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Full Research Article

OCCURRENCE OF NEONATAL DIARRHEA IN CALVES WITH IRON-DEFICIENCY ANEMIA

PRODANOVIĆ Radiša[1*], NEDIĆ Sreten[1], RADANOVIĆ Oliver[2], MILIĆEVIĆ Vesna[2], VUJANAC Ivan[1], BOJKOVSKI Jovan[1], KURELJUŠIĆ Branislav[2], ARSIĆ Sveta[1], JOVANOVIĆ Ljubomir[3], KIROVSKI Danijela[3]

[1]University of Belgrade, Faculty of Veterinary Medicine, Department of Ruminants and Swine Diseases, Belgrade, Serbia; [2]University of Belgrade, Scientific Veterinary Institute of Serbia, Belgrade, Serbia; [3]University of Belgrade, Faculty of Veterinary Medicine, Department of Physiology and Biochemistry, Belgrade, Serbia

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Abstract

Introduction. Neonatal calves are often deficient in iron. Accumulating evidence indicates that iron status is associated with disease pathologies including diarrhea. Our objective was to examine the association between iron status and gut function in neonatal calves with and without a history of calf diarrhea.

Materials and Methods. Calves were divided into two groups based on their history of diarrhea; the first group were diarrheic calves (n=6) and the second group were non-diarrheic healthy calves (n=6). Blood samples (n=12) were collected at day 12 of age and erythrogram determination and measurements of serum iron and total iron binding capacity were performed. Hematological values were measured using an automatic analyzer, and biochemical properties were determined spectrophotometrically. Fecal samples were obtained from all calves and pH measured using semi quantitative test strips as well as being examined by bacterial cultivation for enterotoxigenic *Escherichia coli, Salmonella spp.* and *Clostridium perfringens*, by RT-PCR for the presence of bovine rotavirus, bovine coronavirus and bovine viral diarrhea virus, and by microscopy for the presence of *Cryptosporidium parvum*.

Results and Conclusions. There were significant iron-related changes for most hematological indices in diarrheic calves; and iron (Fe) deficiency and microcytic, hypochromic anemia were diagnosed. The pH of the feces was significantly higher in diarrheic calves than in the non-diarrheic healthy group ($P<0.01$). All fecal samples were negative for the analyzed enteric pathogens. According to the results obtained, calves experiencing iron

*Corresponding author – e-mail: prodanovic@vet.bg.ac.rs

deficiency anemia exhibit changes in gut function leading to diarrhea as compared with a matched group of healthy calves.

Key words: iron-deficiency anemia, calves, diarrhea

INTRODUCTION

Calf diarrhea is the most common health problem and still a major cause of mortality in calves below one month of age (Uetake, 2013). Both infectious and noninfectious factors are known to be involved in the etiology of neonatal calf diarrhea (Scott et al., 2004). Numerous epidemiological studies have indicated a greater incidence of *Escherichia coli*, coronavirus and rotavirus infections than the other causative agents worldwide. In addition, *Cryptosporidium parvum* has been isolated with greater frequency in Serbia (Mišić et al., 2002). Although single infections can be detected in some cases, mixed infections or interactions between noninfectious factors and infection were often reported (Paré et al., 1993).

Among a wide variety of interrelated noninfectious factors, iron deficiency has also been implicated in development of calf diarrhea (Okabe et al., 1996; Pare et al., 1993; Bosted et al., 1990; Blaxter et al., 1957). The monitoring of iron status in newborn calves is essential not only for the achievement of good health but also for the achievement of adequate weight gain of young calves due to the involvement of the insulin-like growth factor (IGF) system in proper utilization of consumed nutrients (Prodanovic et al., 2014). A high incidence of diarrhea is a commonly detected in iron-deficient piglets (Larkin and Hannan, 1983). Changes in the morphology, function and bacterial microbiota of the small intestine have also been recorded (Larkin and Hannan, 1985). Although a significant incidence of anemia in neonatal calves has been reported by many investigators (Prodanovic et al., 2014; Morel 1996; Okabe et al., 1996; Pare et al., 1993; Bosted et al., 1990), no attention has been devoted to their possible involvement in diarrhea outbreaks of these animals.

The current study was, therefore, conducted in order to examine the possible consequences of iron deficiency on gut function in neonatal dairy calves.

MATERIALS AND METHODS

The study was performed on a commercial dairy farm with 12 Holstein calves of which four were male. The animal-related component of the study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Belgrade in accordance with the National Regulation of Animal Welfare (01-19/4). Calves were divided into two equal groups based on clinical symptoms of diarrhea up to two weeks of age. The calves of the first group had clinical symptoms of mild diarrhea; the calves of the second group were clinically normal. The diarrheic calves had pale mucous membranes and increased heart rate while body temperature was in the normal range. The two groups of calves were matched by age (12±1.41 days), sex, housing and feeding system. The calves were single, born of cows unvaccinated against calf enteral pathogens.

Blood samples were collected from all calves under the following conditions: 2.0 mL of total venous blood was collected in vacuum tubes containing EDTA/K2 to determine hematological indices, and 5.0 mL was collected in improvacuter gel and clot activator tubes (Improvacuter™, China) to obtain serum for iron (Fe) and total iron binding capacity (TIBC) measurements. All blood samples were then transported to the laboratory at 4°C and were analyzed within 60 min after blood collection. At the same time, fecal samples were collected from all calves by digital rectal retrieval and separated in two parts. One part of the feces samples was used to determinate fecal pH immediately, and the second part was stored in a refrigerator.

Whole blood samples were analyzed for number of red blood cells (RBC), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) using an automated veterinary analyzer (Phoenix NCC-Vet 30, Neomedica, Serbia). The concentrations of serum Fe and TIBC were measured by commercial kits (BioSystems, Spain) using an auto analyzer (BioSystems A15, Spain).

In fresh fecal samples, pH was measured immediately using semi quantitative test strips (Sigma-Aldrich, Germany) with ranges from 4.5 to 10.0 with intervals of 0.5 units. Within 24 h of sampling, fecal samples were inoculated on Zeissler agar plates (Torlak, Serbia) and into Tarozzi broth (Torlak, Serbia) and incubated anaerobically for 48 h at 37 °C. They were also plated onto 5% sheep blood agar, MacConkey agar (HiMedia, India) and incubated aerobically for 24 h at 37 °C. For the isolation of *Salmonella spp*., the cultural procedure as described by standard SRPS EN ISO 6579-1/2017 was used. Colonies recovered after anaerobic and aerobic incubation were identified on the basis of colonial and cellular morphology, cultural and biochemical characteristics, and commercial tests (BBL Crystal E/N ID Kit, Anaerobe Kit, Becton Dickinson). Fecal samples were tested for the presence of bovine viral diarrhea virus (BVDV) by real time RT-PCR using previously published primers (Applied Biosystems, USA) by Hoffmann and coworkers (2006). For bovine rotavirus and bovine coronavirus detection, molecular tests were also used (Decaro et al, 2008; Falcone et al, 1999). The fecal samples were also examined by microscopy for the presence of *Cryptosporidium* and coccidian as described by (Mišić et al., 2002).

Data analyses were performed using STATISTICA v.8. Software package (StatSoft, Inc., Tulsa, Ok, USA). Student's T test was used to evaluate the differences of means between two groups. Data were presented as mean±SE (standard error) for all investigated parameters.

RESULTS

The results of erythrogram, Fe and TIBC concentrations as well as fecal pH in relation to occurrence of diarrhea are summarized in Table 1 and 2. With the exception of MCH, the differences of individual indicators between groups were significant. The RBC, Hb, HCT, and MCHC were lower ($P < 0.01$), whereas MCV and TIBC were higher ($P < 0.01$) in calves from the diarrheic group compared to calves from the healthy group (Table 1). Serum Fe concentrations were significantly lower ($P < 0.01$) in calves from the diarrheic group compared to calves from the healthy group (Table 2). The pH of the feces was significantly greater ($P < 0.01$) in calves from the diarrheic group compared to calves from the healthy group (Table 2). All grouped data were homogenous ($Cv \leq 30\%$), and in all cases, the data distribution was according to the normal distribution model. Variance was homogenous.

No pathogenic bacteria were isolated. No BVDV was detected in fecal samples and neither were rotavirus or bovine coronavirus detected using molecular tests. Fecal samples were negative for *Cryptosporidium* and coccidia.

Table 1. Hematological indices in groups of diarrheic and healthy calves

Parameters	Diarrheic calves	Healthy calves
RBC (10^6 µl)	6.02 ± 0.48^a	8.93 ± 0.41^b
Hb (g/dl)	5.63 ± 0.31^a	9.20 ± 0.36^b
HCT (%)	20.89 ± 1.77^a	27.79 ± 1.26^b
MCV (fl)	34.70 ± 1.28^a	31.12 ± 0.67^b
MCH (pg)	10.39 ± 0.35^a	11.42 ± 0.41^a
MCHC (g/dl)	26.95 ± 0.73^a	36.70 ± 0.68^b

RBC – red blood cell, Hb – hemoglobin concentration, HCT – hematocrit value, MCV – mean corpuscular volume, MCH – mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration
$^{a, b}$ –Values in the same row with different superscripts are significantly different ($P < 0.01$)

Table 2. Serum iron concentration, TIBC and the fecal pH in groups of diarrheic and healthy calves

Parameters	Diarrheic calves	Healthy calves
Iron (µmol/l)	7.02 ± 0.53^a	26.58 ± 3.11^b
TIBC (µmol/l)	129.31 ± 1.94^a	92.18 ± 2.78^b
Fecal pH	7.08 ± 0.24^a	6.00 ± 0.18^b

TIBC – total iron binding capacity; $^{a, b}$ –Values in the same row with different superscripts are significantly different ($P < 0.01$)

DISCUSSION

As expected, there were marked differences in the examined variables between diarrheic calves as compared with the healthy group of calves. On day 12 after birth, the low values for RBC count, HCT percentage, and Hb and Fe concentrations in the group of diarrheic calves indicated the occurrence of severe iron deficiency anemia. In addition, decreased MCV, MCHC and increased TIBC values are consistent with the development of microcytic, hypochromic anemia (Harvey, 2008). Furthermore, these findings also indicate that the iron requirements of neonatal calves are greater than those of mature ruminants. On the other hand, erythrocytes of the matched healthy calves showed microcytic and normochromic changes. However, these calves did not show any anemic symptoms. This finding is consistent with that of Miyata and coworkers (1984), indicating that MCV is not specific to iron deficiency *per se*. The greatly different iron reserves at birth can be expected to be responsible for differences in erythrograms between two groups of calves. Investigation by Morel (1996) showed significantly different amounts of iron can be stored in the liver and other organs of calves at birth. Many other studies have also implicated prenatal development of iron deficiency as an important cause of the anemia in neonatal calves (Lind and Blum, 1994; Bosted et al., 1990; Tennant et al., 1975).

Selected bacteriological, virological and parasitological examination in anemic calves revealed no infectious disease agents which could have caused the diarrhea in this group of calves. However, findings from fecal examination in anemic calves do not exclude other infectious agents that can play a role in enteric diseases, although their anemia was not consistent with that of inflammatory disease. Anyway, both infectious agents and factors other than infectious disease agents are implicated as causes of calf diarrhea (Scott et al., 2004). Among others, iron deficiency anemia should also be considered to be a predictor of neonatal calf diarrhea on some dairies (Okabe et al., 1996; Pare et al., 1993; Bostedt et al., 1990; Blaxter et al., 1957). Investigation of calves illustrated that low HCT was associated with an increased risk of both the age of onset and length of the first episode of diarrhea (Pare et al., 1993). Moreover, Bostedt and coworkers (1990) reported that calves with Fe values less than 18 μmol/l at or shortly after birth are at greater risk for development of gut disorders (i.e. severe diarrhea). However, except for anemia prevention and immunity, the role of iron in the maintenance of intestinal homeostasis is not still completely clear in calves. In line with this, inadequate morphological and functional adaptation of the gastrointestinal tract is considered to have a central role in the etiology of gut diseases during the neonatal period (Blum, 2006). Consequently, it is tempting to speculate that diminished IGF-I status associated with iron deficiency (Prodanovic et al., 2014) might be one of the predisposing factors for the increased incidence of diarrhea in anemic calves. The major findings indicate that IGF-I could influence the growth and development of intestines, respectively their morphological and functional maturation after binding to respective receptors as also shown in neonatal calves (Georgiev et al., 2003; Hammon and Blum, 2002). Additionally, malabsorptive changes and the incidence of diarrhea in anemic calves could have resulted from changes in the IGF-I induced nutrient uptake (Donovan et al., 2004) or from a lack of iron-dependent enzymes (Nadadur et al., 2008; Larkin and Hannan, 1985). However, gut growth and function are also modified by blood hormones and growth factors and by postprandial release of intestinal and pancreatic humoral factors (Blum and Baumrucker, 2002; Guilloteau et al., 2002; Blum and Hammon, 2000).

The results of this study revealed that fecal pH was higher in anemic calves than in healthy calves. The most likely scenario points to the necessity of iron for normal gastric secretion. In the other words, the incidence of neonatal diarrhea in anemic calves may be a reflection of the alteration in gastric function and consequently imbalance in the gastrointestinal tract microbiota, as was previously described in iron-deficient piglets (Larkin and Hannan, 1985; 1983). A similar explanation may exist for the observation that acidification of milk with dilute hydrochloric acid was effective in reducing diarrhea in anemic calves (Blaxter et al., 1957). Besides, hypochloridia associated with the iron-deficient state, in turn, could result in decreased availability of iron from the diet in milk-fed neonates (Nadadur et al., 2008). However, the potential roles of altered iron status and, consequently, diminished IGF-I status as a contributory factor in the development and/or delayed healing of the gastric mucosal alteration have yet to be elucidated in calves.

CONCLUSIONS

In conclusion, an increase in the fecal pH in anemic calves could be related to their impaired iron status. This relationship could be responsible, at least in part, for the increased incidence of neonatal diarrhea observed in this population of calves. However, further research into the implications of iron deficiency on calf gut health is needed, since calves are often exposed to subnormal iron levels during their first weeks of age.

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Authors contributions

RP and DK conceived of the presented idea and wrote the manuscript with support from IV and JB. OR, BK and VM carried out bacteriological, virological and parasitological examinations. SA and LjJ carried out the samples collection and haematological examination. SN performed the statistical analyses.

Competing interests

The authors declare that they have no competing interests.

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NEONATALNA DIJAREJA KOD TELADI SA ANEMIJOM USLED NEDOSTATKA GVOŽĐA

PRODANOVIĆ Radiša, NEDIĆ Sreten, RADANOVIĆ Oliver, MILIĆEVIĆ Vesna, VUJANAC Ivan, BOJKOVSKI Jovan, KURELJUŠIĆ Branislav, ARSIĆ Sveta, JOVANOVIĆ Ljubomir, KIROVSKI Danijela

Kratak sadržaj

Uvod. Neonatalna telad su često deficitarna u gvožđu. Brojni podaci ukazuju na povezanost nedostatka gvožđa sa različitim patološkim stanjima, uključujući i pojavu dijareje. Cilj rada bio je da se ispita povezanost statusa gvožđa i promena u funkciji digestivnog trakta novorođene teladi sa i bez znakova dijareje.

Materijal i Metode. Telad su podeljena u dve grupe na osnovu pojave dijareje; prva grupa (n=6) telad sa dijarejom i druga grupa (n=6) klinički zdrava telad bez znakova dijareje. Uzorci krvi su uzeti od sve teladi 12. dana života i određeni su parametri crvene krvne slike, sadržaj serumskog gvožđa i ukupni kapacitet vezivanja gvožđa. Parametri crvene krvne slike određeni su automatskim hematološkim analajzerom, dok su biohemijski parametri određeni spektrofotometrijski. Uzorci fecesa uzeti su od sve teladi i izmerena je pH vrednost semikvantitativno pomoću test tračica. Bakteriološkim pregledom uzorci fecesa su ispitani na prisustvo enterotoksogenih *Escherichia coli, Salmonella spp. i Clostridium perfringens* vrsta; RT-PCR metodom ispitani su na prisustvo goveđeg rotavirusa, coronavirusa i virusa goveđe virusne dijareje, dok su mikroskopski pregledani na prisustvo *Cryptosporidium parvum*.

Rezultati i zaključak. Promene u koncentraciji gvožđa dovele su do pojave značajnih promena u hematološkim indeksima teladi sa dijarejom i pojavi mikrocitne hipohromne anemije. Vrednost pH fecesa je bila značajno veća kod teladi sa dijarejom u odnosu na zdravu telad. Svi uzorci fecesa bili su negativni na ispitivane enteropatogene uzročnike. U poređenju sa zdravom teladi, pretpostavka je da kod teladi sa anemijom koja je uzrokovana deficitom gvožđa nastaju promene u funkciji digestivnog trakta koje dovode do pojave dijareje.

Ključne reči: hipohromna anemija, dijareja, telad