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SHORT COMMUNICATIONS

Dairy calf rearing unit and infectious diseases: diarrhea outbreak by bovine coronavirus as a model for the dispersion of pathogenic microorganisms

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Abstract
Dairy calf rearing unit is a management system that is only recently being implemented by some milk producer's cooperatives in southern Brazil. However, aspects related to the health profile of the heifer calves that arrive in the rearing unit as well as about biosecurity practices and microbiological challenges have not yet been evaluated in this rearing system in a tropical country. Diarrhea is the main and most frequent consequence of enteric infections in newborn calves. This study, through some etiological and epidemiological characteristics of an outbreak of neonatal diarrhea, has the aim to alert to the possibility of pathogenic microorganism spread in a dairy heifer calf rearing unit. The diarrhea outbreak presented some non-regular characteristics observed in bovine coronavirus (BCoV) enteric infections in dairy calves. The spread of infection was extremely rapid (1 week); the attack rate (> 50%) was much higher than that observed in calves subjected to conventional rearing; and the age range (5 to 90 days) of the affected heifer calves was much broader than that often observed in the BCoV diarrhea worldwide. These unusual epidemiological characteristics observed in this BCoV diarrhea outbreak raise awareness of the health threat present in calf rearing units as well as of the easy and rapid viral spread in a population of young animals from different dairy herds and, therefore, with very distinct immunological status.

Keywords Dairy calf · Biosecurity · Risk infection · Enteric infection · BCoV

Introduction

One of the aims of dairy herds is the rearing of high-quality heifers for the replacement of adult animals to improve milk production which is the main activity. In order to accelerate the development of the calves and heifers and the genetic improvement of cows, some dairy farmer's cooperatives have adopted the calf rearing system where dairy heifer calves are received up to 5 days old and returned to the original farm as pregnant heifer. However, calves from distinct herds with different health management and immunological status are grown in the same rearing unit which can be a great threat for the occurrence of several infectious health problems (Walker et al. 2012).

Dairy calf rearing unit is a management system that is only recently being implemented by some milk producer's cooperatives in southern Brazil. Aspects of management and nutrition of dairy heifer calves in this rearing system are being evaluated with relative frequency in Brazil. However, regarding the health status, very little is known about biosecurity practices and the microbiological challenges in Brazilian calf rearing units.

Neonatal diarrhea is considered as an important health problem in calves worldwide (Ammar et al. 2014; Lorenzetti et al. 2013; Park et al. 2006). Calf diarrhea is a multifactorial syndrome with predisposing factors (age, genetics, nutritional, and immunological) and mainly the causal agent such as virus,

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bacteria, and protozoa in single or mixed infections (Barry et al. 2009; Coura et al. 2015).

Worldwide, the rotavirus A (RVA) and bovine coronavirus (BCoV) are considered the most important enteric viruses involved in calf diarrhea (Ammar et al. 2014; Jerez et al. 2002; Martella et al. 2010). The BCoV commonly affects calves less than 30 days old causing neonatal diarrhea (Ammar et al. 2014; Lorenzetti et al. 2013). Most studies show that BCoV may be involved in an average of 10 to 30% of cases of neonatal diarrhea (Ammar et al. 2014; Coura et al. 2015; Park et al. 2006; Stipp et al. 2009).

Through the description of a diarrhea outbreak with unusual epidemiological characteristics caused by BCoV, the aim of this study was to describe the possibility of pathogenic microorganism spread in a dairy calf rearing unit.

Materials and methods

The dairy heifer calf rearing unit is located in the city of Medianeira (25° 17′ 43″ S and 54° 05′ 38″ W), Western Parana State, Southern Brazil. The rearing unit consists of 5 barns (5 m × 3 m) containing 20 to 25 heifer calves per barn. The animals are housed until 60 days of age and subsequently placed in pickets located near the barns where they remained from weaning period until the breeding by artificial insemination. This calf rearing unit receives Holstein female calves and crossed Holstein breed up 5 days old from 42 small dairy farms. Until weaning, the calves are fed calf milk replacer. Besides the compulsory control of brucellosis and tuberculosis and vaccination against foot and mouth disease, the health profile of each small dairy farming was unknown. However, vaccination of cows at the end of gestation to control neonatal calf diarrhea was not performed by any of the dairy herds. Additionally, the colostrum quantity, quality, and immune passive transfer were not monitored at the original 42 small dairy farms. Thus, dairy heifer calves with possible distinct immunological and health status were housed together in the same rearing unit. The dairy calf rearing unit has been regularly operating at 18 months. During this period, the average rate of diarrhea episodes varied from 17 to 22%, with the highest frequency of occurrence in calves between 2 and 3 weeks old.

The diarrhea outbreak affected approximately 73 of the 112 heifer calves encompassing the animal unit with 5 to 90 days old. This outbreak resulted in a morbidity rate of 65%, with 8% lethality. The evolution of the clinical signs such as depression, anorexia, dehydration, and watery diarrhea occurred during 2 to 5 days within the 25 to 30 days of the outbreak. The animals were unresponsive to broad-spectrum antibiotic therapy (tulathromycin and tildipirosin) and 6 calves died 5 to 7 days after the onset of diarrhea.

Thirty-three diarrheic fecal samples were randomly selected according to the age group being 7, 9, and 17 diarrheic fecal samples collected from calves that were 5 to 30, 31 to 60, and 61 to 90 days old, respectively, so that the animals from all barns and pickets showed clinic signs of diarrhea. In addition, 15 fecal samples from 5 asymptomatic calves of each age group were collected. The samples were transported at 4 °C and stored at − 80 °C until use.

All diarrheic fecal samples were submitted for parasitological evaluation to detect *Cryptosporidium* sp. and *Eimeria* sp. oocysts using modified Ziehl-Nelseen (Henriksen and Pohlenz 1981) and Gordon and Whitlock techniques, respectively.

For virological analysis fecal suspensions at 10–20% (*w*/*v*) were prepared and the nucleic acid extraction was realized as described by Alfieri et al. (2006). The RNA of enteric viruses in the fecal samples was investigated by molecular techniques such as reverse transcription-polymerase chain reaction (RT-PCR) and semi-nested PCR (SN-PCR) for partial amplification of BCoV N gene (Takiuchi et al. 2006), bovine RVA VP4 and VP7 genes (Gentsch et al. 1992; Gouvea et al. 1990), bovine rotavirus B (RVB) NSP2 gene (Gouvea et al. 1991), and bovine rotavirus C (RVC) VP6 gene (Alfieri et al. 1999). The amplified products were analyzed by electrophoresis on a 2% agarose gel in TBE buffer, pH 8.4 (89 mM Tris; 89 mM boric acid; 2 mM EDTA), stained with ethidium bromide (0.5 μg/ml), and visualized under UV light.

Three BCoV-amplified products, each representing an age group, were sequenced in an ABI3500 Genetic Analyzer sequencer with the Big Dye Terminator v3.1 Cycle Sequencing Kit using the forward and reverse primers used in the SN-PCR assay (Applied Biosystems, Foster City, CA, USA). Sequence quality analysis was performed using Phred software and the contig assembly was obtained using the CAP3 software (http://asparagin.cenargen.embrapa.br/phph/). Sequence similarity searches were performed using the BLAST software (http://blast.ncbi.nlm.nih.gov/). The sequence identity matrix was realized in BioEdit version 7.1.3.0.

Results and discussion

The RNA of RVA, RVB, and RVC were not identified by RT-PCR assay in diarrheic fecal samples evaluated. The sampling was also negative for oocysts of *Cryptosporidium* sp. and *Eimeria* sp. Fecal samples from 15 asymptomatic calves were also negative for all enteropathogens evaluated. In this study, the attempt of bacterial isolation was not performed because at the time of fecal collection, the diarrheic calves were undergoing to an intense treatment with broad-spectrum antibiotics.

BCoV RNA was the single nucleic acid amplified by the SN-PCR assay in 54.5% (18/33) of the diarrheic fecal samples evaluated in this outbreak. Between age groups evaluated, BCoV was identified in 28.6, 66.7, and 58.8% of the fecal samples in the age groups 5 to 30, 31 to 60, and 61 to 90 days

old. Through the sequencing reaction was possible to confirm the specificity of the amplified products. The three nucleotide (nt) sequences presented 100% of nt identity to each other and exhibited 98.1 to 100% of nt identity with other BCoV sequences deposited in GenBank.

Calf rearing unit is a management practice that has been employed in some Brazilian regions that are traditionally recognized by milk production. However, regarding the health status, very little is known about biosecurity practices and the microbiological challenges in this calf rearing system. Compared to traditional rearing, this system has many advantages particularly with regard to management but may represent a sanitary threat when not performed properly. Heifer calves generally from small dairy farms of different geographical origins and herds with different health status maintained in the same rearing unit increase the chance of neonatal infections including diarrhea that is one of the major health problems that affects suckling calves.

In this study, the calves were unresponsive to broad-spectrum antibiotic therapy and all diarrheic fecal samples analyzed were negative for *Cryptosporidium* sp. and *Eimeria* sp., two important protozoan parasites that cause diarrhea in calves and for three enteric RNA virus (RVA, RVB, and RVC) (Koutny et al. 2012; Suler et al. 2016). So, this diarrhea outbreak was selected as a model to describe the rapid dispersion of potentially pathogenic microorganisms in a calf rearing unit.

The SN-PCR assay for BCoV detection amplified a product with 251 bp of the N gene in 54.5% (18/33) of the diarrheic fecal samples. Only single infections associated with BCoV were identified during this diarrhea outbreak, suggesting that this virus participated in the development of the clinical manifestations observed during this investigation. The diarrhea outbreak was characterized by the high morbidity rate including an unusual age group, particularly animals with 60 to 90 days of age.

The diarrhea caused by BCoV is often reported as a self-limiting enteric disorder in young animals with insufficient intake of colostrum, not ensuring adequate passive transfer of immunoglobulin (Al Mawly et al. 2015; Meganck et al. 2014). Throughout the world, most of diagnostic reports of BCoV involved in calf neonatal diarrhea reported frequencies of positive samples ranging from 10 to 30%. Additionally, the infection is also more frequent in calves less than 30 days old (Ammar et al. 2014; Rocha et al. 2017).

BCoV in single and in mixed infection have already been detected in calves from Brazilian beef and dairy cattle herds in transversal and longitudinal studies (Coura et al. 2015; Lorenzetti et al. 2013). However, this diarrhea outbreak presented some non-regular characteristics in infections by BCoV in dairy cattle herds. The spread of infection was extremely rapid (1 week); the attack rate (> 50%) was much higher than that observed in calves subjected to conventional rearing; and the age range (5 to 90 days) of the affected heifer calves was much broader than that often observed in the BCoV diarrhea. Finally, this is the first description of BCoV single infection causing a diarrhea outbreak in a Brazilian calf rearing unit with a high infection rate in calves over 30 days old.

In this study, BCoV-positive diarrheic fecal samples were found in all age groups analyzed. However, the calves between 31 to 60 days old showed the highest rate (66.7%) of infection and also in calves with 61 to 90 days of age (58.8%), in which the BCoV infection is not frequently detected. The high concentration of heifer calves in a calf rearing unit and the presence of animals with distinct immunological status may have facilitated the BCoV widespread and compromised calves of all age groups in the rearing unit at the time of the diarrhea outbreak.

The rearing of heifer calves in groups from different origins in calf rearing units is a type of calf management that only recently has been introduced in the Brazilian dairy production system. However, aspects related to the health management of heifer calves are still neglected in most of the rearing units. The risk mitigation actions for infectious diseases are rarely implemented and mainly adequately monitored. Additionally, standardized health protocols in dairy herds of origin of the calves are sporadic.

The neonatal diarrhea outbreak described in this study was characterized by a high attack rate and rapid dispersion and compromised calves of different ages. These characteristics of the infection are not frequently found in BCoV diarrhea in calves reared in the conventional system. Thus, this report highlights the importance of health management, including laboratory diagnosis, in all health emergencies in dairy calf rearing units.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This study was approved by the Ethics Committee on the Use of Animals in Teaching and Research of the Universidade Estadual de Londrina (UEL) under number 6371.2013.43. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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