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Seroepidemiologic Study of Horses Leptospirosis in Khorramabad, west Iran

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Abstract

Leptospirosis in horses has been considered a relatively uncommon infection. However, recent data suggest that the infection is widespread, with the incidence and infecting serovars varying considerably in different geographical regions. This study was conducted on 105 horses in Khorramabad region in the West of Iran to determine seroprevalence of leptospira infection. Sera were initially screened at dilution of 1:100 against 7 live serovars of Leptospira interrogans: Pomona, Grippotyphosa, Icterohaemorrhagiae, Canicola, Hardjo, Ballum, and Australis using the microscopic agglutination test. The prevalence of leptospiral infection was 7.62% in horses. 12.5% of male horses and 87.5% of female horses were positive. There was significant difference between males and females prevalence ($P<0.05$). There was no significant relationship between aging ($P=0.067$) and the incidence of leptospiral infection and between breed ($P=0.097$) of the horses. The highest number of reactors in horses (45%) was due to serovar Canicola, followed in descending order by Grippothyphosa (33%) and Icterohaemorrhagiae (22%). 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: Seroprevalence, Leptospira, Horse, Iran, Khorramabad.

Introduction

Leptospirosis is a widely spread zoonosis of global concern (Bharti et al. 2003 ; Levett, 2001). 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 significant occupational hazard in the cattle and pig industries in certain areas. Clinical signs in equine leptospirosis resemble those seen in other animal species. The majority of infections remain asymptomatic. However, leptospirosis as a cause of acute respiratory distress is becoming more frequently recognized. Uveitis is the most frequently encountered clinical manifestation of leptospirosis in horses, which appears to be mediated by autoimmune mechanisms involving cross reactivity between ocular tissues and leptospiral membrane proteins; however, abortion and stillbirth are serious problems (Bernard et al. 1993 ; Ellis et al. 1983 ; Faber et al. 2000 ; Hartskeeri et al. 2004 ; Matthews et al. 1987 ; Sheoran et al. 2001). Renal dysfunction in a stallion and neonatal mortality has also been reported (Divers et al. 1992 ; Hogg, 1974). Nonspecific 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 (Barwick et al. 1998 ; Levett, 2001). 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 (Barwick et al. 1998 ; Hogg, 1974). 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 (Bernard et al. 1993).

Diagnosis of leptospirosis can be difficult and may involve antigen detection (PCR), serological evaluation, histological examination, culture, and/or dark field microscopy (Ellis, 1998 ; Roth and Gleckman, 1985). 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 (O.I.E., 2000). 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 (Donahue et al. 1991 ; Hataway et al. 1981 ; Park et al. 1992 ; Piligrim and Threifall, 1999). This study attempted to determine the prevalence of *leptospira interrogans* antibodies in horses in Khorramabad region in Iran. This is the first report of *leptospiral infection* in these animals in the region.

Material and Methods

Blood samples were taken from 105 horses (50 males and 55 females) (60 Arabian, 5 Thoroughbred, 15 Kurd and 25 Crossbreed horses) from 5 horse growing centers and one race clubs of Khorramabad, West of Iran, during the period August 2013 to April of 2014. On the basis of age, these horses were divided in 5 groups (<1, 1 - 4, 4 - 7, 7 - 10 and over 10 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 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 7 live serovars of *leptospira interrogans*: Pomona, Grippothyphosa, Icterohaemorrhagiae, Canicola, Hardjo, 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 microtitration 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 (Park et al. 1992 ; Radostits et al. 2007). The results were analysed by chi-square and Fisher's exact test to determine the difference between two sexes and different groups of age and breeds of horses was significantly related to the prevalence of leptosprial antibodies.

Results

8 (7.62%) from 105 horses that tested were positive for at least one leptospiral antigen. Some samples were positive for two leptospiral antigens. 1 male (12.5%) horses and 7 female (87.5%) horses were positive in MAT test. There was significant difference between male and female seroprevalence ($P<0.05$) (Table 1). 3 Arabian (37.5%), 1 Kurd (12.5%) and 4 Crossbreed (50%) horses were positive and no positive case was observed in Thoroughbred and there was no significant difference between them ($P=0.097$) (Table 2). On the base of age, 1 horses (12.5%) in the 1-4 years group, 2 horses (25%) in the 4 – 7 years group, 2 horses (25%) in the 7 - 10 years group and 3 horses (37.5%) in the over 10 years group were positive for leptospira. no positive case was observed in less than one year. There was no significant relationship between aging and the incidence of *leptospiral infection* ($P=0.067$) (Table 3). The highest number of reactors in horses (45%) was due to serovar Canicola, followed in descending order by Grippothyphosa (33%) and Icterohaemorrhagiae (22%) (Table 4). As shown in Table 5, titer levels between 100 and 200 were positive for Leptospira (77.78% and 22.22% respectively). Out of the horses that were seropositive for leptopirosis, one sample (12.5%) was positive for more than one serotype.

Table 1: Sex distribution in leptospiral seropositive horses.

Sex	Tested	Positive	Percent (%)
male	50	1	12.5
female	55	7	87.5
Total	105	8	7.62

Table 2: Breed distribution in leptospiral seropositive horses.

Breed	Tested	Positive	Percent (%)
Arabian	60	3	37.5
Thoroughbred	5	0	0
Kurd	15	1	12.5
Crossbreed	25	4	50
Total	105	8	7.62

Table 3: Age distribution in leptospiral seropositive horses.

Age groups (year)	Tested	Positive	Percent (%)
<1	15	0	0
1-4	25	1	12.5
4-7	23	2	25
7-10	22	2	25
>10	20	3	37.5
Total	105	8	7.62

Table 4: Prevalence of different leptospiral serovars in horses.

Serovar	Numbers	Percent (%)
Pomona	0	0
Grippotyphosa	3	33
Icterohaemorrhagiae	2	22
Canicola	4	45
Hardjo	0	0
Ballum	0	0
Australis	0	0
Total	9	100

Table 5: Prevalence of leptospiral antibody titers to different antigens in horses.

Titer Numbers		Percent (%)
100	**7**	**77.78**
200	**2**	**22.22**
Total	**9**	**100**

Discussion

No previous observation study of the seroprevalence of leptospiral study in horses in Khorramabad region has been attempted. This seroprevalence survey was based on the MAT, the test is usually used in serodiagnosis of leptospirosis. In this work, 7.62% from 105 horses were tested positive for leptospira. This is because some stables in this region were moist and some horses were in contact with other animals, such as sheep, goat, cattle and dog being the reservoir of leptospirae (Radostits et al. 2007). 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 (Park et al. 1992 ; Piligrim and Threifall, 1999 ; Seshagiri et al. 1985 ; Verma et al. 1977). In Iran, The prevalence of leptospiral infection were 41.05% in horses in Tabriz, 27.88% in horses in ahwaz and 7.77% in horses in Ardabil (Hajikolaei et al. 2005 ; Hassanpour et al. 2009 ; Khousheh et al. 2012). From this study, it is evident that *leptospiral infection* may exist in the horse population in Khorramabad. Whether the presence of the infection or merely persistent antibodies in the absence of infection, exposure to the organism must be acknowledged. In seropositive horses, there was significant difference between males and females prevalence ($p<0.05$), which is in agreement with the reports by Park et al. in horses in Ohio (Park et al. 1992). This may not be true for horses in general, since the number of animals used for this study were too small. In this study there was no significant relationship between aging and the incidence of *leptospiral infection* and between breed of the horses. The highest number of reactors in horses (45%) was due to serovar Canicola. Since, serovar Canicola is considered as widely extended serovar among dogs and these animals are the main host for this serovar, it is possible the cattle will play important role in maintenance of serovar Canicola in nature. So, it can be state that the presence of dog and cattle in the adjacent of horses is an important factor for increasing serovar Canicola among horses in Khorramabad region. The predominant leptospira serovars giving rise of serological reaction vary somewhat between countries and even in different regions of a country. 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 ; Piligrim and Threifall, 1999 ; Seshagiri et al. 1985 ; Verma et al. 1977). In Ireland serovar Bratislava is identified as causing about 25% of leptospiral abortions (Egan and Yearsley, 1989). Haji Hajikolahi et al. reported that serovar Grippothyphosa is present in 33.33% of positive horses in Ahavaz area in Iran (Hajikolaei et al. 2005). Hassanpour et al. reported that serovar Bratislava is present in 41.05% of positive horses in Tabriz region in Iran (Hassanpour et al. 2009). Khousheh et al. reported that serovar Hardjo is present in 7.77% of positive horses in Ardabil region in Iran (Khousheh et al. 2012). Titer levels between 100 and 200 were positive for leptospira (77.78% and 22.22% of positive horses, respectively). Haji Hajikolahi et al. 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 (Hajikolaei et al. 2005). Most researchers found that positive leptospira cases were with titers between 100 and 200 and this agrees with the titers found in our study (Piligrim and Threifall, 1999). In this study one sample (12.5%) was 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 ; Hataway et al. 1981 ; Piligrim and Threifall, 1999 ; Seshagiri et al. 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. 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 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 (O.I.E., 2000). 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 Khorramabad region 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. There are no leptospiral vaccines licensed for use in horses, with no prospect for any becoming available in the foreseeable future. Accordingly, prevention of equine leptospirosis must rely on good hygiene practices, minimisation of rodent contact, and vaccination of other species of production and companion animals.

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