

Full Length Research Paper

Seroprevalence of leptospiral infection in horses in Tabriz - Iran

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This study was conducted on 95 horses in Tabriz area in Iran in order to seroprevalence of leptospiral infection. Sera were initially screened at dilution of 1:100 against 6 live serovars of *Leptospira interrogans*: Pomona, Canicola, Hardjo, Ballom, Icterohaemorrhagiae and Grippothyphosa using the microscopic agglutination test. The prevalence of leptospiral infection was 41.05% in horses. 42.68% of male horses and 30.77% of female horses were positive. There was significant difference between males and females ($P < 0.05$). 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 (46.15%) was due to serovar Pomona, followed in descending order by Grippothyphosa (41.03%), Icterohaemorrhagiae (17.95%), Canicola (12.82%) and Hardjo (2.56%). The majority of titre levels were between 100 and 200 for all the serovars. These results confirm that the majority of leptospiral infections is 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.

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 leptospires were formerly classified as members of the species *Leptospira interrogans*; the genus has recently been reorganised and pathogenic leptospires 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 (Bernard, 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 Non-specific disease characterized by fever, jaundice, anorexia and lethargy may also occur. Leptospirosis has also been reported (Divers et al., 1992; Hogg, 1974) to be readily transmitted between species, including between 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 (Ellis, 1998; Roth and Gleckman, 1985). Headache, myalgia, nausea, and vomiting are common complaints; however, neurologic, respiratory, cardiac, ocular, and gastrointestinal manifestations can occur (Ellis, 1998; Roth and Gleckman, 1985). In rare instances, leptospirosis can be fatal. 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 (Ellis, 1998).

Diagnosis of leptospirosis can be difficult and may involve antigen detection (PCR), serological evaluation, histological examination, culture, and/or dark field

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microscopy (Ellis, 1998). 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 (Manual of standards of diagnostic tests and vaccines, 2000). 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 (Donahue et al., 1991; Egan and Yearsley, 1989; Hathaway et al., 1981; Park et al., 1992; Pilgrim and Threifall, 1999; Seshagiri et al., 1985; Verma et al., 1977). The study attempted to determine the prevalence of *L. interrogans* antibodies in horses in Tabriz area in Iran. This is the first report of leptospiral infection in these animals in the area.

MATERIAL AND METHODS

Blood samples were taken from 95 horses (82 males and 13 females) (55 Arabian, 7 Thoroughbred, 14 Kurd, 17 Crossbred and 2 Pony horses) from 5 race clubs of Tabriz, North-west of Iran, during May to September of 2008. On the bases of age these horses were divided in 4 groups (1 - 3, 3 - 6, 6 - 9 and over 9 years). None of these animals had been vaccinated against leptospires and there was no history of leptospirosis-related symptoms or signs of the disease at the time of sampling. Ten millilitres 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 6 live serovars of *L. interrogans*: Canicola, Grippothyphosa, Hardjo, Pomona, Icterohaemorrhagiae 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. 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 darkfield microscopy. The results were considered positive when 50% or more of agglutination of leptospires at dilution of 1:100 or greater were found (Park et al., 1992; Radostits et al., 2007).

The results were analysed 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 leptospiral antibodies.

RESULTS

39 (41.05%) from 95 horses that tested were positive for at least one leptospiral antigen. Some samples were positive for two leptospiral antigens. 35 male (42.68%) horses and 4 (30.77%) female horses were positive in MAT test. There was significant difference between males and females ($P < 0.05$) (Table 1). 21 Arabian (38.18%), 2 Thoroughbred (28.57%), 7 Kurd (50%) and 9 Crossbred (52.94%) horses were positive and there was no significant difference between them (Table 2). On the

base of age, 19 horses (26.31%) in the 1-3 years group, 11 horses (40.74%) in the 3 - 6 years group, 17 horses (45.94%) in the 6 - 9 years group and 6 horses (50%) in the over 9 years group were positive. There was no significant relationship between aging and the incidence of leptospiral infection (Table 3). The highest number of reactors in horses (46.15%) was due to serovar Pomona, followed in descending order by Grippothyphosa (41.03%), Icterohaemorrhagiae (17.95%), Canicola (12.82%) and Hardjo (2.56%) (Table 4). As shown in Table 5, the majority of titre levels were between 100 and 200 for all the serovars (57.45 and 40.42% respectively). Out of the horses that were seropositive for leptospirosis, 8 samples (20.51%) were positive for more than one serotype.

DISCUSSION

No previous observation study of the seroprevalence of leptospiral study in horses in Tabriz area has been attempted. The seroprevalence survey was based on the MAT, the test usually used in serodiagnosis of leptospirosis. From this study, it is evident that leptospiral infection may exist in the horse population in Tabriz. Whether the presence of the infection or merely persistent antibodies in the absence of infection, exposure to the organism must be acknowledged. 41.05% from 95 horses that tested were positive. 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 leptospires (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; Pilgrim and Threifall, 1999; Seshagiri et al., 1985; Verma et al., 1977). The prevalence of leptospiral infection was 27.88% in horses and 40% in donkeys in Ahvaz area in Iran (Haji Hajikolahi et al., 2005).

In seropositive horses, there was significant difference between males and females ($p < 0.05$), which was in agreement with the reports by Park et al. (1992) in horses in Ohio. 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 (46.15%) was due to serovar Pomona. The predominant leptospira serovars giving rise of 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, Copenhageni, 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:

Table 1. Sex distribution in leptospiral seropositive horses.

Sex	Tested	Positive	Percent
Male	82	35	42.68
Female	13	4	30.77
Total	95	39	41.05

Table 3. Age distribution in leptospiral seropositive horses.

Age group (years)	Tested	Positive	Percent
1-3	19	5	26.31
3-6	27	11	40.74
6-9	37	17	45.94
Over 9	12	6	50
Total	95	39	41.05

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

Titre	Numbers	Percent
100	27	57.45
200	19	40.42
400	1	2.13

1989; Park et al., 1992; Pilgrim and Threifall, 1999; Seshagiri et al., 1985; Verma et al., 1977). Haji Hajikolahi and et al. reported that serovar grippothyphosa was present in 33.33% of positive horses and in 49.51% of positive donkeys in Ahavaz area in Iran (Haji Hajikolahi et al., 2005). In Ireland serovar Bratislava was identified as a cause of about 25% of leptospiral abortions (Egan and Yearsley, 1989). This study making it the most prevalent of all serovars for which we tested and it is probable that this serovar may be adapted to and maintained by the horse in Tabriz.

The majority of titre levels were between 100 and 200 for all the serovars (57.45% and 40.42% of positive horses, respectively). 2.13% of positive horses had 400 titre (Haji Hajikolahi et al.) In Ahvaz - Iran reported that the titre levels in 23.81% of positive horses were 100, 47.62% of positive horses were 200, 19.04% of positive horses were 400 and 9.52% of positive horses were 800 (Haji Hajikolahi et al., 2005). Most researchers found that titres ranged between 100 and 200 and this agreed with the titres found in our study (Pilgrim and Threifall, 1999). In this study 8 samples (20.51%) 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 (Egan and Yearsley, 1989; Hathaway et al., 1981; Pilgrim 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

Table 2. Breed distribution in leptospiral seropositive horses.

Breed	Tested	Positive	Percent
Arabian	55	21	38.18
Thoroughbred	7	2	28.57
Kurd	14	7	50
Crossbreed	17	9	52.94
Pony	2	0	0
Total	95	39	41.05

Table 4. Prevalence of different leptospiral serovars in horses and donkeys.

	G	P	I	C	H	B	Total
Numbers	16	18	7	5	1	0	47*
Percent	41.03	46.15	17.95	12.82	2.56	0	100

G - Gryppothyphosa, P - Pomona, I - Icterohaemorrhagiae, C -Canicola, H - Hardjo, B - Ballum * Some samples were positive for two leptospiral antigens.

excluded from this interpretation.

Laboratory procedures 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 (Manual of standards of diagnostic tests and vaccines, 2000). Therefore, the demonstration of leptospires in the genital tract and or urine only must be interpreted with full consideration of the serological results and culture or detection of leptospires in blood or body fluids, as these findings may indicate that the animals were carriers.

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

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