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Clinico-haemato-biochemical, Peritoneal Fluid and Rumen Fluid Alterations in Buffaloes with Peritonitis

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ABSTRACT

This study was designed to investigate the clinico-haemato-biochemical, rumen fluid and peritoneal fluid alteration in buffaloes with peritonitis. Buffaloes with peritonitis had anorexia, dehydration, abdominal distension, ruminal atony, pain, fever, reduced milk yield and loss of defecation. Pungent smelling peritoneal fluid was yielded on abdominocentesis. Hemato-biochemical alterations revealed absolute neutrophilia, increased levels of plasma aspartate aminotransferase, alkaline phosphatase, bilirubin, glucose, blood urea nitrogen, creatinine, globulin and fibrinogen and decreased levels of plasma sodium, potassium, calcium and chloride. Peritoneal fluid analysis showed increased total leucocyte count, neutrophil count and total protein level. Whereas, rumen liquor of diseased buffaloes had microbial inactivity, increased methylene blue reduction time, increased ammonia nitrogen and decreased total volatile fatty acids.

Keywords: Buffaloes, Haemato-biochemical, Peritonitis, Peritoneal fluid, Ruminal fluid

Peritonitis is an important economic disease of buffaloes, it causes significant reduction in milk production and deaths in bovine. It is relatively common disease in bovine caused by ingestion of penetrating metallic foreign bodies in the reticulum and rarely by haematogenous route. Buffaloes are more likely to ingest foreign bodies than small ruminants since they do not use their lips for prehension and are more likely to eat chopped feed (Mousavi *et al.,* 2007). Swallowed metallic objects such as nails or wire pieces fall directly into the reticulum or pass into the rumen and perforation of the wall of reticulum allows leakage of ingesta along with bacteria, which contaminate the peritoneal cavity; resulting in local or diffuse peritonitis (Gokce *et al.,* 2007). This syndrome is very common in India possibly due to the unorganized farming and poor management and feeding practices. Laboratory tests such as survey radiology or contrast radiography and ultrasonography of the reticulum can be helpful in distinguishing cases of peritonitis from other gastrointestinal diseases. Most of the previous studies on detailed hemato-biochemical changes in peritonitis have been established in cattle and there is paucity of literature in buffaloes. Thus, various aspects of haemato-biochemical, rumen liquor and peritoneal fluid profiles were investigated as an aid to diagnose peritonitis in buffaloes.

MATERIALS AND METHODS

Selection of animals

Out of 56 clinical cases of gastro-intestinal disorders, presented at Large Animal Clinics of Veterinary Teaching Hospital, Department of Teaching Veterinary Clinical Complex, GADVASU, Ludhiana, the present study was conducted in 6 buffaloes. All the buffaloes were examined clinically according to the methods described by Radostits *et al.,* (2000). The diagnosis of peritonitis was made on the basis of peritoneal fluid examination.

Haematological parameters

Blood samples (2 ml) were collected aseptically from jugular vein in EDTA coated vials. Haemoglobin (Hb, g/

dL), Packed Cell Volume (PCV, %), Total Leukocyte Count (TLC, $10^3\mu L^{-1}$) and Differential Leukocyte Count (DLC, % and $10^3\mu L^{-1}$) were estimated by standard methods (Jain 1986).

Blood Biochemical Analysis

For plasma separation, blood samples (10 ml) were collected aseptically from the jugular vein into heparinized vials (1:1000) and centrifuged at 3000 rpm for 10 min, within 6 hrs of collection. The following blood biochemical parameters were estimated using Bayer Diagnostic Kit with the help of an RA50 Autoanalyser: total plasma protein (TP), plasma albumin, plasma glucose, plasma BUN, plasma creatinine, plasma calcium, plasma chloride, total bilirubin, aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Inorganic phosphorus was estimated by the ammonium molybdate method of Taussky and Sharr (1953). Plasma sodium and potassium were estimated by flame photometry. Fibrinogen was estimated by the heat precipitation method (Jain, 1986). The concentration of total globulin was calculated by subtracting the albumin concentration from the total protein concentration.

Peritoneal fluid analysis

Peritoneal fluid was collected in Na_2EDTA @ 2 mg/ml containing vials using 16 gauge, 1.5 inch long hypodermic needle for cytological and biochemical studies. Peritoneal fluid samples were analysed for total leucocyte count (TLC, $10^3\mu L^{-1}$) and differential leucocyte count (DLC, %).

Rumen fluid analysis

Rumen liquor samples were collected by using a 16-gauge, 6-inch long needle from left paralumber fossa. A part of the sample was filtered through a double layer of muslin cloth and centrifuged. The supernatant was divided into two parts; one was preserved with few drops of saturated mercuric chloride solution for total volatile fatty acid (TVFA) and total ammonia-nitrogen (NH_3-N) estimation, and the other for sodium and chloride estimation. Physical and microscopic examinations were done for colour, odour, consistency, pH and protozoal motility (Garry, 2002). Rumen chloride was estimated using Bayer Diagnostic Kits (colorimetric method) with the help of an RA50 autoanalyser. Sodium was estimated by flame photometry (Hawk *et. al*., 1954). Sedimentation activity time (SAT), Methylene Blue Reduction Test (MBRT), TVFA and Total NH_3-N were assayed by standard methods as described by Annison, 1954; Convay, 1957 and Garry, 2002.

RESULTS AND DISCUSSION

All the six buffaloes diagnosed for peritonitis were females between 3-12 years of age. All cases had parturated 15 days to 2 months back and were in 2^{nd} to 5^{th} lactation. Two cases had history of inappetence, whereas four cases were anorectic. All cases had fever (>103 ^oF), reduced water intake and reduced milk yield. Defecation was absent in four cases, whereas two animals were passing constipated feces. Persistent tympany was observed only in two cases. Similar historical finding was reported by Hussain *et al.*, (2011) in cattle affected with peritonitis. Other important clinical signs included depression (100%), suspended rumination (100%), dry muzzle (50%), 6-8% dehydration (100%), distended abdomen (50%), fever (50%), increased heart rate (100%) and respiratory rate (33%), rumen atony (100%) and constipation (100%). These clinical signs may be due to involvement of the fore stomach, peri-reticular adhesions and reduced gastrointestinal motility due to diffused peritonitis. On abdomino-centesis, watery peritoneal fluid with pungent odour was obtained in five cases; whereas one case had slightly viscous peritoneal fluid. These clinical signs were almost similar to earlier reported in bovine with peritonitis (Kumar *et al.,* 2008; Athar *et al.,* 2010; Hussain *et al.,* 2011, Sadeghian *et al.,* 2011, Tharwat *et al.,* 2012).

Haematology

The haematological parameters are depicted in Table 1. Present haematological study revealed normal TLC with neutrophilia and increased ESR. High ESR appeared to be most sensitive indicator of the inflammatory reaction; it can be used as non-specific indicator of inflammation process in this species (Khan *et al.,* 1997). However, TLC is not a reliable indicator of inflammation in large animals (Coles, 1974). Relative neutrophilia and lymphopenia indicated stress induced lymphocytic destruction (Jain, 1986). In contrast to present study, Gokce *et al.* (2007) and Athar *et al.* (2010) reported high levels of PCV and TLC in bovine with peritonitis.

Biochemical analysis

The various biochemical parameters are presented in Table 1. The increased AST level in present study may be attributed to necrosis of liver due to toxaemia from peritonitis (Garry, 2002). The high ALP activity detected in peritonitis may be associated with cholestasis and thus, with the disruption of normal hepatobiliary circulation.

Table 1. Haemato-biochemical changes in buffaloes with peritonitis

Parameters	Mean± SE values (n=6)	Reference Range
Hb (gm%)	11.50±0.64	8-15
PCV (%)	39.16±2.38	24-46
TLC ($x10^3$ μL^{-1})	8.45±1.18	4-12
Neutrophils **($x10^3$ μL^{-1})**	5.12±1.79*	0.6-4
Lymphocytes **($x10^3$ μL^{-1})**	2.66±0.30	2.5-7.5
Eosinophils **($x10^3$ μL^{-1})**	0.08±0.04	0-2.4
Monocytes **($x10^3$ μL^{-1})**	0.01±0.00	0-2.4
ESR (mm in one hour)	118.33±19.90*	18-80[B]
AST (IU/L)	214.66±31.68*	78-132
ALP (IU/L)	214.83±23.1*	27-107[C]
Bilirubin (mg/dl)	2.61±0.54*	0.01-0.47 [C]
Glucose (mg/dL)	95.00±4.28*	45-75
BUN (mg/dL)	73.76±14.97*	6-27
Creatinine (mg/dL)	2.43±0.41*	1-2
TP (gm/dL)	8.81±0.19	5.7-8.1
Albumin (gm/dL)	3.38±0.26	2.1-3.6
Globulin (gm/dL)	5.43±0.34*	2.9-4.9
Fibrinogen (mg/dL)	1633.33±255.1*	200-700[A]
TP:F ratio	6.12:1	>15[A]
Calcium (mg/dL)	6.59±0.62**	9.7-12.4 [C]
Inorganic phosphorus (mg/dL)	6.64±0.31	5.6-6.5 [C]
Sodium (mmol-l^{-1})	115.16±3.52**	132-152 [C]
Potassium (mmol-l^{-1})	3.09±0.08**	3.9-5.8 [C]
Chloride (mmol-l^{-1})	81.06±2.22**	97-111 [C]

****Mean value less than reference range**

***Mean value higher than reference range**

Reference ranges: [A] from Jain (1986), [B] from Brar *et al.* (2000), [C] from Smith (2009) and others from Radostits *et al.* (2000)

The increased bilirubin level in present study may be due to liver damage as a result of toxins absorbed from rumen (Pienkowski, 1969). Impaired uptake and excretion of bilirubin due to impaired liver function, as evident from increased liver enzymes, may have resulted in increased serum bilirubin concentration. The increased bilirubin may also be due to constipation and starvation (Kaneko *et al.,* 2008).

Hyperglycaemia could be due to the glycogenolytic effect of released adrenocorticosteroids as a result of stress of peritonitis (Mann and Boda, 1966). The increased BUN and creatinine levels could be attributed to hypo-perfusion of renal system as a part of compensatory mechanism to maintain circulation in hypovolemia associated with dehydration, leading to azotemia (Kaneko *et al.,* 2008).

Hyperglobulinaemia might be due to increased synthesis of alpha, beta or gamma globulin as a result of chronic inflammation and tissue damage. In bovines, elevation of fibrinogen is a more sensitive indicator of inflammatory processes than total leukocyte count as bovines have greater capacity to produce fibrinogen in response to different stimuli in comparison to other species (Prathaban and Gnanaprakashan, 1990). The increased fibrinogen in present study may be due to tissue inflammation. The normal total plasma protein:fibrinogen ratio (TP:F) in bovines is >10 (Feldman *et al.,* 2000) and this ratio gives more realistic picture of increased fibrinogen in inflammatory conditions and rules out any alterations due to dehydration. So, the lower TP: F ratio in present study indicated marked increase in fibrinogen.

Hypocalcemia might occur in the dairy animals that had anorexia for a couple of days (Radostits *et al.,* 2000). In the present study, decreased plasma calcium level was due to prolonged anorexia. Similarly, hyponatremia, hypokalemia and hypochloremia may be due to less assimilation of feed materials as a result of long standing anorexia (Radostits *et al.,* 2000). Dezfouli *et al.* (2011) and Hussain *et al.* (2011) also observed similar results in cattle with peritonitis.

Peritoneal fluid analysis

On abdomino-centesis, watery peritoneal fluid with pungent odour was obtained in five cases, whereas one case had slightly viscous peritoneal fluid. The mean TLCs

(5.19±1.15x 10^3 μL^{-1}) was on higher side of normal range (0.30 – 5.30 x 10^3 μL^{-1}). The mean relative neutrophil count (72±3.41%) and mean total protein (4.48±0.44 gm%) values were higher than normal reference range (50% and 0.10 – 3.10 gm%, respectively) . Whereas, the mean relative lymphocyte count (28.0±3.41%) was lower than normal reference range (50%). The present peritoneal fluid analysis findings concurred with finding of Hussain *et al.* (2011).

Rumen liquor analysis

Evaluation of rumen fluid revealed characteristic alterations in physical, microbiological and biochemical parameters (Table 2). The color of rumen fluid was greenish-brown and greenish black. The consistency was watery and odour was pungent in all the cases. The protozoa motility was nil in all cases. The SAT was nil in all cases i.e., there was quick sedimentation of particulate matter. Chronic anorexia and ruminal dysfunction might have caused rumen microbial inactivity, which could have attributed to poor to nil motility of protozoa and rapid sedimentation of particulate matter (Garry, 2002). Increased MBRT indicated reduced redox potential of rumen fluid due to dysfunction of the anaerobic fermentative metabolism of the bacterial population. Increased ruminal NH3-N might be due to failure of urea recycling. No literature reports could be traced for comparison of the effect of peritonitis on ruminal liquor variables.

Table 2. Rumen fluid profile of buffaloes suffering from peritonitis

Parameters	Mean± SE values (n=6)	Reference values
pH	7.66±0.33	7
MBRT (min)	55.83±2.38*	3-6
TVFA (mEq/L)	62.16±3.96	60-120
NH_3–N (mg%)	33.33±6.29*	6-25
Na (mmol-L^{-1})	105.16±7.31	-
Chloride (mmol-L^{-1})	21.03±1.99	<30

*Mean value higher than reference range
Reference ranges: taken from Garry (2002).

In conclusion, the results of this study indicate that peritonitis causes significant clinico-haemato-biochemical alterations and rumen fluid abnormalities in buffaloes. To date, there is paucity of available published report on the rumen fluid profile in buffaloes with peritonitis; this study is the first effort to investigate the rumen fluid abnormalities in buffaloes with peritonitis.

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REFERENCES

Annison, E. F. 1954. Some observations in volatile fatty acids in sheep's rumen. *Biochem. J.* **57** : 400-05

Athar, H., Mohindroo, J. K., Kumar, A. and Randhawa, C. S. 2010. Clinical, haematobiochemical, radiographic and ultrasonographic features of traumatic reticuloperitonitis in bovines. *Indian J. Anim. Sci.,* **80**: 608–612.

Brar, R. S., Sandhu, H. S. and Singh, A. 2000. Veterinary Clinical diagnosis by laboratory method, 1st ed. Kalyani Publishers, Ludhiana.

Coles, E. H. 1974. Veterinary Clinical Pathology, 2nd ed. W.B. Saunders Co, Philadelphia, USA.

Convay, E. J. 1957. Microdiffusion analysis and volumetric error. 4th Revised ed. Crossby Lockwood and Son Lond.

Dezfouli, M. M., Lotfollahzadeh, S., Sadeghian, S., Kojouri, G. A., Eftekhari, Z., Khadivar, F. and Bashir, A. 2011. Blood electrolytes changes in peritonitis of cattle. *Comp. Cl. Path.* DOI 10.1007/s00580-011-1312-6.

Feldman, B. F., Zinkl, J. G. and Jain, N. C. 2000. Schalm's Veterinary Haematology. 5th ed. Lee and Febiger, Philadelphia.

Garry, F. B. 2002. Indigestion in ruminants. In: Smith P (ed). Large Animal Internal Medicine, 3rd ed. Mosby Inc, Philadelphia, USA.

Gokce, H. I., Gokce, G. and Cihan, M. 2007. Alterations in Coagulation Profiles and Biochemical and Haematological Parameters in Cattle with Traumatic Reticuloperitonitis. *Vet. Res. Commn.,* **31:** 529-37.

Hawk, P. B., Oser, B. L. and Summerson, W. H. 1954. Practical physiological chemistry. 14th edn. McGraw Hill Book Co., Inc. N.Y.

Hussain, S. A., Uppal, S. K., Sood, N. K., Randhawa, C. S. and Prabhakar, S. 2011. Clinical, Haemato-biochemical findings and Therapeutic management of Peritonitis in Cattle. *Intas Polivet* **12:** 283-289.

Jain, N. C. 1986. Schalm's Veterinary Haematology. Lea and Febiger, Philadelphia, USA.

Kaneko, J. J., Harvey, J. W. and Bruss, M. L. 2008. Clinical Biochemistry of Domestic Animals. 6th ed. Academic Press, London.

Khan, Z., Muhammad, G., Umar, A. and Khan, S. A. 1997. Preliminary comparison of plasma fibrinogen concentration, leucocyte no. and ESR as non-specific indicator of inflammatory condition in buffalo (*Bubalus bubalis*). *Vet. Res. Commn.,* **21**: 265-71.

Kumar, M., Mohindroo, J., Kumar, A. and Singh, S. S. 2008. Clinical, haematobiochemical and ultrasonographic features of diffuse peritonitis in bovines. *Indian J. Vet. Surg.*, **29**: 34–36.

Mann, J. G. and Boda, J. M. 1966. The influence of age of lambs on the ketogenicity and butyrate and tolerance to exogenous in vivo. *J. Agri. Sci.,* **6**7: 377–80.

Mousavi, G., Hassanpour, A., Tabrizi, B. A. and Rezaie, A. 2007. Electrocardiographic changes in buffaloes with Traumatic reticuloperitonitis. *Ital J. Ani. Sci.,* **6 (Suppl. 2):** 1029-1031.

Pienkowski, M. 1969. Estimation of the functional state of liver in cows with acid indigestion. *Annals Universitatis, Marial Curie-Skalodowska, Lublin Pulonia, Section dd* **24** : 209-22.

Prathaban, S. and Gnanaprakashan, V. 1990. Studies on plasma fibrinogen level of Indian crossbred cow in health and disease. *Indian Vet. J.*, **67:** 453–56.

Radostits, O. M., Gay, C. C., Hinchcliff, K. and Constable, P. D. 2000. Veterinary Medicine. A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats 9th ed. Saunders Elsevier, Philadelphia.

Sadeghian, S., Kojouri, G., Eftekhari, Z., Khadivar, F. and Bashiri, A. 2011. Study of blood levels of electrolytes of infected cattle with peritonitis in comparison with healthy cattle. *Intl. J. Appl. Res. Vet. Med.* **9:** 204-10.

Smith, B. P. 2009. Large Animal Internal Medicine. 4th ed. Philadelphia: Mosby Inc, St. Louis, Missouri.

Taussky, H. H. and Sharr, E. 1953. A micro-colorimetric method of determination of inorganic phosphorus. *J. Biol. Chem.*, **202:** 675–685.

Tharwat, M., Ahmed, A. F. and El-Tookhy, O. S. 2012. Chronic peritonitis in buffaloes and cattle: clinical haematological, ultrasonographic findings and treatment. *J. Ani. Vet. Adv.*, **11:** 2775-81.