Journal of Zoonotic Diseases
2022, 6 (1): 17-24
doi: 10.22034/jzd.2022.14483
https://jzd.tabrizu.ac.ir/article_14483.html

Original Article

Seroepidemiological analysis of leptospirosis in sheep in Meshginshahr city, Iran using microscopic agglutination test

Mostafa Jedi[1], Ali Hassanpour[2*], Sina Moghaddam[3]

1- Faculty of Veterinary Medicine, Tabriz Medical Science Branch, Islamic Azad University, Tabriz, Iran.
2- Department of Clinical Sciences, Faculty of Veterinary Medicine, Tabriz Medical Science Branch, Islamic Azad University, Tabriz, Iran.
3- Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
*__Corresponding author:__ *alihassanpour53@gmail.com*
(Received 13 March 2022, Accepted 16 April 2022)

Summary
Leptospirosis is a zoonotic disease that has spread worldwide. The primary origin of infection is rodents and wild animals that excrete *Leptospira* in their urine. This study was performed on 200 sheep in Meshginshahr to determine the serum prevalence of *Leptospira* infection. Serums were analyzed at a dilution of 1:50 and above on six *Leptospira interrogans* serovars, including Pomona, Conicola, Hardjo**,** Icterohemorrhagica, Australis, and Grippotyphosa and by using microscopic agglutination test (MAT). Among 200 samples, 22 samples were positive (11%), and 178 samples were negative (89%); There was a significant difference between positive and negative cases ($P < 0.05$). The most common strains were Icterohemorrhagica, and the lowest strains were Conicola and Pomona, and the Australis strain was not observed. There were five positive samples (13.51%) in the age group of 1.5 to 2 years old, ten positive samples (10.75%) in the age group of 2 to 4 years old, and seven positive samples (11.62%) in the age group of 4 to 6 years old. Also, a positive sample was not observed among the over six years old. One hundred fifty samples were related to ewe, and 50 samples were related to ram, that were positive 20 samples of ewe (13.33%) and two samples of ram (4%), there was a significant difference between different sexes in serum infection with *Leptospira*. The final result is that *Leptospira* infection is present in sheep in Meshginshahr city, and preventive measures should be taken to prevent its further spread.
Keywords: Sheep, Leptospirosis, Microscopic agglutination test, Iran.

Introduction

Leptospirosis is a complex global disease shared between livestock and humans that is caused by the pathogenic species *Leptospira*. It is also considered a disease with a significant economic impact on livestock (Trimble et al., 2018). *Leptospira* A variety of spirochetes belonging to the family *Leptospira*cea A are causative agents, and they are forced aerobic, gram-negative. Their optimum growth temperature is from 28 to 30 °C, and also the presence of a warm and humid environment is required (Adler et al., 2010). The disease has a wide geographical distribution due to the wide range of mammalian hosts that harbor and excrete the causative agent from their renal tubules (Ko et al., 2009). The prevalence of infection in the host population is usually around 30 to 50%, which is often spread through direct contact between livestock (Brown et al., 1999). Various pathogens in sheep and goats cause reproductive failure that

one of the most important causes of leptospirosis in herds that can cause infertility, miscarriage, and stillbirth in these animals (Martins et al. 2014). In rural areas, leptospirosis can be considered dangerous for farmers and ranchers in hot and rainy seasons. On the other hand, urban areas close to rural areas can cause leptospirosis in humans if hygiene is not observed. Although cases of leptospirosis infection in humans are asymptomatic or subclinical, and in developing countries, late diagnosis of the disease can cause death in humans (Lau et al., 2014; Thayaparan et al., 2013). The study performed on sheep in Khoy city showed that 28.5% of them had a positive serum titer against at least one of the *Leptospira* serotypes, and the highest amount was related to Conicola serotype, and the lowest was related to the Icterohemorrhagica serotype (Hassanpour et al., 2011).

However, leptospirosis in sheep can cause significant damage. Although the incidence of the disease is low in sheep, its mortality is on average 20% in sheep and more than 45% in offenses. *Leptospira* Pomona is the most common infectious agent and is the leading cause of clinical disease in sheep. Sheep in the wild do not host survival for Pomona and Hardjo**,** and infection is likely to be relatively short-lived but has severe effects. However, the presence of *Leptospira*'s urinary incontinence due to Hardjo disease has been reported in areas where sheep have not been in contact with cattle. This article suggests that sheep may be the survival hosts for this ranch. This complicates the control of Hardjo infection in cattle in areas that are free of this disease. In addition, infected sheep are a potential health hazard for slaughterhouse workers, sheepherders, and furriers who have not been seriously considered before. Hardjo infection is common in South Australian Merino rams and may infect up to 40% of herds (Quinn et al., 2002). The main source of infection is a sick animal that infects pasture, drinking water, and food through infected urine, aborted fetus and uterine secretions (Carter and Chenagappa, 1991; Quinn et al., 2002). In the subacute form of leptospirosis, the breast tissue of cows is often affected, and in cattle and goats, and vermicomposts due to different serotypes with varying intensities have been observed. However, if the animal is infected, with or without vermicompost, the causative agent enters the milk. However, contamination through milk seems to occur infrequently due to the acidity of the milk and the inhibitory factor present in the milk. However, if milk is consumed immediately after milking, the risk of infection increases, especially if the milk is obtained from goats or cows with mildly alkaline mammals. Contaminated milk that is spread on livestock in the soil can also be a source of infection (Arthure et al., 2001). *Leptospira* microorganisms enter the liver and multiply after the organism penetrates the skin, causing bacteremia, which begins about a week after the entry of the organism and from the peripheral blood until the fever subsides. The organism can be isolated. Also, in the early period of sepsis can cause lysis of red blood cells and intravascular hemolysis occurs, and bloody urine can be observed (Aghamohammadzadeh et al., 2015).

Stable leptospirosis caused by Hardjo in sheep, having no contact with cattle suggests that sheep may be host hosts for this serovar. This can complicate the control of Hardjo infection in cattle and in areas devoid of these serovars, and sheep infection is a zoonotic hazard for farmworkers, owners of sheep farms, and mowers who have not been there before (Constable et al., 2017).

This study was performed to investigate the serological infection of the disease in sheep in Meshginshahr city and determine the dominant Leptospira strains in this region.

Materials and methods

From May to September 2020, 200 samples from Meshginshahr city were collected in 4 geographical regions: north, south, west, and east. Of these, 150 samples were female sheep (75%), and 50 samples were male sheep (25%). The age groups of 1.5-2 years, 2-4 years, 4-6 years, and over six years have been determined. The number of samples taken from the northern region was 46

samples (34 females and 12 males), the southern region 47 samples (31 females and 16 males), the western region 52 samples (39 females and 13 males), and the eastern region was 55 samples (46 females and nine males). Blood samples were taken randomly from all male and female sheep to prepare serum samples. The blood sampling site was the dorsal vein, which was used to draw blood from an animal using a 9-tube tube, a needle, and an injector. About 5-9 cc of blood was collected from each sheep, from which 1-3 cc of serum was prepared in the laboratory. After taking the sample on the label of the tube, the specified number was inserted to make it easier to access the sample information in the later stages of the research. At the same time as sampling, information such as sex, age, and history of related diseases was recorded for each sheep. Ensuring the accuracy of the information was achieved by following the health records registered by the veterinarian and asking the sheep care officials. After blood sampling, the tubes containing blood were placed at room temperature for 1 to 2 hours to form a complete blood clot, then stored in the refrigerator's refrigerator until the next morning. The next morning, the tubes containing the clotted blood were removed from the refrigerator and serum was removed using a sterile pasteurized pipette, and the serums were transferred into a microtube (Fahimipour et al., 2021).

In the present study, the MAT test was performed according to the WHO recommendation with some modifications. Using a special sampler, 10 microliters of the desired antigen were removed and emptied in the center of each of the eight squares on a microscope slide. Using the same method, 10 µl of the serum sample was taken from a dilution of 1:50 and above and drained near the antigen droplet and mixed uniformly and carefully at the same time as the antigen so that the droplet did not deviate from the desired square. In order to avoid mixing serum samples, a separate micropipette was used to collect each sample. Each day of the test, to control the accuracy of the test, three types of controls were prepared as follows: positive control (positive standard serum), negative control (negative standard serum) and third control in which dilute solution was used instead of serum to control spontaneous agglutination. The slides were carefully placed in special containers with the desiccant paper described earlier, then incubated at 30 ° C for 90 minutes. The slides were then removed from the incubator and examined under a dark microscope at 100x magnification. If agglutination was observed in any of the samples, while noting the sample number in the record sheet, the amount of agglutination in each sample was graded from +1 to +4 as follows. According to the WHO standard, samples whose agglutination was +1 were considered negative. Only positive +4 samples were considered, and the rest were counted as suspicious (Maleki et al., 2021).

Statistical analysis

The results of the contamination rate were described descriptively. SPSS24 statistical software was used to compare the level of infection between groups. To compare the level of infection between males and females, the statistical method t-test, between different ages and evaluation of different geographical areas, ANOVA, and Kruskal_wallis method were used to evaluate the history of the disease and the presence of serovars.

Results

Among 200 samples, 22 samples were positive (11%), and 178 samples were negative (89%), which was evaluated by the Chi-square method and there was a significant difference ($P < 0.05$).

The rate of infection with different *Leptospira* serovars was in the order of Icterohaemorrhagiae (n = 12, 50%), Hardjo (n = 4, 16.66%), Grippotyphosa (n = 4, 16.66%), Canicola (n = 2, 8.33%) and Pomona (n = 2, 8.33%), which was the highest strain of Icterohaemorrhagiae and the lowest strains were Canicola and Pomona. Australis was not observed.

Among 200 samples, 150 samples were related to female sheep, and 50 samples were related to male sheep, among which 20 samples were female sheep (13.33%), and two samples were male sheep (4%). The results were analyzed by t-test; significant differences were observed between different sexes

in serum infection with *Leptospira* (P < 0.05) (Table 1).

Among 20 positive females, 10 cases (45.4%) were related to Icterohaemorrhagiae serovar, four cases (18.1%) were related to Hardjo serovar, four cases (18.1%) were related to Grippotyphosa serovar, two cases (9.09 %) was related to Canicola serovar and two cases (9.09%) were related to Pomona serovar. Among the two positive males, both cases were 100% related to serovar Icterohaemorrhagiae. Serova Australis was also not among the positive samples. The results were analyzed by the Kruskal-wallis test, in which the Icterohaemorrhagiae was more common in positive sheep and showed that the incidence of serum infection in this strain was significant compared to other strains (P < 0.05) (Table 2).

Table 1. Frequency and comparison of serum contamination with different strains of leptospirosis in sheep understudy.

Sex	Total samples	Positive cases	*P-value*
Male	50 (25%)	2 (4%)	0.012
Female	150 (75%)	20 (13.33%)	

Table 2. Comparison of the frequency of infection with different *Leptospira* serovars in the studied sheep.

Sex	Strain	Positive cases	*P-value*
Female	Icterohaemorrhagiae	10 (45.4%)	0.024
	Grippotyphosa	4 (18.1%)	
	Canicola	2 (9.09%)	
	Hardjo	4 (18.1%)	
	Pomona	2 (9.09%)	
Male	Icterohaemorrhagiae	2 (100%)	

In the age group of 1.5 to 2 years (37 samples), five positive samples (13.51%), in the age group 2 to 4 years (93 samples) that the highest number of positive samples with 10 (10.75%), and in the age group of 4 to 6 years (43 samples) were seven positive samples (16.27%). Also, no positive sample was observed among the over six years old (27 samples). There was no significant difference between age groups (P > 0.05) which indicates that age had no effect on serum contamination of different strains of *Leptospira* in sheep in Meshginshahr (Table 3).

Table 3. Frequency of sheep infected with different strains of *Leptospira* based on age group and comparison of infection rate among age groups.

Age group (year)	No. of samples		Positive cases		Total positive cases	*P-value*
	Male	Female	Male	Female		
< 2	20	17	0	5 (29.41%)	5 (13.51%)	0.0621
2-4	15	78	0	10 (12.82%)	10 (10.75%)	
4-6	35	8	2 (25%)	5 (14.28%)	7 (16.27%)	
> 6	20	7	0	0	0	

Out of 22 positive samples in the last three months, 13 cases (59.09%) had a history of abortion, and five cases (22.72%) had jaundice symptoms, of which 81.81% were positive samples, and four cases were asymptomatic. It was evaluated by the Kruskal-wallis test and was significant (P < 0.05).

Of 200 samples, a total of 55 samples were taken from the east, 52 samples from the west, 46 samples from the north, and 47 samples from the south; Of these, seven samples (12.72%) in the eastern region, three samples (5.76%) in the western region, 10 samples (21.7%) were in the northern region and two samples (4.25%) in the region south became positive. There was no significant difference between *Leptospira* infection between sheep in different areas of Meshginshahr ($P < 0.05$) (Table 4).

Table 4. Frequency of positive serum sheep based on different areas of Meshginshahr city.

Region	No. of samples	Positive cases	Negative cases	*P-value*
East region	55	7 (12.72%)	48 (87.27%)	0.0854
Western region	52	3 (5.76%)	49 (94.23%)	
Northern region	46	10 (21.7%)	36 (78.26%)	
Southern region	47	2 (4.25%)	45 (95.74%	

Discussion

Leptospirosis is a worldwide infectious and zoonotic disease. This disease is one of the most important infectious diseases in humans and animals, caused by spirochetes of the genus *Leptospira*. This disease causes a lot of damage to the economic and health system of the country, which is due to livestock death, abortion, and other reproductive disorders. On the one hand, the aspect of leptospirosis being zoonotic and transmitting the disease to humans on the other hand. The common leptospirosis between humans and animals doubles the importance of devising strategies to control infection and ensure public health. Humans become infected through skin scratches and mucosal surfaces following contact with water and soil contaminated with the urine of animals with leptospirosis (Karpagam et al., 2020; Haake and Levett, 2015).

Records in scientific centers of the country indicate the spread of infection in most parts of Iran. However, so far, no study has been done on sheep to determine the seroprevalence of leptospirosis in Meshginshahr city, so to determine the disease status in sheep population of Meshginshahr city sampling from four regions Geographical and serological analysis of samples for the presence of anti-*Leptospira* antibodies against six important *Leptospira* serotypes. During this study, which lasted from May to September 2020, 200 blood samples from herds in the region were prepared and serologically tested. In this study, the seroprevalence of leptospirosis was assessed based on the MAT test, which is the usual method for diagnosing serum leptospirosis. This study revealed that there might be *Leptospira* infection among sheep populations in Meshginshahr. To detect the presence of infection or antibodies alone and without symptoms, the organism itself must be identified.

Over the past years, several studies on livestock have been conducted in the provinces of East and West Azerbaijan, all of which indicate the presence of *Leptospira* infection in these two provinces. In a study performed on 359 sheep in Tabriz, 40 and 30% of them had positive serum titers against serotypes of Grippotyphosa and Canicola, respectively. Another study conducted on goats in Khoy city showed that 13.3% of goats had a positive titer against at least one *Leptospira* serotype, and the most positive cases were related to the Grippotyphosa serotype (Tooloei et al., 2008). In Chaharmahal and Bakhtiari and Isfahan provinces, a study was performed to estimate the presence of leptospirosis DNA in blood samples of cattle and sheep by PCR. One hundred ninety five blood samples were randomly collected (92 from cows and 103 from sheep) from a collection of clinically healthy animals. The extracted DNA was stored at -20 °C until the experiment. The results show that 18.63% of the samples were positive. Leptospirosis DNA

was found in 20 of 92 bovine samples (21.73%) and 16 of 103 sheep samples (15.53%). Nine cases (9.78%) out of 92 cases of cattle samples and 5 cases (4.85%) out of 103 cases of sheep samples were positive, infected with Hardjo serovar (Khamesipour et al., 2014). Another study compared the prevalence of *interrogans* leptospirosis antibodies between sheep and goats. Blood samples were taken from 246 sheep and 210 goats from 12 herds in eight geographical areas in Ahvaz, which were kept in one place. Serums were first diluted against eight strains of *Leptospira interrogans*; Pomona, Canicola, Hardjo, Balum, Icterohemorrhagica, Grippotyphosa, Tarasovi and Australis, using the MAT method. The prevalence of *Leptospira*l infection was 8.53% in sheep and 10.95% in goats. The highest rate of conflict between both (sheep and goats) with 68.18% in sheep and 56% in goats, was related to Pomona serovar (Haji Hajikolaei et al., 2016). Another study looked at the prevalence of leptospirosis in cattle, sheep, and goats in a small area of Terzina, Piau, and northeastern Brazil. Serum samples were analyzed in 336 sheep, 292 goats, and 253 cows using a microscopic agglutination test (MAT). Overall, 378 samples were positive for MAT (42.9%). The prevalence in cattle, sheep, and goats was 50.5%, 40.5% and 34.6%, respectively. All herds had at least one positive animal. Common serovars were most common in Hardjo in cattle and Icterohemorrhagica in goats and sheep (Campos et al., 2017). Of the 200 samples, 150 were for females and 50 for males; 20 samples from females and two samples from males were reported positive. Of note, 13.33% of females and 4% of males were *Leptospira* samples positive that there was a significant difference between serological infection of male and female sheep ($P < 0.05$). In this study, the most recorded serovars were Icterohemorrhagica and the least were Canicola and Pomona, and the Hardjo and Grippotyphosa strains were in the middle rows; Serovar Australis was not found among the positive samples. Specific serotypes of *Leptospira* are common in every geographical area. Some types of *Leptospira* are found in most countries, but some are limited to certain areas. In general, the distribution of serum varieties of the pathogen in livestock varies from country to country and even between different regions in a country and depends on environmental and host factors. A study on sheep in Khoy showed that 28.5% of them had a positive serum titer against at least one of the *Leptospira* serotypes, and the highest amount was related to the Canicola serotype and the lowest was related to Icterohemorrhagica serotype (Hassanpour et al., 2011).

In different areas of Meshginshahr city, the highest rate of *Leptospira* infection was observed in the northern region and the lowest in the southern region. No significant difference was observed between *Leptospira* infection sheep in different areas of Meshginshahr ($P < 0.05$).

Conclusion

The results of this study indicate that *Leptospira* infection may be present in the sheep population of Meshginshahr city and the presence of antibodies in the absence of infection necessitates the detection and identification of this pathogen. In addition, these results indicate that some *Leptospira* infections are latent and asymptomatic; Necessary measures should be taken to prevent and control this disease in different regions of the country and Meshginshahr city. Given rodents' proven role in maintaining and transmitting *Leptospira*emia, the most important step in controlling the disease is to fight rodents decisively. Due to the role of litter and its moisture in the survival of pathogenic mass and its prevalence, it is recommended that farmers carefully manage the litter and provide a dry and clean bed to reduce the survival of the mass in the environment and the prevalence of infection.

Acknowledgments

Not applicable.

Conflicts of Interest

The authors declare no conflicts of interest statement.

Ethical approval

No ethical approval was obtained because this study did not involve a prospective evaluation, did not involve laboratory animals, and only involved non-invasive procedures (e.g., serum samples).

References

Adler B. & de la Peña Moctezuma A. Leptospira and leptospirosis. *Veterinary Microbiology,* 2010, 140(3-4), 287-96.

Arthure G.H., Noakes D.E. Pearson H. and Parkinson T.J. Veterinary reproduction and obstetrics. 8th edition, Bailliere Tindall, London. 2001, 474, 484-487, 518-519, 772, 763, 874.

Aghamohammadzadeh M., Fartashvand M., Abdollahpour G., Hassanpour A. and Anzabi A. Relation between Specific Anti-Leptospira Antibodies in the Aqueous Humor of the Eye and Seroprevalence of Leptospirosis in Stallion in Veterinary Faculty of Islamic Azad University- Tabriz Branch. *Journal of Veterinary Microbiology,* 2015, 11, 135-43.

Brown C. Emerging diseases of animals. *Emerging Infections,* 1992, 3, 153-63.

Carter G.R. & Chengappa M.M. 1991, Essential of veterinary bacteriology and mycology. 4th edition, Philadelphia (Pa.): Lea & Febiger, 220-23.

Constable P.D., Hinchcliff K.W., Done S.H. and Grunberg W. 2017, Leptospirosis *In: Veterinary Medicine* (a textbook of the diseases of cattle, horses, sheep, pigs, and goats), 11th ed., Saunders Ltd.

Fahimipour A., Khaki P., and Moradi Bidhendi S. Seroepidemiological Analysis of Leptospiral infection using MAT in Stray Dogs in Alborz, Iran. *Archives of Razi Institute,*2021, 76(2), 391.

Haake D.A., & Levett, P.N. Leptospirosis in humans. *Leptospira and leptospirosis,* 2015, 387, 65-97.

Haji Hajikolaei M., Rezaei S., Ghadrdan Mashhadi A., Ghorbanpour M., and Abdollahpour G. Comparison of Leptospira interrogans infection in the goats and sheep. *Iranian Journal of Veterinary Medicine,* 2016, 10(2), 113-9.

Hassanpour A., Imandar M., Abdollahpour G., and Mahsayekhi M. Seroprevalence of leptospiral infection in ewes in Khoy-Iran. *Advances in Environmental Biology*, 2011, 5(8), 2033-9.

Karpagam K. B., and Ganesh B. Leptospirosis: a neglected tropical zoonotic infection of public health importance—an updated review. *European Journal of Clinical Microbiology & Infectious Diseases* 2020, 39(5), 835-46.

Khamesipour F., Doosti A., and Omrani E. Molecular study of the prevalence of Leptospira spp. Serovar Hardjo in blood samples of Iranian cattle and sheep. *The Thai Journal of Veterinary Medicine,* 2014, 44(1), 141.

Ko A.I., Goarant C., and Picardeau M. Leptospira: the dawn of the molecular genetics era for an emerging zoonotic pathogen. *Nature Reviews Microbiology,* 2009, 7(10), 736-47.

Lau C.L., Smythe L.D., Craig S.B., and Weinstein P. Climate change, flooding, urbanisation and leptospirosis: fuelling the fire? *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 2010, 104(10), 631-8.

Maleki S., Zakian, A. and Abdollahpour G. Seroepidemiology of Leptospira interrogans Infection in Ruminants of Lorestan Province: A Cross-Sectional Study. *Journal of Veterinary Research,* 2021, 75(4), 486-97.

Martins G., and Lilenbaum W. Leptospirosis in sheep and goats under tropical conditions. *Tropical Animal Health and Production,* 2014, 46(1), 11-7.

Quinn P.J., Markey B.K., Carter M.E., Donnelly W.J. and Leonard F.C. 2002, Veterinary microbiology and microbial disease. Blackwell publishing. 175-184, 453-455, 484.

Thayaparan S., Robertson I., Fairuz A., Suut L., and Abdullah, M. Leptospirosis, an emerging zoonotic disease in Malaysia. *Malaysian Journal of Pathology*, 2013, 35(2), 123-32.

Tooloei M., Abdollahpour G., Karimi H. and Hasanpor A. Prevalence of Serum Antibodies Against Six Leptospira Serovars in Sheep in Tabriz, Northwestern

Iran. *Journal of Animal and Veterinary Advances*. 2008, 7, 450-5.

Trimble A.C., Blevins C.A., Beard L.A., Deforno A.R., and Davis, E.G. Seroprevalence, frequency of leptospiuria, and associated risk factors in horses in Kansas, Missouri, and Nebraska from 2016-2017. *PLoS One*, 2018, 13(10), e0206639.