



Original Research Article

Seroprevalence of leptospirosis in aborted dairy cattle in Peshawar district suburb, Khyber Pakhtunkhwa Pakistan

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ABSTRACT

Keywords

Leptospirosis;
Abortion;
Cattle;
Micro-
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Peshawar;
Seroprevalence.

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 Peshawar district Khyber Pakhtunkhwa Pakistan was studied. A total of 60 blood samples were taken from aborted cows and Sera were stored at -20⁰C until analyzed. They were initially screened at serum dilution of 1:100 against six live antigens of *Leptospira interrogans* serovars Pomona, canicola, hardjo, ballum, icterohaemorrhagiae, grippityphosa using microscopic agglutination test (MAT). The samples were considered positive if $\geq 50\%$ of agglutination of leptospira in a dilution test serum of $\geq 1:100$ were observed. Sera with positive results were titrated against reacting antigens in serial two-fold dilutions from 1:100 to 1:800. Out of total 60 samples antibodies were detected in 38 (63.33%) of the aborted cows, including 15 (39.47%) for Pomona followed by grippityphosa 6 (15.78%), canicola, icterohaemorrhagiae and ballum 5 (each of which 13.15%) and 2 (5.26%) against *L. hardjo*.

Introduction

Leptospirosis is a common global zoonotic disease of man and in all farm animal species especially in sub-tropical and tropical regions of the world, 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 Pena Moctezuma, 2010).

derived from wildlife reservoirs while serovar hardjo is a cattle-adapted serovar (Ellis *et al.*, 1981). 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. Most leptospiral infections in sheep and

goat are asymptomatic but may result in high fever, abortion, stillbirth, agalactiae and prenatal death. Affected calf, lambs and kids may manifest fever, jaundice and haemoglobinuria which may result in death (Cousing and Robertson, 1986; Radostits *et al.*, 2000). Two tests have a role in veterinary diagnosis: the microscopic agglutination test (MAT) and enzyme linked immunosorbent assay (ELISA) (OIE, 2000).

Previous serologic surveys in Ahvaz were carried out on cattle, buffalo, horse and donkey (Haji Hajikolaei *et al.*, 2006, 2005a, 2005b). These surveys showed that zoonotic leptospiral infection is common in these animals. Because, there was no study on leptospiral infection in cattle in Peshawar district, this study was carried out to determine the seroprevalence of leptospiral infection in cattle.

Materials and Methods

Electrochemical Treatment

A total of 60 blood samples were collected from aborted cows by coccygeal venipuncture between November, 2012 to May, 2013 in Peshawar district of Khyber Pakhtunkhwa Pakistan suburb dairy farms. No vaccination program against leptospirosis has been applied in these dairy farms. At the time of blood collection, all animals appeared healthy with no clinical signs suggestive for leptospirosis. Twenty five ml of blood was collected from jugular vein of each cow. The samples were allowed to clot and centrifuged for $1800 \times g$ for 10 minutes. After centrifugation, the serum was removed and stored at -20°C until analyzed. All serum samples were examined and tested by the standard microscopic agglutination test (MAT) at the Standardization and Quality Control

Section (Veterinary Research Institute, Peshawar KPK Pakistan). The sera were tested for antibodies against six live antigens of *Leptospira interrogans* (*L.interrogans* serovar pomona, canicola, hardjo, ballum, icterohaemorrhagiae, grippityphosa) using the Microscopic Agglutination Test (MAT). The density of Leptospire was assessed using a counting chamber (Petroff-Hausser, USA) that was adjusted to about 2×10^8 cells/ml. According to the methods of OIE (2000), 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 hrs. The plates were then examined by dark-field microscopy. Results were considered positive when $\geq 50\%$ of agglutination of leptospire at the test serum dilution of $\geq 1:100$ were observed (OIE, 2000). Sera with positive results were titrated against reacting antigens in serial two-fold dilutions from 1:100 to 1:1800. Obtained results were statistically analyzed using chi-square and Fisher's exact test with significance level at 0.05.

The purpose of this study was to investigate the seroprevalence of leptospirosis in aborted cattle in dairy farms of Peshawar district of KPK, Pakistan.

Result and Discussion

The result of this study showed that antibodies against one or more serovars were detected in 38 (63.33%) cows. The highest number of reactors was for Pomona (39.47%) followed by grippityphosa (15.78%), canicola, icterohaemorrhagiae and ballum (each of

Table.1 Distribution of serovar specific antileptospiral antibodies and their titration in seropositive cow

Serovar	1:100	1:200	1:400	1:800	Total
Grippotyphosa	2 (5.26%)	0 (0%)	1(2.63%)	3(7.89%)	6 (15.78%)
Hardjo	1 (2.63%)	1(2.63%)	0(0%)	0(0%)	2 (5.26%)
Canicola	1(2.63%)	3 (7.89%)	0(0%)	1(2.63%)	5 (13.15%)
Pomona	7 (18.42%)	4 (10.52%)	3(7.89%)	1(2.63%)	15 (39.47%)
Icterohaemorrhagiae	2 (5.26%)	1(2.63%)	2 (5.26%)	0(0%)	5(13.15%)
Ballum	1(2.63%)	3(7.89%)	1(2.63%)	0(0%)	5(13.15%)
Total	14 36.84%)	12 (31.57%)	7(18.42%)	5 13.15%)	38 (100%)

which 13.15%) and (5.26%) against *L. hardjo* (Table 1). The highest titer was 1:100 (36.84%) followed by 1:200 (31.57%), 1:400 (18.42%) and (13.15%) for 1:800, respectively (Table 1). No sample was found positive for more than one server. The prevalence of different serovar

No previous observational study of the frequency of leptospirosis in cattle in Peshawar district of Khyber Pakhtunkhwa, or an examination of aborted fetuses for leptospires, has been attempted. The seroprevalence survey was based on the Micro Agglutination Test (MAT), the test usually used in serodiagnosis of leptospirosis. The results of this study indicate the importance of leptospirosis as a possible cause of bovine abortion in dairy farms of Peshawar suburbs. We found that the seroprevalence of leptospiral infection in cattle in Peshawar district was 63.33%. The reported results of seroprevalence of leptospiral infection in cow are different from country to country.

Serovars Pomona and grippotyphosa were, as expected found to be the most prevalent leptospires in the present study. Such a finding has apparantly been reported by Haji *et al.*, (2007). Several Micro human beings. Long-term survival of

Agglutination Test (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) has been reported to be due to hardjo serovar infection. Our findings reported sixty three percent of bovine abortion in Peshawar district of Khyber Pakhtunkhwa Pakistan. Such a finding has apparently not been reported in other countries, contrary in results and such a higher prevalence in Peshawar relates to differences in husbandry methods and natural mating practices in Pakistan rather than artificial insemination in other countries. The most prevalent leptospira serovars in aborted cattle were *L. grippotyphosa* and *L. hardjo* in Turkey (Genc *et al.*, 2005), *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. These differences may be the consequence of environmental factors and control efforts. The environmental factors have been shown to have influential effects on development of leptospiral infection in animal and pathogenic leptospires outside the host

requires a warm, moist environment with a near neutral pH (Miller *et al.*, 1991). In contrast to the previous studies in Iran, the prevalence of antibodies to one or more serovars of *L. interrogans* was 53.8, 58.7, 27.9 and 40% in cattle, buffalo, horse and donkey, respectively (Haji Hajikolaei *et al.*, 2006, 2005a, 2005b). Although the significance of these differences was not defined, but it may be due to difference in susceptibility of these animals. Leptospirosis occurs in sheep and goats with less frequency than in cattle. So that the prevalence of leptospiral infection in cattle, buffalo and sheep in Egypt was 34.5, 26.1 and 4.2%, respectively (Maronpot and Barsoum, 1972). According to the report of Rocha (1988), the prevalence of leptospiral infection in cattle, sheep, goat, and horse in Portugal was 15.3, 3.3, 5.0 and 43.3%, respectively. In Turkey, 44.77% of cattle and 8% of sheep reacted to one or more serovar of *L. interrogans* (Ozdemir and Erol, 2002). In Malaysia 40.5, 31 and 10% of cattle, buffalo and sheep reacted to one or more serovar of *L. interrogans*, respectively (Bahaman *et al.*, 1987).

Pomona was present as the predominant serovar in seropositive cattle in this study. On the other hand, in a previous study in Ahvaz Iran, the predominant serovars were grippotyphosa in cattle, horse and donkey and canicola in buffalo (Haji Hajikolaei *et al.*, 2006, 2005a, 2005b). It is probable that these serovars may be adapted to and maintained by these farm animals in Ahvaz. There is a need for further investigation on clinical cases of leptospirosis to determine whether this serovar is the main cause of leptospirosis in this area. The predominant leptospira serovars in serological reaction varies somewhat from country to country. For example, poi and pomona in Bolivia

(Ciceroni *et al.*, 1997), wolffi, pomona and ballum in Argentina (Draghi *et al.*, 1984), hebdomadis in the UK (Hathaway *et al.*, 1981), pomona in India (Manickavel *et al.*, 1991), autumnalis in Egypt (Maronpot and Barsoum, 1972), castellonis in Italy (Ciceroni *et al.*, 2000) and pomona in Malaysia (Bahaman *et al.*, 1987) were the predominant serovars in ruminant. In addition, one serovar may be predominant in a country but none of the animal reacted with this serovar in another country. This emphasizes the need for regional surveys for leptospirosis, since host-parasite relationship may change depending on the ecology of the region. Antibodies against more than one serovar were found nil in our present study. In serologic tests for leptospirosis such as Microscopic agglutination test (MAT), the results often indicate infection with more than one serovar (Hathaway *et al.*, 1981; Egan and Yearsley, 1989; Firouzi and Vandyousefi, 2000; Haji Hajikolaei *et al.*, 2006, 2005a, 2005b). This may be the result of mixed serovar infection or cross-reactivity among serovars. The high prevalence of infection and dominant titer of 1:100 reveal that leptospiral infection in cattle in Peshawar district is endemic and occurs mostly in subclinical form. The present study results showed that dogs may have an important role in epizootic leptospiral abortion in dairy farms around Peshawar district of Khyber Pakhtunkhwa. The high prevalence of rate of Pomona in this study is not similar to that of the serological surveys conducted in different parts of Hamedan Iran (Bahari *et al.* 2011) and also not in line with the results observed by (Ebrahimi *et al.*, 2004; Khaki *et al.*, 2005; Haji Hajikolaei *et al.*, 2007; Abdollahpour *et al.*, 2009).

It is speculated that canicola and Pomona infection of dairy farms occurs in the

regions where dogs (stray and shepherd) 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 *Pomona* and *canicola* serovar in nature (Abdollahpour *et al.*, 2009).

In Britain, where *Leptospira hardjo* is a major cause of abortion in cattle, whereas our data resulted *Leptospira pomona* is a major cause of abortion in 63.33% cases, this contrast with the results of isolations from positive samples possibly support the cross infection between cattle and dogs, and may be due to difference in susceptibility of these animals.

Recommendations

There is no or little 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 server *Pomona* is dominant, the serovar should be added to the multivalent vaccine. As the vaccination for leptosporosis 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 elimination access to potentially contaminated food and water supplies.

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