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Original Article

Serologic prevalence of leptospiral infection in the horses in Kurdistan Province in Iran

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Summary

Leptospirosis is a zoonotic disease with global distribution, the main source is rodents and wild animals that excrete leptospira in their urine. This study was conducted on 167 horses to determine the serologic prevalence of leptospiral infection in Kurdistan Province, Iran. Sera were examined at 1:100 dilution using six live serovars of *Leptospira interrogan*s, including Pomona, Canicola, Hardjo, Icterohaemorrhagiae, Australis, and Grippotyphosa, through microscopic agglutination test (MAT). Of 167 samples, there were 16 (9.6%) positive and 151 (90.4%) negative samples with significant differences ($P < 0.05$). Icterohaemorrhagiae was the most abundant strain, and Grippotyphosa and Australis had the least numbers. Hardjo and Pomona were not observed here. The grouping was based on population and determined by dental pattern. Age groups of < 2 years, 2-4 years, and 4-6 years presented one (2.94%), seven (10.60%), and eight (18.6%) positive samples. No positive samples were found in samples aged over six years, with no significant differences among the age groups. Of 91 mares and 76 stallions, 12 (13.18%) mares, and 4 (5.26%) stallions were positive. The sexes were not significantly different in terms of serologic leptospiral infection. Of 16 positive samples, Kurdish, Arab, and hybrid races comprised seven (43.8%), five (31.3%), and four (25%) of the horses, respectively. Significant differences were observed among the genera and races of the horses infected with different *Leptospira* serovars ($P < 0.05$). It can, therefore, be concluded that serologic infection with different *Leptospira* serovars is found in the horses studied in Kurdistan Province, which necessitates preventive measures against its further prevalence.
Keywords: Horse, Serologic prevalence, Leptospirosis, MAT (Microscopic Agglutination Test), Kurdistan

Introduction

Leptospirosis is a global zoonotic disease occurring in a wide range of animals and humans (Trimble et al. 2018). In the United States and India, the prevalence of leptospirosis in horses has been reported 20.6-33.6% and 13.5%, respectively (Roth and Gleckman, 1985; Park et al., 1992; Sheoran et al., 2000). In Iran, in Tabriz, Ardabil, and Ahvaz the prevalence of leptospirosis in horses has been reported, 41.05%, 7.77%, and 27.88%, respectively (Haji Hajikolaei et al., 2005; Hassanpour et al., 2009; Khousheh et al., 2012). Although there are many known leptospiral serovars, the infection always occurs by regionally native serotypes that are dependent on environmental and ecological factors (Khousheh et al., 2012; Trimble et al., 2018). In addition to mammals, the pathogenic microorganism has also been isolated from reptiles, birds, amphibians, and fish (Ellis et al., 1983; Constable, 2017), some of which acting as the disease reservoir, with rodents and wild animals being of the prime importance. Some domestic animals, such as dogs, cows, and pigs, are also involved in the spread of infection and act as an infection reservoir for human and livestock (Constable, 2017).

This zoonotic disease can be transmitted to humans who contact livestock (Faber et al., 2000). Leptospirosis is generally important for two reasons, *viz*. threats to public health and disease-related damage to livestock (Hassanpour et al., 2009). Leptospiral infections happen due to direct or indirect exposure of human or livestock to water, soil, of food contaminated with infected urine of multiple carrier wild or domestic animals. The disease is transmitted through direct contact with urine or tissues of infected animals. Leptospiral pathogenic microorganisms penetrate the body through wounds or scratches on the skin, mucosal surfaces, conjunctival tissue, inhalation of droplets, and aerosols containing leptospira microorganisms (Aghamohammadzadeh et al., 2015).

The risk of this disease in humans is determined by the incidence of rice-field fever or Weil's syndrome associated with jaundice, renal failure, bleeding, and myocarditis with arrhythmia. Meningitis and meningoencephalitis form is associated with neurological symptoms, and the pulmonary hemorrhage form is related to the respiratory failure (Imandar et al., 2011).

Leptospira microorganisms immediately enter the bloodstream and start to proliferate, thereby triggering the leptospiremia stage lasting 7-10 days after the disease onset. In this stage of the disease, leptospira microorganisms can be easily isolated from the blood. When the antibodies can be measured after ten days of the disease onset, leptospira microorganisms nested in renal tubules are excreted through leptospiruria. If infected individuals are not cured in this stage, it may persist for a long time (few days to several months), during which leptospira microorganisms can be isolated from urine or kidney tissue (dead animals) (Aghamohammadzadeh et al., 2015).

In horses, the disease causes such problems as abortion, recurrent uveitis, acute respiratory distress, and pyelonephritis (Constable, 2017; Malalana et al., 2017) , which can be lethal if not treated. Multiple risk factors influence the disease, including a shared stall of mares and stallions, contaminated delivery site, carnivore presence on horse farms, and the usage of well water (Bengtsson, 2018). In addition, favorable weather conditions, soil pH, rainfall and moisture levels, stagnant water or bogs, increased populations of rodents (rats and rabbits) and bat, and domestic and wild (Dogs, foxes, wolves, etc.) carnivores in the environment can increase the disease prevalence (Trimble et al., 2018). Leptospira bacterium can penetrate the body through tiny skin scratches or injured skin and via healthy mucus (lips, mouth, and eyes) (Aghamohammadzadeh et al., 2015). Swimming in stagnant waters contaminated with the urine of infected animals, working at rice fields where wild animals and rodents live, and exposure to infected animals amplify the possibility of human infection (Jahed-Dashliboron et al., 2013).

Although leptospirosis is a relatively uncommon infection in horses, recent studies indicate various and widespread serovars of the infection throughout different geographical regions (Aghamohammadzadeh et al., 2015). Most infections remain with no symptoms and diagnosis, and clinical indications in horses appear to be similar to those of other species, but equine leptospirosis is detected as a cause of acute dyspnea (Verma et al. 2013). A leptospiral feature specific to equines is recurrent uveitis (periodic ophthalmia) and moon blindness, which apparently consists of autoimmune mechanisms including the ocular tissue response and membrane proteins of *Leptospira* (Verma et al., 2013; Constable, 2017). There is no permissible vaccine for use in horses, and vaccine development cannot be predicted in the future (Correia et al., 2017). Accordingly, leptospirosis prevention is contingent on environmental hygiene, physical exercise, minimizing rodent contact, and vaccination of other animal species in contact with horses(Hassanpour et al., 2009).

The present study was conducted to investigate serologic disease infection and to determine dominant *Leptospira* species in horses of Kurdistan Province.

Materials and methods

A total of 167 horse samples was collected from five cities in Kurdistan Province from February 2018 to June 2019, consisting of 91 (54.49%) mares and 76 (45.50%) stallions. According to the horse population in the area, the age grouping of the horses was done, and it was determined by the dental pattern. To prepare serum samples, blood samples were taken randomly from jugular veins of all mares and stallions using a 10 cc special syringe. Of 7-10 cc blood taken per horse, 1-3 cc was used to prepare serum in the laboratory. After collecting of blood samples, determining numbers were written on the tube labels to facilitate access to the sample data in the next stages. The determined age groups were < 2 years (n = 34, 20.4%), 2-4 years (n = 66, 39.5%), 4-6 years (n = 43, 25.7%), and > 6 years (n = 24, 14.4%). Samples were collected from cities Sanandaj (42 mares, 27 stallions), Bijar (24 mares, 18 stallions), Ghorveh (13 mares, 13 stallions), Kamyaran (5 mares, 11 stallions), and Marivan (7 mares, 7 stallions), with total numbers of 69, 42, 26, 16, and 14 for both sexes in each city, respectively. Three Kurdish, Arab, and hybrid races were also investigated each comprising 118 (70.65%), 14 (8.38%), and 35 (20.95%) horses, respectively.

At the same time with sampling horses, such data as gender, age, race, and history of related diseases were recorded for individual horses. Accuracy of data was ensured through follow up of recorded health information by a veterinarian and inquiries from responsible horse fancier.

After serum isolation, samples were kept frozen and sent to the leptospirosis laboratory of the Veterinary Hospital, Faculty of Veterinary Medicine, University of Tehran, for examinations. The infection with different leptospira serovars was examined by MAT to determine average contamination.

Statistical analysis

Results of infection level were expressed descriptively and were compared between groups using SPSS 24 software. Infection level was compared between male and female horses by t-test and between age groups, races, and cities with analysis of variance (ANOVA). Racial differences, disease history, and the presence of various serovars were assessed through the Kruskal-Wallis test.

Results

According to chi-square test, there were 16 (9.6%) positive and 151 (90.4%) negative samples (of 167 samples) with significant differences ($P < 0.05$).

Among positive samples, 15 cases (93.75%) and one case (6.25%) were infected with one and two serotypes, respectively.

The frequency of infection with different *Leptospira* serovars was in order of Icterohaemorrhagiae (n = 9, 56.3%), Canicola (n = 5, 31.3%), Grippotyphosa (n = 1, 6.3%), and Australis (n = 1, 6.3%). Icterohaemorrhagiae was the most abundant strain, Grippotyphosa and Australis were of the least abundance, and Hardjo and Pomona were not observed here.

Of 91 mares and 76 stallions from a total of 167 samples, 12 (13.18%) mares and four (5.26%) of stallions were positive, with no significant differences between the sexes in terms of serum leptospiral infection according to t-test results (Table 1).

Of 12 positive mares, the Grippotyphosa, Icterohaemorrhagiae, Canicola, and Australis serovars comprised 8.33%, 33.33%, 50%, and 8.33%, respectively. All four cases of positive cases (100%) belonged to Icterohaemorrhagiae. Pomona and Hardjo were absent among positive samples. According to the Kruskal_Wallis test, Icterohaemorrhagiae was more prevalent among positive horses, and serologic infection with this strain was significant in comparison to the other strains ($P < 0.05$) (Table 2).

Age groups of < 2 years, 2-4 years, and 4-6 years presented one (2.94%), seven (10.60%), and eight (18.6%) positive samples. No positive samples were found in samples aged over six years. There were no significant differences among the age groups indicating that age had no effects on levels of serologic infection with different leptospirosis strains in the studied horses of Kurdistan Province (Table 3).

Horses of the Kurdish race had the highest abundance (118 out of 167) among the samples. Horses of the Arab race were 14 cases. A total of 35 outbred and hybrid horses were assessed for leptospirosis, and of 16 positive samples, there were seven (43.8%) Kurdish, five (31.3%) Arab, and four (25%) hybrid races. There were all-mare, one stallion and four mares, and three stallions and one mare among leptospira-positive Kurdish, Arab, and hybrid races, respectively. Kruskal_Wallis test revealed significant differences among the genera and races of horses infected with different leptospira serotypes ($P < 0.05$) (Table 4).

History of urogenital diseases, abortion, or ocular infections were observed in 12 out of 16 positive samples, which accounted for 75% of positive specimens, as confirmed by the Kruskal-Wallis test ($P < 0.05$).

A total of 167 samples were collected from Sanandaj (69), Bijar (42), Ghorveh (26), Kamyaran (16), and Marivan (14), of which positive samples were recorded in Sanandaj (n = 9, 13.04%), Bijar, Ghorveh, and Kamyaran each with two samples (4.76%, 7.69%, and 12.5%, respectively), and in Marivan with one sample (7.14%). Leptospiral infection was not significantly different among different cities of the province (P > 0.05) (Table 5).

Table 1. Frequency and comparison of serologic infection with different leptospirosis strains in the experimental horses based on sex.

Sex	Total samples	Positive cases	*P-value*
Stallion	76 (45.5%)	4 (5.26%)	0.072
Mare	91 (54.5%)	12 (13.18%)	

Table 2. Comparison of serologic infection with different leptospirosis strains in the experimental horses.

Sex	Strain	Positive cases	*P-value*
Stallion	Icterohaemorrhagiae	4 (33.33%)	0.048*
	Canicola	6 (50%)	
Mare	Australis	1 (8.33%)	
	Grippotyphosa	1 (8.33%)	
	Icterohaemorrhagiae	4 (100%)	

*Significant at P < 0.05

Table 3. Frequency of horses infected with different leptospirosis strains based on age groups and infection levels among them.

Age group (year)	No. of samples		Positive cases		Total positive cases	P-value
	Stallion	Mare	Stallion	Mare		
< 2	22	12	0	1 (8.33%)	1 (2.94%)	0.084
2-4	27	39	2 (7.40%)	5 (12.82%)	7 (10.60%)	
4-6	16	27	2 (12.5%)	6 (22.22%)	8 (18.6%)	
> 6	11	13	0	0	0	

Discussion

Although the literature available at Iranian scientific centers suggest the spread of infection in most regions of the country, no research has so far been conducted on the serologic prevalence of leptospirosis in horses of Kurdistan Province. To determine the disease status in the equine population of the region, samples were collected from horse-riding clubs in this region to examine the presence of leptospira antibodies serologically against six important leptospira serotypes.

Table 4. Frequency of serum-positive horses based on races.

Race	Total No.	Sex	No.	Total infection level	Infection level by sex	P-value
Kurdish	118	Stallion	51	7 (5.93%)	0	0·008*
		Mare	67		7 (5.93%)	
Arab	14	Stallion	7	5 (35.72%)	1 (7.14%)	
		Mare	7		4 (28.57%)	
Hybrid	35	Stallion	18	4 (11.37%)	3 (8.57%)	
		Mare	17		1 (2.8%)	

*Significant at P < 0.05

Table 5. Frequency of serum positive horses based on different cities of Kurdistan province

City	Total	Positive	Negative	P-value
Sanandaj	69	9 (13.04%)	60 (86.95%)	0.833
Bijar	42	2 (4.76%)	40 (95.23%)	
Ghorveh	26	2 (7.69%)	24 (92.30%)	
Kamyaran	16	2 (12.5%)	14 (87.5%)	
Marivan	14	1 (7.14%)	13 (92.85%)	

A total of 167 blood samples was prepared from horse farms in the above regions and undergone the serologic tests for serologic prevalence of leptospirosis based on MAT test as a commonly used method for serologic diagnosis of leptospirosis. According to our observations, the leptospiral infection may be present in the equine population of Kurdistan Province. The microorganism itself has to be identified to detect the presence of either infection or asymptomatic antibody alone.

The presence of 16 (9.6%) and 151 (90.4%) positive and negative samples, respectively, out of 167 samples can be attributed to the presence of moisture and rats in some local stables and contact of some horses with such animals as goats, sheep, and cows as reservoirs of leptospira (Pilgrim and Threlfall, 1999).

In serologic-based examinations at different regions, leptospiral infections of 20.6-33.6% and 13.5% were reported in the USA and India, respectively (Roth and Gleckman, 1985; Park et al., 1992; Sheoran et al., 2000). A study in Ahvaz reported leptospiral infections of

27.88% in horses and of 40% in donkeys (Haji Hajikolaei et al., 2005). Serologic leptospiral infection level was found to be 41.05% in horses of Tabriz, and 42.68% of stallions and 30.77% of mares were positive serologically with significant differences between the two sexes, but the ages and races of horses were not significantly correlated with leptospiral infection levels (Hassanpour et al., 2009). The study also reported that the highest infection (46.15%) was caused by Pomona serovar, followed by Grippotyphosa (41.03%), Icterohaemorrhagiae (17.95%), Canicola (12.82%), and Hardjo (2.56%). In research in Ardabil, a leptospiral infection prevalence of 7.77% was noticed in horses of the region (Khousheh et al., 2012).

The dominant leptospira serovars inducing serologic responses are different in various countries. For example, Pomona with 30.5% and 12.47% in Queensland and California, respectively, Australis with 16.2, 16.6, 53.3, and 22.3% in Ohio, England, Northern Ireland, and the USA, respectively, Bratislava, Copenhagen, and Pyogenes with 21.3% in the Republic of Ireland, and Pomona with 48.7% in India were reported as the most prevalent serovars among horses (Verma et al., 1977; Egan and Yearsley, 1986; Park et al., 1992; Pilgrim and Threlfall, 1999). Grippotyphosa serovar reportedly accounted for 33.33% and 49.51% of serum positive cases among horses and donkeys studied in Ahvaz region (Haji Hajikolaei et al., 2005). Bratislava-related abortion was found to account for about 25% of abortions caused by *Leptospira* (Egan and Yearsley, 1986).

Studies have been conducted in various countries concerning *Leptospira* serovars through such methods as MAT and PCR. In Brazil, for instance, a study on the prevalence of *Leptospira* serovars in horses indicated an antibody presence of 32.7% in general infection, with Serjoe being the most abundant serovar (15.9%). Research has shown relationships between serum response with age, sex, activity (exercise, rest, or both), nutrition, complement use, waste depot, and the presence of felines in the environment (Peixoto Ribeiro et al., 2018). In England, 30 horses with equine recurrent uveitis (ERU) and 43 controls were analyzed to determine recurrent EUR inducing serovars. In the ERU group, only two cases were diagnosed with a c-value > 4 (a *Leptospira* induced uveitis event, 6.7%), along with the detection of serovars Hardjo and Javanica. There was no serologic difference between uveitis prevalence (65.5%) and control group (41.9%; P = 0.11). No significant difference was observed between *Leptospira*'s positive group (100%) and *Leptospira*-free uveitis (P = 0.52); (Malalana et al., 2017).

In a post-flood study, 276 thoroughbred horses (aged 2-5 years) were examined in Rio de Janeiro, Brazil. The animals abided in flood for 72 hours, after which blood samples were collected for serologic tests. Assuming the flood incidence as day zero, the horses were sampled on days 20 and 35. On Day 20, 132 (47%) horses were seroactive with 200 titers, and 23 (31%) animals had increased antibody titers on Day 35. In addition, 34 urine samples for culture and PCR analysis were collected from serum positive horses on Day 35. The Copenhagen serovar had the highest incidence (88.8%). Even so, none of the urine samples were positive in the culture, and 12 (35.2%) samples were positive in PCR analysis. This seems to be the first report of leptospirosis incidence in urban horses (Hamond et al., 2012).

Some reports suggest the presence of leptospirosis-induced hemolytic anemia in horses. In a blood profile analysis, serum biochemistry and mineral factors were analyzed on days zero, 7, and 21. Significant reductions were observed in RBC, MCHC, Hb, PCV, platelets, RDW, and TLC (total leucocyte count, including neutrophils, eosinophils, basophils, lymphocytes, and monocytes). MCV also increased significantly indicating the incidence of hemolytic anemia in the samples (Sohail, 2017). Biochemical assays revealed significant rises of ALT, AST, ALP, BUN, and creatinine anemia in the samples, as evidence of liver and kidney damages. Significant decreases were recorded in serum concentrations of sodium, potassium, calcium, magnesium, phosphorus, copper, and zinc, indicating renal tubular injury (Sohail, 2017).

A general 5-week survey confirmed the presence of five leptoserovars in horses of a northern island in New Zealand. Three different groups of mares (non-pregnant, pregnant, or foal-bearing) were investigated in each farm(Bengtsson, 2018).

For the first time in Belgium, the presence of equine *Leptospira* was confirmed and compared using MAT and real-time PCR methods in 2018. Of 66 eyes, *Leptospira* strains were prevalent in 20 (30.33%) samples in the ERU group. DNA was positive in both 11 aqueous humors and 17 vitreous bodies of 8 *Leptospira* positive horses. Vitreous bodies and aqueous humors were separately positive in 9 and 3 horses, respectively. A correlation of 0.47 was obtained between aqueous humors and vitreous bodies in the PCR diagnosis of *Leptospira*, which was interpreted as a low association. All the eyes were negative in the control group (Sauvage et al., 2019).

Of 91 mares and 76 stallions from a total of 167 samples herein, 12 (13.18%) mares and four (5.26%) stallions were *Leptospira* positive, with no significant differences between serologic infections of mares and stallions (P > 0.05). Age groups of < 2, 2-4, and 4-6 years presented one (2.94%), seven (10.60%), and eight (18.6%) positive samples. No positive samples were found in horses aged over six years, with no significant differences among the age groups.

The Kurdish race was the most abundant among the sampled horses due to its high abundance in the region. The Arab and hybrid horses were also samples in limited numbers. Among *Leptospira*'s positive horses, there were all-mare, one stallion and four mares, and three stallions and one mare among leptospira-positive Kurdish, Arab, and hybrid races, respectively. Significant differences were recorded among the genera and races of horses infected with different leptoserovars (P < 0.05).

In this study, different sexes were not significantly different in terms of serologic infection with *Leptospira*. In a study in Tabriz, on the other hand, significant differences were observed in serologic infection between stallions (42.68%) and mares (30.77%) (Hassanpour et al. 2009).

In the current investigation, Icterohaemorrhagiae and Australis were the most and least abundant strains, respectively, and Canicola and Grippotyphosa were of intermediate abundance. Hardjo and Pomona were not observed among positive samples. The dominant leptospira serovars inducing serologic responses are different in various countries. For instance, Pomona with 30.5% and 12.47% in Queensland and California, respectively, Australis with 16.2, 16.6, 53.3, and 22.3% in Ohio, England, Northern Ireland, and the USA, respectively, Bratislava, Copenhagen, and Pyogenes with 21.3% in the Republic of Ireland, and Pomona with 48.7% in India were reported as the most prevalent serovars among horses (Ellis et al., 1983; Roth and Gleckman, 1985; Park et al., 1992; Sheoran et al., 2000). Grippotyphosa serovar reportedly accounted for 33.33% and 49.51% of positive serum cases among horses and donkeys studied in Ahvaz region(Haji Hajikolaei et al., 2005). Hassanpour et al. (2009) reported that Pomona serovar was present in 38.96% of positive serum cases among horses, donkeys, and mules in East Azerbaijan Province (Hassanpour et al., 2009). The same authors reported that 46.15% of positive serum cases were related to Pomona serovar among horses in the Tabriz region. In Ardabil, Hardjo serovar comprised 43% of serum positive cases among horses (Hassanpour et al., 2012). Bratislava-caused abortion was detected to be responsible for about 25% of abortions induced by *Leptospira* (Ellis et al., 1983).

The highest and lowest *Leptospira* infection levels were recorded in Sanandaj and Bijar, respectively, with no significant differences among the sampled cities of Kurdistan Province ($P > 0.05$).

Among positive samples, 15 (93.75%) and one (6.25%) cases were infected with one and two serotypes, respectively. Hassanpour et al. (2009) noticed that eight cases of experimental samples (20.51% of positive serum cases) were positive for more than one serotype (Hassanpour et al., 2009). In serologic tests of leptospirosis such as MAT, the results usually determine infection about more than one serovar (Ellis et al., 1983; Roth and Gleckman, 1985; Park et al., 1992; Hartskeerl et al., 2004). This may result from simultaneous infection with several serovars, but the presence of cross- reactivity between serovars can deviate the report of the MAT test.

History of urogenital diseases, abortion, or ocular infections were observed in 12 out of 16 positive samples, which accounted for 75% of positive specimens, with significant differences ($P < 0.05$).

Conclusion

Our findings demonstrate that leptospiral infection may be present in the equine population of Kurdistan Province, and the presence of antibodies in the absence of infection necessitates the detection of this pathogen. The results also indicate that some leptospiral infections are hidden without clinical symptoms; hence preventive measures are necessary to control the disease in different regions of western Iran and Kurdistan Province.

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).

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Conflicts of Interest

The authors declare no conflicts of interest statement.

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