

Research Article

Haematological Profile and Blood Chemistry in Diarrhoeic Calves affected with Collibacillosis

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ABSTRACT

Among the multiple causes, diarrhoea in neonatal calves is one of the most challenging clinical syndromes encountered by large animal veterinary practitioners due to multiple etiologies and its severity. Moreover, the preexisting factors and the intricacy of calf diarrhoea due to bacterial pathogens like *E. coli*, enforced to conduct research work on some important clinical aspects of the disease with the objectives to study haematological and haemochimistry alterations in positive cases of collibacillosis. Isolation and then identification of bacterial pathogens and confirmation of *E. coli* strains associated with calf diarrhoea along with their clinical examination of common clinical parameters such as rectal temperature, pulse rate and respiratory rate revealed non-significant difference in body temperature; and significant difference in pulse rate and respiration rate in diarrheic calves in comparison to apparently healthy calves. When haematological parameters were compared, there was a significant increase in parameters like packed cell volume and Total Leukocyte counts which was suggestive of dehydration. The increased number of neutrophils and leukocytosis in early stage of diarrhoea clearly indicated involvement of bacteria. Biochemical profile revealed significant increase in chloride level in diarrheic calves as compared to apparently healthy calves and suggested the presence of hyperchloremia in diarrheic calves.

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INTRODUCTION

Diarrhoea during early stage of life in neonates is one of the most severe clinical conditions (Kumar, et al., 2012a, 2013 and Malik et al., 2012). The treatment of diarrheic calves is required at the earliest with the attention to restore all physiological parameters. However, it is not always possible to perform routine blood profile at the door step of owner prior to start the treatment. In such conditions information of haematology may be of great value in the investigations of animal diseases and restoration of normalcy (Mahima et al., 2013). This also helps in suggesting the prognosis and to improve the efficacy of a particular therapeutic regimen, in a particular disease condition like diarrhea. Thus haematological parameters are always helpful for not only giving prognosis but also to improve efficacy of a particular selected therapeutic regimen irrespective of causal agents (Kumar et al., 2012a, b). Diarrhoeic calves showed increased packed cell volume (PCV), indicated fluid losses from vascular compartment (Naylor, 1987), and oral rehydration solution therapy along with other therapeutic measure were effective in bringing back the hydration status of diarrhoeic calves to normal (Dhaliwal, 1993) hence packed cell volume (PCV) were good reflection of underlying change in extra cellular fluid (ECF) volume during diarrhoea. Moreover, many times ignored imbalance of minerals and dehydration are the major cause of calves mortality (Malik et al., 2012). Under such conditions the estimation of packed cell volume is of utmost importance to monitor hydration status of animals and

is a sensitive indicator for assessing the severity of dehydration as compared to other parameters indicating dehydration. It has been observed that in general significant elevation in haematological parameters as Haemoglobin (Hb), packed cell volume (PCV) and total erythrocyte counts (TEC) due to haemoconcentration are always associated with dehydration (Schalm et al. 1975). Moreover, the clinico-biochemical alterations in diarrhoea are complex in nature characterized by imbalances of fluid altering the electrolyte and acid base status which ultimately endanger the life of patients. The effects of diarrhoea on such parameters as fluid loss, imbalance of electrolyte and acid base are always governed by the type, duration and severity of diarrhea. Other than these common alterations diarrheic calves are also found to have biochemical alterations such as hypoglycemia (Fettman et al. 1986), Hyperproteinaemia and hyperalbuminaemia (Dubey et al. 1992), hyperkalaemia, hyperchloraemia and hyponatraemia, (Sridhar et al. 1988). Keeping all these points in view, the present study was conducted to study haematological and haemochimistry alterations in positive cases of collibacillosis.

MATERIALS AND METHODS

The present study was undertaken on calves up to three months of age exhibiting symptoms of diarrhoea in Western Uttar Pradesh, India. The study included a total of 109 samples (blood and faeces), collected from the calves showing diarrhoea and brought to the laboratory on ice.

A total of 109 cases of calf diarrhea from different locations were physically examined and clinical parameters viz., temperature through rectum, pulse rate through coccygeal vein and respiration rate were recorded along with grossly evident clinical signs. The information collected in the form of temperature, pulse rate and respiration rate were pooled and mean were calculated for comparison with normal data as per Benzamin (2007).

The faecal samples of diarrhoeic calves of less than 3 months of age were collected directly from rectum. Approximately 20 grams of faecal contents were collected in sterilized containers (Edwards and Ewing, 1972).

Five to ten ml of blood from each of the 109 diarrheic calves was collected from the jugular vein in aseptic conditions using labeled sterile disposable syringes (Dispovan). About half of the blood was transferred immediately after collection to the sterilized vial containing ethylene di-amine tetra acetic acid (EDTA) solution and half of the blood was transferred to sterilized labeled test tubes for serum. Blood and serum samples were transported to laboratory on ice. In laboratory blood samples were processed immediately for haematological parameters viz., packed cell volume, Haemoglobin, total erythrocyte count, total leukocyte count and differential leukocyte count as per standard procedures (Schalm, 1975).

Haemoglobin was determined by Sahli's haemoglobinometer. In this method 20 cubic millimeter blood was drawn in Sahli's pipette and then was transferred to haemometer tube containing 3-4 drops of 0.1N HCl. After proper mixing tube was then allowed to stand still for 4 minutes for the haemoglobin to change into acid haematin. This final fluid was then diluted with distilled water drop-by-drop mixing after each drop until it matched to the colour of the standard comparison tubes. Finally the readings of haemometer tube measured the amount of haemoglobin in g/100 ml of blood.

The RBC pipette was filled up to 0.5 mark with the blood and then diluting fluid (Hayem's fluid) was drawn up to 101 mark before mixing and finally tube was kept stand still for three minutes. The counting chamber of the haemocytometer was carefully charged with the diluted blood after placing a cover slip. It was ensured that blood cells were evenly distributed over the counting chamber avoiding overloading. The red blood corpuscles present in the four corners small squares and one small central square of the large central square were counted under high power of the microscope.

Numbers of erythrocytes per cubic millimeter of blood were calculated by multiplying the total erythrocyte count by factor 10,000, as per the formula:

$$\text{Total erythrocytes} = \text{Cells counted} \times 200 \times 10 \times 5/\text{mm}^3$$

Where 200 stands for dilution

10 stands for depth

5 stands for 1/5th of square millimeter counted

The WBC pipette was filled up to 0.5 mark with blood was taken in tube and then WBC diluting fluid was drawn up to 11 mark. Then pipette was shaken to mix properly. The pipette was allowed to stand for three minutes, and fluid in its stem was discarded. Finally to count the cells counting chamber of the haemocytometer was carefully charged with diluted blood after placing cover slip. The cells were counted under low power of the microscope in the large four corner squares of the haemocytometer. The numbers of leucocytes in one cubic millimeter of blood were calculated by multiplying the total leucocytes counted by factor 50, as per the formula:

$$\text{Leucocytes}/\text{mm}^3 = \text{cell counted} \times 20 \times 10/4$$

Where

20 stands for dilution

10 stands for depth

4 stands for the number of square millimeters counted

Packed cell volume (PCV) was determined by using Wintrobe's haematocrit tube by filling them with blood by the help of pasteur pipette and centrifuged for 30 minutes at 3000 rpm to note the final reading. Final values were recorded as a percentage.

Thin smear of blood were prepared on dust, lint and grease free clean and dry microscopic slides. These slides were placed on a standing rack and flooded with Leishman's stain and allowed to act for 2 minutes then dilute the stain on the smear with double the amount of buffered distilled water and mixed gently and allowed to act for 12-15 minutes. To observe in microscope the smear were washed with distilled water, air-dried and examined under oil emersion lens for Differential leucocyte count. Finally under the microscope neutrophils, lymphocytes, monocytes, eosinophils and basophils were differentiated and expressed in percentage.

$$\% \text{ of each type of cell calculates} = \frac{\text{Number of cells}}{\text{Total Number}} \times 100$$

Sodium and potassium estimation was done by Flame photometer as per method described by Oser (1965). The results were expressed in mEq/l.

Estimation of chloride was done by Thiocyanate method (Bhattacharya *et al.*, 1978).

$$\text{Serum chloride (mEq/L)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

The faecal samples collected from diarrheic calves were processed in laboratory for isolation of *Escherichia coli*, according to procedure described by Edwards and Ewing (1972).

Statistical analysis of data:

Mean of parameters obtained were calculated and the data were subjected to test of significance (t test) analyzed against and standard values as described by Snedecor and Cochran (1975).

RESULTS

The clinical examination data viz., temperature, pulse rate and respiration rate were pooled and mean calculated (Table 1). These data were compared with normal standards described by Benzamin (2007). On clinical examination of 109 cases of calf diarrhea taken in present study revealed that the diarrhoeic calves showed similar clinical signs as weak, lethargic, anorexic and dehydrated irrespective of age, sex and breed. Clinical parameter viz., body temperature, pulse rate, respiratory rate were also recorded at the time of sample collection.

Non-significant difference in body temperature was found in diarrheic calves with early stage of diarrhea (on the basis of history). Whereas, the mean value of temperature recorded for sick calves was 102.88 ± 0.36 degree Fahrenheit (Table 1). There was significant difference in pulse rate and respiration rate in diarrheic calves in comparison to normal values of healthy calves. The mean value of pulse rate in diarrheic cases was 109.57 ± 1.32 per minute (Table). Increased respiratory rate were found in all the diarrheic calves. The mean value of respiration rate was 34.27 ± 0.93 per minute (Table). All the faecal samples collected were processed for the isolation and confirmation of *E. coli* with standard protocol (Quinn *et al.*, 2002). Out of 109 faecal samples from diarrheic calves only 41 revealed *E. coli*.

The blood samples collected for the study from diarrheic cases (41 cases) were subjected to hematological parameters (Haemoglobin, packed cell volume, total erythrocyte count,

total leukocyte count and differential leukocyte count) irrespective of species. All the information collected has been compiled in Table 2.

On hematological examination, there was no significant difference in the haemoglobin concentration between normal

and diarrheic calves. On calculation average mean value of Haemoglobin was obtained as 12.2 ± 0.221 g/dl whereas average Hb value of the normal calves is 11.3 g/dl.

Parameters	Normal range (Calves)	Diarrheic calves
Body temperature (°F)	101.98 ± 0.136	102.88 ± 0.36
Pulse rate (per minute)	95.87 ± 1.14	109.57 ± 1.32*
Respiration rate (per minute)	26.87 ± 0.854	34.27 ± 0.93*

* Values differ significantly (P<0.05)

Table 2: Hematological profiles of diarrheic calves

Parameters	Normal range (Calves) [#]	Diarrheic calves
Na (mEq/ litre)	148 ± 13	126.16 ± 1.815
K (mEq/ litre)	5.4 ± 0.8	5.6 ± 0.084
Cl (mEq/ litre)	95 ± 5.0	122.47 ± 1.943 ¹

[#]Butler et al., 1971 ¹ Values differ significantly (P<0.05)

The PCV Values in all the diarrheic calves were higher than the normal range. Finally average mean value of packed cell volume was recorded as 44.2 ± 0.692 percent (Table 2). Statistically, there was no significant difference.

The TEC Values in all the diarrheic calves were higher than the normal range. The mean value of the total erythrocytic cell count was 8.54 ± 0.316 million per cm^3 (Table 2). Statistically, there was no significant difference. As shown in Table 2, the TLC values in all the diarrheic calves were significantly higher than the normal range. The mean value of Total leukocyte count was 12.4 ± 0.295 thousands per cm^3 .

From the Table 2, it can also be seen that the neutrophils count was significantly higher in diarrheic calves as compared with the normal values and present study revealed average mean value of neutrophil count as 46.8 ± 0.748 percent. It can be seen that the lymphocyte count was lower in diarrheic calves as compared with the normal values and the average mean value in the present study appeared to be 51.6 ± 0.514 percent for lymphocytes. The monocyte count in all the diarrheic calves was slightly lower in comparison to that of normal values of healthy calves whereas, the mean value of monocytes was 2.72 ± 0.687 percent (Table 2). Statistically no significant difference was observed. The eosinophils percentages, in diarrheic calves were slightly decreased (1.7 ± 0.482) in comparison to that of normal value (Table 2). Statistically, there was no significant difference. It is thus evident from the hematology of diarrheic cases of calves that significantly higher PCV indicative of dehydration and leukocytosis with neutrophilia are the characteristic changes in early stage of diarrhea.

Three common electrolytes (sodium, potassium and chloride) as a measure of homeostasis and water balance were studied and the mean values of the results have been shown in Table 3. It can be seen that the sodium value in all diarrheic

Table 1: Physical examination profiles of diarrheic calves

Parameters	Normal range (Calves) [#]	Diarrheic calves
Haemoglobin (g/dl)	11.3 (9.5-13.5)	12.2 ± 0.221
PCV (%)	37.5 (36.0-49.0)	44.2 ± 0.692
TEC (million/ cm^3)	8.1	8.54 ± 0.316
TLC (thousands/ cm^3)	9.7	12.4 ± 0.295 ¹
Neutrophils (%)	37 (12-38)	46.8 ± 0.748 ¹
Eosinophils (%)	1.9 (2-30)	1.7 ± 0.482
Basophils (%)	0.6 (0-2)	0.5 ± 0.147
Lymphocytes (%)	68.6 (33-87)	51.6 ± 0.514
Monocytes (%)	3.4 (1-5)	2.72 ± 0.687

[#]Benzamin, 2007; ¹ Values differ significantly (P<0.05)

Table 3: Biochemical profile of therapeutic regimens on calves' diarrhea

calves was lower (126.16 ± 1.815 mEq/l) than that of normal values (148 ± 13 mEq/l) but statistically, there was no significant difference. For potassium, it can be seen that the potassium values in all diarrheic calves were slightly higher than that of normal values. The mean value of potassium in diarrheic calves was 5.6 ± 0.084 mEq per litre. Statistically, there was no significant difference. Similarly for chloride, there was significant increase in the chloride values in all diarrheic calves as compared to that of normal values. The mean value of chloride in diarrheic calves was 122.47 ± 1.943 mEq per litre.

DISCUSSION

Diarrhea in calves during early age has mixed etiology and irrespective of etiological agent the physiological conditions, age, breed, sex, stress, nutritional status and treatment always affects mortality (Kumar et al., 2012 a, b). In the present study when all the collected samples (blood and faeces) were analyzed as per the standard protocols the percent positivity of 37.61% was recorded in diarrheic calves due to *E. coli*. These findings indicated the high percentage of *E. coli* in diarrheic calves as suggested by Malik et al. (2012). The blood was estimated for haematological parameters (Haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count and differential leukocyte count). On haematological examination, there was an increase in haemoglobin concentration in all the calves as shown in Table 2. The increase in hemoglobin was due to haemoconcentration associated with dehydration. Our finding was in concurrence with the findings of Schalm et al. (1975) and Sridhar et al. (1988). PCV revealed a significant elevation of PCV in all the calves as shown in Table 2. Naylor (1987) reported that increased PCV values in diarrheic calves indicated fluid loss from vascular compartment. Dhaliwal (1993) and Rajora and Pachauri (2000) reported

PCV as indicator of dehydration. Thus, estimation of PCV is of utmost importance to monitor hydration status of animal and is a sensitive indicator for assessing the severity of dehydration. Tennant et al., 1972; Boyd et al., 1974; Bijwal et al., 1985 Sridhar et al., 1988 and Bukhari (2002) have already reported mean PCV value increased upto significant level in diarrhoeic calves. TEC revealed significant elevation of TEC in all the calves as shown in Table 2. Similar to earlier findings, Schalm et al., 1975 and Sridhar et al., 1988, who reported significant elevation of TEC in scouring calves. The cause of this might be the haemoconcentration of blood due to diarrhoea. TLC values of diarrhoeic calves revealed significant elevation of TLC values as shown in Table 2. This finding has supported the finding of Boyd et al. (1974). Sridhar et al. (1988) also reported significant leucocytosis which might have occurred due to normal reaction of body defense mechanism against infection and also due to haemoconcentration due to dehydration. Differential leucocyte count (DLC) includes the count of neutrophils, eosinophils, basophils, lymphocytes and monocytes, which are the suggestive of infectious agents of different origin. The present study revealed increase in neutrophils i.e. neutrophilia and decreased lymphocyte i.e. lymphopenia in diarrhoeic calves (Table 2). These finding had close agreement with the findings of 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.

Water and electrolytes balance is an integral component of homeostasis. Change in water balance is accompanied by changes in electrolyte concentration in all body fluids. Thus, it is obvious that water and electrolyte imbalance associated with certain clinical status like neonatal calf diarrhea need to be suitably compensated at the earliest to avoid life-threatening situation. In the present study, an attempt was made to evaluate the nature and magnitude of electrolyte imbalance and relative efficacy of various therapeutic regimens with suitable infusion. The present study revealed significant reduction of Na values in diarrhoeic calves (Table 3). These findings were in agreement with observations of Bijwal et al., 1985 and Sridhar et al., 1988. Similarly, Aly et al. 1996 and Dubey and Rao (1997) also reported hyponatraemia in diarrhoeic calves due to an excessive secretion of sodium along with water into intestinal lumen. These finding were contrary to that of Kaur et al. (2006), who recorded hypernatraemia in diarrhoeic calves as compared to healthy calves. The present study revealed elevation of potassium levels in all the diarrhoeic calves (Table 3). Hyperkalaemia was due to increased potassium retention by kidney and also due to cellular damage (Fisher et al, 1983). Similar observations have been reported by other workers such as Sridhar et al., 1988, Groutides and Michell (1990), and Kaur et al., (2006). Serum chloride values of diarrhoeic calves showed elevation of chloride values in diarrhoeic calves (Table 3). These finding were similar to observations of Sridhar et al., (1988). They reported hyperchloraemia in scouring calves, it might be due to liver dysfunction leading to an increase in capillary permeability with the loss of colloidal protein into tissue and resulting increased serum chloride. Gross (1988) reported metabolic acidosis resulting due to the excess of H⁺ and deficit of NaHCO₃, in the pathogenesis of this condition, due to overproduction of H⁺ and Cl⁻ released from the RBCs to maintained the electro neutrality and to cope with the loss to anion (HCO₃⁻) and the results eventually in hyperchloraemia.

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