Serodiagnosis of leptospirosis in Cattle in Khorramabad, west Iran

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ABSTRACT. Leptospirosis is an acute infectious, systemic, septisemic and zoonotic disease which had resent outbreaks in some parts of Iran. Cattle populations may be infected with serovars Grippotyphosa, Pomona and Hardjo. Infection with Icterohaemorrhagiae, Canicola, Bratislava, Hebdomadis, Autumnalis, Australis, Sejroe and Bataviae serovars also occurs. For this study, a total of 200 serum samples were randomly collected from five districts in Khorramabad province in November 2013 to May of 2014. None of these animals had been vaccinated against leptospirosis and there was no history of leptospirosis-related symptoms or signs of the disease at the time of sampling. On the bases of age these cattles were divided in five groups. All serum samples were serologically tested by the standard MAT using live antigens representing the following leptospira interrogans serovars: Grippotyphosa, Pomona, Hardjo, Icterohaemorrhagiae, Canicola and Australis. The lowest dilution that each serum was considered positive was 1:100. The results of this study showed that 40 (20%) animals had a positive reaction against one or more serovars. The most prevalent leptospira serovars were Grippotyphosa representing 18(45%) samples and Canicola 16(40%) samples, and the least prevalent leptospira serovars were Hardjo representing 4 (10%) sample and Australis 2(5%) samples. 90% of female cows and 10% of male cows were positive. Also the most seropositive cases were observed in 3 to 4-year-old cows. There was significant difference between in two kind of sex and aging prevalence (P<0.01). The most prevalent reciprocal titer was 100 and the highest titer was 400. According to our history taken from each farms, the main risk factors were the presence of rodents and low hygienic conditions in farms. Also The results of this study may support that cows may have a role in maintaining Canicola serovar. As mostany of the visited herds had at least one unvaccinated dog for guarding the herds, it has been is concluded that the high prevalence of Canicola serogroup can be associated with close contact between dogs and cows. The results of this study revealed that cow could have a major role in maintaining Grippotyphosa, Canicola and Hardjo serovars in cows, indeed they are a potential zoonotic risk to slaughter house workers, meat inspectors, milkers and farmers.

INTRODUCTION

Leptospirosis affects most mammals throughout the world and is a common zoonotic disease and known as a global public health problem because of its increased mortality and morbidity in different countries. It causes financial loss to the cattle industry from decreased milk production, abortion, stillbirth, infertility and mortality (1, 14). The aspects of bovine leptospirosis are still inadequately defined, particularly in the cattle population of west of Iran, where published reports indicate widespread serological evidence of infection. Diagnosis of leptospirosis is based on laboratory confirmation because its clinical signs are nonspecific and may be mistaken with other febrile diseases (15). The carrier cows secrete leptospires in their urine without clinical signs of disease because of the tendency of bacteria to accumulate in kidneys. Therefore, they have an important role in the epidemiology of disease (3, 14). The earliest recognised report of leptospirosis in Iran is published by Rafyi and Magami (1968). Since then the most prevalent leptospiro serovars reported in Iran includes: Grippotyphosa, Canicola, Hardjo, Pomona and Icterohaemorrhagia (1). More recent published data in Iran indicated that serovar Canicola is widely
prevalent in the cattle population in different provinces (2, 5, 7). As West of Iran, especially Khorramabad province, has a humid temperate climate with plenty of annual rainfall, is a suitable environment for maintaining of leptospires. In order to investigate the seroprevalence of leptospirosis in the cattle population, a serological study was conducted in Khorramabad province.

MATERIALS AND METHODS

Study Population

For this study, Blood samples were taken from 200 cows from 10 cow herds in five districts in Khorramabad, west of Iran, during the period November 2013 to May of 2014. According to dental formula, these cows were divided into five age groups (<1, 1-2, 2-3, 3-4 and over 4 years old). 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. The numbers of samples from suburb one to five were forty one, forty three, forty, thirty eight and thirty eight, respectively.

Serum Samples

Ten ml of blood were collected from the jugular vein of each cattle. The blood samples were allowed to clot and centrifuged for 10 min at 3000 g. After centrifugation, the serum was removed and stored at -20°C until ready for test. The sera were tested for antibodies to six live serovars of Leptospira interrogans: Pomona, Grippotyphosa, Icterohaemorrhagiae, Canicola, Hardjo and Australis using the Microscopic Agglutination Test (MAT) in the Leptospira Research Laboratory, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. According to the methods of O.I.E (9), sera were initially screened at a dilution of 1:100 against these antigens. At first, a serum dilution at 1:50 was made and a volume equal to the diluted serum volume of each antigen was added to each well of micro-titration plates, making the final serum dilution of 1:100. The micro-titration plates were incubated at 29°C for two hours. The plates were then examined by dark-field microscopy. The results were considered positive when ≥ 50% of agglutination of leptospires at the test serum dilution of ≥ 1:100 were observed (9). Sera with positive results were titrated against reacting antigens in serial two-fold dilutions from 1:100 to 1:1600.

The results were analysed by Chi-Square and Fisher’s exact test to determine the difference between two sexes and different groups of age was significantly related to the prevalence of leptospiral antibodies.

RESULTS

The results of this study showed that 40 (20%) cows had a positive reaction against one or more serovars. Two samples (5%) showed serological reaction with more than one serovar. The most prevalent leptospira serovars were Grippotyphosa 18(45%) and Canicola 16(40%), respectively. The less prevalent leptospira serovars were Hardjo 4(10%) and Australis 2(5%), respectively (Table 1). All of sera were seronegative for other serovars (Table 1). The most 36 female (90%) cows and 4 male (10%) cows were positive in MAT test. There was significant difference between seropositives and sex (P<0.01) (Table 2). On the base of age, 1 cow (2.5%) in less than one year group, 4 cows (10%) in the 1–2 years group, 10 cows (25%) in the 2-3 years group, 16 cows (40%) in the 3-4 years group and 9 cows (22.5%) in the over 4 years group were positive for leptospira (Table 3). There was significant relationship between aging of the cows (P<0.01) and increased of seropositive animals that they were observed in 3-4 years old (Table 3). Prevalent serological titer was 1/100 and the highest titer was 400 (Table 4). The highest seropositive reactors were belonged to the South and North respectively.

DISCUSSION

Leptospirosis is a zoonosis of worldwide distribution, caused by Leptospira interrogans. It is one of the well-known causes of bovine reproductive losses such as abortion, infertility, stillbirth, birth of weak calves, weight loss and decreased milk production. The reported results of seroprevalence of leptospiral infection in cattle are different from country to country. In Portugal, 15.3% of cattle reacted to one or more serovar of L. interrogans (12). According to the report of Ozdemir and Erol (2000), the prevalence of leptospiral infection in cattle and sheep in Turkey was 44.77 and 8%, respectively. In Malaysia, 40.5% of cattle reacted to one or more serovar of L. interrogans (14). In Turkey, 25.42% of cattle reacted to one or more serovar of L. interrogans (4).

The earliest study (1967) on leptospirosis prevalence in Iran indicated that there are 31% serum positive titer against L. interrogans in cattle and 17% in sheep (6). Another study showed that the prevalence of serum positive titer against leptospiral antigen has been about 24.6% in Tehran suburb dairy farms (8). Results of studies on leptospirosis prevalence in other regions in Iran include: between 3 to 30.7% in Tehran suburb, 24.24% in Mashhad suburb, 32% in Shiraz suburb, 46.8% in Karadj suburb, 22% in Gilan province (13) and finally 53.73% in Ahwaz suburb (6). The results of this study showed that the seroprevalence of leptospirosis in cattle in West of Iran (Khorramabad) was 20%.

Results of previous studies about prevalence of each serovar of leptospiros in Iran has shown that Leptospira hardjo was the most (67.7%) and Leptospira icterohaemorrhagiae the least (0.8%) prevalent serovars in Tehran suburb (8), Leptospira icterohaemorrhagiae was the most and Leptospira pomona the least prevalent serovars in...