

Seroprevalence of *Leptospira* Infection in Horses in Ardabil-Iran

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Abstract: This study was conducted on 90 horses in Ardabil area to determine seroprevalence of leptospira infection. Sera were initially screened at dilution of 1:100 against 7 live serovars of *Leptospira interrogans*: Pomona, Canicola, Hardjo, Ballom, Icterohaemorrhagiae, Australis and Grippotyphosa using the microscopic agglutination test. The prevalence of leptospiral infection was 7.77% in horses. 71.43% of male horses and 28.57% of female horses were positive. There was significant difference between males and females prevalence ($P < 0.05$). The highest number of reactors in horses (43%) was due to serovar Hardjo, followed in descending order by Icterohaemorrhagiae (29%), Pomona (14%) and Grippotyphosa (14%). Titer levels between 100 and 200 were positive for *Leptospira*. 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.

Key words: Horse • Seroprevalence • *Leptospira* • Iran • Ardabil

INTRODUCTION

Leptospirosis is a widely spread zoonosis of global concern [1, 2]. It is caused by spirochetes belonging to the genus *Leptospira*. All the pathogenic leptospirae were formerly classified as members of the species *Leptospira interrogans*; the genus has recently been reorganized and pathogenic leptospirae are now identified in several species of *Leptospira*. Leptospirosis is a significant occupational hazard in the cattle and pig industries in certain areas. Uveitis is the most frequently encountered clinical manifestation of leptospirosis in horses; however, abortion and stillbirth are serious problems [3- 8]. Renal dysfunction in a stallion and neonatal mortality has also been reported [9, 10]. Non-specific disease characterized by fever, jaundice, anorexia, and lethargy may also occur. Leptospirosis can be readily transmitted between species, including animals and humans through infected urine, contaminated soil or water, or other body fluids [11]. 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 [9-11]. 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 [12].

Diagnosis of leptospirosis can be difficult and may involve antigen detection (PCR), serological evaluation, histological examination, culture, and/or dark field microscopy [13]. 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 [14]. A number of serological studies have indicated a wide-spread evidence of leptospiral infection in horses in several countries, but there is only one study dealing with the infection in donkeys [15-17]. This study attempted to determine the prevalence of *L. interrogans* antibodies in horses in Ardabil area in Iran.

MATERIALS AND METHODS

Blood samples were taken from 90 horses (68 males and 22 females) at 5 race clubs of Ardabil, North-west of Iran, during the period December 2011 to May 2012. On the basis of age these horses were divided in 4 groups (1-3 years, 3-6 years, 6-9 years and over 9 years). None of these animals had been vaccinated against *Leptospira* and there was no history of leptospirosis-related symptoms or signs of the disease at the time of sampling. Ten ml of blood were collected from the jugular vein of each horse. 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 7 live serovars of *L. interrogans*: Canicola, Grippothyphosa, Hardjo, Pomona, Icterohaemorrhagiae, Ballum and Australis 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 micro titration plates were incubated at 29°C for 2 hours. The plates were examined under dark field microscopy.

The results were considered positive when 50% or more of leptospirae at dilution of 1:100 or greater were agglutinated [18, 19].

The results were analyzed by chi-square test to determine the significance of difference between two sexes and different groups of age of horses related to the prevalence of leptospiral antibodies.

RESULTS

Seven (7.77%) out of 90 horses were positive for at least one leptospiral antigen. Some samples were positive for two leptospiral antigens. 5 male (71.43 %) and 2 (28.57%) female horses were positive in MAT test. There was significant difference between male and female seroprevalence (P<0.05) (Table 1). On the base of age, 1 horse (14.28%) in the 1-3 years group, 3 horses (42.85%) in the 3-6 years group, 2 horses (28.57%) in the 6-9 years group and 1 horse (14.28%) in the over 9 years group were positive for *Leptospira*. There was no significant relationship between aging and the incidence of leptospiral infection (Table 2). The highest number of reactors in horses (43%) was due to serovar Hardjo, followed in descending order by Icterohaemorrhagiae (29%), Grippothyphosa (14%), and Pomona (14%) (Table 3). As shown in table 4, titer levels between 100 and 200 were positive for *Leptospira* (71.43 and 28.57%, respectively).

Table 1: Sex distribution in leptospiral seropositive horses

Sex	Tested	Positive	Percent
Male	68	5	71.43
Female	22	2	28.57
Total	90	7	7.77

Table 2: Age distribution in leptospiral seropositive horses

Age group	Tested	Positive	Percent
1-3 years	15	1	14.28
3-6 years	42	3	42.85
6-9 years	23	2	28.57
Over 9 years	10	1	14.28
Total	90	7	7.77

Table 3: Prevalence of different leptospiral serovars in the tested horses

Serovar	H	I	P	G	C	B	A	Total
Numbers	3	2	1	1	0	0	0	7
Percent	43	29	14	14	0	0	0	100

G - Grippothyphosa , P - Pomona, I - Icterohaemorrhagiae , C - Canicola, H - Hardjo, B – Ballum, A - Australis

* Some samples were positive for two leptospiral antigens

Table 4: Distribution of leptospiral antibody titers to different antigens in horses

Titer	100	200	400
Numbers	5	2	0
Percent	71.43	28.57	0

DISCUSSION

This seroprevalence study was based on the MAT, the test is usually used in serodiagnosis of leptospirosis [20]. In this work, 7.77% of 90 horses were tested positive for *Leptospira*. This is because some stables in this area were moist and some horses were in contact with other animals, such as sheep, goat, and cattle being the reservoir of leptospirae [20, 21]. The prevalence of leptospiral infection based on serological testing has been reported to be 20.6-33.6% in USA, 13.5% in India horse population [22] and 27.88% in horses in Ahvaz area in Iran [23].

In seropositive horses, there was significant difference between males and females prevalence ($p < 0.05$), which is in agreement with the report by Park *et al.* [18] concerning horses in Ohio. This may not be true for horses in general, since the number of animals used for this study was too small. The highest number of reactors in horses (43%) was due to serovar Hardjo. The predominant *Leptospira* serovars giving rise serological reaction vary somewhat between 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, Copenhageni, and Pyogenes (21.3%) in the Republic of Ireland, and Pomona (48.7%) in India were the most common serovars in the horse [16, 18- 20]. Haji Hajikolahi *et al.* [23] reported that serovar grippothyphosa is present in 33.33% of positive horses in Ahavaz area in Iran [23]. In Ireland serovar Bratislava is identified as causing about 25% of leptospiral abortions [16].

Titer levels between 100 and 200 were positive for *Leptospira* (71.43 and 28.57% of positive horses, respectively). Haji Hajikolahi *et al.* [23] in Ahvaz – Iran reported that the titer levels in 23.81, 47.62, 19.04 and 9.52% of positive horses were 100, 200, 400 and 800, respectively [23]. Most researchers found that positive *Leptospira* cases were with titers between 100 and 200 and this agrees with the titers found in our study [19].

In serological tests for leptospirosis such as MAT, the results often indicate infection with more than one serovar [21]. 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.

Laboratory procedures are 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 [14]. 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 are carriers.

These results confirmed that leptospiral infection may exist in the horse population in Ardabil area and the presence of antibodies in the absence of infection indicates exposure to the organism and must be acknowledged. In addition, these results confirmed that the majority of leptospiral infections are asymptomatic.

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