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ORIGINAL ARTICLE

Frequency of group A rotavirus in diarrhoeic calves in Brazilian cattle herds, 1998–2002

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Abstract The frequency of group A bovine rotavirus (gpA BRV) in calves from 1998 to 2002 was determined by the polyacrylamide gel electrophoresis technique in 2177 faecal samples, of which 1898 samples were diarrhoeic and 279 were of normal consistency (control group) that were collected from asymptomatic calves for comparative purposes. The animals were from beef and dairy cattle herds ($n = 321$) from 158 counties in seven States (Paraná, São Paulo, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Goiás and Rondônia) and four Brazilian geographical regions (south, south-east, centre-west, and north). GpA BRV was detected in 19.4% (369/1898; $p = 0.0001$) of the samples collected in calves with diarrhoea and in only 2.2% (6/279; $p = 0.0001$) of the faeces with normal consistency. The proportion of positive samples collected from beef and dairy cattle herds was 22.8% (205/899; $p = 0.0001$) and 16.4% (169/999; $p = 0.0005$), respectively. In relation to age, a higher prevalence of infections was found in calves up to 30 days old, where 33.0% (189/573; $p = 0.0001$) and 20.2% (138/683; $p = 0.0001$) of the diarrhoeic faecal samples from beef and dairy cattle herds, respectively, were positive for gpA BRV. These results show the possible importance of inclusion of gpA BRV in the management of neonatal calf diarrhoea in Brazilian cattle herds.

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Keywords Calves · Diarrhoea · Group A bovine rotavirus · Polyacrylamide gel electrophoresis

Abbreviations

BRV	bovine rotavirus
gpA	group A
SS-PAGE	silver-stained polyacrylamide gel electrophoresis

Introduction

Acute diarrhoea is common and is one of the most important diseases in newborn calves from both dairy or beef herds throughout the world; it can cause considerable economic loss. A significant increase in morbidity and mortality and in treatment costs, poor growth and greater susceptibility to other infections, especially respiratory, are the most frequent consequences of neonatal diarrhoea in calves (Janke, 1989; Paul and Lyoo, 1993).

Diarrhoea is defined as having a complex multifactorial aetiology, influenced by infectious, nutritional and environmental factors as well as management practices. Among its suggested causes are toxins, bacteria,

protozoa, viruses and management/environmental factors such as overfeeding, low temperature, poor hygiene, colostrum deprivation and individual animals' susceptibility. Infectious agents, either singly or in combination, may be associated with field outbreaks with considerable economic burden (Reynolds *et al*., 1986; Snodgrass *et al*., 1986; Janke, 1989; Takiuchi *et al*., 2006).

Regarding viral aetiology, the rotaviruses are considered a common, widespread cause of enteric illness in the newborn of many mammalian and poultry species throughout the world (Gentsch *et al*., 1996; Barreiros *et al*., 2003; Tamehiro *et al*., 2003). Rotaviruses belongs to the family *Reoviridae* and the virus particle consists of a genome of 11 double-stranded RNAs (dsRNA) enclosed in a triple-layered capsid. There are at least seven rotavirus antigenic groups (A–G) and the groups A, B and C occur in mammals (Teixeira *et al*., 1998; Alfieri, *et al*., 1999; Estes, 2001).

Group A bovine rotaviruses (gpA BRV) are among the enteropathogenic agents more commonly associated with neonatal diarrhoea in calves up to 30 days old (Luchelli *et al*., 1992; Ishizaki *et al*., 1995; Vende *et al*., 1999). In Brazil, the presence of gpA BRV has been reported in calves but there are few reports in which epidemiological studies of both beef and dairy calves have been conducted (Jerez *et al*., 1987; Buzinaro *et al*., 2000, 2003; Brito, 1994; Alfieri *et al*., 2004).

The purpose of the study reported here was to estimate the prevalence of gpA BRV using silver-stained polyacrylamide gel electrophoresis (SS-PAGE) in diarrhoeic and normal faeces from beef and dairy calves from four Brazilian geographical regions and to present epidemiological data of the infection.

Materials and methods

Herds

The faecal samples included in this study were collected from calves from 1 to 90 days old from 321 cattle herds from south (Paraná state) south-east (São Paulo and Minas Gerais states) centre-west (Mato Grosso do Sul, Mato Grosso and Goiás states) and north (Rondônia state) geographical regions of Brazil. Calves' ages were determined from producers' records and for dairy calves up to 30 days old the age group could be stratified in terms of weeks of life.

Faecal samples

From 1998 to 2002, faecal samples were collected from 2177 calves and stored at −20°C. A total of 1898 faecal samples were from calves with diarrhoea and 279 from asymptomatic calves (control group). Diarrhoeic faecal samples were collected from beef ($n = 899$) and dairy ($n = 999$) cattle herds and in this group were included 116 samples that presented some evidence of blood in the faeces.

Virus and cells

The gpA BRV Lincoln strain, propagated in MA 104 (African Monkey kidney) cells according to the standard procedure described by Estes and colleagues (1981), was used as positive control in the SS-PAGE technique.

RNA extraction

The faecal suspension (10–20% w/v) in buffer pH 7.4 (50 mmol/L Tris-HCl; 10 mmol/L NaCl; 1.5 mmol/L 2-mercaptoethanol; 3 mmol/L $CaCl_2$) was vigorously homogenized, and centrifuged at 1500*g* for 10 min, and the supernatant was collected and stored at −20°C. A combination of the phenol-chloroform-isoamyl alcohol (25:24:1) and silica-guanidine isothiocyanate methods described by Alfieri and colleagues (2004), with modifications, was used to extract viral dsRNA. A 500 µl aliquot of the faecal suspension was treated with sodium dodecyl sulphate (SDS) at a final concentration of 1% and incubated in a water bath at 56°C for 30 min. Later, the nucleic acid was extracted with an equal volume of phenol-chloroform-isoamyl alcohol (Sambrook *et al*., 1989). After homogenization, incubation in a water bath at 56°C for 15 min and centrifugation at 10,000*g* for 10 min, the aqueous phase was processed with silica-guanidine isothiocyanate (Invitrogen, Life Technologies, Carlsbad, CA, USA) according Boom and colleagues (1990). The dsRNA was eluted in 30 µl ultrapure (MilliQ, Bedford, MA, USA) autoclaved water and stored at −20°C until use. Aliquots of ultrapure autoclaved water were included as negative controls in all the nucleic acid extraction procedures.

Polyacrylamide gel electrophoresis

The dsRNA was analysed by PAGE at 7.5% and silver stained according to the techniques described by Herring and colleagues (1982) and Pereira and colleagues (1983).

Statistical analysis

The chi-squared test was used at 5% significance to assess the differences among the proportions of faecal samples positive or negative to gpA BRV and variables such as faecal characteristic (diarrhoea/normal), herd category (beef/dairy) and age group of the animals studied. The EpiInfo 6.04B program (Dean *et al*., 1997) was used to perform the statistical calculations.

Results

The SS-PAGE technique showed faecal samples positive for gpA BRV in all the years (1998–2002) and Brazilian geographical regions included in this study. The proportions of gpA BRV-positive diarrhoeic faecal samples according to year of sample collection were 18.9% (1998), 25.2% (1999), 15.8% (2000), 16.2% (2001) and 21.2% (2002), and by Brazilian state were 12.8% (Paraná), 24.1% (São Paulo), 32.7% (Mato Grosso do Sul), 24.5% (Mato Grosso), 17.3% (Goiás), 10.5% (Rondônia) and 18.6% (Minas Gerais).

Of the 1898 diarrhoeic faecal samples, 369 (19.4%) (95%CI 17.68 to 21.29) were positive for gpA BRV, while among the 279 samples of faeces with normal consistency collected from asymptomatic calves, only 6 (2.2%) (95%CI 0.79 to 4.62) were positive. The identification of gpA BRV in diarrhoeic faeces was significant ($p = 0.0001$) compared to the result obtained in faeces with normal consistency (Table 1).

Table 1 Bovine group A rotavirus detection by silver-stained polyacrylamide gel electrophoresis from diarrhoeic and asymptomatic calves in Brazilian cattle herds (1998–2002)

	No. of samples (%)		
Faecal consistency	Positive	Negative	Total
Diarrhoeic	369 (19.4)	1529 (80.6)	1898
Normal (control)	6 (2.2)	273 (97.8)	279
Total	375 (17.2)	1802 (82.8)	2177

$\chi^2 = 49.80$ ($p = 0.0001$)

Table 2 Bovine group A rotavirus detection by silver-stained polyacrylamide gel electrophoresis from diarrhoeic beef and dairy calves in Brazilian cattle herds (1998–2002)

	No. of samples (%)		
Herd category	Positive	Negative	Total
Beef	205 (22.8)	694 (77.2)	899
Dairy	164 (16.4)	835 (83.6)	999
Total	369 (19.4)	1529 (80.6)	1898

$\chi^2 = 11.92$ ($p = 0.0005$)

The 22.8% frequency (205/899) of diarrhoeic faecal samples positive for gpA BRV collected from calves in beef herds was statistically significant ($p = 0.0005$) in relation to the positive rate (16.4%) detected in faecal samples collected in calves from dairy herds (164/999) (Table 2).

The highest percentage (36%) of positive diarrhoeic faecal samples (327/1256) was found in calves up to 30 days of age ($p = 0.0001$) and in samples collected in beef herds (33%) (189/573; $p = 0.0001$). There was no significant association for the age groups 30–60 days and 60–90 days for herd type (beef or dairy) (Table 3).

In dairy herds, where the age group could be stratified in weeks of age, in faecal samples from calves up to 1 month old the highest frequency ($p = 0.0001$) of positive results occurred in the diarrhoeic faecal samples collected from animals in the 2-week-old (25.3%) to 3-week-old (36.0%) age group (Table 4).

In normal faeces (control group) from asymptomatic calves, gpA BRV was identified only in the calf category up to 30 days old from dairy herds. Only 3.4% (4/116) of the diarrhoeic faecal samples that showed blood in varied degrees were positive for gpA BRV gpA.

Discussion

Most studies on the gpA BRV frequency in calves from Brazilian cattle herds were carried out in specific geographical regions and with relatively small samples (Jerez *et al*., 1987; Barbosa *et al*., 1998; Buzinaro *et al*., 2000, 2003; Alfieri *et al*., 2004; Brito, 1994). The present study examined the presence of BRV over a 5-year period (1998–2002) in several Brazilian states from different geographical regions. The proportion of 19.4% (369/1898) of gpA BRV-positive samples found in faeces of calves with diarrhoea showed the presence

Table 3 Bovine group A rotavirus detection by silver-stained polyacrylamide gel electrophoresis from beef and dairy diarrhoeic faecal samples, distributed according to age group, in Brazilian cattle herds (1998–2002)

	No. of samples (%)							
	Beef herds			Dairy herds			Statistical analysis	
Age group (days)	Positive	Negative	Total	Positive	Negative	Total	χ^2	p
<30	189 (33.0)	384 (67.0)	573	138 (20.2)	545 (79.9)	683	25.77	0.0001
30–60	14 (10.0)	126 (90.0)	140	23 (10.5)	197 (89.5)	220	0	0.968
61–90	2 (1.1)	184 (98.9)	186	3 (3.1)	93 (95.6)	96	*	0.341
Total	205	694	899	164	835	999		

*Exact Fischer test

Table 4 Bovine group A rotavirus detection by silver-stained polyacrylamide gel electrophoresis from diarrhoeic dairy calves up to 30 days old in Brazilian cattle herds (1998–2002)

	No. of samples (%)		
Age group (weeks)	Positive	Negative	Total
First	16 (18.2)	72 (81.8)	88
Second	36 (25.3)	106 (74.6)	142
Third	40 (36.0)	71 (64.0)	111
Fourth	46 (13.4)	296 (86.6)	342
Total	138 (20.2)	545 (79.8)	683

$\chi^2 = 29.49$ ($p = 0.0001$)

($p = 0.0001$) of this virus in neonatal diarrhoea in beef and dairy calves in all the periods, regions and states studied, confirming its possible importance in the aetiology of this enteric disease that has implications for calf rearing throughout the world (Luchelli *et al*., 1992; Brenner *et al*., 1993; Ishizaki *et al*., 1995; La Fuente *et al*., 1998; Verdier and Svensson, 1998; Fukai *et al*., 1999; Constantini *et al*., 2002).

The gpA BRV frequency in diarrhoeic faeces was greater, and statistically significant ($p = 0.0005$), in calves from beef herds. This result may have been influenced by the different management practices that the calves are subjected to in beef and dairy herds. In beef herds, the widespread practice of reproductive management in a mating season of 90 to 120 days' duration results in a concentration of calf births in a relatively short period, which could facilitate transmission of the enteropathogen.

GpA RV is the main aetiological agent of diarrhoea in young animals, especially in the age groups in which passive immunity is declining (Conner *et al*., 1994). This immunological aspect, together with great resistance of the rotaviruses to environmental conditions and to disinfectants routinely used in animal production and the very large number of viral particles (10^{10}–10^{12}) eliminated during the acute period (Estes, 2001), favours the onset of the infection in the neonatal period, since the animals are challenged early. The highest gpA BRV frequency occurred in the first month of life of calves from both beef (33.0%) and dairy (20.2%) cattle herds. After 60 days, the frequency of positive diarrhoeic faecal samples was statistically very similar to that found in faeces with normal consistency. This result indicates that greater care in animal health management and the prophylactic programmes for bovine rotaviruses should aim to protect this age group (Paul and Lyoo, 1993; Barreiros *et al*., 2004).

In the samples of diarrhoeic faeces collected in dairy herds, where the age group could be stratified in weeks of life, 2- and 3-week-old calves were the most susceptible to gpA BRV infection. Passive immunity and increase in natural resistance against infection could account for the lower proportion of positive results found in calves aged 1 and 4 weeks.

Only 3.4% (4/116) of diarrhoeic faecal samples with some evidence of blood were positive for gpA BRV. This low rate of positive results is due to the pathogenesis of rotavirus infection, with virus replication only in the apical and intermediate portions of the enterocytes. In calves, the episodes of diarrhoea with blood are more related to other infections, such as those caused by *Clostridium perfringens* type C, *Salmonella* spp. or *Eimeria* spp. (Snodgrasss *et al*., 1986; Janke, 1989).

The results of this study showed that gpA BRV is involved in episodes of neonatal diarrhoea in calves from beef and dairy cattle herds in several Brazilian regions, and confirms the greater frequency of rotavirus infection in calves in the first 30 days of life. Considering that neonatal diarrhoea is the main cause of calf

morbidity and mortality and with the objective of increasing cattle herd productivity, control (hygiene and management) and prophylactic (vaccination) measures should be adopted for prevention of rotavirus infection in beef and dairy cattle herds in all Brazilian geographical regions.

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Fréquence du rotavirus de groupe A chez les veaux diarrhéiques dans le bétail brésilien

Résumé – La fréquence du rotavirus bovin de groupe A (gpA BRV) retrouvé chez les veaux de 1998 à 2002 a été déterminée par la technique d'électrophorèse au gel de polyacrylamide dans 2177 échantillons fécaux, dont 1898 échantillons étaient diarrhéiques et 279 étaient de consistance normale (groupe témoin), recueillis chez des veaux asymptomatiques pour des objectifs de comparaison. Les animaux provenaient de troupeaux de bovins et de vaches laitières ($n = 321$) provenant de 158 pays de sept états (Paraná, São Paulo, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Goiás et Rondônia) et de quatre régions géographiques brésiliennes (Sud, Sud-Est, Ouest central et Nord). Le gpA BRV a été détecté dans 19.4% (369/1898; $p = 0.0001$) des échantillons recueillis chez des veaux présentant une diarrhée et chez 2.2% (6/279; $p = 0.0001$) seulement du fèces à consistance normale. La proportion des échantillons positifs recueillis des troupeaux de bovins et de vaches laitières a été de 22.8% (205/899; $p = 0.0001$) et de 16.4% (168/999; $p = 0.0005$) respectivement. En relation à l'âge, une prévalence plus élevée d'infections a été observée chez les veaux de plus de 30 jours, où 33.0% (189/573; $p = 0.0001$) et 20.2% (138/683; $p = 0.0001$) des échantillons fécaux diarrhéiques de troupeaux de bovins et de vaches laitières respectivement ont été positifs au gpA BRV. Ces résultats mettent en évidence l'importance possible de l'inclusion du gpA BRV dans la gestion de la diarrhée néonatale des veaux dans les troupeaux de bétail brésilien.

Frecuencia de Rotavirus del grupo A en terneras diarreicas del ganado de Brasil

Resumen – Se determinó la frecuencia de rotavirus bovino del grupo A (gpA BRV) en terneras desde 1998 hasta 2002 mediante la técnica de electroforesis en gel de poliacrilamida en 2177 muestras fecales, de las cuales 1898 eran diarreicas y 279 de consistencia normal (grupo control), recogidas de terneras asintomáticas para propósitos de estudios comparativos. Los animales eran de ganado vacuno y ganado lechero ($n = 321$) provenientes de 158 países en siete estados distintos (Paraná, São Paulo, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Goiás y Rondônia) y cuatro regiones geográficas brasileñas (sur, sureste, centro-oeste, y norte). El rotavirus bovino de grupo A se detectó en 19.4% (369/1898; $p = 0.0001$) de las muestras recogidas en terneras con diarrea y en sólo 2.2% (6/279; $p = 0.0001$) de las heces con consistencia normal. La proporción de muestras positivas recogidas de manadas de ganado vacuno y ganado lechero fue 22.8% (205/899; $p = 0.0001$) y 16.4% (169/999; $p = 0.0005$), respectivamente. En relación con la edad, se encontró una incidencia más alta de infección en terneras de hasta 30 días de edad, en donde el 33.0% (189/573; $p = 0.0001$) y el 20.2% (138/683; $p = 0.0001$) de las muestras fecales diarreicas de las manadas de ganado vacuno y ganado lechero, respectivamente, fueron positivas para gpA BRV. Estos resultados muestran la posible importancia de la inclusión del gpA BRV en el manejo de la diarrea de ternera neonatal en el ganado brasileño.