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Serological Study of Leptospirosis in Horses in Gonbad, Iran

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Abstract: This study was conducted on 200 horses in Gonbad area in Iran in order to determine seroprevalence of leptospiral infection. Sera were initially screened at dilution of 1:100 against 7 live serovars of *Leptospira interrogans*: Pomona, Canicola, Hardjo, Ballom, Icterohaemorrhagiae, Automenalis and Grippotyphosa using the microscopic agglutination test. The prevalence of leptospiral infection (At titers 100 and 200) was 12% in horses. 11.12% of male horses and 12.73% of female horses were positive. There was significant difference between males and females prevalence ($P<0.05$). There was significant relationship between aging and the incidence of leptospiral infection ($P<0.05$) and there was no significant relationship ($P>0.05$) between breed of the horses and the incidence of leptospiral infection. The highest number of reactors in horses (58.34%) was due to serovar Canicola, followed in descending order by Grippothyphosa (41.67%) and Pomona (8.34%). The majority of titer levels were between 100 and 200 for all the serovars. These results confirmed that the majority of leptospiral infections are asymptomatic and the presence of antibodies in the absence of infection indicates exposure of these animals to the organism.

Key words: Horse · Seroprevalence · *Leptospira* · Iran

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 and neonatal mortality have also been reported [7].

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 [2, 9]. 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 [10].

Diagnosis of leptospirosis can be difficult and involve antigen detection (PCR), serological evaluation, histological examination, culture and/or dark field microscopy [10]. 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 [11, 12]. 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 [13-19]. This study attempted to determine the prevalence of *L. interrogans* antibodies in horses in Gonbad area in Iran.

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MATERIALS AND METHODS

Blood samples were taken from 200 apparently healthy horses (90 males and 110 females) (116 Cross breed, 20 Thoroughbred and 64 Turkeman) in 5 race clubs of Gonbad, North-east of Iran during yhe period from August to November 2012. On the bases of age; these horses were divided into 1-4 groups (1-3, 3-6, 6-9 and over 9 years, respectively). None of these animals was vaccinated against Leptospira and there was no history of leptospirosis-related symptoms or signs of the disease at the time of sampling. Ten milliliters of blood were collected from the jugular vein of each horse. The blood samples were allowed to clot and centrifuged for 10 min at 3000g. After centrifugation, the serum was removed and stored at – 20°C until used. Serum samples were tested for antibodies to 7 live serovars of *L. interrogans*: Canicola, Grippothyphosa, Hardjo, Pomona, Icterohaemorragiae, 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 agglutinated leptospirae at dilution of 1:100 or greater were obtained [16, 20].

The results were analyzed by chi-square 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

Twenty four (12%) out of 200 tested horses were positive for at least one leptospiral antigen. Some samples were positive for two leptospiral antigens. Ten male (11.11%) and 14 (12.73%) female horses were positive in MAT test. There was significant difference between males and females prevalence ($P<0.05$) (Table 1). Twenty cross breed (17.24%), 0 thoroughbred (0%) and 4 Turkeman (6.25%) horses were positive for leptospiral infection and there was no significant difference ($P>0.05$) between them (Table 2). On the base of age, 4 horses (4.87%) in the 1-3 years group, 18 horses (20%) in the 3-6 years group, 2 horses (20%) in the 6-9 years group and 0 horses (0%) in the over 9 years group were positive. There was significant ($P<0.05$) relationship between aging and the incidence of leptospiral infection (Table 3). The highest number of reactors in horses (58.34%) was due to serovar Canicola, followed in descending order by Grippothyphosa (41.67%) and Pomona (8.34%).

Table 1: Sex distribution in leptospiral seropositive horses

Sex	Tested	Positive	Percent
Male	90	10	11.11
Female	110	14	12.73
Total	200	24	12

Table 2: Breed distribution in leptospiral seropositive horses

Breed	Tested	Positive	Percent
Cross breed	116	20	17.24
Thoroughbred	20	0	0
Turkeman	64	4	6.25

Table 3: Age distribution in leptospiral seropositive horses

Age group	Tested	Positive	Percent
1-3 years	82	4	4.87
3-6 years	90	18	20
6-9 years	10	2	20
Over 9 years	18	0	0
Total	200	24	12

Table 4: Prevalence of different leptospiral serovars in horses

	G	P	I	C	H	B	A	Total
Number	10	2	0	14	0	0	0	26*
Percent	41.66	8.33	0	58.33	0	0	0	100

G - Gryppothyphosa, P - Pomona, I - Icterohaemorrhagiae , C - Canicola, H - Hardjo, B - Ballum, A - Automenalis

* Two samples were positive for two leptospiral antigens

Table 5: Prevalence of leptospiral antibodies in horses to different antigens

Titer	100	200	400
Number	18	6	0
Percent	9	3	0

Serovars Icterohaemorrhagiae, Hardjo and Automenalis were not detected among reactors (Table 4). As shown in Table 5, the presence of leptospiral antibodies at 9 and 3% was obtained at titer levels 100 and 200 for all the serovars, respectively. Out of the horses that were seropositive for leptopirosis, 2 samples (8.34%) were positive for more than one serotype.

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 horse population in Gonbad. Whether the infection or merely persistent antibodies in the absence of infection were evident exposure to the organism must be acknowledged.

12% of the examined 200 horses were positive for leptospiral antibodies at titers 100 and 200. This is because the 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]. Higher prevalence of leptospiral infection in horses based on serological testing has been reported by several investigators; 20.6-33.6% in USA[16].13.5% in India [19]. 27.88% in Ahvaz area in Iran [21], 39.23% in East Azarbiajan in Iran [15] and 41.05% in horses in Tabriz area in Iran [13].

In seropositive horses, there was significant difference between males and females ($p<0.05$), which is in agreement with the report by Park *et al*. [16] in horses in Ohio. This may not be true for horses in general, since the number of animals used for this study was too small. In this study there was significant relationship between aging and the incidence of leptospiral infection and there was no significant relationship between breed of the horses and the incidence of leptospiral infection.

The highest number of reactors in horses (58.34%) was due to serovar Canicola. The predominant leptospiral serovars giving rise to serological reaction varies 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, Copehageni and Pyogenes (21.3%) in the Republic of Ireland and Pomona (48.7%) in India were the most common serovars in horse [14, 16-19]. Haji Hajikolahi *et al*. [21] reported that serovar grippothyphosa is present in 33.33% of positive horses in Ahavaz area in Iran. In Ireland serovar Bratislava was identified as a cause of about 25% of leptospiral abortions [14].

In this work, the titer levels in 9 and 3% of positive horses respectively were 100 and 200 for all the serovars. Haji Hajikolahi *et al*. [21] 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.

In this study 2 samples (8.33%) 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 [14, 15, 17, 18]. 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 titers may fall to undetectable levels while animals remain chronically infected[12].To overcome this problem, sensitive methods are needed to detect the organism in urine or the genital tract of chronic carriers [12, 22]. 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 horse population in Gonbad 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 are asymptomatic.

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