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ORIGINAL ARTICLE

Comparison of electrocardiographic parameters and serum electrolytes and microelements between single infection of rotavirus and coronavirus and concurrent infection of *Cryptosporidium parvum* with rotavirus and coronavirus in diarrheic dairy calves

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Abstract To study the effects of concurrent infection of *Cryptosporidium parvum* with rotavirus and coronavirus in comparison to single infection of rotavirus and coronavirus, electrocardiographic parameters and serum electrolytes and microelements are compared between eight calves with concurrent infection and ten calves with single infection. Calves with single infections had higher serum concentrations of sodium and calcium, and less potassium, copper, and iron, which was not statistically significant. They also had significantly shorter P and QRS wave amplitudes and longer T wave duration in electrocardiogram patterns.

Keywords Electrocardiographic parameters · Serum electrolytes and microelements · Rotavirus and coronavirus · *Cryptosporidium parvum* · Diarrheic dairy calves

Introduction

Neonatal calf diarrhea is one of the most devastating diseases of the dairy industry worldwide. Although infectious causes of calves' scours and their prevalence may vary in different regions, often enterotoxigenic *Escherichia coli*, rotavirus, coronavirus, and *Cryptosporidium* sp. together account for more than 75–95% of all cases of neonatal calf diarrhea in calves less than 1 month of age (Bartels et al. 2010).

At least four *Cryptosporidium* species, including *Cryptosporidium parvum*, *Cryptosporidium bovis*, *Cryptosporidium andersoni*, and *Cryptosporidium* deer-like genotype, infect the cattle. The occurrence of this *Cryptosporidium* spp. in cattle is age-related (Thompson et al. 2007). *C. parvum* is responsible for about 85% of the *Cryptosporidium* infections in preweaned calves, and is considered to be the most commonly found enteropathogen in calves during their first weeks of life (Thompson et al. 2007; de Graaf et al. 1999).

Concurrent infections of *C. parvum* with rotavirus and coronavirus have been described (Naciri et al. 1993). Bartels et al. (2010) found concurrent infections of *C. parvum* with rotavirus in 7.8% of diarrheic calves, and increased mortality due to concurrent infections of *C. parvum* with other enteric pathogens has been reported (Naciri et al. 1993).

Cryptosporidiosis, similar to rotavirus and coronavirus infections, causes destruction of intestinal epithelia resulting in a reduction of enzymatic activity and a decrease in the absorptive surface, finally leading to maldigestion and malabsorption followed by diarrhea (Foster and Smith 2009).

Although it is generally believed that the losses due to diarrhea are more severe when concurrent infections occur (de Graaf et al. 1999), the effect of concurrent infection of *Cryptosporidium* sp. with rotavirus and coronavirus in

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comparison to the single infection of rotavirus and coronavirus on blood serum electrolytes and microelements of diarrheic calves has not yet been clarified.

Materials and methods

In a 2,000-Holstein cow dairy herd in Fars province, south 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 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. An additional 1.2-kg pooled colostrum was fed within approximately 12 h of the first colostrum feeding. Then, the calves were 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 free choice.

Evaluation of fecal consistency was performed using the following criteria—0 or 'normal': firm consistency, brown color, and perineum and tail of the calf are clean and dry; 1: a paste-like consistency, yellow color, and perineum and/or tail of the calf is smeared with feces; and 2: watery consistency and perineum and/or tail of the calf is smeared with watery feces. Fecal scores 1 and 2 were considered as diarrheic. The diarrheic calves, before any treatment, were clinically examined, and clinical data (rectal temperature, fecal consistency score, and presence of visible blood in feces) as well as data on sex, age, and days between disease onset and sampling were recorded per calf. After clinical examination, rectal fecal samples and jugular vein blood samples in tubes containing EDTA and plane tubes, free from anticoagulant, were collected from the calves. Also, the electrocardiogram (ECG) was recorded from each of the calves on a bipolar base apex lead using limb lead. Animals were kept in a standing position in a stock without any sedation. The ECG was recorded when the animals were thought to be in a quiet state using an alligator-type electrode attached to the skin. The positive electrode of lead I (left arm) was attached to the skin of the fifth intercostal space just caudal to the olecranon and the negative electrode (right arm) on the jugular furrow about the lower 1/3 of the left side of the neck, and the earth was attached away from these two electrodes (Rezakhani et al. 2004). All ECGs were obtained on a triple-channel ECG machine (Cardioline, Delta 1 Plus, Remco, Italy) with a paper speed of 25 mm/s and calibration of 10 mm equal to 1 mV.

The blood serum was separated after centrifugation at 1,800×*g* for 10 min, and serum and fecal samples were stored at −18°C until analysis. Also, blood packed cell volume (%) was measured using microhematocrit method.

The presence of *E. coli* (k99), rotavirus, coronavirus, and *C. parvum* in fecal samples were diagnosed using commercial ELISA antigen detection kits (BGVV B-290, Bio-X Diagnostics, Belgium).

Two groups were defined according to the results of fecal tests: group A (presence of rotavirus or coronavirus without *C. parvum*) and group B (concurrent presence of rotavirus or coronavirus with *C. parvum*).

The serum samples were analyzed for calcium, magnesium, copper, zinc, and iron by atomic absorption spectrophotometry (Shimadzo AA- 670, Kyoto, Japan). Also, concentrations of sodium and potassium in the serum samples were measured using automatic electrolyte analyzer (Convergys ISE NG, GmbH, Germany).

For measuring ECG parameters, the ECGs were analyzed using a magnifying glass. In this method, precision of duration is 0.02 s. and amplitude is 0.05 mV. To describe the QRS complex in this lead, the first positive wave was named R and the negative deflection after R was designated S. (Rezakhani et al. 2004). The heart rate was calculated by measuring the average six R–R intervals of each trace.

Statistical analysis was performed using SPSS12 (Illinois, Chicago). Kolmogorov–Smirnov tests were used to assess normality of distribution for each variable. Two sample *t* tests were used to detect differences in parameters between the two groups. Chi-square tests were used for the comparison of proportion of bloody stool and fecal score between the two groups. Differences were considered significant at $P<0.05$.

Results

Fecal samples were obtained from 50 diarrheic calves. The calves were 1–30 days old. Single presence of *E. coli* (k99), rotavirus, coronavirus, and *C. parvum* were detected in three, seven, three, and six out of 50 samples, respectively. Concurrent infection of rotavirus and *C. parvum* was found in six of the calves, and two calves had concurrent infection of *C. parvum* and coronavirus. No enteropathogen was detected in 23 out of the 50 samples. Therefore, group A consisted of ten calves while group B was consisted of eight.

Median age for group A was 9.2 days and for group B 10.6 days, and there was no significant difference. Median number of days between disease onset and sampling for group A was 1.9 days and for group B 3.5 days. The difference between the two groups was not statistically significant.

Group A, in comparison to group B, had higher serum concentrations of sodium and calcium and less potassium, copper, and iron; however, statistical analysis showed no significant differences. Also, rectal temperature, heart rate, and blood packed cell volume had no significant differences between the two groups. The data regarding the physical examination and measured factors in the blood serum of both groups are depicted in Table 1.

ECGs showed shorter P and QRS wave amplitudes in group B ($P=0.058$ and $P=0.081$, respectively) and a longer T wave duration in group B ($P<0.05$). Amplitude and duration of ECG waves are presented in Table 2.

There were no significant differences between the two groups for the fecal consistency score. Two cases of bloody diarrhea were diagnosed in group A, but no case was found in group B. Fisher's exact test showed no significant difference between the two groups about the presence of visible blood in the fecal samples.

Discussion

The present study, although with a limited number of diarrheic calves, showed that concurrent infection of *C. parvum* with rotavirus and coronavirus in comparison with single infection of rotavirus and coronavirus may deteriorate changes of blood serum electrolytes, microelements, and electrocardiographic parameters in diarrheic calves. The severity of diarrhea and its subsequent changes in serum influence the morbidity and mortality of the diarrheic calves (O'Handley et al. 1999).

Diarrhea caused by *Cryptosporidium* sp., rotavirus, or coronavirus has a similar pathophysiology and is due to the destruction of the intestinal epithelial cells and villous atrophy and the resultant malabsorption and maldigestion. Additionally, the crypt enterocytes and the colonocytes can be affected by coronavirus; therefore, the resultant diarrhea may have a longer duration (Foster and Smith 2009).

Table 1 The results of physical examination and concentrations (mean±SEM) of serum electrolyte and microelements in both groups of diarrheic calves

Measured factor	Group A	Group B
Rectal temperature (°C)	39.5±0.23	38.6±0.58
Blood packed cell volume (%)	23.7±5.6	23.6±4.5
Sodium (mmol/L)	138.5±2.6	131±5.584
Potassium (mmol/L)	5.13±0.17	5.83±0.61
Calcium (mmol/L)	1.283±0.09	1.232±0.042
Magnesium (mmol/L)	0.753±0.016	0.763±0.057
Copper (μmol/L)	7.13±2.83	10.15±0.306
Zinc (μmol/L)	27.27±7.33	27.33±5.04
Iron (μmol/L)	8.67±2.92	9.94±2.3

Table 2 Electrocardiographic values (mean±SEM) of both groups of diarrheic calves

ECG parameters	Group A	Group B
Heart rate/min	117±7	96.25±12.53
P duration (ms)	0.049±0.004	0.044±0.008
P amplitude (mV)[a]	0.3±0.03	0.194±0.045
PRI	0.148±0.006	0.13±0.02
QRS amplitude (mV)[a]	2.11±0.091	1.8±0.15
S	1.96±0.13	1.69±0.14
T duration (s)[a]	0.088±0.01	0.12±0.01
T amplitude (mV)	0.85±0.106	0.8125±0.09
QT	0.26±0.014	0.285±0.016

[a] Significant difference between two groups

Concurrent infection of *Cryptosporidium* sp. with rotavirus and coronavirus deteriorates the malabsorption in comparison to single infection (Foster and Smith 2009), and can lead to more severe illness. Some authors believe that the presence of concurrent infections of *Cryptosporidium* spp. and other causes of diarrhea affects the severity of diarrhea (O'Handley et al. 1999). According to our results, the concurrent infection of *Cryptosporidium* sp. with rotavirus and coronavirus in comparison with the single infection of rotavirus and coronavirus had no significant effect on fecal consistency, but it does increase the severity of the changes in the serum electrolytes and ECG parameters.

Although different infectious microorganisms cause scours in calves and all three enteropathogens included in this study may also be found in healthy calves, the detection of them cannot be interpreted as a proof of cause; however, enterotoxigenic E. coli, rotavirus, coronavirus and *Cryptosporidium* sp. together often account for more than 75–95% of all cases of neonatal calf diarrhea, and a strong association between the presence of one of these intestinal agents and diarrhea has been shown (Bartels et al. 2010). Because of the importance and high prevalence of enterotoxigenic *E. coli*, the presence of *E. coli* (k99) in fecal samples was also evaluated, and *E. coli* (K99) positive samples were deleted from both groups.

In diarrheic calves, electrolyte imbalance and acidosis affect cardiac function, and cardiac arrhythmia is one of the main causes of death in diarrheic calves (Naylor et al. 2006). In the current study, ECG records were used for the evaluation and comparison of cardiac function between the two groups. From our results, significant differences there were seen between the two groups for the amplitude of P and QRS waves and the duration of T wave. Group B had a lower mean heart rate, which seems to cause a longer T wave duration in this group. Although differences of serum electrolytes between the two groups were not statistically significant, the difference of the P wave amplitude may be due to the different serum concentrations of some electrolytes. Hyperkalemia is the first suspect for ECG changes during calf diarrhea. The serum potassium level of group B was above the normal (3.9–5.8 mmol/L) (Kaneko et al. 1997). ECG patterns during the hyperkalemia are largely comparable between different species, and are characterized by a widening and flattening of P wave, broadening of QRS complex, increased P-R interval, shortening of the QT interval as well as tall, symmetric, peaked T waves (Pourjafar et al. 2008). No probable cause for the change in the QRS complex amplitude was found.

According to our results, ECG patterns during calf diarrhea may be suitable as an indicator of the severity of serum electrolyte changes and can be used to estimate the severity of illness in diarrheic calves. Although some researchers observed no significant correlation between blood electrolytes and ECG parameters in diarrheic calves (Dalir Naghadeh and Yaresmaeil 2001), Gertsch (2009) believes that although the correlation between definitively pathologic electrolyte serum levels and the ECG is poor (10–30%), severe or extreme electrolyte imbalance is detectable in the ECG up to 90%.

According to the results of present study, it is recommended that the calves with concurrent infection of *Cryptosporidium* sp. with rotavirus and coronavirus receive more intensive therapeutic attention, although additional studies with greater sample size are needed to confirm the results.

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