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ETIOLOGY OF DIARRHOEA AMONG ADULT BUFFALOES (*Bubalus bubalis*) AND THEIR IMPACT ON HAEMATO-BIOCHEMICAL AND MINERAL PROFILE

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ABSTRACT

80 adult buffaloes suffering from diarrhea from sub-tropical and temperate zones of Jammu division were selected to establish etiology and to evaluate hemato-biochemical and mineral alterations. Clinico-haemato-biochemical,mineral and faecal evaluation was carried to relate alterations with respect to etiologies of diarrhoea. Strongyle infection was recorded among 32.5% buffaloes followed by amphistomiasis (18.75%), coccidiosis (7.5%), salmonellosis (7.5%) and balantidiasis (7.5%). 23.45% of affected buffaloes were placed under miscellaneous group as definite etiology could not be established. Season-wise, analysis revealed maximum prevalence of diarrhoea during rainy season (47.5%) followed by summer (35%) and winter (17.5%). Animals of 1 to 3 years age group had higher prevalence of coccidiosis (50%), strongyle infection (46.1%) and salmonellosis (37.5%) whereas, >6 years age group had higher prevalence amphistomiasis (66.7%) and balantidiasis (50%). Significant reduction ($P<0.05$) in hemoglobin, TEC, TPP, albumin, sodium, chloride, calcium and copper levels was recorded along with significant increase in plasma fibrinogen level among the diarrheic buffaloes. The results of blood gas analysis revealed significant ($P<0.05$) decrease in pH, pCO_2, HCO_3 and base excess and significant ($P<0.05$) increase in anion gap. Since, diarrhoea is a multifactorial disease leading to varying clinical signs, haemato-biochemical, mineral and blood gas changes which needs to be evaluated before recommending therapeutic regimen for recovery.

Keywords: *Bubalus bubalis*, buffaloes, diarrhoea, fibrinogen, haematology, mineral

INTRODUCTION

Buffaloes (*Bubalus bubalis*) in India constitute 57% of world population and contribute near half of the country's milk production and one-third of meat export (DADF 2019; APEDA 2018). Buffaloes are preferred for rearing in India due to its sustenance on poor feed and forage quality, better feed conversion efficiency, adaptability to harsh environment and high disease resistance. Diarrhoea is a multifactorial disease entity having infectious and non-infectious origin. Enteric diseases

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resulting in diarrhoea continues to have a severe economic impact observed at neonatal stage and continuing into adulthood. Strongyle is a nematode parasite of *Strongyloides* spp. Amphistomiasis is caused by digenean trematodes that in general belong to the family: Paramphistomatidae, which in their early stage are located in the small intestine and abomasum, from where they move to the rumen to finally lodge as adult trematodes. Among the protozoan diseases, coccidiosis caused by *Eimeria* spp. is an opportunistic pathogen which becomes more prevalent when animals are malnourished, the environment has poor sanitation and is overcrowded (Constable, 2015). Balantidiasis caused by *Balantidium coli* (a largest ciliate protozoan), is a natural inhabitant of the caecum, colon and rectum of apparently healthy animals, produces clinical disease under certain circumstances. Salmonellosis has assumed major importance because of its frequent out breaks in adult dairy animals and its ability to establish persistent infections, which serve as reservoirs for transmission. Keeping in view the above facts, the present study was envisaged to establish the etiologies of diarrhoea among adult buffaloes.

MATERIALS AND METHODS

Eighty buffaloes presented with the history of diarrhoea over 12 months period at university clinics and various unorganized farms were selected. Each selected animal was evaluated for clinical parameters (rectal temperature, heart rate, respiration rate, color of mucus membrane) and faecal characteristics (consistency and odour). Body condition scoring (BCS) was performed as per 1 to 5 scale reported by Rebhun (2008). Fecal samples were collected directly from the rectum of diarrhoeic animals in sterile fecal collection vials and stored at -20ºC for microscopic examination, culture and PCR. The floatation, sedimentation and direct wet smear methods were used to find the parasitic eggs and protozoan cysts (Soulsby, 2006). Fecal samples were subjected to EPG/OPG (eggs per gram/ oocysts per gram) by using Stoll's technique.

Blood samples (2 ml) were collected from diarrheic animals in EDTA coated vacutainer vials and examined for Hb, PCV, TEC and TLC using Mythic 18 Vet Hematology Analyser, Compact Diagnostic Pvt. Ltd. India and TEC and TLC using Mythic 18 Vet Hematology analyser, Compact Diagnostics Pvt. Ltd. India and DLC as per Weiss *et al.* (2021). For estimation of biochemical constituents and mineral profile, blood samples were collected by jugular venipuncture into 30 ml mineral free heparinized glass vials (dipped overnight in 2N HCL). Plasma was separated within 2 h and stored at -20°C. Total plasma protein (TPP), albumin, sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphorous (Pi) and magnesium (Mg) were estimated using diagnostic kits procured from Erba Mannheim and Agappe diagnostics Ltd. Fibrinogen was estimated by heat precipitation method (Weiss *et al*., 2021). Estimation of trace minerals was done by digesting 3 ml of plasma sample in distilled concentrated nitric acid AR (15 ml). Digestate (approx. 1 ml) was diluted to 10 ml with double glass distilled water. The concentration of micro elements *viz*. Cu, Zn and Fe were measured by Polarised Zeeman Atomic Absorption Spectrophotometer (Z-2300, HITACHI). Data was subjected to analysis of variance (ANOVA) and independent t-test using statistical software SPSS.

RESULTS AND DISCUSSIONS

Adult diarrhoeic dairy buffaloes were diagnosed with 5 different etiologies. Buffaloes, whose ethology could not be confirmed, were placed in miscellaneous group.

Strongyle infection

Overall prevalence of strongyle infection among diarrhoeic adult buffaloes was 32.5%. Sreedhar *et al*. (2009); Chaudhri *et al*. (2014) reported 7.6 and 29% prevalence of strongyle infection in diarrhoeic cattle and buffaloes from Andhra Pradesh and Haryana, respectively. Seasonwise higher prevalence recorded during rainy season (55%) which could be due to the favorable environmental conditions for the development and survival of pre parasitic stages (Table 1). Study reported maximum prevalence among 1 to 3 years (47.5%) age group which can be attributed to age related responsiveness and improper development of immunity causing higher worm fecundity and susceptibility to new infection. Sreedevi and Hafeez (2014); Vanisri *et al*. (2016) also reported higher prevalence of strongyle infection during rainy season and among similar age group. Clinical signs exhibited by buffaloes included, anorexia (90%), decreased milk production (52.5%), dullness (30%), weight loss (66.67%), pallor mucosae (60%) and moderate degree of dehydration (57.5%). Reduced digestion and absorption of nutrients from the intestines due to the damaged intestinal mucosa were the probable cause.

Fecal examination revealed watery consistency in 57.5% followed by semi liquid/ liquid (27.5%), pasty (7.5%) and hemorrhagic feces (7.5%) (Table 2). 15% positive cases had foul smelling feces. BCS 3 (moderate body condition) was recorded among 45% buffaloes whereas BCS 2 (Poor body condition) and 4 (good body condition) were observed in 35 and 20%, respectively. The average value of EPG of diarrhoeic feces was 857.69±76.08.

Hb, PCV and TEC levels among diarrhoeic buffaloes were significantly ($P<0.05$) lower whereas, the value of TLC was significantly higher (Table 3). Strongyle is recognized as active and gregarious blood sucker in stomach and intestine. The increase in TLC count may be attributed to eosinophilia and neutrophilia in response to tissue damage and inflammation. Significant ($P<0.05$) increase in eosinophil count recorded could be attributed to the hypersensitivity state resulting from penetration and migration of the parasitic larval stages into the gastric mucosal lining epithelial cells. Findings corroborate with the earlier reports of Debbarma *et al*. (2014) in cattle. Decrease in TPP and albumin can be attributed to inappetance, malabsorption, plasma losses from damaged intestinal mucosa and gastroenteropathy induced by the parasites (Radostits *et al*., 2007). The increased fibrinogen level is attributed to the inflammatory changes produced by the developing stages of the parasites.

Levels of Na, K, Cl, Ca, Pi, Mg, Cu, Zn and Fe showed significant ($P<0.05$) decrease (Table 4). Electrolytes are lost in the inflamed gastrointestinal tract due to hypersecretion of mucus and protein leakage (Abouzeid *et al*., 2010) also reported significant decrease in Ca, P, and Mg levels among strongyle infested animals. Decreased appetite and absorption along with rapid absorption and utilization of soluble carbohydrates from gut by the parasites could be contributing to these changes. Parasites cause oxidative stress and thus lipid peroxidation, which decreases the concentrations of the trace minerals that prevent destructive

effects. Gastrointestinal nematodes interfere with copper metabolism by causing an increase in the pH of abomasal and duodenal digesta which leads to reduced absorption of copper from GI tract. The reduced level of plasma iron might also be due to rapid depletion of iron stores by the bone marrow for Hb production.

Amphistomiasis

Amphistomiasis was confirmed among 18.75% of diarrhoeic adult buffaloes. The findings are also in consonance with the findings of Chaudhri *et al*. (2014). Rainy season provides conducive environment for intermediate host snail, dispersal of the infective stages wherein the metacercariae infect the host as maximum prevalence (59.09%) was recorded (Table 1). Age-wise, >6 years group had higher (63.64%) prevalence. The probable reason for higher prevalence in older animals could be poor immunological response, longer time of exposure and heavy grazing habit in submerged areas. The observations are in consonance with the findings of Sreedevi and Hafeez (2014). The clinical signs exhibited by affected buffaloes included anorexia and decreased milk production (86.36%), weight loss (81.82%), submandibular edema (50%), dullness (36.36%), congested mucosae (40.91%) and moderate dehydration (45.45%). Similar observations were reported by Chauhan *et al*. (2015); Chaudhri *et al*. (2014) in diarrhoeic buffaloes. Liver injury caused by the toxins released by the flukes along with hemato-biochemical alterations could be contributing for the clinical signs. Fecal consistency revealed watery feces among 68.18% and semi liquid/liquid feces among 31.82% animals (Table 2). 13.64% had foul smelling feces. BCS 2 was present in 50% whereas score of 3 and 4 in 31.82 and 18.18% buffaloes, respectively. Average value of EPG of diarrhoeic feces was 600±51.68.

Erythrocytic indices were significantly ($P<0.05$) lower among affected buffaloes (Table 3). Finding corroborates with the observation of Malik *et al.* (2017), Yogeshpriya *et al.* (2017); Chauhan *et al.* (2015). Loss of blood due the hemorrhages caused by the migration of immature flukes from the intestine to their predilection site rumen or migration to hepatic parenchyma and blood sucking activity of adult flukes contributes to these changes. Eosinophilia and neutrophilia were observed among affected buffaloes whereas the value of lymphocytes showed significant ($P<0.05$) decrease. Biswas *et al*. (2013) reported circulating eosinophilia to be an important feature of amphistomiasis in cattle. In contrary to this, Sinha *et al*. (2008) reported neutropenia and lymphocytosis in buffaloes suffering from amphistomiasis. TPP and albumin levels significantly decreased whereas, fibrinogen level increased significantly ($P<0.05$).

Average values of Na, Cl, Ca, Pi, Mg and Cu showed significant ($P<0.05$) decrease whereas, K level showed significant ($P<0.05$) increase (Table 4). Thakur *et al*. (2007) also reported significantly decreased level of electrolytes in cattle affected with amphistomiasis. However, Ahmed and Hassan (2007) reported non-significant changes in Na and Cl levels along with significantly increased K level in buffalo calves. Hypersecretions of mucous due to inflamed intestinal epithelium leads to hampered absorption of the electrolytes from the gut. Elevated K level among affected animals is suggestive of cellular damage or increased permeability of cell membrane. The decrease in Ca and P values coincided with the protein loss. 30 to 50% of total blood Ca in domestic ruminants is bound to proteins, including albumin (Kaneko *et al*., 2008), and thus a loss of albumin automatically

leads to a loss of Ca. Inappetance/anorexia with hypersecretions and less absoption due to damaged intestinal mucosa could justify the decreased levels.

Coccidiosis

Coccidiosis was recorded among 7.5% of diarrhoeic adult buffaloes. Haque *et al*. (2011); Reddy *et al*. (2013) reported 13.42 and 8.1% prevalence of coccidiosis in diarrhoeic dairy cattle from Punjab and Andhra Pradesh, respectively. Sreedevi and Hafeez (2014) reported 1.15% prevalence among diarrhoeic buffaloes from Andhra Pradesh. Seasonal prevalence revealed maximum occurrence in rainy season (75%) (Table 1). The findings are in accordance with the observations of Sreedevi and Hafeez, (2014); Reddy *et al*. (2013); Haque *et al*. (2011). Increased humidity and temperature favor the development of coccidian oocysts (Soulsby, 2006). Maximum prevalence was in the age Group 1 to 3 years (50%) followed by 3 to 6 years (41.67%) and >6 years (8.33%). Similar observations were reported among cattle by Reddy *et al*. (2013); Vanisri *et al*. (2016). The reason for higher prevalence of coccidiosis in 1 to 3 years age group was lack/absence of previous exposure and immunity. Clinical signs exhibited by affected buffaloes included weight loss and anorexia (83.33%), moderate dehydration (58.33%), decreased milk production (50%), dullness and pale mucosae (41.67%). Similar observations were reported by Reddy *et al.* (2013). Fecal consistency revealed hemorrhagic feces among 75% buffaloes whereas 25% had watery feces (Table 2). 66.67% positive cases had foul smelling feces whereas, 33.33% had normal fecal odour. BCS 2 was present in 66.67% affected buffaloes whereas BCS 3 and 1 (very poor body condition) were present in 25 and 8.33%, respectively. Average value of OPG of diarrhoeic feces was 1800±338.55.

Ssignificantly ($P<0.05$) lower average values of Hb and TEC and higher levels of PCV and TLC were recorded (Table 3). Finding corroborates with the observations among lambs by Kockaya and Ozsensoy (2016); Al-dujaily *et al.* (2017). Anumol *et al.* (2012) reported decreased value of PCV in coccidiosis affected goats. Haemorrhagic enteritis associated with coccidiosis was attributed to be the contributory factor. Increased PCV might be attributed to dehydration. Leukocytosis is attributed to inflammatory changes in intestine and to eosinophilia and neutrophilia observed in the present study. TPP and albumin levels decreased significantly ($P<0.05$) whereas, the fibrinogen levels increased significantly. Findings are in agreement with the observations of Anumol *et al.* (2012). Decreased absorption of nutrients from infection sites at intestinal mucosa due to damage and cell sloughing caused by coccidian was responsible for these changes.

Levels of plasma Na, Cl^-, Ca, Pi, Mg, Cu and Fe showed significant ($P<0.05$) decrease (Table 4). Bangoura and Daugschies (2007) reported significant decrease in Na and Cl^- in diarrhoeic calves suffering from coccidiosis. This reduction in the electrolyte concentrations is attributed to an elevated fecal loss due to an increased intestinal loss of water and nutrients. Similarly, Kockaya and Ozsensoy (2016) reported decreased levels of Ca, Pi and Mg in animals suffering from coccidiosis. Al-dujaily *et al.* (2017); Ahmed and Hassan (2007) reported significant decrease in Cu and Fe level in coccidiosis affected lambs and buffalo calves, respectively. Similar to our study, Kockaya and Ozsensoy (2016) reported significant decrease in the level of iron in lambs suffering from coccidiosis. Ghanem and Abd El-Raof (2005) reported non-significant decrease in Cu and significant decrease

in Zn level in coccidiosis affected lambs. The decreased level of iron level could be attributed to the bloody diarrhea and the inappetance occurring concurrently. The decreased level of zinc is probably due to the secondary bacterial infection and the malabsorption syndrome occurring subsequently to damage of intestinal mucosa and loss of surface epithelial cells and villous atrophy associated with first-generation meronts, crypt destruction and crypt hyperplasia (Taylor *et al*., 2003).

Salmonellosis

Bacterial infection was diagnosed among 7.5% diarrhoeic adult buffaloes. Earlier studies by Murugkar *et al.* (2005); Hassan (2015) recorded 9.7 and 6.9% prevalence of salmonellosis in adult diarrheic cattle, respectively. Seasonal prevalence revealed 35.71% cases during rainy and summer season and 28.57% in winter season (Table 1). Study is also in collaboration with Murugkar *et al*. (2005); Jadidi *et al.* (2012). High humidity and temperature of the study area provides congenial environment for the growth of microorganisms along with the environmental stress caused to buffaloes was contributing to this observation. Maximum prevalence was in the age Group 1 to 3 years (42.86%) followed by 3 to 6 years (35.71%) and >6 years (21.43%). Our findings are in congruence with Murugkar *et al*. (2005); Sychanh *et al*. (2013). Clinically affected buffaloes manifested weight loss (100%), anorexia (100%), dullness (71.43%), decreased milk production (50%) and severe dehydration (50%) and pale mucosae (42.86%). Faecal examination revealed haemorragic faeces in 57.14% buffaloes, watery faeces in 42.86% and foul-smelling feces in 5.71% buffaloes (Table 2). BCS 2 was present in 57.14% buffaloes whereas, BCS 3 and 1 were present in 28.57 and 14.29% animals, respectively.

Average values of Hb, PCV and TEC were significantly ($P<0.05$) lower whereas the value of TLC was significantly ($P<0.05$) higher (Table 3). Significant ($P<0.05$) increase in the neutrophil and monocyte count along with significant decrease in lymphocyte and eosinophil count was observed. The findings are in corroboration with the observation of Hassan (2015). Loss of blood through diarrhoeic feces due to immunological cell damage probably contributed for declined levels. Leukocytosis is attributed to increased neutrophil count. The release of tissue breakdown products from the inflammed tissue, activates colony stimulating factors, which in turn lead to enhanced release of neutrophils from bone marrow to blood (Weiss *et al*., 2021). Lymphocytopenia could have resulted from acute inflammation, focal necrosis, and focal depletion of lymphocytes from lymph node and spleen. Increased monocyte count could be attributed to body's response to either bacteremia or endotoxemia as tissue lesions of salmonellosis have numerous macrophages which are derived from monocytes. Eosinopenia observed could be attributed to release of steroids into the circulation in affected animals (Weiss *et al*., 2021).

The average values of TPP and albumin showed significant ($P<0.05$) decrease whereas the value of fibrinogen showed significant ($P<0.05$) increase (Table 4). Salmonellosis causes severe fibrinopurulent necrotizing enteritis leading to intestinal protein loss. Na, K, Cl-, Ca, Mg and Cu levels of plasma showed significant ($P<0.05$) decline among affected animals (Table 4). The findings are in substantiation with the observation of santos *et al*. (2002); Hassan (2015) who reported significant decrease in Na, K and Cl- levels. Inflammation of the intestinal wall along with increased vascular permeability and loss of epithelial integrity in salmonella infected animals progresses to necrosis

Table 1. Age-wise and Seasonal prevalence of different etiologies of diarrhoea.

Group	Age-wise			Season-wise		
	1-3 years	3-6 years	>6 years	Summer (Mar-Jun)	Rainy (Jul-Oct)	Winter (Nov-Feb)
Strongyle infection (n=26)	12 (46.1)	10 (38.5)	4 (15.4)	8 (30.8)	14 (53.8)	4 (15.4)
Amphistomiasis (n=15)	1 (6.7)	4 (26.7)	10 (66.7)	3 (20.0)	9 (60.0)	3 (20.0)
Balantidiasis (n=6)	1 (16.7)	2 (33.3)	3 (50.0)	2 (33.3)	3 (50.0)	1 (16.6)
Salmonellosis (n=8)	3 (37.5)	3 (37.5)	2 (25.0)	3 (37.5)	3 (37.5)	2 (25.0)
Coccidiosis (n=6)	3 (50)	2 (33.3)	1 (16.7)	0 (0)	4 (66.7)	2 (33.3)
Miscellaneous (n=19)	5 (26.3)	7 (36.8)	7 (36.8)	12 (63.1)	5 (26.3)	2 (10.5)
Total (n=80)	25 (31.2)	28 (35.0)	27 (33.7)	28 (35.0)	38 (47.5)	14 (17.5)

Figures in parenthesis refer to percentage.

Table 2. Evaluation of fecal consistency and odour in relation to etiology among adult diarrhoeic animals.

Group	Fecal consistency				Fecal odour		EPG/OPG
	Pasty feces	Semi liquid to liquid feces	Watery feces	Hemorrhagic and/or with tissue in feces	Foul smelling	Normal	
Strongyle infection (n=26)	2 (7.7)	7 (26.9)	15 (57.7)	2 (7.7)	4 (15.4)	22 (84.6)	857.69±76.08
Amphistomiasis (n=15)	0 (0)	5 (33.3)	10 (66.7)	0 (0)	2 (13.3)	13 (86.7)	600±51.68
Balantidiasis (n=6)	2 (33.3)	3 (50.0)	1 (16.7)	0 (0)	0 (0)	6 (100.0)	416.67±47.72
Salmonellosis (n=8)	0 (0)	0 (0)	3 (37.5)	5 (62.5)	7 (87.5)	1 (12.5)	-
Coccidiosis (n=6)	0 (0)	0 (0)	1 (16.7)	5 (83.3)	4 (66.7)	2 (33.3)	1800±338.55
Miscellaneous (n=19)	6 (31.5)	8 (42.1)	5 (26.3)	0 (0)	9 (47.4)	10 (52.6)	-
Total (n=80)	10 (12.5)	23 (28.7)	35 (43.7)	12 (15.0)	26 (32.5)	54 (67.5)	-

Figures in parenthesis refer to percentage.

Table 3. Hematological parameters of diarrhoeic buffaloes with varying etiologies.

Hematology	Control (n=6)	Strongyle positive (n=26)	Amphistomiasis positive (n=15)	Balantidiasis positive (n=6)	Salmonellosis positive (n=8)	Coccidiosis positive (n=6)
Hemoglobin (g/dl)	11.48±0.17^{a}	8.31±0.09^{b}	8.64±0.12^{b}	9.43±0.33^{b}	7.99±0.16^{b}	8.27±0.14^{b}
Packed Cell Volume (%)	35.78±0.22^{a}	25.14±0.29^{b}	25.09±0.16^{b}	36.83±0.25^{b}	24.76±0.25^{b}	38.41±0.20^{b}
Total Erythrocyte count (×10^{6}/µl)	7.38±0.09^{a}	4.68±0.08^{b}	5.71±0.12^{b}	5.98±0.26^{b}	5.43±0.13^{b}	5.35±0.11^{b}
Total Leucocyte count (×10^{3}/µl)	11.12±0.22^{a}	15.61±0.11^{b}	11.40±0.01^{a}	12.8±0.97^{a}	14.49±0.17^{b}	15.75±0.18^{b}
Lymphocytes (%)	59.17±1.40^{a}	57.0±0.67^{a}	55.4±0.48^{b}	58±0.58^{a}	55.88±0.64^{b}	54.67±0.84^{b}
Neutrophils (%)	32.67±1.20^{a}	33.65±0.52^{a}	35.2±0.51^{b}	33.83±0.95^{a}	36.25±0.59^{b}	35.33±1.80^{a}
Monocytes (%)	2.83±0.31^{a}	2.62±0.18^{a}	2.4±0.27^{a}	1.83±0.40^{a}	4.13±0.40^{b}	2.83±1.08^{a}
Eosinophils (%)	4.17±0.31^{a}	5.46±0.28^{b}	5.8±0.20^{b}	5.17±0.17^{b}	2.5±0.33^{b}	5.83±0.17^{b}
Basophils (%)	1.17±0.17^{a}	1.27±0.09^{a}	1.2±0.11^{a}	1.16±0.16^{a}	1.25±0.16^{a}	1.33±0.21^{a}

Different superscripts ^{a,b} indicate significant difference within row at $P<0.05$.

Table 4. Biochemical and mineral status of diarrhoeic buffaloes with varying etiologies.

Electrolytes and Minerals	Control (n=6)	Strongyle positive (n=26)	Amphistomiasis positive (n=15)	Balantidiasis positive (n=6)	Salmonellosis positive (n=8)	Coccidiosis positive (n=6)
Biochemical parameters						
Total Protein (g/dL)	7.2±0.06^{a}	6.38±0.04^{b}	6.5±0.15^{b}	6.22±0.31^{b}	5.95±0.22^{b}	6.22±0.14^{b}
Albumin (g/dL)	3.55±0.06^{a}	2.86±0.07^{b}	3.13±0.09^{b}	2.98±0.09^{b}	2.83±0.08^{b}	2.92±0.09^{b}
Globulin (g/dL)	3.65±0.10^{a}	3.52±0.08^{a}	3.31±0.09^{a}	3.23±0.26^{a}	3.12±0.23^{a}	3.30±0.20^{a}
Fibrinogen (mg/dL)	329.5±22.64^{a}	458.54±21.15^{b}	503.93±29.27^{b}	410.67±4.31^{b}	675.5±30.28^{b}	765.50±32.57^{b}
Electrolyte and mineral status						
Na (mEq/L)	144.23±3.43^{a}	133.21±1.60^{b}	128.65±1.47^{b}	130.9±1.65^{b}	129.54±2.13^{b}	131.87±2.10^{b}
K (mEq/L)	4.93±0.16^{a}	4.01±0.10^{b}	5.19±0.02^{b}	5.37±0.12^{a}	3.95±0.15^{b}	5.13±0.14^{a}
Cl (mEq/L)	104.83±2.97^{a}	95.67±1.50^{b}	96.27±1.35^{b}	93.32±2.45^{b}	95.2±1.64^{b}	95.10±2.19^{b}
Ca (mg/dL)	8.73±0.15^{a}	7.57±0.10^{b}	7.64±0.11^{b}	7.68±0.48^{a}	7.95±0.17^{b}	7.97±0.14^{b}
Pi (mg/dL)	5.68±0.13^{a}	4.77±0.10^{b}	5.04±0.10^{b}	4.87±0.44^{a}	5.56±0.13^{a}	4.97±0.13^{b}
Mg (mg/dL)	1.9±0.14^{a}	1.43±0.10^{b}	1.35±0.10^{b}	1.2±0.29^{a}	1.21±0.11^{b}	1.23±0.12^{b}
Cu (µg/dL)	89.17±3.27^{a}	79.32±1.22^{b}	76.59±1.60^{b}	79.02±2.07^{b}	77.50±1.96^{b}	78.82±2.32^{b}
Zn (µg/dL)	107.43±4.77^{a}	98.57±1.12^{b}	106.93±1.32^{a}	98.95±5.34^{a}	106.69±3.54^{a}	97.87±11.34^{a}
Fe (µg/dL)	133.88±5.42^{a}	95.18±1.29^{b}	118.89±10.18^{a}	114.97±11.51^{a}	92.22±16.77^{a}	87.39±2.21^{b}

Different superscripts ^{a,b} indicate significant difference within row at $P<0.05$.

of uppermost mucosa along with loss of intestinal villi/ crypts structures contributed for such changes. Santos *et al.* (2002); Tsolis *et al.* (2000) also recorded decreased levels of Ca, Pi and Mg in salmonella affected diarrhoic calves.

Balantidiasis

Protozoal disease was diagnosed in 7.5% of diarrhoeic adult buffaloes. Seasonal prevalence revealed maximum occurrence in rainy season (43.75%) followed by summer (37.5%) and winter (18.75%) (Table 1). Age-wise >6 years age group had high (56.25%) prevalence followed by 3 to 6 years (31.25%) and 1 to 3 years (12.5%). The details of the clinical, haemato-biochemical, and mineral alterations observed among buffaloes during balantidiasis are published earlier (Singhal *et al*., 2019).

Among the 80 adult diarrheic buffaloes, 19 animals (23.75%) were placed into miscellaneous group. 14 animals had a history of sudden change in diet which probably contributed for diarrhoea and got corrected after some modifications in their daily diet. On the basis of herd mortality, persistent fever, no response to antibiotics and absence of parasites in fecal samples, 5 diarrhoeic buffaloes were suspected of viral diarrhoea.

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