Journal of Animal Research: v.5 n.1, p. 31-35. April. 2015

DOI Number: 10.5958/2277-940X.2015.00006.6

Seroprevalence of Leptospirosis in Clinically Ailing Bovine

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Received: 19 January, 2015 **Accepted:** 23 March, 2015

ABSTRACT

The purpose of this study was to carried out seroepidemiological study of leptospirosis in clinically ailing cattle and buffaloes of South Gujarat where cases of leptospirosis in human is increase every monsoon since last decade. To determine clinical pattern of bovine Leptospirosis in this area a total of 130 serum samples of cattle and buffaloes were collected randomly from different age groups of cattle and buffaloes of either sex reared in this area and tested by microscopic agglutination test (MAT) using different serovars of *Leptospira* spp. The prevalence of leptospiral antibodies was detected in 15.84 and 17.24 % in clinically ailing cattle and buffaloes, respectively. In clinically ailing seropositive cattle history of mastitis/agalactia/oligolactia was recorded in maximum number of cases (24.00 %) followed by abortion (20.00 %), fever (14.81%), repeat breeding (11.76%) and anorexia (5.88%) in different combinations. In seropositive clinically ailing buffaloes the clinical signs included mastitis (30.00%), fever (16.66 %) and abortion (14.28%). In clinically ailing cattle highest prevalence was noted in animals above 4 years of age (20.00%) followed by 1-4 years of age (4.76%) whereas in clinically ailing buffalo seropositivity was recorded only in above 4 years of age (20.00%). The most prevalent serovar pomona has been reported from different clinical conditions in both cattle and buffaloes. Based on present and few past investigation reports the sero-epidemiological condition of bovine leptospirosis that need due attention as South Gujarat region is endemic zone for leptospirosis.

Keywords: Bovine, Clinical cases, Leptospirosis, MAT, Seroprevalence

Leptospirosis is an economically important worldwide zoonotic bacterial infection of livestock caused by pathogenic spirochetes *Leptospira*. The disease causes a wide spectrum of clinical manifestations in animals and is responsible for economical losses due its reproductive impacts like mastitis, repeat breeding, early embryonic death and abortion (Vinetz, 2001). The clinical signs associated with bovine leptospirosis are variable and depend upon the infecting serovar as well as the susceptibility of the individual animals and so its presentation may varies from subclinical infection to a severe illness (Adler and de la Pena Moctezuma, 2010). Clinically bovine Leptospirosis is difficult to diagnose because the expressions are non-specific and may be easily confused with other infectious diseases (Ellis, 1984). So to know the spectrum of clinical sign of bovine leptospirosis along with infecting serovars as well as keeping in view the sporadic reports of leptospirosis in human (Shivakumar, 2008) and in animal (Balakrishnan *et al*., 2011) from South Gujarat region the present seroprevalence study of leptospirosis has been carried out.

Table 1. Prevalence of leptospirosis in clinically ailing cattle (N=101) and buffaloes (N=29).

Particulars	Cattle		Buffaloes	
	Total cases	Seropositive cases	Total cases	Seropositive cases
A. Overall prevalence				
Total Clinically ailing animals	101	16 (15.84%)	29	5 (17.24%)
Mastitis/Agalactia/ Oligolactia	25	6 (24.00%)	10	3 (30.00%)
Abortion	15	3(20.00%)	7	1(14.28%)
Repet breeder	17	2(11.76%)	2	0 (00%)
Fever	27	4(14.81%)	6	1(16.66%)
Anorexia	17	1(5.88%)	4	0(00.00%)
	$\chi^2 = 9.49$ NS(P<0.05)		$\chi^2 = 9.49$ NS(P<0.05)	
B. Age wise prevalence in clinically ailing animals				
<1 year	5	0 (00%)	1	0(00%)
1-4 years	21	1(4.76%)	3	0(00%)
>4 years	75	15(20.00%)	25	5 (20.00%)
	$\chi^2 = 5.99$ NS(P<0.05)		$\chi^2 = 5.99$ NS(P<0.05)	
C. Sex wise prevalence in clinically ailing animals				
Male	5	1(20.00%)	1	0(00.00 %)
Female	96	15(15.63%)	28	5(17.85%)
	$\chi^2 = 3.84$ NS(P<0.05)		$\chi^2 = 3.84$ NS(P<0.05)	

Note: NS-Non significant at $P < 0.05$

MATERIALS AND METHODS

Collection of blood and serum samples

A total of 130 blood/serum samples were collected randomly from clinically ailing (cattle = 101; buffaloes=29) bovines reared in villages of various districts (Navsari, Surat, Tapi, Valsad) of South Gujarat having history of anorexia, fever, abortion, repeat breeding and mastitis. The age and sex of individual animals were also recorded during sample collection.

Whole blood samples were collected from jugular vein directly in cattle and or during slaughter in some of the buffaloes in sterile 9.0 ml plain vacutainers. To obtain serum whole blood was kept in slanting position in 9.0 ml plain vacutainers until serum oozed out from clotted blood. The 9.0 ml plain vacutainers were centrifuged at 7000 rpm for 10 minutes. The straw coloured serum was collected into of 1.5 ml sterile cryovials and stored at – 20°C for carrying out Microscopic Agglutination Test (MAT).

Microscopic Aggutination Test (MAT)

All the sera collected were tested for antibodies against live antigens of *Leptospira* sp. (serovars Pyrogenes, Australis, Bankinang, Grippotyphosa, Patoc, Pomona, Icterohaemorrhagiae, Hebdomadis, Canicola, Hardjo, Bellum, Bataviae, Tarassovi, Shermani, Kaup, Hurstbridge and Javanica) following MAT at Leptospirosis Reference Laboratory, Government Medical College, Surat (Vijayachari *et al.,* 2001) and Project Directorate on Animal Disease Monitoring and Surveillance (PD-ADMAS), Bengaluru using standard procedure (WHO-OIE, 2013).

Warthin-Starry and H&E Staining Method

Tissues were preserved in 10 per cent neutral buffered formalin for at least 24-48 hours for Warthin-starry special staining technique for demonstration of leptospires in microsections (5 µ). Briefly, the steps followed were (i) Deparaffinize and hydrate the tissue section in distilled

Table 2. Distribution of *Leptospira* serovars among clinically ailing cattle and buffaloes.

Different Serovars of leptospira	Clinical expressions									
	Mastitis		Abortion		Repeat breeder		Fever		Anorexia	
	C	B	C	B	C	B	C	B	C	B
Pyrogen	1	-	-	-	-	-	-	-	-	-
Australis	1	-	-	-	1	-	-	-	-	-
Autumnalis/Bankinang	-	-	-	-	-	-	1	-	-	-
Grippotyphosa	-	-	-	-	-	-	-	-	-	-
Patoc	-	-	-	-	2	-	-	-	-	-
Pomona	3	1	2	1	2	-	4	1	-	-
Icterohaemorrhagiae	1	-	1	-	-	-	2	-	-	-
Hebdomadis	-	-	1	-	-	-	2	-	-	-
Canicola	3	1	1	-	-	-	2	-	-	-
Hardjo	3	-	3	-	-	-	2	-	-	-
Bellum	-	-	-	-	-	-	-	-	-	-
Bataviae	-	1	-	-	-	-	-	-	-	-
Sejroe	-	-	-	-	-	-	-	-	-	-
Tarassovi	-	-	-	-	-	-	-	-	-	-
Shermani	-	-	-	-	-	-	-	-	-	-
Kaup	-	-	-	-	-	-	-	-	-	-
Hurstbridge	-	-	-	-	1	-	-	-	1	-
Javanica	-	-	-	-	1	-	-	-	1	-

C=Cattle, B=Buffalo

water. (ii) Impregnate in 1% silver nitrate solution at 43ºC for 30 minutes. (iii) Then place slides on staining rack using glass rods and flood with the developing solution. Allow to develop yellow brown colouration. (iv) Wash quickly and thoroughly in hot tap water to stop reaction. (v) Dehydrate and clear the slide through 95% isopropyl alcohol, absolute isopropyl alcohol and xylene, 2 changes each for 2 minutes. (vi) And finally mount the slide with DPX (Prophet *et al.,* 1994). Hematoxylene and Eosin Staining method (H&E) was performed on paraffin embedded microsections (5 µ) of tissue according to Luna (1968).

Statistical Analysis

Chi-square test was used according to WEB AGRI STAT PACKAGE software developed by Jangam and Wadekar, ICAR research complex, Goa for statistical analysis of data (Jangam and Wadekar, 2012).

RESULTS AND DISCUSSION

The prevalence of leptospiral antibodies was detected in 15.84 and 17.24 % clinically ailing cattle and buffaloes, respectively. In clinically ailing seropositive cattle history of mastitis/ agalactia/ oligolactia was recorded in maximum number of cases (24.00%) followed by abortion (20.00%) fever (14.81%) repeat breeding (11.76%) and anorexia (5.88%) in different combinations. In seropositive clinically ailing buffaloes the clinical signs included mastitis (30.00%), fever (16.66%) and abortion (14.28%) (table 1).

Clinically ailing seropositive cases were having history of mastitis/ agalactia/ oligolactia in maximum number both in cattle (24.00%) and buffaloes (30.00%) and supported the observations of earlier worker (Sakhaee *et al.,* 2007). Our present findings related to reproductive (abortion, repeat breeding, mastitis/agalactia/oligolactia) and systemic ailments (fever and anorexia) were also in agreement with

such observations made in cattle and buffaloes in past (Mariya *et al.,* 2007; Balakrishnan *et al*., 2011).

Reproductive problems seen in cattle and buffaloes in the present study could be due to localization of leptospires in reproductive tract/uterus and supported earlier observations (Ellis and Michna, 1977; Ellis *et al.,* 1982). Further, Ellis and Michna (1977) mentioned that leptospires are demonstrated in placenta for 14-60 days and renal tubules up to 174 days. Ellis *et al.* (1982) isolated leptospires from bovine uterus having the history of impaired fertility. To support these conjuncture in the present study further studies are needed because morbid material (liver, kidneys, lung and spleen) collected from slaughter house did not revealed any leptospira like structure in microsections stained by H&E and Warthin Starry special staining technique. However, Vegad and Katiyar (2001) have mentioned about occult form of disease which is characterized by absence of clinical symptoms. Eaglesome *et al.* (1992) mentioned that subclinical/occult disease is the main form occurring in areas where leptospirosis is endemic.

In respect of age among clinically ailing cattle highest prevalence was noted in animals above 4 years of age (20.00%) followed by 1-4 years of age (4.76%) whereas in clinically ailing buffaloes seropositivity was recorded only in those above 4 years of age (20.00%). Clinical expressions among cattle were seen in more number of males (20.00%) than in females (15.63%). Conversely in buffaloes, females showed higher prevalence (17.85%) in comparison to males (0.0%).

Agrawal *et al*. (2005) reported that frequency of leptospirosis increase with increasing age of the animals. Ramin and Azizzadeh (2013) in their latest study could not observe any sex bias in respect of seropositivity. Contrary to the observations of Balakrishnan *et al*. (2011) noted significantly higher seropositivity in males than females in cattle. In brief, there is absence of unanimity about sex bias in bovine leptospirosis.

Serovars reported from clinically ailing cattle comprised of Pyrogen, Australis, Pomona, Icterohaemorrhagiae, Canicola, Hardjo, Hebdomadis, Patoc, Hurstbridge, Javanica and Autumnalis. On the other hand in buffaloes, serovars Pomona, Canicola and Bataviae were detected (Table 2).

The reported serovars in this study were in agreement with the observations made in past in ailing cattle and buffaloes from India (Sachan *et al.*, 2011; Mariya *et al.*, 2007). From Iran, Bahari *et al*. (2011), Sakhaee *et al*. (2007) and Hamali *et al*. (2012) also reported serovars like Canicola, Icterohaemorrhagiae, Pomona and Hardjo from cattle having history of mastitis, abortion, jaundice and haemoglobinuria. Further Hamali *et al.* (2012) mentioned that Pomona and Canicola serovars were involved in equal number of abortion cases while Bahari *et al*. (2011) indicated that Canicola alone was predominant in cattle having history of abortion. In cases of mastitis and abortion serovar Hardjo was mostly involved (Durfee and Allen, 1980).

Based on the world literature available on distribution of various leptospiral serovar from different countries it has been concluded that serovar distribution in clinical cases varies from countries to countries and from areas to areas in the same countries (Patel, 2014). These variations could be due to the fact that the survival of leptospires outside their hosts depends on a complex interaction involving climatic variables, soil salinity and other factors (Radostits *et al*., 2009).

ACKNOWLEDGEMENTS

The authors are highly thankful to the Dean & Principal, Vanbandhu College of Veterinary science & Animal Husbandry, Navsari Agricultural University, Navsari for providing facilities and fund. Authors also wish to thank Dr. Sumaiya Mulla, Professor & Head, Department of Microbiology, Government Medical College, Surat and The director, National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Indian Council of Agricultural Research, formerly PD_ADMAS, Hebbal, Bengaluru, Karnataka, India for their help in screening serum samples.

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