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Original Article
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# Neonatal diarrhea and rotavirus A infection in beef and dairy calves, Brazil, 2006-2015[1]

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**ABSTRACT.-** Medeiros T.N.S., Lorenzetti E., Massi R.P., Alfieri A.F. & Alfieri A.A. 2020. **Neonatal diarrhea and rotavirus A infection in beef and dairy calves, Brazil, 2006-2015**. *Pesquisa Veterinária Brasileira 40(1):7-11*. Laboratório de Virologia Animal, Departamento de Medicina Veterinária Preventiva, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, Campus Universitário, Cx. Postal 10011, Londrina, PR 86057-970, Brazil. E-mail: alfieri@uel.br

Calf diarrhea causes substantial economic losses in the cattle industry worldwide. Bovine rotavirus A (RVA) is the main viral agent that leads to enteric infection and diarrhea outbreaks in calves throughout the world. The aim of this retrospective (2006-2015) study was to determine the frequency of RVA detection in diarrheic fecal samples from beef and dairy calves from the three main cattle-producing regions of Brazil. Diarrheic fecal samples ($n$=1,498) of 124 beef and 56 dairy cattle herds from the Midwest, South, and Southeast geographical regions of Brazil were evaluated using the silver-stained polyacrylamide gel electrophoresis (ss-PAGE) technique. RVA double stranded-RNA was identified by the ss-PAGE technique in 410 (27.4%) fecal samples. The frequency of positive samples found in beef calves (31.9%; 328/1,027) was higher than the frequency found in diarrheic fecal samples from dairy calves (17.4%; 82/471). RVA infection was identified in calves from the three Brazilian geographical regions analyzed. However, the frequency of positive diarrheic calves in the Midwest region (39.4%), predominantly beef calves, was higher than in the South (19.4%) and Southeast (17.6%) regions. The temporal distribution of RVA-infected calves evaluated by two five-year periods (2006-2010, 24.5%; 2011-2015, 28.8%) demonstrated a very similar frequency of RVA in both periods. Considering the wide regional and temporal scope of this study, it can be concluded that RVA remains an important etiology of neonatal diarrhea in calves of Brazilian cattle herds.

INDEX TERMS: Newborns, diarrhea, rotavirus A, infection, beef cattle, dairy cattle, calves, Brazil, cattle, enteric infection, RVA, ss-PAGE, epidemiology.

**RESUMO.-** [**Diarreia neonatal e infecção por rotavírus A em bezerros de corte e leite, Brasil, 2006-2015.**] A diarreia neonatal ocasiona perdas econômicas importantes na pecuária bovina em todo o mundo. Rotavírus A (RVA) é o principal agente etiológico viral de infecções entéricas e surtos de diarreia em bezerros de rebanhos de corte e leite. O objetivo deste estudo retrospectivo (2006-2015) foi determinar a frequência de detecção de RVA em amostras de fezes diarreicas de bezerros de corte e leite das três principais regiões produtoras de bovinos do Brasil. Amostras de fezes diarreicas ($n$=1.498) de 124 rebanhos bovinos de corte e 56 rebanhos bovinos de leite das regiões Centro-Oeste, Sul e Sudeste do Brasil foram avaliadas utilizando a técnica de eletroforese em gel de poliacrilamida (EGPA). O genoma segmentado de RVA foi identificado pela técnica de EGPA em 410 (27,4%) amostras de fezes. A frequência de amostras positivas encontrada em bezerros de rebanhos de corte (31,9%; 328/1.027) foi maior que a frequência identificada em amostras de fezes diarreicas de bezerros de rebanhos leiteiros (17,4%; 82/471). A infecção por RVA foi identificada em bezerros das três regiões geográficas brasileiras analisadas.

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No entanto, a frequência de bezerros com diarreia positivos para RVA na região Centro-Oeste (39,4%), predominantemente de bezerros de rebanhos de corte, foi maior que nas regiões Sul (19,4%) e Sudeste (17,6%). A distribuição temporal dos bezerros infectados com RVA avaliados por dois períodos de cinco anos (2006-2010, 24,5%; 2011-2015, 28,8%) demonstrou uma frequência muito semelhante em ambos os períodos. Considerando a amplitude regional e temporal deste estudo, pode-se concluir que RVA continua sendo uma importante etiologia de diarreia neonatal em bezerros de rebanhos bovinos brasileiros.

TERMOS DE INDEXAÇÃO: Diarreia neonatal, infecção, rotavírus A, bezerros de corte, bezerros de leite, Brasil, bovinos, infecção entérica, RVA, EGPA, epidemiologia.

## INTRODUCTION

Neonatal diarrhea is a major health problem in livestock production worldwide (Smith 2012). Diarrhea outbreaks in calves have severe direct and indirect economic consequences due to morbidity, mortality, reduced growth rates, increased age at first calving, treatment costs, and time spent caring for the affected calves (Alfieri et al. 2006, Windeyer et al. 2014).

Diarrhea in calves has a multifactorial etiology (Blanchard 2012). Virus, bacteria, and protozoa infection, as well as immunological status and management factors (housing, feeding, and hygienic conditions) play an important role as determinants and predisposing factors, respectively (Alfieri et al. 2006, Blanchard 2012, Windeyer et al. 2014). With regard to the determinant factors, enteropathogens such as *Escherichia coli* K99, *Cryptosporidium parvum*, *Clostridium perfringens*, bovine coronavirus, and rotavirus A (RVA) have been widely recognized in cases of intestinal infections in young calves, including in Brazil (Alfieri et al. 2006, Oliveira Filho et al. 2007, Bartels et al. 2010, Blanchard 2012, Lorenzetti et al. 2013, Coura et al. 2015).

Rotavirus is one of the most important etiological viral agents of severe gastroenteritis in young humans and many mammalian and avian species (Estes & Greenberg 2013). In Brazil, several studies related to the etiology of neonatal diarrhea point to infection with RVA as one of the major causes of enteric infections in newborn calves (Langoni et al. 2004, Alfieri et al. 2006, Oliveira Filho et al. 2007, Coura et al. 2015).

Rotaviruses belong to the Reoviridae family, genus *Rotavirus*. The virus is 70-100nm in diameter and is characterized by a non-enveloped triple-layered protein capsid with a genome composed of 11 segmented double-stranded RNA (dsRNA) translated into six structural (VP1-VP4, VP6-VP7) and six non-structural (NSP1-NSP5/6) proteins (Estes & Greenberg 2013). Based on the antigenic properties and genetic characteristics of the VP6 gene that composes the middle layer of the viral capsid, rotaviruses are classified into nine species that are designated RVA to RVI (Mihalov-Kovács et al. 2015, ICTV 2017). A new species described in bats, named RVJ, have been suggested (Bányai et al. 2017).

Infections by RVA, B, and C have already been reported in cattle (Ghosh et al. 2010, Otto et al. 2015). However, RVA is the most common RV species that causes neonatal diarrhea outbreaks in calves in a number of different countries, including Brazil (Papp et al. 2013, Medeiros et al. 2014, Otto et al. 2015).

For the diagnosis of rotavirus, both the viral particle and proteins and genomes identification can be performed by several laboratory methods (Alfieri et al. 2004, 2006, Buzinaro et al. 2009, Medeiros et al. 2014, Otto et al. 2015, Rocha et al. 2017).

Silver-stained polyacrylamide gel electrophoresis (ss-PAGE) is a simple, fast, and low-cost method for RV dsRNA detection in diarrheic feces. This technique is commonly used for RV identification in acute infections due to specificity and sensitivity (Herring et al. 1982, Alfieri et al. 2006, Medeiros et al. 2014, Coura et al. 2015, Rocha et al. 2017).

This retrospective study (2006-2015) aimed to describe the frequency of RVA diagnosis in diarrheic fecal samples from beef and dairy calves in the three main cattle-producing regions of Brazil.

## MATERIALS AND METHODS

**Ethics statement.** The study was submitted to Ethics Committee on Animal Experiments of the "Universidade Estadual de Londrina" (UEL) and approved under the identification number 6371.2013.43. All applicable institutional guidelines for the care and use of animals were followed.

**Study population.** The diarrheic fecal samples included in this study were obtained from a collection of fecal samples that were sent to the Laboratory of Animal Virology, UEL, Londrina, Paraná, Brazil. For the analysis, all fecal samples collected from January 2006 to December 2015 in beef and dairy herds from the three main cattle-producing regions of Brazil (Midwest, South, and Southeast) were selected. A total of 1,498 diarrheic fecal samples (beef calves *n*=1,027; dairy calves *n*=471) from 124 beef and 56 dairy cattle herds were included in the study. Only samples collected from diarrheic calves up to 60 days of age were included in the study. Based on the number of diarrheic fecal samples evaluated each year, the temporal distribution of bovine RVA infection in calves was not uniform. To reduce this sampling bias, we chose to analyze the results in two five-year periods, represented by the years 2006-2010 (first) and 2011-2015 (second). Additional information about the origin of the fecal samples is presented in Table 1. The fecal samples were stored at -80°C until analysis.

**Table 1. Diarrheic fecal samples for rotavirus diagnosis according to the origin (geographical region) and type (beef/dairy) of cattle production, Brazil, 2006-2015**

| Region | State | County (*n*) | Herds | | | Samples | | |
|---|---|---|---|---|---|---|---|---|
| | | | Beef | Dairy | Total | Beef | Dairy | Total |
| South | RS/SC/PR | 43 | 38 | 32 | 70 | 250 | 327 | 577 |
| Southeast | SP/MG | 33 | 20 | 20 | 40 | 174 | 122 | 296 |
| Midwest | MS/GO/MT | 44 | 66 | 4 | 70 | 603 | 22 | 625 |
| Total | | 120 | 124 | 56 | 180 | 1,027 | 471 | 1,498 |

RS = Rio Grande do Sul, SC = Santa Catarina, PR = Paraná, SP = São Paulo, MG = Minas Gerais, MS = Mato Grosso do Sul, GO = Goiás, MT = Mato Grosso.

**Table 2. Rotavirus A identified by ss-PAGE in diarrheic fecal samples from calves according to the geographical origin of the cattle herds, Brazil, 2006-2015**

| Region | Fecal samples | | |
|---|---|---|---|
| | Positive (%) | Negative | Total |
| South | 112 (19.4) | 465 | 577 |
| Southeast | 52 (17.6) | 244 | 296 |
| Midwest | 246 (39.4) | 379 | 625 |
| Total | 410 (27.4) | 1,088 | 1,498 |

**Table 3 Temporal distribution of rotavirus A identified by ss-PAGE in diarrheic calves, Brazil, 2006-2015**

| Period | Fecal samples | | Total |
|---|---|---|---|
| | Positive (%) | Negative | |
| 2006-2010 | 123 (24.5) | 378 | 501 |
| 2011-2015 | 287 (28.8) | 710 | 997 |
| Total | 410 (27.4) | 1,088 | 1,498 |

**Nucleic acid extraction and ss-PAGE.** Fecal suspensions 20% (w/v) in buffer Tris-$Ca^{2+}$ pH7.4 (50mM Tris-HCl; 10mM NaCl; 1.5mM 2-mercaptoetanol; 3mM $CaCl_2$) were homogenized and centrifuged at 2,000 x *g* for 5min at 4°C. Aliquots of 500µL of supernatant were collected and treated with SDS (sodium dodecyl sulfate) at a final concentration of 1%. The nucleic acid was extracted using a combination of the methods phenol/chloroform/isoamyl alcohol (25:24:1) and silica/guanidinium isothiocyanate (Alfieri et al. 2006). The nucleic acid was eluted in 50µL of ultrapure diethylpyrocarbonate (DEPC)-treated water (Invitrogen Life Technologies, Carlsbad/CA, USA) and briefly stored at -20°C. The cell culture adapted bovine RVA NCDV-Lincoln strain and aliquots of Tris-$Ca^{2+}$ buffer were included as positive and negative controls, respectively, in all nucleic acid extraction procedures. The presence of RVA dsRNA in diarrheic fecal samples was evaluated by the ss-PAGE technique (Herring et al. 1982, Pereira et al. 1983).

## RESULTS

The dsRNA of bovine RVA was identified in 27.4% (410/1,498) of the diarrheic fecal samples included in this study. The rate of positive samples was higher in calves from beef (31.9%, 328/1,027) than in calves from dairy (17.4%, 82/471) herds.

RVA infection was identified in calves from three geographical regions in Brazil. However, the frequency of positive diarrheic calves in the Midwest region (39.4%) was higher than that in the South (19.4%) and Southeast (17.6%) regions (Table 2).

The frequencies of RVA diagnosis by ss-PAGE in diarrheic calves according to the temporal distribution (2006-2010 and 2011-2015) is presented in Table 3.

## DISCUSSION

The current retrospective study evaluated over a period of 10 years (2006-2015) the frequency of RVA diagnosis in diarrheic fecal samples of beef and dairy calves from three geographical regions that represent approximately 65% of the Brazilian cattle industry (Brasil 2015). The detection rate of ss-PAGE RVA-positive fecal samples (27.4%) was similar to that identified in other studies performed in Brazil (Alfieri et al. 2006, Buzinaro et al. 2009), Argentina (Badaracco et al. 2012), and Iran (Madadgar et al. 2015). Therefore, the present information highlights the similar frequency of RVA infection in calves from different countries, despite differences in the management system, temperature, humidity, breed, and other calf diarrhea risk factors.

The frequency of RVA-positive fecal samples in diarrheic calves from beef herds (31.9%) was higher than in dairy herds (17.4%). The use of fixed-time artificial insemination during the two- or three-month breeding season is widespread in extensive beef cattle herds and herds with the highest number of cows. Due to this reproduction management practice, there is a temporal concentration of calves born and the number of animals susceptible to infection is higher. Thus, the challenge, and consequently the risk of RVA infection, is even greater, and in the field, outbreaks of diarrhea in beef calves are common in the main Brazilian cattle producing regions (Buzinaro et al. 2003, Medeiros et al. 2014, 2015).

Cattle herds from three Brazilian geographical regions with different management systems were evaluated in this study. Although in the South region was evaluated a higher number of diarrheic fecal samples in relation to the Southeast region, the frequency of RVA-positive samples identified in these two regions was similar.

The frequency of diarrhea caused by RVA infection identified in the Midwest region was higher than in the other regions. Some herd and management characteristics that are present in most of the farms in this region may have contributed to the higher frequency of diarrhea. In this context, we highlight several potential risk factors: I) a short breeding season with a concentration of calving; II) herd size, as this region is characterized by the presence of large herds with fixed-time artificial insemination; III) greater frequency of crossbreeding producing calves (*Bos indicus* x *Bos taurus*) with lower resistance than Nelore (*B. indicus*) calves; IV) use of maternity pens; and V) population density (Aono et al. 2013, Pereira et al. 2013, Alfieri & Alfieri 2017).

Although more fecal samples (*n*=997) were evaluated in the second period (2011-2015) compared to the first period (2006-2010) (*n*=501), the frequency of RVA-positive results found in both time periods was very similar.

A study performed from 1998-2002 by our research group detected bovine RVA in 19.4% (369/1,898) of the samples collected in calves with diarrhea from beef and dairy cattle herds in four Brazilian geographical regions (South, Southeast, Midwest, and North). The proportion of positive samples collected was 22.8% (205/899) and 16.4% (164/999) from beef and dairy cattle herds, respectively (Alfieri et al. 2006). Comparing the results, it can be observed that in nearly 20 years, the frequency of RVA diagnosis in Brazilian dairy cattle herds has remained practically the same. However, in beef cattle herds, the frequency of RVA diagnosis has increased considerably, possibly due to the reproductive management system, including reproduction biotechniques, used in the beef cattle herds.

It is likely that the highest rate of diagnosis of RVA in beef herds in the Midwest region is due to changes in reproductive management practices, such as fixed-time artificial insemination, which leads to an increased risk of enteric infections in neonates.

Protocols for the control of neonatal diarrhea in calves have been implemented in some countries, and studies indicate that the commercial vaccines currently in use are appropriate for providing protection against RVA infection in cattle (Collins et al. 2014).

Considering the quantitative and qualitative increase in the Brazilian cattle industry, measures to control and prevent bovine neonatal diarrhea outbreaks are necessary and urgent. The mitigation of risk factors and the implementation of a vaccination program against calf diarrhea are two ways to achieve this goal.

## CONCLUSIONS

The frequencies of RVA detection when evaluating two five-year periods (2006-2010 and 2011-2015) were similar.

The RVA infection rate was higher in calves of beef herds than in those of dairy cattle herds.

In the Midwest region, where predominately diarrheic fecal samples from beef herds were evaluated, the rate of RVA-positive diagnosis was higher than in the other Brazilian geographical regions (South and Southeast) evaluated in the study.

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**Conflict of interest statement.-** The authors declare that they have no conflict of interest.

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