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**Short Paper**

# A serological survey on leptospirosis in aborted dairy cattle in industrial farms of Hamedan suburb, Iran

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## Summary

Leptospirosis is a zoonosis of worldwide distribution, caused by *Leptospira interrogans*. It is a well known cause of bovine reproductive losses such as abortion, infertility, stillbirth and birth of weak calves. In this research, the relationship between the seroprevalence rate of *Leptospira* spp. infection and abortion in industrial dairy farms of Hamedan province, Iran was studied. A total of 80 blood samples were taken from aborted cows in six dairy farms. Sera were tested for antibodies against 6 serovars of *Leptospira interrogans* (*hardjo*, *pomona*, *canicola*, *grippotyphosa*, *icterohaemorrhagiae* and *ballum*) using microscopic agglutination test (MAT). Antibodies were detected in 18 (22.5%) of the aborted cows, including 17 (21.25%) against *L. canicola* and 1 (1.25%) against *L. pomona*. It is concluded that dogs (shepherd and stray) and wild carnivores may have an important role to maintain and transmit the *L. canicola* infection to the cattle population in this region, therefore, vaccination of cattle and shepherd dogs should be applied.

**Key words:** Leptospirosis, Abortion, Cattle, Microagglutination test

## Introduction

Leptospirosis is a worldwide zoonotic disease caused by pathogenic *Leptospira* species. Although *Leptospira* was divided into several species on the basis of DNA, the term *Leptospira interrogans* is still widely used in reference to pathogenic leptospires (Adler and de la Peña Moctezuma, 2010).

Leptospirosis is a well known cause of reproductive losses in cattle. Typically, the disease occurs through bacterial exposure to mucous membranes and generally results in occult form or relatively mild acute clinical signs. Abortion, stillbirth, or birth of weak calf occurs as a result of *Leptospira* infection. Abortion may occur several weeks after infection of the dam and is usually not associated with any obvious illness in the cow. Abortions due to serovar *hardjo* infection tend to occur sporadically as opposed to abortion "storms" which may occur as a result of infection with serovars *pomona* or *grippotyphosa* (Grooms, 2006).

Recently published data indicate that serovar *canicola* is widespread in the cattle population in different provinces of Iran (Abdollahpour *et al*., 2004; Ebrahimi *et al*., 2004; Khaki *et al*., 2005; Haji Hajikolaei *et al*., 2007; Abdollahpour *et al*., 2009). In spite of this fact, accurate data on the frequency of abortion attributable to leptospirosis is very limited.

The purpose of this study was to investigate the seroprevalence of leptospirosis in aborted cows in dairy farms of Hamedan suburbs, Iran.

## Materials and Methods

A total of 80 blood samples were collected from aborted cows by *coccygeal* venipuncture between October 2008 and May 2009 in Hamedan suburb dairy farms.

No vaccination program against leptospirosis has been applied in these farms. The farm data are summarized in Table 1. The blood serum was separated after centrifugation at 1,800 × g for 10 min, and serum samples were stored at -20°C until analysed. All serum samples were examined by the standard micro-agglutination test (MAT) at the Leptospira Research Laboratory, Faculty of Veterinary Medicine, University of Tehran. A 7–10-day-old culture of six *Leptospira interrogans* serovars: *icterohaemorrhagiae, grippotyphosa, canicola, hardjo, ballum* and *pomona* grown in EMJH liquid medium was used as live antigen in the MAT. The density of Leptospires was assessed using a counting chamber (Petroff-Hausser, USA) that was adjusted to about $2 \times 10^8$ cells/ml. Serial dilution of test sera was added to an equal volume of antigen suspension on a microscope slide. Following incubation at 28-30°C for 1.5 h the slide was examined under a Dark field microscope, using a long working distance objective lens at a final magnification of ×200. Agglutination was noted by observing clumps of leptospires. The lowest dilution in which each serum was considered significant was 1:50. The end point titer was the highest dilution of serum in which 50% of Leptospiral cells were agglutinated; so that a titre of 1:100 was considered the cut off point for MAT.

## Results

The results of this study showed that a total of 18 (22.5%) sera were positive, including 17 (21.25%) against *L. canicola* and 1 (1.25%) against *L. pomona* (Table 1). The highest titer was 1:100. No sample was found positive for more than one serovar.

**Table 1: Distribution of leptospiral infection in aborted cows in 6 dairy farms of Hamedan suburbs**

| Farm No. | Herd size | No. of samples | Positive samples n (%) |
|---|---|---|---|
| 1 | Medium | 10 | 2 (20%) |
| 2 | Medium | 6 | 1 (16.7%) |
| 3 | Large | 45 | 12 (26.7%) |
| 4 | Medium | 8 | 1 (12.5%) |
| 5 | Small | 4 | 0 (0%) |
| 6 | Medium | 7 | 2 (28.6%) |
| Total | | 80 | 18 (22.5%) |

Large herd >500, medium herd 100-500 and small herd <100 dairy cows

## Discussion

The results of this study indicate the importance of leptospirosis as a possible cause of bovine abortion in dairy farms of Hamedan suburbs. Moreover, our results showed that *Leptospira interrogans* serovar *canicola* was the most prevalent serovar in aborted cows in the sampled farms. However, the mentioned serovar has been considered as a common serovar in dogs; and dog is the main reservoir for this serovar.

Several MAT-based studies have revealed various reactive serovars in cattle and serovar *hardjo* has been reported as the most important throughout the world (Grooms, 2006). Ten percent of bovine abortion in the United States (Kirkbride and Johnson, 1989), 6% in Canada (Prescott *et al*., 1988) and 50% in Northern Ireland (Ellis *et al*., 1985) have been reported to be due to *hardjo* serovar infection. The most prevalent *leptospira* serovars in aborted cattle were *L. grippotyphosa* and *L. hardjo* in Turkey (Genc *et al*., 2005) and, *L. bratislava* and *L. hardjo* in Brazilian dairy cattle (Langoni *et al*., 1999). Regarding these studies, there are some differences between our findings and other serological surveys.

The results of the present study showed that dogs may have an important role in epizootic leptospiral abortion in dairy farms around Hamedan. The high prevalence rate of *canicola* serovar in this study is similar to that of the serological surveys conducted in different parts of Iran (Ebrahimi *et al*., 2004; Khaki *et al*., 2005; Haji Hajikolaei *et al*., 2007; Abdollahpour *et al*., 2009). It is speculated that *canicola* infection of dairy farms occurs in the regions where dogs (shepherd and stray) act as the main reservoir. Moreover, these findings may possibly support the cross-infection occurrence between cattle and dogs, and cattle may play a role in the maintenance of *canicola* serovar in nature (Abdollahpour *et al*., 2009). According to this theory, the control of *canicola* infection in dairy cattle is more complicated.

Control of leptospirosis generally involves a multi-pronged attack. The first

step is to reduce exposure to the pathogen. Antibiotic treatment of cattle infected with leptospirosis may eliminate the carrier stage of this disease. In addition, control of close contact with other reservoirs and contaminated environments is necessary. The second step is to institute an appropriate vaccination program that is designed to reduce the risk of reproductive loss.

It is concluded that the high prevalence rate of *canicola* serovar in aborted cattle of Hamedan suburbs can be associated with close contact between dogs and cows. All visited farms had a history of frequent access to stray dogs and/or wildlife carnivorous to the herds. Thus, limiting stray dogs and wild carnivores contact with cattle and their feed and water reduces the potential for transmission of this serovar. On the other hand, *canicola* serovar is not included in the present multivalent vaccine which is used in the bovine population in Iran. There is little or no cross-protection between the serovars that affect cattle, so the multivalent vaccine is used for the vaccination program for susceptible cattle. Therefore, it is suggested that in regions which serovar *canicola* is dominant, this serovar should be added to the multivalent vaccine. As the vaccination for leptospirosis is not fully effective, it must be used in combination with other control methods such as limiting exposure to stray dogs and wildlife, control of rodents, and eliminating access to potentially contaminated food and water supplies.

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