ASSOCIATION OF *LEPTOSPIRA* SEROVARS WITH DIFFERENT CLINICAL CONDITIONS OF LEPTOSPIROSIS IN BUFFALOES

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ABSTRACT

The present study was undertaken to understand the involvement of different leptospiral serovars with different clinical conditions of leptospirosis among buffaloes. A total of 468 serum samples were collected from buffaloes with the history of abortion, repeat breeding, jaundice and haemorrhagic mastitis subjected to Microscopic agglutination test. Out of 468 serum samples, 329 (70.30 per cent) were found positive for 5 serovars namely *pomona* (28.27 per cent) *hardjo* (27.64 per cent), *hebdomadis* (23.00 per cent), *australis* (13.50 per cent) and *ballum* (7.59 per cent). Among the five serovars detected from buffaloes, reports are available only about serovar *pomona* which was found to be associated with abortion and jaundice. In the present study, it is observed that the influence of clinical signs over the seropositivity to leptospoirosis was statistically significant in buffaloes.

KEY WORDS: Leptospirosis-*Leptospira*-Clinical conditions-Buffaloes

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 and small ruminants has been reported earlier (Thierman, 1982; Slee *et al*., 1983; Venugopal *et al*., 1986 and Ramakrishna and Venkataraman, 1994 and paramasivam *et al.*, 2014). 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). Hence a detailed study on the distribution of different leptospiral serovars from clinically suspected buffaloes was undertaken in the present study.

MATERIALS AND METHODS

Collection of serum samples and Microscopic agglutination test (MAT)

A total of 468 serum samples were collected from cattle and buffaloes with different clinical conditions and suspected for leptospirosis which included abortion (150), repeat breeding (140), jaundice (80), haemorrhagic mastitis (98). The serum samples were subjected to Microscopic agglutination test.

A 5-8 day old liquid culture of live leptospires incubated at 29 ± 1^0C, containing density of $2x10^8$ leptospires 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.

Table 1 : Reference strains of Leptospires used in the study

S.No	Serogroup	Serovar	Strain
1	Australis	*Australis*	Ballico
4	Canicola	*Canicola*	HondUtrecht IV
5	Grippotyphosa	*Grippotyphosa*	Moskva V
6	Hebdomadis	*Hebdomadis*	Hebdomadis
7	Icterohaemorrhagiae	*icterohaemorrhagiae*	RGA
8	Javanica	*Poi*	Poi
9	Pomona	*Pomona*	Pomona
10	Pyrogenes	*Pyrogenes*	Salinem
11	Sejroe	*Hardjo*	Hardjoprajitno
12	Tarassovi	*Tarassovi*	Peripellicin

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

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 µl of PBS, 20 µl (1:50) of serum samples were added in individual wells. Serum dilutions (25 µl 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 µl was added to all the wells which served as antigen control. Thus each row is corresponding to each sample. Twelve antigens (25 µl) 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 1 in 100. The plates were closed with lids and incubated at 37^0C for 2 h. A drop (5 µl) 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 leptospires in the test sample comparable with the respective antigen control was considered positive with or without agglutination.

RESULTS AND DISCUSSION

A total of 468 serum samples were collected from buffaloes with the history of abortion, repeat breeding, jaundice and haemorrhagic mastitis (Table - 2). Out of 468 serum samples, 329 (70.30 per cent) were found positive. The 5 serovars namely *pomona* (28.27 per cent) *hardjo* (27.64 per cent), *hebdomadis* (23.00 per cent), *australis* (13.50 per cent) and *ballum* (7.59 per cent) were found to be involved. Venugopal *et al*. (1986) reported *autumnalis, pomona, grippotyphosa* and *icterohaemorrhagiae* among buffaloes with the history of abortion. The involvement of multiple

Table 2 : Seroprevalence of leptospirosis in clinically suspected buffaloes

Clinical signs	Total screened	Total positive (MAT)		Serovars reacted					
		No.	Percent	*australis*	*ballum*	*Hardjo*	*hebdomadis*	*pomona*	Total
Buffaloes $x^2 = (9.52)^*$									
Abortion	150	95	63.33	13 (20.31%)	9 (25.00%)	30(22.90%)	21(19.27%)	25(18.66%)	**98**
Repeat breeding	140	98	70.00	25 (39.06%)	11(30.55%)	54(41.22%)	45(41.28%)	60(44.78%)	**195**
Jaundice	80	56	70.00	12 (18.75%)	11(30.55%)	23(17.56%)	16(14.68%)	25(18.66%)	**87**
Haemorrhagic Mastitis	98	80	81.63	14 (21.88%)	5 (13.89%)	24(18.32%)	27(24.77%)	24(17.91%)	**94**
Total	**468**	**329**	**70.30**	**64**	**36**	**131**	**109**	**134**	**474**

** Statistically significant at 5 per cent level (P<0.05)*

serovars namely *tarassovi, pomona, grippotyphosa, icterohaemorrhagiae* and *autumnalis* in leptospiral jaundice among buffaloes was reported earlier (John *et al*. 1980) and also jaundice by *javanica* (Venkataraman and Jagannathan, 1961). In the present study serovar *australis* was in association with abortion (20.31 per cent), repeat breeding (39.06 per cent), jaundice (18.75 per cent) and haemorrhagic mastitis (21.88 per cent). Meanwhile *ballum* was found to be associated with abortion (25.00 per cent), repeat breeding (30.55 per cent), jaundice (30.55 per cent) and haemorrhagic mastitis (13.89 per cent). Similarly the serovar *hardjo* was found in association with abortion (22.90 per cent), repeat breeding (41.22 per cent), jaundice (17.56 per cent) and haemorrhagic mastitis (18.32 per cent). The serovar *hebdomadis* was observed in association with abortion (19.27 per cent), repeat breeding (41.28 per cent), jaundice (14.68 per cent) and haemorrhagic mastitis (24.77 per cent). The serovar *pomona* was found to be associated with abortion (18.66 per cent), repeat breeding (44.78 per cent), jaundice (18.66 per cent) and haemorrhagic mastitis (17.91 per cent). From this study, it was found that serovar *ballum* was more involved with abortion (25.00 per cent) and jaundice (30.55 per cent) while *pomona* with repeat breeding (44.78 per cent) and *hebdomadis* with haemorrhagic mastitis (24.77 per cent), compared to other serovars. Among the five serovars detected from buffaloes reports are available only about serovar *pomona* which was found to be associated with abortion and jaundice (Venugopal *et al*., 1986 and John *et al*., 1980). The association of clinical signs with the seropositivity to leptospirosis was analysed by chi-square test. The association between the clinical signs and leptospiral seropositivity was found to be statistically significant at 5 per cent level in buffaloes (Table - 2).

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