooloei, M., Abdollapour, G., Karimi, H., Hasanpor, A. (2008). Prevalence of serum antibodies against six leptospira serovars in sheep of Tabriz Iran, J. Anim. Vet. Adv. 7, 4505.

Zakeri, S., Khorami, N., Ganji, Z.F. (2010). Leptospira wolffii, a potential new pathogenic leptospira species detected in human, sheep and dog, Inf. Gen. Evolul. 10, 273-277