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Seroprevalance of leptospirosis in dairy cows in Mianeh-Iran

[1]H. Vakili, [*2]A. Hassanpour and [3]M. Khakpour

[1]Department of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[3]Department of Pathobilogy, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

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ABSTRACT

This study was conducted on 210 dairy cows in Mianeh 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 7.14% in dairy cows. There was significant relationship between aging and the incidence of leptospiral infection (P<0.05) and There was no significant relationship between breed of the dairy cows and the incidence of leptospiral infection. The highest number of reactors in dairy cows (40%) were due to serovar Pomona and serovar Icterohaemorrhagiae, followed in descending order by Grippothyphosa (20%). All off the sera were seronegatives for Hardjo, Canicola, Ballum and Autominalise. 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: Dairy cow, 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 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 [3-8]. Renal dysfunction in a stallion 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 between 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 dairy cows in Mianeh area in Iran.

MATERIALS AND METHODS

Blood samples were taken from 210 dairy cows from herds of Mianeh, North-west of Iran, during April to September of 2012. On the bases of age these dairy cows were divided into 1-5 groups (1-2 years, 2-3 years, 3-4 years, 4-5 years, 5-6 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 dairy cow. The blood samples were allowed to clot and centrifuged for 10 min at 3000g. After centrifugation, the serum was removed and stored at – 20^{o}C until ready for used. serum samples were tested for antibodies to 7 live serovars of *L. interrogans:* Canicola, Grippothyphosa, Hardjo, Pomona, Icterohaemorrhagiae, 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 agglutination of leptospires at dilution of 1:100 or greater were obtained [16, 20].

The results were analysed by chi-square test to determine the difference between different groups of age of dairy cows was significantly related to the prevalence of leptosprial antibodies.

RESULTS

Fiffteen (7.14%) out of 210 dairy cows that tested were positive for at least one leptospiral antigen. None of samples weren′t positive for two leptospiral antigens. On the base of age, 2 dairy cows (11.90%) in the 1-2 years group, 5 dairy cows (35.71%) in the 2-3 years group, 4 dairy cows (28.57%) in the 3-4 years group, 3 dairy cows (19.04%) in the 4-5 years group, 1 dairy cows (4.76%) in the 5-6 years group were positive. There was significant relationship between aging and the incidence of leptospiral infection (table 1). The highest number of reactors in dairy cows (40%) were due to serovar Pomona and serovar Icterohhaemorrhagiae, followed in descending order by Grippothyphosa (20%), Ballum, Hardjo, canicola and Automenalis were not detected among reactors (Table 2). As shown in Table 3, the presence of leptospiral antibodies at 60% and 40% was obtained at titer levels 100 and 200 for all the serovars, respectively.

Table 1: Age distribution in leptospiral seropositive dairy cows

Age group	tested	positive	percent
1-2 years	25	2	11.90
2-3 years	75	5	35.71
3-4 years	60	4	28.57
4-5 years	40	3	19.04
5-6 years	10	1	4.76
Total	210	15	7.14

Table 2: Prevalence of different leptospiral serovars in dairy cows

	G	**P**	**I**	**C**	**H**	**B**	**A**	**Total**
Numbers	3	6	6	0	0	0	0	15*
Percent	20	40	40	0	0	0	0	100

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

Table 3: Prevalence of leptospiral antibody titres to different antigens in dairy cows

Titre	**100**	**200**	**400**
Numbers	9	6	0
Percent	60	40	0

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

7.14% from 210 dairy cows that tested were positive for leptospiral antibodies at titers 100 and 200. This is because the some stables in this area were moist and some dairy cows 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 to be 20.6-33.6% in USA [16]. 13.5% in India[19]. The prevalence of leptospiral infection was 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 this study there was significant relationship between aging and the incidence of leptospiral infection and the incidence of leptospiral infection.

The highest number of reactors in dairy cows (40%) were due to serovar Pomona and serovar Icterohhaemorrhagiae. The predominant leptospiral 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, Copehageni, and Pyogenes (21.3%) in the Republic of Ireland, and Pomona (48.7%) in India were the most common serovars in the horse [14, 16-19]. Haji Hajikolahi and et al. [21] reported that serovar grippothyphosa was present in 33.33% of positive horses and Ahavaz area in Iran. In Ireland serovar Bratislava was identified as acouse of about 25% of leptospiral abortions [14]. 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 dairy cows in Mianeh area.

In this work, the titer levels in 60% and 40% of positive horses respectively were 100 and 200 for all the serovars. Haji Hajikolahi and et al. [21] In Ahvaz – Iran reported that the titre levels in 23.81%, 47.62% and 9.52% of positive horses were 100, 200, 400 and 800, respectively.

In this study any samples were not 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 titres 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 dairy cow population in Mianeh 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 is asymptomatic.

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