

## Involvement of *Leptospira* serovars with Different Clinical Conditions of Leptospirosis in Cattle

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### ABSTRACT

Leptospirosis is a worldwide zoonosis affecting domestic animals, pet animals, wild animals and human beings. It causes abortion, repeat breeding, jaundice and haemorrhagic mastitis in bovines. The present study was undertaken to understand the involvement of different leptospiral serovars with different clinical conditions of leptospirosis among bovines. A total of 1122 serum samples collected from cattle with different clinical conditions which included abortion (247), repeat breeding (429), jaundice (215), haemorrhagic mastitis (231) were subjected to Microscopic agglutination test. The study revealed the association of *hardjo* with abortion (21.98 per cent), repeat breeding (47.25 per cent), jaundice (15.38 per cent) and haemorrhagic mastitis (15.38 per cent). The serovar *pomona* was associated with abortion (19.67 per cent), repeat breeding (39.34 per cent), jaundice (21.31 per cent) and haemorrhagic mastitis (19.67 per cent). Serovar *hebdomadis* was associated with abortion (29.48 per cent), repeat breeding (37.17 per cent), jaundice (16.67 per cent) and haemorrhagic mastitis (16.67 per cent). Serovar *ballum* was found to associate with abortion (18.75 per cent), repeat breeding (46.87 per cent), jaundice (15.62 per cent) and haemorrhagic mastitis (18.75 per cent). In addition, the serovar *australis* was found to associate with abortion (19.29 per cent), repeat breeding (42.10 per cent), jaundice (19.29 per cent) and haemorrhagic mastitis (19.29 per cent). However from this study, serovar specificity with regard to clinical conditions in cattle could not be established as all the five serovars were involved with different clinical conditions.

**Key words:** *Leptospira* serovars, Clinical conditions, Leptospirosis, Cattle.

### INTRODUCTION

Leptospirosis is a worldwide zoonosis affecting domestic animals, pet animals, wild animals and human beings. It causes abortion, repeat breeding, jaundice and haemorrhagic mastitis in bovines. The involvement of different leptospiral serovars with different clinical conditions of leptospirosis among bovines has been reported earlier by many workers (Venkataraman and Jaganathan, 1961; Rao and Surendran, 1970; Thierman, 1982; Slee, *et al.*, 1983; Venugopal, *et al.*, 1986 and Ramakrishna and Venkataraman,

1994). All these studies recorded only few serovars namely *hardjo*, *hebdomadis*, *pomona*, *autumnalis*, *javanica*, *grippotyphosa* and *icterohaemorrhagiae* in association with different clinical conditions of leptospirosis among bovines. Whereas the association of serovars *australis*, *ballum* etc., has not been available in the perusal of the literature. But the serological evidences of leptospirosis due to *australis* and *ballum* have been documented in the literature (Srivastava and Kumar, 2003; Selvaraj, *et al.*, 2005; Koteeswaran, 2006; Thiyageeswaran, 2007 and Sekar, *et al.*, 2008). Hence a detailed study on the distribution of different leptospiral serovars

from clinically suspected cattle was undertaken in the present study.

## MATERIALS AND METHODS

### Collection of serum samples

A total of 1122 serum samples were collected from cattle with different clinical conditions and suspected for leptospirosis which included abortion (247), repeat breeding (429), jaundice (215), haemorrhagic mastitis (231). The serum samples were subjected to Microscopic agglutination test.

### Microscopic agglutination test (MAT)

#### Preparation of antigens for MAT

A 5-8 day old liquid culture of live leptospire incubated at  $29 \pm 1^\circ\text{C}$ , containing density of  $2 \times 10^8$  leptospire per ml was used (OIE, 2004). The leptospiral cultures without clumps were used as antigens in MAT. The panel of antigens used in MAT is presented in Table 1.

### Microscopic agglutination test

This test was conducted as per OIE (2004) in 96 well 'U' bottom titration plates (M/s. Laxbro, India). Serum dilutions were made in deep well (96 well) dilution plates (M/s. Laxbro, India). To 980  $\mu\text{l}$  of PBS, 20  $\mu\text{l}$  (1:50) of serum samples were added in individual wells. Serum dilutions (25  $\mu\text{l}$  of 1:50) were added to each of the 12 wells in the A to G

rows of 'U' bottom microplates. In the last row, only PBS 25  $\mu\text{l}$  was added to all the wells which served as antigen control. Thus each row is corresponding to each sample. Twelve antigens (25  $\mu\text{l}$ ) were added in all the wells of respective columns (antigen 1 in column 1, antigen 2 in column 2 and so on) including in the respective antigen control wells, so that the final serum dilution was in 1 in 100. The plates were closed with lids and incubated at  $37^\circ\text{C}$  for 2 h. A drop (5  $\mu\text{l}$ ) of mixture (final dilution of 1:100) was placed on grease-free slide and the wet preparation without cover slip was screened using 20X objective of the dark field microscope (M/s. Nikon, 200E Japan) for the presence of agglutination and/or reduction in number of organisms in comparison with the respective antigen control. A 50 per cent reduction in the number of free leptospire in the test sample comparable with the respective antigen control was considered positive with or without agglutination.

## RESULTS AND DISCUSSION

A total of 1122 serum samples were collected from cattle with the history of abortion, repeat breeding, jaundice and haemorrhagic mastitis (Table - 2). Out of 1122 serum samples, 351 were found positive (31.28 per cent). The five serovars namely *hardjo*, *hebdomadis*, *ballum*, *australis* and *pomona* were found to be involved. The prevalence rate of *hardjo* (25.93 per cent) was more, followed by *hebdomadis* (22.22 per cent), *ballum* (18.23 per

Table 1: Reference strains of Leptospire\* used in the study

S.No	Serogroup	Serovar	Strain
1	Australis	<i>australis</i>	Ballico
2	Autumnalis	<i>rachmati</i>	Rachmati
3	Ballum	<i>ballum</i>	Mus127
4	Canicola	<i>canicola</i>	HondUtrecht IV
5	Grippotyphosa	<i>grippotyphosa</i>	Moskva V
6	Hebdomadis	<i>hebdomadis</i>	Hebdomadis
7	Icterohaemorrhagiae	<i>icterohaemorrhagiae</i>	RGA
8	Javanica	<i>Poi</i>	Poi
9	Pomona	<i>pomona</i>	Pomona
10	Pyrogenes	<i>pyrogenes</i>	Salinem
11	Sejroe	<i>hardjo</i>	Hardjoprajitno
12	Tarassovi	<i>tarassovi</i>	Peripellicin

\*Obtained from National Reference laboratory, Indian Council of Medical Research, Andaman and Nicobar Islands, India.

Table 2: Seroprevalence of leptospirosis in clinically suspected cattle

Clinical signs	Total screened $x^2 = (5.82)^{NS}$	Total positive (MAT)					Serovars reacted					Total
		No.	Percent	Australis	ballum	hardjo	hebdomadis	pomona				
Abortion	247	78	31.58	11(19.29%)	12 (18.75%)	20 (21.98%)	23(29.48%)	12(19.67%)	78			
Repeat breeding	429	150	34.97	24 (42.10%)	30 (46.87%)	43 (47.25%)	29(37.17%)	24(39.34%)	150			
Jaundice	215	61	28.37	11(19.29%)	10 (15.62%)	14 (15.38%)	13(16.67%)	13(21.31%)	61			
Haemorrhagic Mastitis	231	62	26.84	11(19.29%)	12 (18.75%)	14 (15.38%)	13(16.67%)	12(19.67%)	62			
Total	1122	351	31.28	57	64	91	78	61	351			

NS – Statistically non- significant at 5 per cent level (P<0.05)

cent), *pomona* (17.38 per cent) and *australis* (16.24 per cent). Slee *et al.* (1983) proved that serovar *hardjo* was associating with bovine abortions in Australia, whereas Thiermann (1982) reported that *hardjo* could produce mastitis and abortions. Sullivan and Callan (1970), Hoare and Claxton (1972), Gordon (1977) and Higgins *et al.* (1980) reported association of clinical mastitis with *hardjo* in cattle. The association of *hardjo* with abortion and infertility in cattle was also reported earlier (Ellis *et al.*, 1983 and Ramakrishna and Venkataraman, 1994). In the present study, *hardjo* was associated with abortion (21.98 per cent), repeat breeding (47.25 per cent), jaundice (15.38 per cent) and haemorrhagic mastitis (15.38 per cent). Knott and Dadsnell (1970) recorded abortion amongst cattle in Australia following *pomona* infection. The association of *pomona* with abortion was recorded earlier by several workers in India (Rao and Surendran, 1970; Venugopal *et al.*, 1986). In addition to abortion, the serovar *pomona* also caused repeat breeding (Venugopal *et al.*, 1986) and jaundice (John *et al.*, 1980). In the present study serovar *pomona* was associated with abortion (19.67 per cent), repeat breeding (39.34 per cent), jaundice (21.31 per cent) and haemorrhagic mastitis (19.67 per cent). The serovar *hebdomadis* was earlier observed in aborted cows (Rao and Surendran, 1970). In the present study, *hebdomadis* was associated with abortion (29.48 per cent), repeat breeding (37.17 per cent), jaundice (16.67 per cent) and haemorrhagic mastitis (16.67 per cent). Serovar *ballum* was found to associate with abortion (18.75 per cent), repeat breeding (46.87 per cent), jaundice (15.62 per cent) and haemorrhagic mastitis (18.75 per cent). In addition, the serovar *australis* was found to associate with abortion (19.29 per cent), repeat breeding (42.10 per cent), jaundice (19.29 per cent) and haemorrhagic mastitis (19.29 per cent). The involvement of serovars *ballum* and *australis* with different clinical conditions of leptospirosis in cattle has not been reported earlier in the available literature. However from this study, serovar specificity with regard to clinical conditions could not be established as all the five serovars were involved with different clinical conditions.

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