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Leptospirosis in Bovines: Haematobiochemical and Urine Analysis Studies

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ABSTRACT

Keeping in view the clinical importance of leptospirosis haematobiochemical and urine analysis were carried out on 500 (cattle-398, buffalo-102) blood/serum and 304 (cattle-232, buffalo-72) urine samples of both seropositive (cattle-51, buffalo-16) and seronegative (cattle-347, buffalo-86) animals from different district of South Gujarat region. A significant decrease in the values of PCV, MCH and MCHC was noted in seropositive group of cattle in comparison to seronegative group. Such difference among seropositive and seronegative groups could not be recorded in buffaloes. The mean values of ALT, AST and bilirubin registered an increase at significant level in seropositive cattle in comparison to seronegative. Among buffaloes, the mean values of ALT increased and total protein decreased significantly ($P < 0.05$) in seropositive buffaloes in comparison to seronegative buffaloes. On urine analyses (n=304; cattle=232, buffaloes=72) hardly any significant difference was noted in various parameter studied in either species in seropositive and seronegative animals. All the urine samples (304) collected were subjected to Dark Field Microscopy (DFM) proved to be negative for leptospires.

Keywords: Bovine, Dark field Microscopy, Haematobiochemical, Leptopsirosis, Urine analysis

Leptospirosis is an important zoonotic disease (WHO, 2000) and recently it has reemerged in India covering a number of states (Andhra Pradesh, Punjab, Orissa, Haryana, Tamil Nadu, Uttaranchal, Uttar Pradesh and Gujarat). There is a paucity of information on various haematobiochemical parameters in Leptospirosis in cattle and buffaloes both in Indian and foreign literature (Balakrishnan *et al.*, 2011). Haematobiochemical alterations and immunological reactions occurring in the body of living organisms are the initial responses which are usually interpreted by the pathologists to study the pathogenesis and diagnosis of the disease especially the infectious ones. Keeping these age old and time tested principles, in the present investigation an attempt has been made to study the various haematobiochemical parameters and urinalysis in bovine Leptospirosis.

MATERIALS AND METHODS

Collection of blood and serum samples

A total of 500 blood/serum samples were collected randomly from clinically ailing (cattle = 101; buffaloes=29) and apparently healthy (cattle = 297; buffaloes = 73) cattle and buffaloes (cattle = 398; buffaloes=102) of both sex reared in villages of various districts (Navsari, Surat, Tapi, Valsad) of South Gujarat. Whole blood samples were collected from jugular vein directly or during slaughter

of buffaloes in sterile 6.0 ml K3 EDTA and 9.0 ml plain vacutainers. Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Total Leucocyte Count (TLC) were estimated with fully auto haematology cell counter (CA-630 Blader) and Differential Leucocyte Count (DLC) was done manually.

To obtain serum, whole blood was kept in slanting position in 9.0 ml plain vacutainers until serum was extracted out of the whole blood. The 9.0 ml plain vacutainers were centrifuged at 7000 rpm for 10 minutes. The straw coloured serum was collected into two sets of 1.5 ml sterile cryo vials and aliquoted. One set was stored at – 20°C for carrying out Microscopic Agglutination Test (MAT) while the other set was used for serum biochemical parameters using Randox Kits (M/S Randox Laboratory Limited., 55 Diamond road, Crumlin, co. Antrim, BT 29 4 QY, United Kingdom) in Auto Serum Analyzer (M/S Chemwell Awareness Technology, INC.).

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) by MAT at Leptospirosis Reference Laboratory, Government Medical College, Surat (Vijayachari *et al.,* 2001) and Project Directorate on Animal Disease Monitoring and Surveillance (PD-ADMAS), Bangalore using standard procedure (WHO-OIE, 2013).

Collection of urine samples

Attempts were made to collect urine samples from all those cattle and buffaloes whose blood samples were collected but out of 500 animals, urine samples were collected from 304 animals only. At Municipal Corporation Slaughter House, Surat the urine samples were aseptically aspirated directly from urinary bladder of slaughtered buffaloes whose blood samples were collected during slaughter. While at Teaching Veterinary Clinical Complex (TVCC), College of Veterinary Science & A. H., Navsari, individual households and Panjarapol just before collection of urine the vulvar region was washed with tap water to avoid dung contamination. The midstream urine samples (30 - 50 ml) were collected into sterile plastic containers. Reagent Strips (10P) (M/S Beacon Diagnostic Pvt. Ltd., Kabilpore, Navsari) were used for qualitative determination of different parameters like nitrite, urobilinogen, protein, pH, ketone bodies, bilirubin, glucose, erythrocytes, leucocytes along with pH and specific gravity of urine samples.

Statistical analysis

Student's T-Test was carried out by using Statistical Packages for Social Science (SPSS) software (version 17).

RESULTS AND DISCUSSION

Haematological Parameters

The detail values of various haematological parameters (mean ± S.E.) analysed from seronegative and seropositive cattle and buffaloes are presented in Table 1. Among cattle, total 398 blood samples (seropositive-51, seronegative- 347) were analysed. Significant decrease ($P<0.05$) in the values of PCV, MCH and MCHC were noted in seropositive group when compared with seronegative group. Remaining other haematological parameters i.e. Hb, TEC, TLC, MCV and DLC did not show any significant variation between seronegative and seropositive groups. Whereas among buffaloes, 16 were seropositive and 86 were seronegative out of 102 serum samples (Table 1). No significant difference was observed in respect of Hb, PCV, TEC, TLC, MCV, MCH, MCHC and DLC values of seropositive buffaloes when compared with seronegative buffaloes.

In-depth study is needed to pinpoint the exact cause of the variations in the parameters studied. Though significant decrease in the values of PCV, MCH and MCHC were noted in seropositive group of cattle. Almost a comparable findings like reduction of hematocrit, hemoglobin, total leukocyte count and total erythrocytes count in induced leptospiral infection in Wistar rats was reported by Tonin *et al*. (2012). In equine Leptospirosis, leucocytosis associated with neutrophilia and lymphocytosis with decreased MCHC (Melissa *et al.,* 2010) and in canine Leptospirosis, leucocytosis associated with neutrophilia (Sophie Hedberg, 2013) have been

Table 1. Hematological parameters in cattle and buffaloes (mean ± SE)

Sr. No.	Parameters Studied	Cattle		Buffaloes	
		Seronegative (n = 347)	Seropositive (n = 51)	Seronegative (n = 86)	Seropositive (n = 16)
1.	Hb (g/dL)	9.29 ±0.09	9.25 ± 0.21 NS	10.87 ± 0.29	10.83±0.39 NS
2.	PCV (%)	26.45± 0.33	24.95 ± 0.66*	31.64±0.85	30.94± 1.49 NS
3.	TEC (xl0^6/µL)	5.81± 0.07	5.66± 0.17 NS	6.45±0.17	5.73±0.24 NS
4.	TLC (x10^3/µL)	7.821± 0.15	7.337 ± 0.356NS	9.033± 0.405	9.503± 0.624 NS
5.	MCV (fl)	44.01± 0.35	43.66± 1.06 NS	50.73± 0.68	49.54± 1.26 NS
6.	MCH (pg)	16.30±0.13	14.94± 0.48**	17.17±0.24	16.54± 0.55 NS
7.	MCHC (g/dL)	34.87± 0.15	33.67± 0.58*	34.73± 0.23	34.27± 0.60 NS
8.	DLC				
	Neutrophils %	34.28± 0.74	32.55± 1.73 NS	35.80± 1.54	33.75±5.07 NS
	Lymphocytes %	58.61± 0.77	60.96± 1.79 NS	56.78± 1.60	58.63± 5.27 NS
	Eosinophils %	4.25± 0.21	3.88± 0.31 NS	4.03± 0.28	4.13± 0.68 NS
	Monocytes %	2.65± 0.07	2.43± 0.17 NS	3.10±0.24	3.25±0.42 NS
	Basophils %	0.21± 0.02	0.18± 0.05 NS	0.26± 0.05	0.25±0.11 NS

Note: ** - Highly Significant at P<0.01 as compared to seronegative animals
* - Significant at P<0.05 as compared to seronegative animals
NS - Non Significant at P < 0.05 as compared to seronegative animals

Table 2. Biochemical parameters in cattle and buffaloes (mean ± SE)

Sr. No.	Parameters	Cattle		Buffaloes	
		Seronegative (n = 347)	Seropositive (n = 51)	Seronegative (n = 86)	Seropositive (n = 16)
1.	ALT (IU/L)	24.57±0.34	27.10±1.07*	25.99±0.67	28.13±2.90*
2.	AST (IU/L)	89.02±0.73	91.49±2.17*	90.56±1.56	93.52±4.56 NS
3.	ALP (IU/L)	69.51±1.24	73.01±3.37 NS	79.27±2.34	81.68±5.00 NS
4.	Bilirubin (mg/dL)	0.99±0.02	1.03±0.07**	0.21±0.014	0.22±0.013 NS
5.	BUN (mg/dL)	20.66±0.19	21.02±0.45 NS	20.56±0.35	20.83±0.92 NS
6.	Creatinine (mg/dL)	1.59±0.02	1.60±0.05 NS	1.60±0.04	1.62±0.08 NS
7.	Total Protein (g/dL)	7.05±0.013	6.99±0.03 NS	7.17±0.04	6.99±0.05*
8.	Albumin (g/dL)	2.81±0.02	2.76±0.06 NS	2.85±0.04	2.77±0.10 NS

Note: * - Significant at P<0.05 as compared to seronegative animals
NS - Non Significant at P < 0.05 as compared to seronegative animals

Table 3. Urine analyses (Mean ± SE) in cattle and buffaloes.

Parameters studied	Total no. of samples tested			
	Cattle (n=232)		Buffaloes (n=72)	
	Seronegative (n = 208)	Seropositive (n =24)	Seronegative (n = 61)	Seropositive (n =11)
Leucocytes (cells/µL)	Negative	Negative	Negative	Negative
Nitrate (µmol/L)	Negative	Negative	Negative	Negative
Urobilinogen (µmol/L)	Negative	Negative	Negative	Negative
Protein (g/L)	Negative	Negative	Negative	Negative
pH	8.19±	8.23±	8.05±	8.00±
	0.021	0.067 ^{NS}	0.05	0.12 ^{NS}
Blood (cells/ µL)	Negative	Negative	Negative	Negative
Specific gravity	1.017± 0.0003	1.016±0.0009^{NS}	1.016± 0.0006	1.015± 0.001 ^{NS}
Ketone Bodies (mmol/L)	Negative	Negative	Negative	Negative
Bilirubin (µmol/L)	Negative	Negative	Negative	Negative
Glucose (mmol/L)	Negative	Negative	Negative	Negative

Note: n = Number of animals,
^{NS} – Non Significant at P<0.05 in comparison to seronegative animals

reported. On the other hand Ananda *et al.* (2008) reported increased level of PCV, TLC and decreased value of haemoglobin in dogs.

Serum Biochemical Parameters

Serum from 500 animals (398 cattle and 102 buffaloes) was subjected to various biochemical parameters (Table 2). The mean values of individual parameters of seronegative (347) and seropositive (51) cattle were compared. Increased activity/level of ALT, AST and bilirubin were noted at significant level (P<0.05) in seropositive animals compared to seronegative cattle. The activity/level of parameters like ALP, BUN, creatinine, total protein and albumin did not differ significantly between seronegative and seropositive groups of cattle. Among buffaloes the mean values of ALT significantly (P < 0.05) increased and total protein (P<0.05) decreased in seropositive buffaloes when compared with seronegative buffaloes. Other biochemical parameters like AST, ALP, bilirubin, BUN, creatinine and albumin did not differ significantly between seropositive (n=16) and seronegative (n=86) groups of buffaloes.

Biochemical profile is an indicative of functional status of major vital organs such as liver and kidneys. In the present study, a significant increase in serum level of ALT, AST and bilirubin and decrease in total protein were observed. Similarly, increased levels of total bilirubin, SGOT and SGPT in bovine (Balakrishnan *et al.*, 2011), ALP, ALT, total bilirubin, direct bilirubin and creatinine in equine (Melissa *et al.*, 2010), ALP, ALT, urea and creatinine in Wistar rats (Tonin *et al.*, 2012) have been reported in different studies in past. Contrary to this Millar *et al.* (1977) could not observe any alteration in the liver function in sheep.

An increase levels of SGPT, SGOT and total bilirubin in serum were noted in seropositive animals in comparison to seronegative animals. Among ruminants SGPT activity is nonspecific but SGOT activity is indicative of liver damage (Benzamin, 1985). Similarly higher total bilirubin

level occurs in liver damage. Thus, the observations of the present study suggested varying degree of hepatic damage and supported the findings of Patel (2014). Hypoproteinaemia was recorded in seropositive buffaloes. A number of nonspecific factors like parasitism, low/ poor protein level in feed and hepatic ailment would have been responsible for hypoproteinaemia. In the absence of complete anamnesis of individual animal it would be only hypothetical to link it with leptospirosis. Biochemical parameters investigated in the present study did not suggest kidney involvement. Thus the biochemical parameters studied presently in a limited way were suggestive of hepatic damage and supported the general consensus that the hepatic damage do occur in leptospirosis.

Urine Analysis

In current study, a total 304 urine samples (cattle-232 and buffaloes-72) were analysed (Table 3). In seronegative (n=208) and seropositive (n=24) cattle, the mean urine pH values was 8.19± 0.021 (mean ± S.E.) and 8.23± 0.067 and the mean urine specific gravity value was 1.017± 0.0003 and 1.016±0.0009, respectively. Whereas in seronegative and seropositive buffaloes the mean pH values was 8.05± 0.05 and 8.00± 0.12, respectively. The mean urine specific gravity values in seronegative and seropositive buffaloes were 1.016± 0.0006 and 1.015± 0.001, respectively. Specific gravity and pH in either species did not differ significantly ($P<0.05$) in seropositive compared to seronegative animals. Whereas leukocytes, nitrite, urobilinogen, RBCs, ketone bodies, bilirubin and glucose were found to be negative both in seronegative and seropositive cattle and buffaloes.

Urine examination findings for various parameters found to be negative and insignificant difference observed in pH and specific gravity between seronegative and seropositive group of cattle and buffaloes (Table 3). However, Sophie Hedberg (2013) and Jamshidi *et al.* (2008) noted proteinuria, bilirubinuria, haematuria, pyuria, presence of granular casts and low specific gravity in clinical cases of dogs suffering from severe leptospirosis.

Dark Field Microscopy

In the present study a total 304 urine samples (cattle-232 and buffaloes-72) were screened under dark field microscope (DFM). Out of these we could not detected leptospires in any urine samples. One of the possible explanations could be discharge of very low number of leptospires in urine samples and supported the findings made by Shivaraj *et al*. (2009) who also could not detect any leptospires from 60 ovine samples (blood, tissue and urine). Contrary to our present findings a number of workers in past (Sakhaee *et al.,* 2007; Lilenbaum, *et al.,* 2008) detected leptospires under DFM from various body fluids like urine and vaginal secretion of cattle, sheep and goat.

No doubt DFM of centrifuged urine samples or other body fluids is a convenient and rapid diagnostic test but requires a skilled observer to differentiate between leptospires and other artefacts (Bolin *et al.,* 1989). On the other hand, Vijayachari *et al.* (2001) opined that DFM has low indices of accuracy due to significant numbers of false positive and false negative results but it is the method of choice for determining leptospires in cultures. Further, Sakhaee *et al.* (2007) mentioned that the success of DFM is also influenced by the number of leptospires discharged in various body fluids (urine, vaginal secretion, milk, etc.). Larger the number of leptospires discharged greater the chance of success of Dark field microscopy.

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