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Research Article

PREVALENCE OF BALANTIDIASIS AMONG DIARRHOEIC ADULT DAIRY ANIMALS OF NORTH WEST HIMALAYAN REGION AND ITS IMPACT ON HEMATO-BIOCHEMICAL AND MINERAL PROFILE

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ABSTRACT

One hundred forty seven adult (>1 year age) dairy animals (67 cattle and 80 buffaloes) suffering from diarrhoea from sub-tropical and temperate zones of Jammu division were screened for prevalence of balantidiasis. The overall prevalence of 10.88 per cent (14.93% in crossbred cattle and 7.5% in buffaloes) was recorded. Hematology revealed significant ($p<0.05$) decrease in Hb and TEC along with significant increase in PCV level and eosinophil count amongst affected animals. Significant ($p<0.05$) decrease in total plasma protein and albumin level along with significant ($p<0.05$) increase in the levels of fibrinogen were recorded. Electrolyte and mineral analysis revealed significant ($p<0.05$) decrease in the levels of Na, Cl and Cu. Therapeutic trial conducted on the affected animals revealed 72% and 100% efficacy of metronidazole and secnidazole, respectively.

Keywords: *Balantidium coli*, Cattle, Diarrhoea, Fibrinogen, Haematology, Mineral

Balantidiais caused by ciliate protozoa *Balantidium coli* is associated with diarrhoea in dairy animals (Islam *et al.*, 2000 and Randhawa *et al.*, 2010). The disease is endemic and prevalent worldwide, mostly prevalent in subtropical and tropical regions (Roy, 2007). *Balantidium coli* a natural inhabitant of the caecum, colon and rectum of apparently healthy animals, produces clinical disease under certain circumstances. The clinical features are manifested by loose feces to watery persistent foetid diarrhoea with dehydration, loss of body condition and appetite, retarded growth and reduced production of the dairy animals. The organism has public health importance and is considered to be the largest protozoal parasite of human. Nitro heterocyclic compounds can be used as therapeutic agents in the treatment of balantidiasis. Limited literature highlighting hemato-biochemical and mineral alterations during balantidia infection in cattle and buffaloes is available. Therefore, this study was undertaken to study the prevalence, hemato-biochemical, mineral alterations in balantidiasis affected adult dairy animals in North West Himalayan Region.

MATERIALS AND METHODS

One hundred forty seven adult (>1 year of age) dairy animals (67 crossbred cattle and 80 buffaloes) presented with the history of diarrhoea over the period from July 2017 to June 2018 were selected for the study. Each animal was evaluated for its faecal characteristics (consistency and odour), clinical parameters (rectal temperature, heart rate, respiration rate, colour of mucus membrane and hydration status. Body condition scoring (BCS) was performed as per 1 to 5 scale adopted by Rebhun (2008). Faecal samples collected directly from the rectum of diarrhoeic animals were examined by Stoll's ova counting technique to determine the count of cysts or trophozoites as described by Soulsby (1982).

Blood samples (2ml) were collected from balantidiasis positive animals (cattle-10; buffaloes-6) and 12 healthy animals (cattle-6; buffaloes-6) in EDTA coated vacutainer vials and examined for Hb, PCV, TEC and TLC using Mythic 18 Vet Hematology analyser, Compact Diagnostics Pvt. Ltd. India and DLC as per Weiss and Wardrop (2010). For estimation of biochemical constituents and mineral profile, blood samples were collected by jugular venipuncture into 30 ml stoppered mineral free heparinised glass vials (dipped overnight in 2N HCL). Plasma was separated within 2-3 hours by centrifugation at 3000 rpm for 15 minutes and was stored at -20 °C. Total plasma protein (TPP), albumin, sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphorus (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 and Wardrop, 2010). Estimation of trace minerals was done by digesting 3 ml of plasma sample in distilled concentrated nitric acid AR (15ml). Digestate (approx. 1-2 ml) was diluted to 10 ml with double glass distilled water. The concentrations of micro elements *viz*. Cu, Zn and Fe were measured by Polarised Zeeman Atomic Absorption Spectrophotometer (Z-2300, HITACHI).

To study therapeutic efficacy, 12 dairy animals suffering from balantidiasis were equally divided into two groups. Group-I (n=6) animals were treated with secnidazole @10 mg/kg body weight orally single dose whereas, Group-II (n=6) animals were given metronidazole @ 10mg/kg body weight orally. Faecal and blood samples from both the groups were collected on day 0

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(pre-treatment), 7th and 14th (post treatment) for CPG count and haematological, biochemical, electrolyte and mineral analysis. The therapeutic efficacy of the drugs was calculated as described by Varady *et al.* (2004).

$$\% \text{ CPG reduction} = \frac{\text{Pre treatment CPG - Post treatment CPG}}{\text{Pre treatment CPG}} \times 100$$

Data was subjected to analysis of variance (ANOVA) and independent t-test using statistical software SPSS.

RESULTS AND DISCUSSION

Out of 147 (67 cattle and 80 buffaloes) adult diarrhoeic dairy animals screened, 10.88% (14.93% cattle and 7.5% buffaloes) were found positive for balantidiasis. Similar prevalence (17%) among diarrhoeic bovines from Bidar, Karnataka was reported earlier by Patil *et al.* (1998). However, Bilal *et al.* (2009), Roy *et al.* (2011) and Hassan (2015) reported prevalence rate of 25 per cent (Lahore, Pakistan), 45.0 per cent (Mymensingh, Bamgladesh) and 7.5 per cent (Punjab, India) among various South East Asian countries, respectively. The variations observed in the prevalence rates could be due to difference in the geographical locations/ topography and climatic conditions as the present study area falls under subtropical and temperate agro-climatic zones having mean height above mean sea level (MSL) ranging from 300 to 1200 meter besides sample size, selection of samples, technique of sample examination, management and nutritional factors of the animals. Average values of CPG among cattle and buffaloes suffering from diarrhoea due to balantidiasis were 450±45.37 and 416.67±47.72, respectively.

Season-wise, maximum prevalence was recorded during the rainy season (43.75%; July-Oct) followed by summer (37.5%; March-June) and winter (18.75%; Nov-Feb). Roy *et al.* (2011) and Wisesa *et al.* (2015) also reported higher prevalence during rainy season. High humidity, heavy rain fall and higher chance of contamination of feeds could enhance the rate of infection. However, Islam *et al.* (2000) reported highest prevalence among buffaloes from Bangladesh during summer season.

Animals >6 years age had higher prevalence i.e., 56.25% (9/16) followed by 31.25% (5/16) and 12.5% (2/16) among 3-6 and 1-3 years age groups, respectively. Islam *et al.* (2000) and Roy *et al.* (2011) have also reported higher prevalence in animals >5 years age. Old age animals are under various stress conditions (pregnancy, lactation, parturition, transportation of goods etc.) and lowered immune response could be responsible for the higher prevalence of balantidiasis.

Table 1

Clinical and haemato-biochemical parameters of balantidiasis positive diarrhoeic animals

Parameters	Cattle		Buffalo	
	Control group(n=6)	Affected group(n=10)	Control group(n=6)	Affected group(n=6)
Clinical parameters				
Rectal temperature (°F)	101.73±0.24	101.38±0.28	100.13±0.30	101.10±0.41
Heart rate (beats/minute)	70.00±2.39	67.90±1.87	69.67±2.87	71.67±1.14
Respiratory rate (/minute)	24.83±1.64	23.60±1.07	26.00±1.06	23.50±1.26
Haematological parameters				
Hb (g/dl)	11.87^{a}±0.13	9.66^{b}±0.31	11.48^{a}±0.17	9.43^{b}±0.33
PCV (%)	35.65^{a}±0.21	36.67^{b}±0.20	35.78^{a}±0.22	36.83^{b}±0.25
TEC (×106/µl)	7.67^{a}±0.10	6.08b±0.17	7.38^{a}±0.09	5.98^{b}±0.26
TLC (×103/µl)	11.85±0.10	12.88±0.51	11.12±0.22	12.80±0.97
Lymphocytes (%)	58.83±0.95	57.50±0.67	59.17±1.40	58.00±0.58
Neutrophils (%)	33.50±1.18	33.90±0.61	32.67±1.20	33.83±0.95
Monocytes (%)	2.67±0.33	2.40±0.40	2.83±0.31	1.83±0.40
Eosinophils (%)	3.83^{a}±0.31	5.10^{b}±0.38	4.17^{a}±0.31	5.17^{b}±0.17
Basophils (%)	1.17±0.17	1.10±0.10	1.17±0.17	1.16±0.16
Biochemical parameters				
Total Protein (g/dL)	7.05^{a}±0.10	6.27^{b}±0.19	7.20^{a}±0.06	6.22^{b}±0.31
Albumin (g/dL)	3.45^{a}±0.10	3.09^{b}±0.04	3.55^{a}±0.06	2.98^{b}±0.09
Globulin (g/dL)	3.60±0.10	3.16±0.16	3.65±0.10	3.23±0.26
Fibrinogen (mg/dL)	323.83^{a}±38.98	460.70^{b}±18.71	329.50^{a}±22.64	410.67^{b}±4.31

Values bearing different superscripts within a row differ significantly at $p<0.05$.

Clinical evaluation of the affected animals revealed mild (6-8%) dehydration in 56.25% and moderate (8-10%) among 43.75% animals. Congested mucous membrane was observed in 12.5% animals. Fecal odour was normal however, consistency evaluation revealed semi liquid/ liquid feces among 56.25%, whereas 37.5 and 6.25% had pasty and watery feces, respectively. Body weight loss was reported among 87.5% with BCS 2 and 3 in 43.75% and BCS 4 in 12.5% animals. 75% affected animals were anorectic and had decreased milk production. Dull behavior was recorded in 18.75% of affected animals. Taylor *et al.* (2013) attributed clinical signs to the lesions inflicted on the mucosal enterocytes and due to hyaluronidase enzyme secreted by the pathogen. Randhawa *et al.* (2010), Roy *et al.* (2011) and Jamil *et al.* (2015) reported similar observations in animals suffering from balantidiasis.

Hb and TEC levels of balantidiasis positive dairy animals were significantly ($p<0.05$) lower whereas the values of PCV and eosinophil count were significantly ($p<0.05$) higher (Table 1). TPP and albumin levels showed significant ($p<0.05$) decrease whereas fibrinogen showed significant ($p<0.05$) increase than control group. Bauri *et al.* (2012) reported significant decrease in Hb, PCV and TEC among pigs suffering from balantidiasis. Chimpanzees suffering from balantidiasis showed mild anaemia and leukocytosis (Miller, 2003). The decreased Hb and TEC levels were probably due to *B. coli* induced blood loss via intestinal hemorrhages and suppressive effect of toxic substances secreted by the protozoa. Increased PCV levels among affected animals could be attributed to dehydration status. Jamil *et al.* (2015) and Khan *et al.* (2013) reported similar haematological changes among balantidiasis affected small ruminants and donkeys. Significant ($p<0.05$) increase in eosinophil count could be due to the protozoal penetration into the gut wall and induction of eosinopoiesis by toxins produced by protozoa. The findings are in unison with the observations of Bauri *et al.* (2012) who recorded significant increase in eosinophil count among balantidiasis affected pigs.

TPP and albumin levels of affected animals showed significant ($p<0.05$) decline whereas fibrinogen was significantly elevated. The mucosal cells damage due to release of toxins of the small and large intestine with penetration of protozoa might have contributed to the reduction of albumin level leading to hypoproteinemia. The inflammatory changes in the intestinal mucosa contribute to the rise in acute phase proteins. Meager literature citing biochemical alterations during balantidiasis is available. Miller (2003) reported mild hypo-albuminaemia in *B. coli* positive chimpanzees.

Significant ($p<0.05$) decline in the levels of Na, Cl, and Cu among balantidiasis positive animals was recorded (Table 2) and could be attributed to the hypersecretory activity of intestinal mucosa along with malabsorption by the villi enterocytes in the intestine under influence of toxins produced by *B. coli*. The reduction in copper level can be attributed to loss of appetite and decreased absorption from inflamed intestinal mucosa.

Results of therapeutic trial revealed significant ($p<0.05$) decrease in CPG values from day 0 to day 14 among Group I (CPG per cent reduction on day 7 and 14 post-treatment were 84.38 and 100, respectively) and day 0 to day 7 among Group II animals (CPG per cent reduction on day 7 and 14 post-treatment were 52 and 72, respectively, Table 3).

Treatment with secnidazole resulted in significant ($p<0.05$) increase in Hb and TEC values from day 0 to 14 whereas metronidazole treated group showed non-significant ($p>0.05$) increase. Treatment resulted in significant ($p<0.05$) decrease in PCV and TLC values among Group-I and non-significant ($p>0.05$) decrease in Group-II (Table 3). TPP and globulin values showed non-

Table 2

Electrolyte and mineral status of balantidiasis positive diarrhoeic animals

Parameters	Cattle		Buffalo	
	Control group (n=6)	Affected group (n=10)	Control group (n=6)	Affected group (n=6)
Sodium (mEq/L)	$146.32^{a}\pm1.18$	$131.34^{b}\pm2.06$	$144.23^{a}\pm3.43$	$130.90^{b}\pm1.65$
Potassium (mEq/L)	4.70±0.15	4.91±0.16	4.93±0.16	5.37±0.12
Chloride (mEq/L)	$103.43^{a}\pm1.49$	$94.20^{b}\pm1.60$	$104.83^{a}\pm2.97$	$93.32^{b}\pm2.45$
Calcium (mg/dL)	8.83±0.13	7.80±0.38	8.73±0.15	7.68±0.48
Phosphorus (mg/dL)	5.85±0.10	4.97±0.42	5.68±0.13	4.87±0.44
Magnesium (mg/dL)	1.82±0.12	1.55±0.14	1.90±0.14	1.20±0.29
Copper (µg/dL)	$86.74^{a}\pm3.54$	$78.91^{b}\pm1.52$	$89.17^{a}\pm3.27$	$79.02^{b}\pm2.07$
Zinc (µg/dL)	110.17±5.24	98.61±5.33	107.43±4.77	98.95±5.34
Iron (µg/dL)	124.96±7.73	113.27±12.05	133.88±5.42	114.97±11.51

Values bearing different superscripts within a row differ significantly at $p<0.05$.

Table 3

Effect of treatment on CPG and haemato-biochemical parameters in balantidiasis affected animals

Parameters	Group-I (n=6)			Group-II (n=6)		
	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment	
	Day 0	Day7	Day 14	Day 0	Day7	Day 14
CPG	533.33^{b}±49.43	88.33^{a}±30.73	0	416.67^{b}±47.72	200.00^{a}±36.51	116.67^{a}±30.73
Haematological parameter						
Hb (g/dl)	9.32^{a}±0.34	9.48^{a}±0.24	11.65^{b}±0.29	9.78^{a}±0.34	9.82^{a}±0.34	10.85^{a}±0.20
PCV (%)	36.57^{b}±0.23	36.15^{ab}±0.50	35.38^{a}±0.33	36.80^{a}±0.25	36.48^{a}±0.33	36.20^{a}±0.22
TEC (×106/µl)	5.92^{a}±0.21	6.20^{a}±0.10	7.63^{b}±0.08	6.12^{a}±0.30	6.25^{a}±0.33	6.58^{a}±0.22
TLC (×103/µl)	12.97^{b}±0.08	12.50^{b}±0.19	11.75^{a}±0.15	12.50^{a}±0.88	12.35^{a}±0.87	11.90^{a}±0.34
Lymphocytes (%)	57.67±1.02	57.83±0.65	58.33±0.88	58.17±0.54	57.83±0.48	58.33± 1.21
Neutrophils (%)	34.67±0.71	34.50±0.67	33.67±1.05	33.17±0.87	33.00±0.73	32.67± 1.05
Monocytes (%)	2.17±0.60	2.50±0.62	2.83±0.31	2.00±0.37	2.83±0.40	3.00 ± 0.48
Eosinophils (%)	4.33±0.42	4.00±0.37	3.83±0.17	5.50±0.34	5.17±0.31	4.83± 0.17
Basophils (%)	1.17 ±0.17	1.17±0.17	1.33±0.21	1.17±0.17	1.17±0.17	1.17±0.17
Biochemical parameter						
Total Protein (g/dL)	6.35±0.29	6.58±0.14	6.98±0.06	6.27±0.31	6.43±0.34	7.03±0.11
Albumin (g/dL)	3.08^{a}±0.05	3.28^{ab}±0.09	3.42^{b}±0.07	3.02^{a}±0.07	3.27^{ab}±0.15	3.48^{b}±0.09
Globulin (g/dL)	3.23^{a}±0.23	3.30^{a}±0.09	3.60^{a}±0.06	3.25^{b}±0.26	3.17^{ab}±0.22	3.55^{a}±0.09
Fibrinogen (mg/dL)	435.83^{b}±16.52	380.00^{ab}±8.56	330.50^{a}±39.51	421.50^{b}±10.55	353.00^{ab}±27.39	338.00^{a}±24.14

Values bearing different superscripts within a row differ significantly at $p<0.05$.

significant ($p>0.05$) increase in both the treated groups. Significant ($p<0.05$) increase in albumin level in both the treated groups was observed. Fibrinogen level declined significantly ($p<0.05$) in both treated groups. Na, Cl and Pi levels increased significant ($p<0.05$) among Group-I animals (Table 4). Thus, secnidazole proved to be the more potent drug compared to metronidazole in the treatment of balantidiasis. The findings corroborate with the earlier observations of Bilal *et al.* (2009) who reported 7.5 and 87.5 per cent comparative efficacy of metronidazole and secnidazole in cattle suffering from balantidiasis, respectively. The findings are also in agreement with Khan *et al.* (2013) who reported 89.51 per cent efficacy of secnidazole on day 14 (post-medication) in donkeys suffering from similar condition. Similarly, Jamil *et al.* (2015) reported higher efficacy of secnidazole in sheep (86.66%) and goats (93.75%).

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Table 4

Effect of treatment on electrolyte and mineral status of balantidiasis affected animals

Parameter	Group-I (n=6)			Group-II (n=6)		
	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment	
		Day7	Day 14		Day7	Day 14
Na (mEq/L)	128.32^{a}±2.29	137.78^{b}±1.46	145.03^{c}±1.52	130.90^{a}±1.65	136.80^{a}±4.63	141.05^{a}±4.36
K (mEq/L)	5.02±0.18	4.98±0.10	4.78±0.18	5.37±0.12	5.17±0.12	5.20±0.14
Cl (mEq/L)	93.32^{a}±2.45	101.32^{ab}±2.79	102.68^{b}±1.49	93.32^{a}±2.45	96.88^{a}±1.63	99.35^{a}±2.36
Ca (mg/dL)	7.62±0.63	7.87±0.27	8.78±0.09	7.68±0.46	8.43±0.28	8.63±0.13
Pi (mg/dL)	4.32^{a}±0.43	5.03^{ab}±0.12	5.77^{b}±0.09	4.87^{a}±0.44	5.58^{a}±0.27	5.53^{a}±0.16
Mg (mg/dL)	1.35±0.18	1.42±0.15	1.77±0.10	1.20±0.29	1.52±0.25	1.73±0.17
Cu (µg/dL)	78.19±2.39	80.53±1.11	84.88±2.23	79.02±2.07	80.38±3.49	84.90±4.18
Zn (µg/dL)	94.26±7.94	97.96±6.01	109.05±4.34	98.95±5.34	103.99±5.82	104.89±4.54
Fe (µg/dL)	109.54±3.80	111.71±3.60	121.43±5.21	114.97±11.51	118.95±11.28	130.59±6.50

Values bearing different superscripts within a row differ significantly at $p<0.05$

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