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RESEARCH ARTICLE

A Preliminary Study of the Correlations of Serum Concentrations of Electrolytes and Trace Elements with Clinical Signs in Diarrheic Dairy Calves

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ABSTRACT

To study the correlations of clinical signs with serum electrolytes and trace elements, 50 diarrheic dairy calves in a dairy herd were examined. The diarrheic calves, before any treatment, were clinically examined, and fecal consistency score, age, and days between disease onset and sampling were recorded per calf. The serum concentrations of calcium, magnesium, copper, zinc, manganese and iron were measured. Serum copper concentration had a significant correlation with PCV (r=-0.56, P<0.001) and had marginally significant correlations with calves age (r=-0.32, P=0.06) and disease length (r=-0.31, P=0.07), and serum calcium concentration had significant correlations with body temperature (r=0.41, P<0.01) and calves age (r=-0.41, P<0.01). Fecal consistency score or diarrhea severity had a significant effect on none of the measured serum factors. Our results showed the importance of diarrhea length and calves age vs. fecal score in estimation of changes in serum parameters in diarrheic calves.

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INTRODUCTION

Neonatal calf diarrhea is one of the most devastating diseases of the dairy industry worldwide (Elhassan *et al*., 2011; Pourjafar *et al*., 2011). Although calf diarrhea has numerous infectious and non-infectious causes, dehydration, electrolyte imbalance and acidosis have been considered as the major systemic changes in diarrheic calves that often result in the death of calves in the absence of correction (Guzelbektes *et al*., 2007;). Different studies have shown that administration of water and electrolytes to diarrheic calves improves survival (Naylor, 1989). During administration of water and electrolytes to diarrheic calves, it is essential to know what disturbance of serum electrolytes is more severe and its correction is more important (Groutides and Michell, 1990). Measurement of the serum electrolytes and evaluation of acid-based disturbance require laboratory tests and are not routinely measured in the field condition.

The degree of dehydration can be estimated by physical examination of the calf, and has been introduced as a reliable method (Naylor, 1989). Electrolyte imbalance in diarrheic calves may cause death in some cases and estimation of the severity of electrolyte imbalance is necessary before administration of electrolytes to diarrheic calves. Although it is believed that serum concentrations of sodium (Na) and potassium (K) are more important in relation to the composition of the fluids used for therapy (Groutides and Michell, 1990), there is little information about the changes of some other electrolytes such as calcium (Ca) and magnesium (Mg) and trace elements, and their relationships with clinical signs in diarrheic calves. Untreated changes in serum concentrations of Ca and Mg and some trace elements may be the cause of some death or post diarrhea complications such as growth retardation.

This study was designed to evaluate the relationships of the clinical signs, age and disease length with the serum concentrations of Ca and Mg, and some trace elements such as copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe) during calf diarrhea.

MATERIALS AND METHODS

In a 600-Holstein cow dairy herd in Khorasan Razavi province, in the northeast of Iran, daily visit and fecal consistency scoring were performed for all born calves until 1 month old. No diarrhea vaccination against calf diarrhea was applied, and the calves were reared under the same husbandry conditions. After birth, the calves were fed 2 kg of dam's colostrum by nipple bottle and moved to individual pens. Additional 1kg pooled colostrum was fed within approximately 12 h of the first colostrum feeding. The calves were then fed milk from buckets thrice daily at approximately 3% of body weight per feeding until 3 days of life. After this time, the calves were fed a milk replacer (thrice daily and approximately 10% of body weight per day) and calf starter (including concentrate (90% DM) and high-quality alfalfa (10% DM) until 90 days of life. Water was offered *ad libitum*.

Evaluation of fecal consistency was performed using the following criteria. Zero or 'normal': perineum and tail of the calf are clean and dry, firm consistency and brown color; 1: perineum and/or tail of the calf is smeared with feces, a paste-like consistency and yellow color; and 2: perineum and/or tail of the calf is smeared with watery feces and watery consistency. Fecal scores 1 and 2 were considered as diarrheic. The diarrheic calves, before any treatment, were clinically examined, and clinical data (heart rate, fecal consistency score, rectal temperature, presence of visible blood in feces, mucous membrane color and moistness) as well as data on sex, age, and days between disease onset and sampling were recorded per calf. Heart rate (beat/min) was measured by thoracic auscultation for a minimum of 30 seconds. Mucous membrane color was categorized as typical, pale, or congested by examining the lower eyelid mucous membrane. Mucous membrane moistness was also categorized as typical or dry at the same location. After clinical examination, jugular vein blood samples in tubes containing EDTA and plane tubes, free from anticoagulant, were collected from the calves. The blood serum was separated after centrifugation at 1800*g* for 10 min and the serum samples stored at −18°C until analysis. Also, packed cell volume (%) was measured using microhematocrit method. The serum samples were analyzed for Ca, Mg, Cu, Zn, Mn and Fe by atomic absorption spectrophotometery (Shimadzo AA- 670, Kyoto, Japan) (Nazifi *et al*., 2003).

Statistical analysis was performed using SPSS12 (Illinois, Chicago). Kolmogorov–Smirnov tests were used to assess normality of distribution for each variable. Correlations of the rectal temperature, heart rate, age, disease length, and blood packed cell volume (PCV) with serum electrolytes and trace elements were analyzed by Pearson's correlation tests. Two sample t-tests were used to compare serum concentration of electrolytes and trace elements between calves with fecal score 1 and 2, and to compare between calves with bloody stool and other calves. Analysis of variance tests were used to compare between calves with different mucous membrane colors. Multiple linear regression analysis tests were also conducted to assess the relationship between the serum concentration of each of the measured serum parameters with age, evaluated clinical signs and disease length. Differences were considered significant at P<0.05.

RESULTS

Serum samples were obtained from 50 diarrheic calves. The calves were 1-30 days old. The results of the measurement of the serum electrolytes and trace elements are shown in Table 1.

Table 1: Serum concentrations of electrolytes and trace elements in diarrheic calves (n = 50)

Electrolytes and trace elements	Mean± SEM
Mg (L/mmol)	0.8145±0.4
Ca (L/mmol)	1.297±0.035
Mn (L/μmol)	0.546±0.18
Cu (L/μmol)	10.86±1.1
Fe (L/μmol)	10.03±1
Zn (L/μmol)	26.01±3.06

Table 2: Correlation of serum concentrations of the measured serum electrolytes and trace elements with the rectal temperature, heart rate, fecal consistency score, age, disease length, and blood packed cell volume in diarrheic calves (n=50)

	Rectal temperature (°C)	Heart rate (beat/min)	Age (day)	Disease length (day)	Blood PCV (%)
Ca	r=0.41*	r=0.234	r=-0.41*	r=-0.11	r=-0.126
(mmol/L)	P<0.01	P>0.05	P<0.01	P>0.05	P>0.05
Mg	r=-0.02	r=-0.15	r=-0.06	r=-0.04	r=0.06
(mmol/L)	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Cu	r=0.05	r=-0.26	r=0.32#	r=0.31#	r=-0.561*
(μmol/L)	P>0.05	P>0.05	P=0.06	P=0.07	P<0.001
Zn	r=0.09	r=-0.12	r=0.175	r=0.02	r=-0.245
(μmol/L)	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Fe	r=0.06	r=-0.12	r=0.25	r=0.13	r=-0.29
(μmol/L)	P>0.05	P>0.05	P>0.05	P>0.05	P=0.09
Mn	r=0.07	r=-0.16	r=0.254	r=0.03	R=-0.274
(μmol/L)	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

* Significant correlation; #marginally significant correlation

Serum copper concentration had a significant correlation with PCV (r=-0.56, P<0.001) and had marginally significant correlations with calf age (r=-0.32, P=0.06) and disease length(r=-0.31, P=0.07). Body temperature and age had significant correlations with the serum concentration of Ca (r=0.41, P<0.01 and r=-0.41, P<0.01, respectively). The results of the measurement of the correlations are shown in Table 2.

Two sample t-tests showed that calves with dry mucous membrane had significantly longer disease length than calves with moist mucous membrane (P=0.001). There was no significant difference in serum concentration of the measured serum electrolytes and trace elements between calves with different fecal scores. Calves with bloody feces (12 calves) had a significantly higher serum concentration of Ca (P<0.01) than calves without visible blood in the feces (38 calves). Linear regression analysis showed that the heart rate was a negative strong predictor of the serum Mg (P<0.01) and serum concentration of Cu could be negatively predicted by the PCV.

DISCUSSION

Fecal losses of electrolytes in combination with losses of water, reduced dietary intake and probable translocation between intracellular and extra cellular compartments are the main causes of serum electrolytes and trace elements changes in diarrheic calves. Proper use of oral and intravenous fluid therapy is one of the most important factors in decreasing mortality associated with diarrhea in calves (Berchtold, 2009). Although the changes in serum concentrations of Na and K in diarrheic neonatal calves and their relationships with clinical signs have been studied (Butler *et al*., 1971; Berchtold, 2009), there is little information about the changes of some other electrolytes such as calcium (Ca) and magnesium (Mg), and their relationships with clinical signs in diarrheic calves. On the other hand, little information exists about the changes of trace elements and, to the best of our knowledge; the current study is the first study regarding the relation of serum concentrations of trace elements with clinical signs in diarrheic calves.

Berchtold (2009) believes that electrolyte disturbances vary among individual cases of calf diarrhea and can not be predicted from clinical findings without laboratory analysis. However, it has been shown that, although there are contradictory reports about the changes of Na and K in blood serum of diarrheic calves (Chalvea and Encheva, 2003), the serum Ca and Mg fall have been reported in more than 75% of cases (Groutides and Michell, 1990).

Although different infectious microorganisms cause scours in calves, more than 75–95% of all cases of neonatal calf diarrhea are due to enterotoxigenic *E. coli*, rotavirus, coronavirus and *Cryptosporidium* spp together. It is believed that because of the different pathophysiology, the severity of diarrhea and its subsequent changes in those serum concentrations of electrolytes and trace elements may differ between different infectious causes (Bartels *et al*., 2010). For example, some authors believe that concurrent infection of *Cryptosporidium* spp. and other causes of diarrhea affect the severity of diarrhea (O'Handley *et al*., 1999; Foster and Smith 2009). However, some clinical studies did not confirm this opinion (Tajik *et al*., 2010). On the other hand, diagnosis of the diarrhea causative agent requires laboratory tests and is not routinely measured in the field condition. The results of the current study showed that calves with different fecal scores had no significant difference in serum concentrations of the measured serum electrolytes and trace elements. It has been shown that fecal consistency did not reflect the loss of water and electrolytes during diarrhea and calves may even die due to loss of fluid and electrolytes loss into gut before the diarrheic feces appears (Brooks *et al*., 1996).

Packed cell volume was used as an index for the estimation and quantification of hydration status in the calves. Although some authors believe that other clinical signs such as enophthalmos have the closest correlation with the degree of dehydration, dehydration of less than 5% of body weight cannot be clinically diagnosed and estimated using enophthalmos (Constable *et al*., 1998). Other authors believe that PCV is the best indicator for the diagnosis of changes in hydration status (Michell *et al*., 1992).

Bostedt *et al*. (2000) reported that abnormal serum values of Ca and Mg persist for a significantly long time after the treatment of diarrheic calves, even if no clinical evidence of calf diarrhea was found. According to our results, serum concentration of Ca had a negative relationship with the calves age, which was similar to that reported by Butler *et al*. (1971). Our results also showed that serum Ca had a positive correlation with body temperature. Different previous studies have shown serum Ca decrement in diarrheic calves (Michell *et al*., 1992; Groutides and Michell, 1990; Cabello and Michel, 1977). The normal serum concentration of Ca in cow has been reported as 2.43-3.1 mmol/L (Kaneko *et al*., 2008) and the mean serum Ca in diarrheic calves in the current study was 1.297 mmol/L. Hypocalcaemia has been diagnosed as a cause of hypothermia in ruminants (Radostits *et al*., 2007), which may explain the observed relationship between Ca and body temperature in the current study. According to our results, calves with bloody stool had significantly higher serum concentration of Ca. With attention to the role of Ca in blood coagulation, higher serum Ca in diarrheic calves with bloody feces may be a physiological response to control the bleeding. Higher serum Ca in this group should be considered in selection of the fluid therapy regime.

In the current study no significant correlation between serum Mg and the measured clinical signs was found and linear regression analysis showed that the heart rate can be used in the estimation of serum Mg. Michell *et al*. (1992) reported that blood Mg had little change during diarrhea in calves. The normal serum concentration of Mg in cow has been reported as 074-0.95 mmol/L (Kaneko *et al*., 2008) and the mean serum Mg in the current study was 0.8145 mmol/L, which confirms the Michell *et al.* (1992) report. Berchtold (2009) believes that magnesium concentrations vary in calves with diarrhea. Butler *et al*. (1971) found that in normal calves, serum Mg tended to fall with increasing age. Another study has shown plasma Mg tended to fall in 80% of the survived diarrheic calves, while dying diarrheic calves were equally likely to show a rise or a fall in Mg, and Mg rise in the terminal stage of diarrhea occurs due to a rise in albumin and total protein (Groutides and Michell, 1990).

Very minute concentration of trace elements such as Mn, Cu, Fe and Zn are needed for many physiological functions, including immune and antioxidant function, growth and reproduction (Cunnane, 1988), and untreated decrement of some trace elements may be the cause of some death or post diarrhea complications such as growth retardation. The decrease in the serum concentrations of Cu and Zn in diarrheic people in comparison to healthy people has been shown (Kilic *et al*., 2003; Arora *et al*., 2007). The results of the current study showed that the serum concentration of Mn, Fe and Zn had no significant correlations with the measured clinical signs. Serum copper concentration had a significant correlation with PCV ($r=-0.56$, $P<0.001$) and linear regression analysis showed that serum concentration of Cu could be negatively predicted by the PCV. Anemia has been proposed as the most frequent sign associated with chronic Cu deficiency. The role of Cu in iron absorption, mobilization, and utilization has been proposed as a cause of anemia. On the other hand, uptake of Cu from a diet can be influenced by the physiological state of the animal (Rucker *et al*., 2008). Higher PCV means more severe dehydration, which prolonged water loss is one of its causes. Prolonged Cu malabsorption can be proposed as the cause of the negative observed relationships between PCV and disease length with the serum Cu in the current study. The serum copper also had a marginally significant correlation with calves age. Ninety percent of the Cu present in plasma/serum is associated with ceruloplasmin (Rucker *et al*., 2008). Okumura *et al*. (1998) reported that the lowest value of serum ceruloplasmin in foals was within the first week of life and then increased rapidly at 1 month, where this level was maintained. On the other hand, in other studies no significant effect of age on the serum ceruloplasmin in horse was seen (Nazifi *et al*., 2003) however, an age-related increase in serum Cu in water buffalo (Tajik and Nazifi, 2010) has been observed.

According to the results of the current study, body temperature, age and bloody feces can be used in selection of Ca concentration in fluid therapy regime, and PCV, calf age and disease length can be used to make a decision about the copper supplementation in treatment of diarrheic calves. Although, additional studies with greater sample size are needed to confirm the results.

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