Clinical and Haematological Studies in Buffaloes Suffering from Diarrhoea

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ABSTRACT

Diarrhoea in buffaloes was most commonly encountered in age group ranged between calf hood to thirteen years and majority of affected animals were more than four years old in their third to fourth lactation with drastic reduction in milk production therefore, causing great economic loss to animal owners. Duration of illness was found to be one day to one year and 57.5% cases were chronic patients. Associated clinical findings revealed slight variations in rectal temperature but an appreciable increase in pulse rate and respiration rate, congested mucus membrane (67.5%) and decreased or absent rumen motility in most of the cases as compared to healthy controls during the course of investigation. Hematological findings in diseased animals revealed significant (p < 0.05) anemia, lower hemoglobin, higher DLC showed neutrophilia along with lymphopenia and absence of any haemoprotozoan parasite.

Keywords: Buffaloes, diarrhoea, clinical findings, haematology

Diarrhoea has been extensively studied in calves; however the research work on diarrhoea in adult buffaloes is scarce. Disorders of gastro-intestinal tract are very important causes of morbidity and mortality in young buffalo calves and cause a great economic loss to buffalo owners (Roy *et al.*, 1997; Rathore, 1998; Saxena *et al.*, 2002). Diarrhoea requires immediate attention of Veterinarians failing which there may be fluid and electrolyte imbalance leading to irreparable damage to vital organs because of dehydration and acid-base imbalance and in severe cases may lead to death of animal (Nadig *et al.*, 2000). Such animals may not be properly diagnosed, undergo incomplete therapy and there may be development of resistance to some drugs (Radostits *et al.*, 2007 and Devkate *et al.*, 2010).

MATERIALS AND METHODS

Clinical and haematological examination

The study was aimed for Clinico-haematological and biochemical studies on buffaloes suffering from diarrhoea on 40 clinical cases brought to Teaching Veterinary Clinical Complex (TVCC), LUVAS Hisar as well as in and around Hisar. For comparison samples were also collected for the apparently healthy control group (n=6).

Detailed history along with symptoms was recorded in each clinical case. History included species, lactation no., stage of lactation and milk yield, duration of illness, appetite, water intake, general appearance of the animal, frequency of defaecation, appearance of faeces and its abnormal contents. Clinical observations included rectal temperature, pulse rate, respiration rate, appearance of mucous membranes and ruminal movements.

Ten ml of blood samples were collected aseptically using EDTA / heparin coated sterile vials from jugular vein of the affected as well as healthy control group animals for estimation of Haemoglobin, TLC, DLC, Haemoprotozoan by standard laboratory procedure (Weiss and Wardrop, 2011). Blood sample was also collected from these animals and serum was separated by centrifuge at 3000 rpm for 15 min. These serum samples were used for analysis of blood glucose, ALT, AST, Ca, P, Mg, Na, K, Cl, BUN, creatinine, ALP, total cholesterol, TGs, Total protein, serum albumin, serum globulins and A/G ratio by standard procedure using fully automated Random Access Clinical Chemistry



Analyzer (EM 180TM Erba Mannheim – Germany) was employed for estimation of biochemical parameters using kits procured from M/s Transasia Biomedical Limited. Fully automated Easylyte® expand Na/K/Cl/Ca/Li analyzer was also employed for estimation of serum Na, K, Cl and Ca parameters using kits procured from same Manufacturer.

Faecal test

Faecal samples collected per-rectally for identification of parasitic ova/oocyst etc. were then processed for microscopic examination by floatation and sedimentation technique (Dryden et al., 2005).

Johnin test

An extract of the culture of Mycobacterium avium subspecies *paratuberculosis* was used in a cutaneous delayed hypersensitivity test for Johne's disease. Positive test revealed a thickening and edema at the site of injection 48 hours later.

Statistical method: The data was analysed statistically using Independent Students' t-test and Duncan test for multiple comparisons using computer software SPSS 16.0.

RESULTS

History of clinical cases

Detailed history of clinical cases included species, lactation no., stage of lactation and milk yield, duration of illness, appetite, water intake, general appearance of the animal, frequency of defaecation, appearance of faeces and its abnormal contents. The age - wise incidence was recorded to be highest in the age group of 4 years (57.50%) followed by 1-4 years (35%) and < 1 year (7.50%) for all 40 clinical cases. Most of the affected animals i.e. 17 (42.5%) were in 3rd-4th lactation followed by 13 (32.5%) animals were in 1st-2nd lactation and seven (17.5%) animals having no lactation while three (7.5%) animals were in $> 4^{\text{th}}$ lactation. Most of the affected animals *i.e.* 25 (62.5%) were non- pregnant while about 37.5% animals were pregnant, out of which 12 (30%) animals were having 1 month and above pregnancy status and three (7.5%) animals were having upto 1 month pregnancy

status. Most of affected animals i.e. 23 (57.5%) animals were having 2 months and above post parturient duration while five (12.5%) had 1 - 2 months and same number of animals had less than 1 month post parturient duration and seven (17.5%) animals were having no parturition history. Most of affected animals i.e. 23 (57.5%) animals were in chronic stage of diarrhoea while 10 (25 %) animals were in subacute stage and seven (17.5%) animals were in acute stage of diarrhoea. Twenty (50%) animals had defaecation frequency more than 10 times per day while 13 (32.5%) animals were having upto 5 times per day and seven (17.5%) animals had 6-10 times per day. Fifteen (37.5%) animals were having inappetance while 12 (30%) animals were having anorexia, rest of the animals i.e.13 (32.5 %) had normal feed intake.

Most of affected animals i.e. 23(57.5%) animals were having less water intake and two (5%) animals were having no water intake while two (5%) animals were having increased water intake and rest of the animals i.e. 13 (32.5%) normal water intake. Out of 40 animals, six (15%) animals were having hematochezia and two (5%) animals had melena. Out of 40 animals, 38 (95%) animals were having foul smell in their faeces and 25 (62.5 %) animals had bubbles in their faeces. There was more than 50% decrease in milk yield of 24 (60%) animals while rest of the animals i.e. 16 (40%) had less than 50% decrease. Twelve (30%) animals were having slight subnormal temperature while 9 (22.5%) were having mild fever and rest of the animals 19 (47.5%) had normal temperature. Most of the affected animals i.e. 27 (67.5%) animals were having congested mucus membrane and four (10%) animals were having pale mucus membrane.

Rest of the animals i.e. nine (22.5 %) had normal mucus membrane. Most of the affected animals i.e. 26 (65%) animals were having absence of ruminal motility while 14 (35%) animals showed reduced ruminal motility. Five (12.5%) animals were having positive for parasitic ova / oocyst while 35 (87.5%) animals had absence of parasitic ova/oocyst. Two (10%) animals were found positive for Johnin test and rest of the animals 18 (90%) were found negative for Johnin test.

Clinical observations

The clinical status of six healthy and 40 diarrhoea affected animals was observed and presented in table 1.

Hhemato-biochemical in buffaloes during diarrhea

The following clinical observations were made i.e. rectal temperature (°F), respiration rate (per min), pulse rate (per min) and ruman motility (per 2 min). Parameters of healthy animals have been compared with diseased animals.

Table 1: Vital clinical parameters of buffaloes affected with diarrhoea (n=40) in comparison to healthy buffaloes (mean \pm S.E.)

Clinical Parameters	Control group (n=6)	Diarrhoeic buffaloes (n=40)
Temperature (°F)	101.66 ± 0.17	101.73 ± 0.23
Respiration rate	(100.8-102.1) $15.33^{b} + 0.98$	(99.0-103.8) $27.02^{a} + 0.67$
(per min)	(8-19)	(15-38)
Pulse rate (per min)	$55.66^b\pm2.41$	$68.77^a \pm 1.69$
	(40-65)	(42-88)
Rumen Motility	$2.66^{a}\pm0.31$	$0.82^b \pm 0.13$
(per 2 min)	(2-3)	(0-3)

Means bearing different superscripts in a row differ significantly (p< 0.05). Figures in parenthesis indicate range of the parameter

Mean temperature of healthy animals was 101.66 ± 0.17 °F. Mean values of temperature of all diarrhoeic buffaloes was 101.73 ± 0.23 °F. Value of mean of respiration rate for healthy animals was recorded as 15.33 ± 0.98 breaths / min. Mean values of respiration rate of diarrhoeic buffaloes was 27.02 ± 0.67 breaths/min. The pulse rate in healthy animals was recorded 55.66 ± 2.41 per min. Mean values of pulse rate of diarrhoeic buffaloes was 68.77 ± 1.69 per min. The rumen motility of healthy animal was observed as $2.66 \pm 0.31/2$ min. Mean values of rumen motility of diarrhoeic buffaloes was 0.82 ± 0.13 /2min.

Haematological Observations

The mean values of Haemoglobin (Hb), Total Leukocyte Count (TLC), Differential Leukocyte Count [Neutrophils (N), Lymphocytes (L), Eosinophils (E), Monocytes (M) and Basophils (B)] was estimated in diseased as well as in healthy control animals.

Estimated Hb of healthy animal was reported as 13.03 \pm 0.76 g/dl. Mean values of Hb for diarrhoeic buffaloes was 9.62 \pm 0.24 g/dl. The TLC for healthy animals was estimated 6.84 \pm 0.55 10³/ µl. Mean values of TLC for diarrhoeic buffaloes was 12.67 \pm 0.33 10³/ µl. Mean neutrophil percentage value for healthy animals was found

to be 29.50 ± 1.76 . Mean percentage values of neutrophil for diarrhoeic buffaloes was 57.87 ± 2.02 . The lymphocyte percentage value for healthy animals was found to be 64.16 ± 1.93 . Mean values of lymphocyte percentage for diarrhoeic buffaloes was 37.32 ± 1.97 .

Table 2: Hematological parameters of buffaloes affected with diarrhoea (n=40) in comparison to healthy buffaloes (mean \pm S.E.)

Haematological Parameters	Control group (n=6)	Diarrhoeic buffaloes (n=40)
Hb (g/dl)	$13.03^{a} \pm 0.76$ (10.10 -14.40)	9.62 ^b ± 0.24 (7.40-14.20)
TLC (1000/µl)	$6.84^{b} \pm 0.55$ (5.44-7.82)	12.67 ^a ± 0.33 (6.43-19.10)
N (%)	29.50 ^b ±1.76 (26-42)	$57.87^{a} \pm 2.02$ (32-81)
L (%)	64.16 ^a ±1.93 (37-72)	37.32 ^b ± 1.97 (12-65)
M (%)	$2.33^{b} \pm 0.21$ (2-6)	$3.35^{a} \pm 0.38 (0-10)$
E (%)	2.66 ± 0.33 (2-4)	$2.10 \pm 0.52 \; (0\text{-}5)$
B (%)	Nil	$0.50 \pm 0.16 \ (0\text{-}1)$

Means bearing different superscripts in a row differ significantly (p< 0.05). Figures in parenthesis indicate range of the parameter

Percentage mean value of monocyte for healthy animal was reported to be 2.33 ± 0.21 . Mean values of monocyte for diarrhoeic buffaloes was 3.35 ± 0.38 . The eosinophil percentage value for healthy animals was found to be 2.66 ± 0.33 . Mean values of eosinophil percentage for diarrhoeic buffaloes was 2.10 ± 0.52 . The basophil percentage value for healthy animals was found to be nil. Mean values of basophil for diarrhoeic buffaloes was 0.50 ± 0.16 .

DISCUSSION

History and clinical observations

The age group of animals affected ranged between calfhood to 13 years and majority of affected animals were more than four years old in their third to fourth lactation. Duration of illness was found to be 1 day - 1 year and majority of cases were chronic patients. Therefore, chronic diarrhoea may also be affecting the reproduction in the buffaloes. In order to avoid the worsening of enteric haemorrhage, COX inhibitors should not be used in the treatment of such



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cases. Milk production was reduced remarkably (more than 50%), therefore, causing great economic loss to the animal owners. Our study is supported by Faheim (1999) who reported that diarrhoeic faeces of calves ranged from whitish to yellow or greenish colour and tinged with blood or mucus in some cases. Calves showed loss of appetite with dehydration and death. Alsaad et al. (2012) reported that calves with BVD showed anorexia (89.39%), profuse watery diarrhoea mixed with mucus/blood (78.78%), dehydration (78.78%), erosive lesions in the oral cavity (65.15%), salivation (60.6%), erosive lesions on muzzle (48.48%), petechial and ecchymotic haemorrhages of the visible mucosa (40.9%) and lacrimation (31.81%).

Similar observations were reported by several workers. Kumar et al. (2010) reported that calves showed dullness, depression with lethargy and anorexia having semisolid to watery faeces with offensive odour, yellowish white in colour and sometimes blood stained. These observations were similar to the earlier reports (Bellamy and Acres, 1979; Radostits et al., 2007). Yellowish white diarrhoeic faeces might be due to high content of salt particularly bicarbonate (Ward, 1976) with heavy secretion of water in intestinal lumen (Szancer, 1980). Devkate et al. (2010) reported that diarrhoeic animals exhibited signs of anorexia, dullness, depression, weakness and suspended rumination. The conjunctivae were normal to pale or congested, consistency of faeces varied from loose to semisolid pasty, sometimes blood tinged suggestive of haemorrhage in the gastro-intestinal tract.

Affected animals were showing subnormal to followed by an appreciable increase in pulse rate and respiration rate, congested mucus membrane and decreased or absent rumen motility in most of the cases during the course of investigation. Similar types of findings were consistently observed by various other researchers. Alsaad et al. (2012) reported that significant increase was detected in body temperature, respiratory and heart rates in diseased calves affected with BVD in comparison with controls. Ramkumar (2012) observed rectal temperature, pulse and respiration rates were within the normal range. Malik et al. (2013) reported non-significant difference in body temperature and significant (P <0.05) difference in pulse rate and respiration rate in diarrhoeic calves in comparison to apparently healthy calves.

Hematology

Hematological values of diarrhoeic buffaloes in the present study exhibited appreciably lower hemoglobin, higher TLC while in DLC showed neutrophilia along with lymphopenia and absence of any haemoprotozoan parasite as compared to healthy control animals. Lower hemoglobin was probably as a result of inappetence or anorexia leading to poor body condition. Leukocytosis might have occurred due to normal reaction of body defense mechanism against infection and also due to dehydration and haemoconcentration (Kumar et al., 2010). These finding had close agreement with the findings of Singh and Singh (1973). Similarly, Sridhar et al. (1988) also reported neutrophilia and lymphopenia in scouring calves.

The marked neutrophilic response with lymphopenia is characteristic of acute bacterial enteritis. Monocyte and eosinophils in diarrhoeic calves did not show any significant change (Malik et al., 2013). Arafa et al. (2008) reported that significant decrease (p<0.05) in total RBCs counts, Hb values, MCH and MCHC than the values of healthy ones. He found that these results might be due to coexistence of clinical marginal anemia with deficiency of minerals. Some different findings were reported by Asati et al. (2008) showing significantly higher (p<0.05) mean value of Hb, PCV, TLC, lymphocytosis and neutropenia and no significant differences in eosinophil, monocyte and basophil percentage. The similar observations were made by other researchers (Fernandes et al., 2009; Sheikh et al., 2012; Malik et al., 2013; Mahboob et al., 2014). Alsaad et al. (2012) showed no significant difference in TEC and Hb, however PCV values were significantly higher in diseased calves along with leukopenia, lymphopenia and thrombocytopenia than in controls. Kumar et al. (2010); Sridhar et al. (1988) reported that mean hemoglobin value in diarrhoeic calves were found to increased, which might be due to haemoconcentration because of dehydration and fluid loss.

Diarrhoea in majority of adult buffaloes occurs in subacute and chronic form resulting in anorexia/inappetence, reduced/absent rumen motility, appreciably increased pulse rate, respiration rate, anaemia, neutrophilic lymphopenic leucocytosis and huge economic losses in terms of production.

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