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Research Article

HAEMATO-BIOCHEMICAL AND MINERAL ALTERATION IN ADULT DIARRHOEIC DAIRY ANIMALS INFECTED WITH SALMONELLOSIS

VINIT SINGHAL, RAJIV SINGH*, RAJESH AGRAWAL, N. SHARMA and A. TIKOO
Division of Veterinary Medicine, Faculty of Veterinary Sciences and Animal Husbandry,
SKUAST-Jammu, R.S. Pura-181102, India

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ABSTRACT

To study the prevalence of salmonellosis in adult dairy animals and its impact on haemato-biochemical parameters, 147 (67 cattle and 80 buffaloes) diarrhoeic adult (>1yr age) dairy animals were screened. Microbial isolation attempted from faecal samples revealed presumptive *Salmonella* colonies from 21 samples of which 14 were confirmed on the basis of biochemical tests and PCR assay, thus revealing overall prevalence of 9.52 per cent. Haemato-biochemical and mineral estimation was done in affected animals and compared with control animals. Hematology revealed significant ($p<0.05$) increase in TLC, neutrophil and monocyte count while Hb, PCV, TEC, lymphocyte and eosinophil count were significantly ($p<0.05$) decreased. Significant ($p<0.05$) increase in fibrinogen while total plasma protein (TPP) and albumin were significantly ($p<0.05$) decreased. Affected animals showed significant ($p<0.05$) decrease in electrolytes (Na, K and Cl) and mineral (Ca, Mg and Cu) plasma levels. Isolates were subjected to antibiotic sensitivity testing which revealed *Salmonella* isolates to be highly sensitive towards ciprofloxacin, enrofloxacin, gentamicin and kanamycin and refractory to ampicillin/cloxacillin, cefixime and ceftriaxone. Study concluded that diarrhoea caused by salmonellosis induces anaemia, leucocytosis with neutrophilia, hypoproteinemia, hyponatremia, hypokalemia, hypochloremia and is of great clinical significance.

Keywords: Biochemical, Buffaloes, Diarrhoea, Fibrinogen, Haematology, Mineral, Salmonella

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Salmonellosis causes diarrhoea in all age groups of dairy animals. It has assumed major importance because of its frequent outbreaks in adult dairy animals and its ability to establish persistent infections, which serve as reservoirs for transmission (Yan *et al.*, 2003). Organism is ubiquitous in the environment and can survive for extended periods of time, which contributes to the transmission and re-infection of animals. Outbreaks of disease are costly for producers because of increased mortality and treatment costs. Although several studies reported fecal shedding of *Salmonella* in dairy farms, however, little information is available regarding the clinical appraisal during diarrhoea in adult dairy animals associated with *Salmonella* infection. Present study reports the prevalence alongwith clinico-haemato-biochemical and mineral alterations in adult diarrhoeic dairy animals infected with salmonellosis.

MATERIALS AND METHODS

A total of 147 (67 cattle and 80 buffaloes) adult dairy animals (>1 year age) presented with the history of diarrhoea at university clinic, during the period from July 2017 to June 2018 were selected. Each animal was evaluated for its fecal characteristics (consistency and odour). The clinical parameters recorded were rectal temperature (°F), heart rate, respiration rate, colour of mucous membrane and per cent dehydration (Radostitis *et al.*, 2007). In order to monitor body condition, body condition scoring (BCS) was performed as per the scale (ranging from 1 to 5) adopted by Rebhun (2008). Blood samples in EDTA were analyzed for determinations of haematological alterations by estimating complete blood count using Mythic 18 Vet hematology analyser, Compact Diagnostics Pvt. Ltd. For biochemical and mineral estimation, plasma was separated from blood collected in mineral free heparinized glass vials and stored at -20°C till further use. Total plasma protein (TPP), albumin, sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphorus (Pi) and magnesium (Mg) were estimated from plasma samples using Erba diagnostic kits. Fibrinogen was estimated by heat precipitation method (Schalm *et al.*, 1996). Estimation of trace minerals *viz.*, Cu, Zn and Fe was done by digesting 3 ml of plasma sample in distilled concentrated nitric acid AR (15ml). Digested (approx. 1-2 ml) was diluted to 10 ml with double glass distilled water and values recorded using Polarised Zeeman Atomic Absorption Spectrophotometer (Z-2300, HITACHI).

Fecal samples collected in sterile swab were pre-enriched using 1% buffered peptone water at 37°C for 24-48 hours, thereafter cultured on MacConkey Agar at 37°C for 24-48 hours. Cultures were transferred to tetrathionate broth and incubated at 37°C for 24-48 hours. The enriched samples were further cultured on XLD agar for 24-48

*Corresponding author: rajivrajiv101@gmail.com

hours and then to Brilliant Green Agar for 24-48 hours. The isolates were subjected to morphological, biochemical (Indole test, methyl red test, voges- proskauer, citrate utilization, catalase, oxidase and triple sugar iron test) and antimicrobial drug susceptibility/resistance by disc diffusion technique (Bauer *et al.*, 1996). Molecular identification of *Salmonella* was done as per Lin and Tsen (1996) using 16SF1 (Primer sequence 5'-3: 'TGTTGTGGTTAA TAACCGCA) and 16SIII (Primer sequence 5'-3': CACAAATCCATCTCTGGA) oligonucleotides. Data were analyzed using analysis of variance (ANOVA) and independent t-test with statistical software SPSS 20.

RESULTS AND DISCUSSION

Presumptive *Salmonella* colonies were isolated from 21 out of 147 fecal samples collected from diarrhoeic animals. Biochemical tests confirmed 14 out of 21 isolates (14/147; 6/67 cattle and 8/80 buffaloes) as colonies were negative for indole, voges-proskauer and oxidase test whereas, positive for methyl red and citrate utilization. Molecular identification for detecting genus specific 16S rRNA gene by PCR confirmed 14 isolates (Fig. 1). Thus, study indicated 9.52% prevalence of *Salmonella* infection. The findings are in congruence with the observations of Hassan (2015) and Murugkar *et al.* (2005) who reported 6.9 and 9.7% prevalence of salmonellosis in adult diarrhoeic animals from Punjab and northeast India, respectively. Sato *et al.* (2011) and Jadidi *et al.* (2012) reported 34.2 % and 3.2 % prevalence of salmonellosis in diarrhoeic cattle from California and Iran, respectively. The variation in the prevalence may be due to method of sample collection, difference in geographical location, immune status and epidemiology of the organism.

Season-wise analysis revealed 35.71% (5/14) prevalence in each rainy (July-October) and summer (March-June) seasons and 28.57% (4/14) in winter (November-February). Murugkar *et al.* (2005) and Jadidi *et al.* (2012) reported higher prevalence during rainy season. High humidity and temperature provide a congenial environment for the growth of pathogenic microorganisms and the environmental stress caused on dairy animals could be the contributing factor (Radostitis *et al.*, 2007).

Age-wise, maximum prevalence (42.86%) was among 1-3 years age group followed by 3-6 (35.71%) and >6 years (21.43%). Younger age group has higher susceptibility towards infections due to poor immunological response. Non-significant ($p>0.05$) changes in the body temperature, heart and respiratory rate were recorded . Clinically 50, 28.57 and 21.43% of positive cases had severe (10-12%), moderate (8-10%) and mild (6-8%) dehydration, respectively. The status of mucous membrane revealed that 42.86 and 35.71% animals had congested and pale mucous membranes, respectively and 21.43% had normal mucous membrane. The clinical signs exhibited by animals with salmonellosis included weight loss (100%), anorexia (100%), dullness (71.43%) and decreased milk production (50%). Fecal examination revealed that 57.14% animals had hemorrhagic whereas, 42.86% had watery feces. 85.71% animals with salmonellosis had foul smelling feces. BCS score of 1, 3 and 2 was observed among 14.29, 28.57 and 57.14 % of diarrhoeic animals, respectively. The findings of the present study are in unison with those of Hassan (2015).

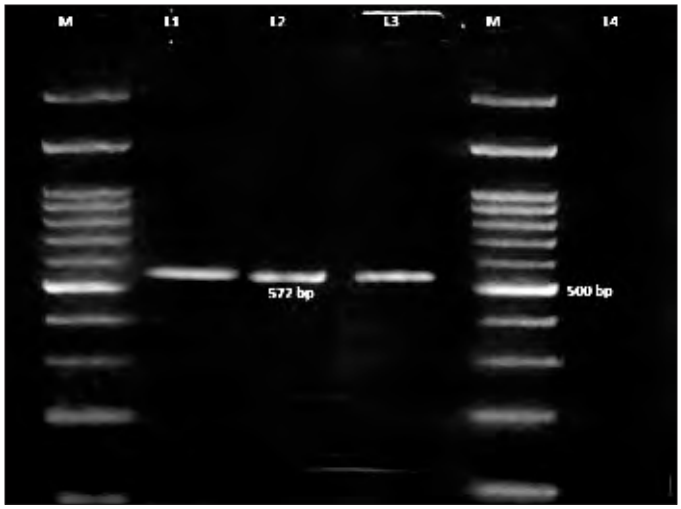

Fig. 1. Amplification of 16S rRNA gene region of *Salmonella* by PCR.
- ❒ Lane M 100 bp DNA ladder
- ❒ Lane 2-3 Positive isolates Size (572bp)
- ❒ Lane 1 Positive control
- ❒ Lane 4 Negative control.

The average values of Hb, PCV and TEC were significantly ($p<0.05$) lower whereas the TLC was significantly ($p<0.05$) higher among affected animals (Table 1). The findings are in corroboration with the observations of Hassan (2015) who reported significant decrease in Hb, PCV and TEC and significant increase in TLC in dairy animals suffering from salmonellosis. In contrary to present study, Santos *et al.* (2002) reported increased levels of Hb, PCV and TEC in bovines suffering from salmonellosis. The reason for decreased Hb, PCV and TEC is probably the blood loss via diarrhoeic feces as a result of immunological cell damage. Leukocytosis is attributed to increased neutrophil count in salmonellosis affected dairy animals. The values of neutrophils and monocytes showed significant ($p<0.05$) increase whereas the value of lymphocytes and eosinophils showed significant ($p<0.05$) decrease than control group. The findings are in

Table 1

Hemato-biochemical, electrolyte and mineral alterations in diarrhoeic dairy animals infected with salmonellosis

Parameters	Buffaloes		Cattle	
	Control (n=6)	Salmonellosis positive (n=8)	Control (n=6)	Salmonellosis positive (n=6)
Hematology				
Hemoglobin (g/dl)	11.48±0.17[a]	7.99±0.16[b]	11.87±0.13[a]	7.68±0.15[b]
Packed cell volume (%)	35.78±0.22[a]	24.76±0.25[b]	35.65±0.21[a]	23.65±0.45[b]
Total erythrocyte count ($\times 10^6/\mu l$)	7.38±0.09[a]	5.43±0.13[b]	7.67±0.10[a]	5.68±0.22[b]
Total leucocyte count ($\times 10^3/\mu l$)	11.12±0.22[a]	14.49±0.17[b]	11.85±0.10[a]	14.57±0.19[b]
Lymphocytes (%)	59.17±1.40[a]	55.88±0.64[b]	58.83±0.95[a]	57.17±0.60[a]
Neutrophils (%)	32.67±1.20[a]	36.25±0.59[b]	33.50±1.18[a]	36.67±0.61[b]
Monocytes (%)	2.83±0.31[a]	4.13±0.40[b]	2.67±0.33[a]	2.83±0.31[a]
Eosinophils (%)	4.17±0.31[a]	2.5±0.33[b]	3.83±0.31[a]	2.17±0.31[b]
Basophils (%)	1.17±0.17[a]	1.25±0.16[a]	1.17±0.17[a]	1.16±0.17[a]
Total Protein (g/dL)	7.2±0.06[a]	5.95±0.22[b]	7.05±0.10[a]	5.97±0.09[b]
Albumin (g/dL)	3.55±0.06[a]	2.83±0.08[b]	3.45±0.10[a]	2.63±0.09[b]
Globulin (g/dL)	3.65±0.10[a]	3.12±0.23[a]	3.60±0.10[a]	3.33±0.03[a]
Fibrinogen (mg/dL)	329.5±22.64[a]	675.5±30.28[b]	323.83±38.98[a]	754.33±41.57[b]
Electrolytes and Minerals				
Sodium (mEq/L)	144.23±3.43[a]	129.54±2.13[b]	146.32±1.18[a]	129.20±1.46[b]
Potassium (mEq/L)	4.93±0.16[a]	3.95±0.15[b]	4.70±0.15v	3.97±0.12[b]
Chloride (mEq/L)	104.83±2.97[a]	95.2±1.64[b]	103.43±1.49[a]	90.48±0.98[b]
Calcium (mg/dL)	8.73±0.15[a]	7.95±0.17[b]	8.83±0.13[a]	7.77±0.14[b]
Phosphorus (mg/dL)	5.68±0.13[a]	5.56±0.13[a]	5.85±0.10[a]	5.82±0.09[a]
Magnesium (mg/dL)	1.9±0.14[a]	1.21±0.11[b]	1.82±0.12[a]	1.18±0.10[b]
Copper (µg/dL)	89.17±3.27[a]	77.50±1.96[b]	86.74±3.54[a]	77.01±1.07[b]
Zinc (µg/dL)	107.43±4.77[a]	106.69±3.54[a]	110.17±5.24[a]	110.09±1.84[a]
Iron (µg/dL)	133.88±5.42[a]	92.22±16.77[a]	124.96±7.73[a]	88.02±18.23[a]

Different superscripts a,b indicate significant difference within row at $p<0.05$

Table 2

Per cent sensitivity and resistance of salmonella isolates to various antimicrobials

Antibiotic	Sensitivity (%)
Amoxicillin/clavulanic acid	28.57 (4)
Ampicillin/cloxacillin	0
Chloramphenicol	42.86 (6)
Cefixime	0
Ceftriaxone	0
Ciprofloxacin	100 (14)
Doxycycline	71.43
Enrofloxacin	100
Gentamicin	100
Kanamycin	100

congruence with the observations of Hassan (2015). Santos *et al.* (2002) reported lymphopenia and neutropenia in bovines suffering from salmonellosis. Activation of colony stimulating factors by tissue breakdown products released from inflammed tissues, could be responsible for enhanced neutrophil release from bone marrow to central pool (Schalm *et al.*, 1996). The monocytosis could have been in response to either bacteremia or an endotoxemia. The probable reason for eosinopenia is the release of steroids into the circulation (Schalm *et al.*, 1996).

Decreased TPP and albumin levels in diarrhoeic dairy animals observed could be attributed to severe intestinal protein loss associated with the profound fibrinopurulent necrotizing enteritis in which a large amount of protein-rich effusion enters the intestinal lumen. A decreased concentration of albumin parallel to that of total protein indicated that the loss of protein was nonselective (Santos *et al.*, 2002). Inflammatory reactions due to fibrinopurulent enteritis among *Salmonella* affected animals could be contributing factor (Thomas, 2000).

The levels of Na, K, Cl, Ca, Mg and Cu showed significant ($p<0.05$) decrease whereas Pi, Zn, and Fe showed non-significant ($p>0.05$) changes (Table 1). The findings of the present study are in substantiation with the observations of Santos *et al.* (2002) who reported decreased level of Na, K and Cl in diarrhoeic bovines

suffering from salmonellosis. The study is in conjuction with the findings of Hassan (2015). Increased vascular permeability accompanying inûammation along with loss of intestinal epithelial integrity due to necrosis of the uppermost mucosa with loss of discernible villi or crypt structures led to such alterations. Santos *et al.* (2002) and Tsolis *et al.* (2000) also reported decreased level of Ca, P and Mg in salmonellosis. Hassan (2015) reported decreased Ca and P level in adult *Salmonella* affected diarrhoeic dairy animals. Decreased level of Ca and Mg is attributed to the electrolyte loss during *Salmonella* induced diarrhoea (Tsolis *et al.*, 2000).

Antibiotic sensitivity assay revealed high sensitivity (100%) of *Salmonella* isolates for ciprofloxacin, enrofloxacin, gentamicin and kanamycin followed by doxycycline (71.43%), chloramphenicol (42.86%) and amoxicillin/ clavulanic acid (28.57%). All isolates were resistant against ampicillin/cloxacillin, cefixime and ceftriaxone (Table 2). In accordance to our findings, McEvoy *et al.* (2003) reported *Salmonella* resistance against ampicillin and cefixime and Berge *et al.* (2006) reported resistance against ampicillin with moderate sensitivity for tetracycline. Abdullah *et al.* (2013) reported that most of the *Salmonella* isolates were susceptible to gentamicin and ciprofloxacin. In contrary to the present findings, Jadidi *et al.* (2012) reported that *Salmonella* isolates showed highest sensitivity towards ceftriaxone and ampicillin. Antibiotic resistance patterns vary among different farms, regions, states and countries depending upon the type of organisms and use of antibiotics in a particular area; therefore, antimicrobial sensitivity is suggested before institution of treatment. Study concluded that *Salmonella* induced diarrhoea causes significant ($p<0.05$) haemato-biochemical, electrolyte and mineral alterations.

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