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G and P genotype profiles of rotavirus A field strains circulating in beef and dairy cattle herds in Brazil, 2006–2015

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ABSTRACT

The aim of this retrospective study was to use RT-PCR and nucleotide sequencing analysis to determine the G (VP7 gene) and P (VP4 gene) genotypes of 155 Brazilian bovine rotavirus A (RVA) wild-type strains detected in diarrheic calves from all Brazilian geographical regions from 2006 to 2015. The RVA strains evaluated belonged to the G6, G10, P[5], and P[11] genotypes. The G6P[5] genotype was prevalent (65.5%; $P < 0.05$) in beef, and the G10P[11] (38.4%) and G6P[11] (30.8%) genotypes were more prevalent in dairy cattle herds. The Midwest was the region with the highest number of genotyped RVA strains, where the genotypes G6, P[5], and P[11] were identified. Genotype combination G6-IV/P[5]-IX, prevalent in beef herds, and G6-III/P[11]-III or G10-IV/P[11]-III, prevalent in dairy herds, were detected. In addition, for the first time in Brazil, we detected the P[5] and P[11] genotype RVA strains that belong to lineage II and VII, respectively.

1. Introduction

Rotaviruses (RVs) are the main viral etiology of diarrhea in children and young animals. They affect a wide variety of species of mammals and birds worldwide [1–3].

Rotaviruses belong to the *Reoviridae* family, genus *Rotavirus*. The virus is 70–100 nm in diameter and characterized by a non-enveloped triple-layered protein capsid with a genome composed by 11 segments of double-stranded RNA (dsRNA) translated into six structural and six non-structural proteins [2]. Based on the antigenic and genetic characteristics of the VP6 protein/gene that composes the middle layer of the viral capsid, RVs can be classified into nine (RVA-RVI) distinct groups or species [4,5]. Recently, Bányai et al. [6] proposed a new RV group/species (RVJ). Rotavirus A (RVA) is the most common cause of acute gastroenteritis in young children and neonatal diarrhea in production animals such as piglets and calves [1,2]. According to the antigenic and molecular characteristics of the two proteins (VP7 and VP4) present in the outer layer of viral capsid, the RVA can be classified into the G and P serotype / genotype, respectively [2]. To date, 36 G genotypes and 51 P genotypes have been described in RVA strains identified in human and animal hosts [7].

Several RVA genotypes have been detected in calves. These include 14 different G genotypes (G1-G6, G8, G10-G12, G15, G18, G21, and G24) and 11 P genotypes (P[1], P[3], P[5]-P[7], P[11], P[14], P[17], P[21], P[29], and P[33]) [8–12]. The G6P[1] (NCDV strain), G6P[5] (UK strain), G6P[11] (KN-4 strain), G8P[1] (A5 strain), and G10P[11] (B223 strain) genotype combinations are considered epidemiologically important in beef and dairy cattle herds worldwide [8,10,13–18].

Based on nucleotide (nt) and deduced amino acid sequences and on the phylogenetic analysis, it is possible to show genetic heterogeneity in the G and P genotypes of wild-type RVA strains that can still be phylogenetically classified into lineages. So far, the G6 and G10 genotypes are classified into six (I-VI) [19] and ten (I-X) [20] distinct lineages, respectively, while the P[5] and P[11] genotypes are classified into nine (I-IX) [21] and six (I-VI) [22] lineages, respectively.

The aim of this study was to describe the G and P genotypes of wild-

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type Brazilian RVA strains identified in diarrheic calves from 2006 to 2015.

2. Materials and methods

2.1. Inclusion criteria

The diarrheic fecal samples included in this study were selected from a fecal sample collection sent to the Laboratory of Animal Virology that is a national reference for the control and diagnosis of animal RV. The collection consisted of fecal samples of dairy and beef calves up to 60 days old from all Brazilian geographical regions (South, Southeast, Midwest, North, and Northeast) from 2006 to 2015. The fecal samples were stored at -80 °C. The sampling, composed of 1589 diarrheic fecal samples, was previously evaluated by a silver-stained polyacrylamide gel electrophoresis (ss-PAGE) technique for RVA dsRNA detection, in which 417 (26.2%) samples were RVA-positive.

To achieve high diversity of genotypes in relation to the geographic regions, states, counties, and cattle herds included in the analysis, one or more fecal samples were selected for analysis per farm. Even as inclusion criteria in those herds with more than two RVA-positive samples, the samples with best intensity of dsRNA bands and distinct migration profile in ss-PAGE were selected. Based on these inclusion criteria, 155 RVA-positive diarrheic fecal samples from beef ($n = 116$) and dairy ($n = 39$) calves were selected to determine their G and P genotypes. The calves were from 70 beef and 30 dairy cattle herds located in five Brazilian geographical regions.

2.2. Nucleic acid extraction

Nucleic acid extraction was performed using 20% (w/v) fecal suspensions in Tris-Ca^{2+} buffer, pH 7.4 (50 mM Tris-HCl; 10 mM NaCl; 1.5 mM 2-mercaptoetanol; 3 mM $CaCl_2$). The mixture was centrifuged at 2000 × *g* for 5 min at 4 °C. Aliquots of 500 µL of supernatant and 50 µL of sodium dodecyl sulfate were homogenized and incubated at 56 °C for 20 min. The nucleic acid was extracted using a combination of the phenol/chloroform/isoamyl alcohol (25:24:1) and silica/guanidinium isothiocyanate methods [1]. The nucleic acid was eluted in 50 µL of ultrapure diethylpyrocarbonate-treated water (Invitrogen Life Technologies, Carlsbad, CA, USA) and immediately stored at −20 °C until use. Aliquots of Tris-Ca^{2+} buffer were included as negative controls in all viral nucleic acid extraction procedures. The cell culture (MA104 cell) adapted bovine RVA NCDV-Lincoln strain was used as a positive control in the experiments.

2.3. RT-PCR assay

The extracted nucleic acid was submitted to RT-PCR assay using RVA VP7 and VP4 consensus primers that amplify 1013 [23] or 1062 bp [24] and 864 (VP5 subunit of the VP4 gene) [23] or 876 bp (VP8 subunit of the VP4 gene) [25,26] to determine G and P genotypes, respectively. The RT-PCR products were analyzed by electrophoresis on 2% agarose gels in TBE buffer, pH 8.4 (89 mM Tris; 89 mM boric acid; 2 mM EDTA), containing 0.5 µg/mL ethidium bromide. After electrophoresis at a constant voltage (100 V) for 40 min, the gel was visualized under UV light.

2.4. Sequencing analysis

RT-PCR products were purified using an illustra GFX PCR DNA and Gel Band purification commercial kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK) and quantified with a Qubit Fluorometer (Invitrogen Life Technologies, Eugene, OR, USA). The RT-PCR products were sequenced with a sequencer ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using a Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the forward and reverse primers used in the RT-PCR assay. The nt sequence quality analysis and contig assembly of the RVA gene sequences were performed with Phred and CAP3 software, respectively, and the sequences were accepted if the base quality score was ≥20. Similarity searches were performed against sequences deposited in GenBank using the BLASTn software. Multiple and pairwise alignments with RVA strains available in GenBank were performed with MEGA software version 7.0.26, and the sequence identity matrix was constructed using the BioEdit software version 7.2.6.1. Phylogenetic trees based on the nt sequences were obtained using the neighbor-joining method with the kimura two-parameter model using MEGA software version 7.0.26. The bootstrapping probabilities were calculated using 1000 replicates.

2.5. Nucleotide sequence accession numbers

The VP7 and VP4 genes of the Brazilian bovine RVA strains described in this study are available in the GenBank database. The accession numbers for the G6 nt sequences are MG452862 - MG452930, those for the G10 nt sequences are MG269482 - MG269496, those for the P[5] nt sequences are MG269497 - MG269512 and MG269513 - MG269539, and those for the P[11] nt sequences are MG269540 - MG269547 and MG269548 - MG269572.

2.6. Statistical analysis

Fisher's exact test, performed using the Minitab 16.1.1.0 software and adopting a value of $P \leq 0.05$, was used to analyze the frequencies of the RVA G and P genotypes identified in diarrheic calves according to cattle exploitation type (beef x dairy).

3. Results

The most frequent G and P genotypes detected in the Brazilian wild-type bovine RVA strains evaluated in this study were G6 (133/155) and P[5] (87/155) (Table 1). The G6 genotype was identified in combination with the P[5] ($n = 85$), P[11] ($n = 34$), and P[X] (P not-determined) ($n = 14$) genotypes. With the exception of two RVA strains, in which the G genotype could not be identified (GX), the P[5] genotype was only found in combination with the G6 genotype. The G10 genotype was identified with P[11] ($n = 19$) and P[X] ($n = 1$) genotypes (Table 1).

A different distribution of the G and P genotype combinations was found according to the exploitation type. G6P[5] (65.5%; 76/116) was the combination that occurred more frequently in beef, while G10P[11] and G6P[11] occurred more frequently, at 38.4% (15/39) and 30.8% (12/39), respectively, in dairy cattle herds ($P < 0.05$) (Table 2).

The RVA G and P genotype distribution showed differences according to the Brazilian geographical regions evaluated. The Midwest region, which is the major region of the Brazilian beef cattle industry, had the highest number of RVA strains genotyped. However, with respect to the G genotype, only the G6 genotype was identified in this region, in association with the P[5], P[11], and P[X] genotypes. The South and Southeast regions presented more genetic diversity in terms

Table 1
G (VP7) and P (VP4) genotypes of wild-type RVA strains found in diarrheic calves of Brazilian cattle herds.2006–2015.

P	G			Total
	G6	G10	GX*	
P[5]	85	–	2	87
P[11]	34	19	–	53
P[X]*	14	1	–	15
Total	**133**	**20**	**2**	**155**

* undetermined genotype.

Table 2
G (VP7) and P (VP4) genotypes of wild-type RVA strains identified in diarrheic beef and dairy calves in Brazil.2006–2015.

RVA genotype	Exploitation type		Total (%)
	Beef (%)	Dairy (%)	
G6P[5]	76 (65.5)[a]	9 (23.1)[ab]	85 (54.8)
G6P[11]	22 (19.0)[b]	12 (30.8)[a]	34 (21.9)
G10P[11]	4 (3.4)[cd]	15 (38.4)[a]	19 (12.3)
G6P[X]*	11 (9.5)[c]	3 (7.7)[b]	14 (9.0)
G10P[X]*	1 (0.9)[d]	–	1 (0.7)
GX*P[5]	2 (1.7)[d]	–	2 (1.3)
Total	**116**	**39**	**155**

Different letters denote significant differences ($P < 0.05$) between genotypes (beef-dairy).

* undetermined genotype.

Table 3
The most common G and P genotypes of wild-type RVA strains identified in diarrheic calves, according to the geographical region of the cattle herds in Brazil.2006–2015.

RVA Genotype	Brazilian geographical region					Total
	South	Southeast	Midwest	North	Northeast	
G6P[5]	17	9	56	–	3	85
G6P[11]	5	10	18	1	–	34
G10P[11]	15	4	–	–	–	19
G6P[X]	4	2	8	–	–	14
G10P[X]	1	–	–	–	–	1
GXP[5]	–	1	1	–	–	2
Total	**42**	**26**	**83**	**1**	**3**	**155**

(–) no bovine RVA strain showed this genotype combination.

of the RVA G and P genotypes. The G6 genotype was identified in RVA strains of all Brazilian regions included in the study. The P[5] and P[11] genotypes were found in the main bovine producing regions of Brazil (Table 3).

G and P genotype combinations of RVA strains distributed according to the year of diarrheic fecal sample collection identified the G6P[5] genotype in RVA strains from all 10 years evaluated. The G6P[11] genotype combination was not found only in two years of this study (2006 and 2008), while G10P[11] was identified only after 2011 (Table 4).

The nt sequences of the Brazilian RVA strains VP7 gene were compared with the G6 and G10 genotype sequences deposited in the GenBank database. The 69 G6 RVA strains characterized in this study clustered together with strains from the G6-III ($n = 12$) and G6-IV ($n = 57$) lineages. The Brazilian G6-III strains exhibited 93.6 to 97.7%, 93.7 to 94.9%, and 93.2 to 99.7% of nt identity with bovine G6-III RVA strains (B61, SI-B17, B609_BA, and B3206_BA), with human G6-III RVA strain (Hun3), and with other G6-III RVA strains detected in cow, buffalo, and rabbit hosts, respectively. The Brazilian G6-IV strains showed 90.7 to 95.4%, 89.8 to 95.8%, 89.2 to 95.8%, and 86.9 to 99.8% of nt identity with the UK prototype strain (G6P[5]), the NCDV prototype strain (G6P[1]), the WC3 prototype strain (G6P[5]), and with other G6-IV RVA strains detected in cow, pig, horse, cat, and turkey hosts, respectively. The Brazilian G6-III and G6-IV RVA strains were found to have 96.4 to 100% and 87.9 to 100% of nt identity to each other, respectively. In the phylogenetic tree, the G6-III strains clustered together, and the G6-IV strains clustered in different branches into lineage IV (Fig. 1).

Table 4
G and P genotype combinations in Brazilian wild-type RVA strains identified in diarrheic calves in beef and dairy cattle herds, distributed according to the year of the diarrheic fecal sample collection.

Year	G and P genotype combinations						Total
	G6P[5]	G6P[11]	G10P[11]	G6P[X]	G10P[X]	GXP[5]	
2006	3	–	–	–	–	–	3
2007	4	4	–	–	–	–	8
2008	2	–	–	2	–	–	4
2009	20	2	–	1	–	–	23
2010	1	3	–	–	–	–	4
2011	5	7	1	1	–	–	14
2012	7	3	1	–	–	–	11
2013	11	6	8	5	1	–	31
2014	28	8	9	2	–	–	47
2015	4	1	–	3	–	2	10
Total	**85**	**34**	**19**	**14**	**1**	**2**	**155**

(–) none bovine RVA strain showing this genotype combination.

The 15 G10 Brazilian RVA strains characterized in the present study were compared with representative RVA strains of the ten G10 lineages. Two G10 Brazilian RVA strains clustered into the G10-III lineage and 13 into the G10-IV lineage (Fig. 2). The G10-III RVA strains exhibited 88.6–90.3%, 92.5–92.6%, and 93.6% of nt identity with human (I321 and N155), bovine (T01TR), and giraffe (GirRV-1) G10-III RVA strains, respectively. The G10-IV RVA strains shared 93.9–96.4% and 91.9–96.8% of their nt identity with the B223 prototype strain (G10P[11]) and with other G10-IV RVA strains detected in bovine hosts, respectively. The Brazilian G10-III and G10-IV RVA field strains shared 99.7% and 89.6–100% of their nt identity with each other.

Two different fragments of the VP4 gene (the VP5 and VP8 subunits) were obtained depending on the primer pair used in the RT-PCR assay. The RVA strains described herein were characterized as the P[5] and P[11] nt sequences and were compared with nt sequences acquired from the GenBank database. The 16 P[5] RVA strains of the VP8 subunit showed 92.8 to 99% nt identity with the prototype of the P[5]-IX lineage strain (BRA1532) and 92 to 100% of nt identity to each other. These P[5] strains grouped together with the prototype P[5]-IX in the phylogenetic tree and in a distant branch of the other P[5] lineages (Fig. 3). One P[5] strain of the VP5 subunit exhibited 95.8 to 98.9% nt identity with the P[5]-II prototype strains (B2376_D_BA and 791_BA) from Argentina. The other 26 P[5] RVA strains of the VP5 subunit showed 86 to 93.5% of nt identity with the representative P[5] strains of lineages I to VIII. The 26 P[5] RVA strains presented 93.5 to 100% of nt identity with each other and clustered together in a branch far from the other P[5] lineages, which suggests that it may represent lineage IX described using the VP8 subunit of the VP4 gene (Fig. 4).

Thirty-one P[11] RVA strains obtained in this study grouped into the P[11]-III lineage in the phylogenetic tree and two P[11] strains formed a new branch separated from the other lineages of P[11] genotype, therefore, we proposed a new lineage, named (P[11]-VII) (Figs. 5 and 6). Eight P[11]-III strains were obtained using a primer pair that amplified the VP5 subunit and that presented 94.4 to 95.9% nt identity with the B223 prototype strain (G10P[11]); 93.1 to 96% of nt identity with P[11]-III RVA strains; and 91.9 to 100% with each other. The 23 P[11]-III strains amplified with the primer pair of the VP8 subunit shared 95.1 to 97.7% nt identity with the B223 prototype strain; 92.9 to 100% with other P[11]-III strains detected in bovine, giraffe, and rabbit hosts; and 93.2 to 100% with each other. The two P[11]-VII strains amplified with the primer pair of the VP8 subunit showed 86.5 to 91.6% of nt identity with the representative P[11] strains of lineages I to VI and 100% of nt identity with each other.

4. Discussion

Even considering the great number of diarrheic fecal samples obtained from beef and dairy herds located in the most important Brazilian geographical regions of cattle breeding, the neonatal diarrhea by RVA infection in calves evaluated in this study was predominantly due to the G6P[5] strains. This genotype combination was also reported

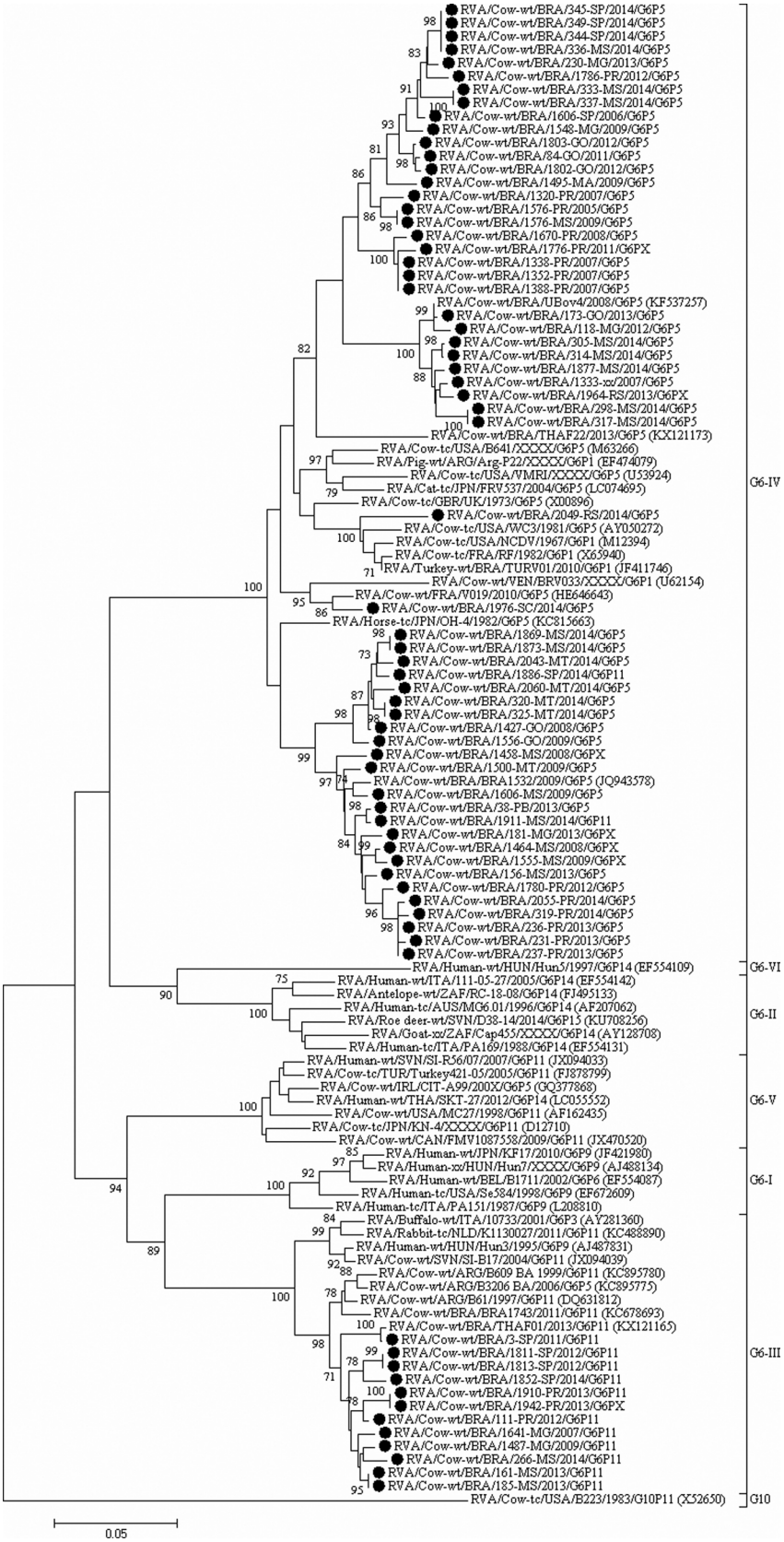

Fig. 1. The VP7 phylogenetic tree with 706 bp amplicon (158–863 nt) of the G6 genotype lineage assignments G6-I to G6-VI. The tree was constructed using the neighbor-joining method and the Kimura two-parameter model for nt substitution. The bootstrap values are shown at the branch nodes (values < 70% are not shown). The scale bar at the bottom of the tree represents the number of nt substitutions per site. The GenBank accession numbers of the strains are provided in parentheses. The Brazilian bovine RVA G6 strains are indicated with a filled circle.

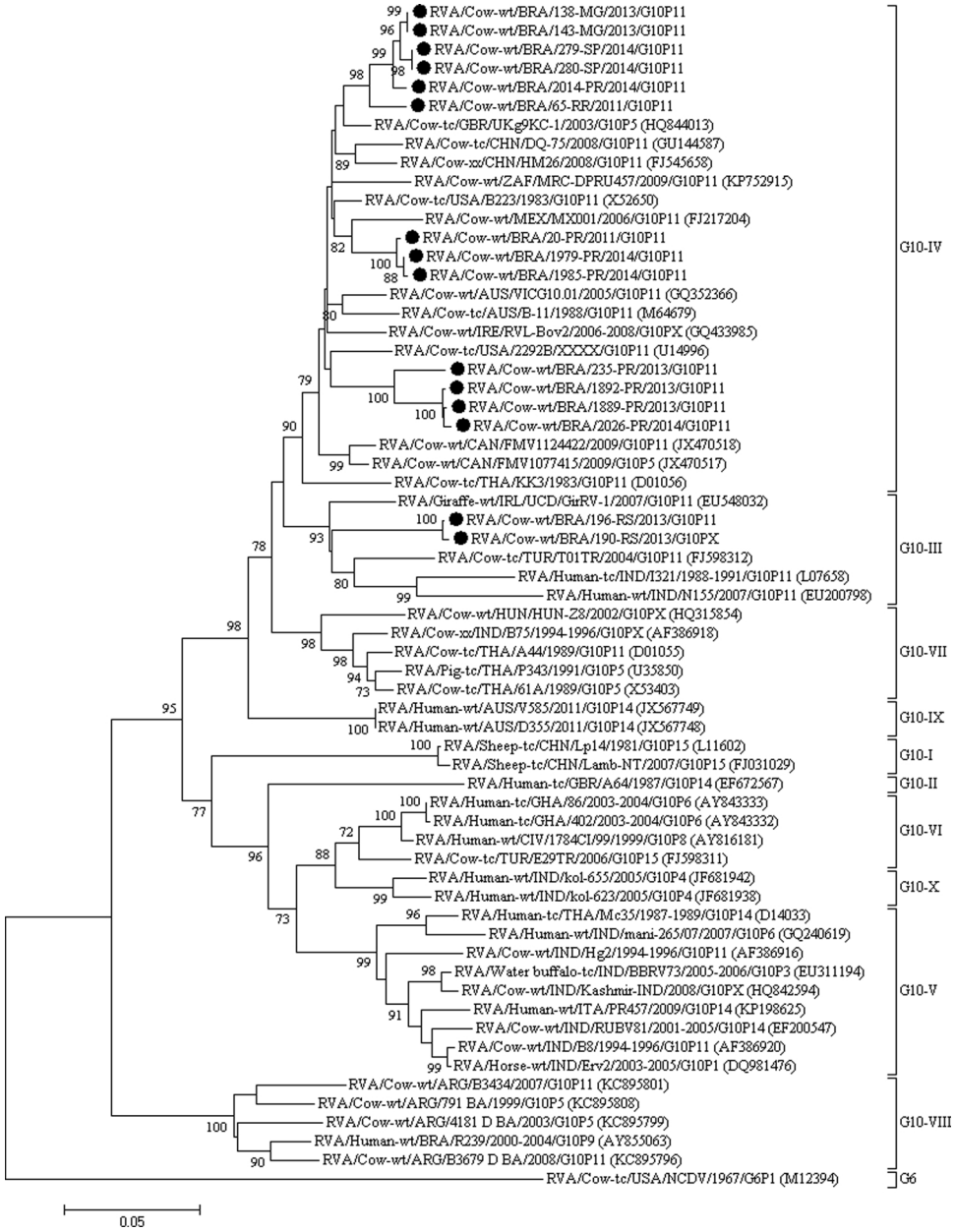

Fig. 2. The VP7 phylogenetic tree with 760 bp amplicon (151–910 nt) of the G10 genotype lineage assignments G10-I to G10-X. The tree was constructed using the neighbor-joining method and the Kimura two-parameter model for nt substitution. The bootstrap values are shown at the branch nodes (values < 70% are not shown). The scale bar at the bottom of the tree represents the number of nt substitutions per site. The GenBank accession numbers of the strains are provided in parentheses. The Brazilian bovine RVA G10 strains are indicated with a filled circle.

as the most common in bovine RVA strains identified in other studies performed in Brazil and around the world [10,13,17,18,27–29].

A small range of genotypes was found in the RVA strains, including two G genotypes (G6 and G10) and two P genotypes (P[5] and P[11]). In contrast, genotyping studies in porcine and human RVA strains demonstrated the occurrence of a wide variety of genotypes circulating simultaneously or causing diarrhea outbreaks with the reemergence and/or the emergence of new genotypes [10–12,30]. The reason for the greater diversity of the G and P genotypes in porcine RVA strains compared to bovine RVA strains is unknown. Some authors suggest that differences in breeding practices and facilities, life cycle, and the trade and transport of animals are important factors to be considered [10]. Furthermore, some authors suggest that the presence of different cattle breeds, different systems of breeding, different ecological areas and different virus biology can be used to explain the diversity of RVA strains detected in bovine RVA strains [22].

A different distribution of G and P genotype combinations was found according to the cattle exploitation type, in which the G6P[5] genotype combination was more frequent (65.5%) in beef, and the G10P[11] (38.4%) and G6P[11] (30.8%) combinations were more frequent in dairy herds. In Argentina (2004–2010), G6P[5] was also the most frequent genotype combination in RVA strains found in beef cattle herds. Although genotype G10P[11] was found mainly in diarrheic fecal samples in dairy calves in the Brazilian study, a different distribution of the G6P[11] (21%), G10P[11] (17%), and G6P[5] (14%) genotypes was found in Argentinean dairy herds [17].

Although the diversity of the RVA genotypes found in dairy herds is considered to be low, it was higher than that in beef herds. Although the number of RVA strains from the Brazilian Midwest exceeded the analyzed number of other regions, the G10P[11] genotype was

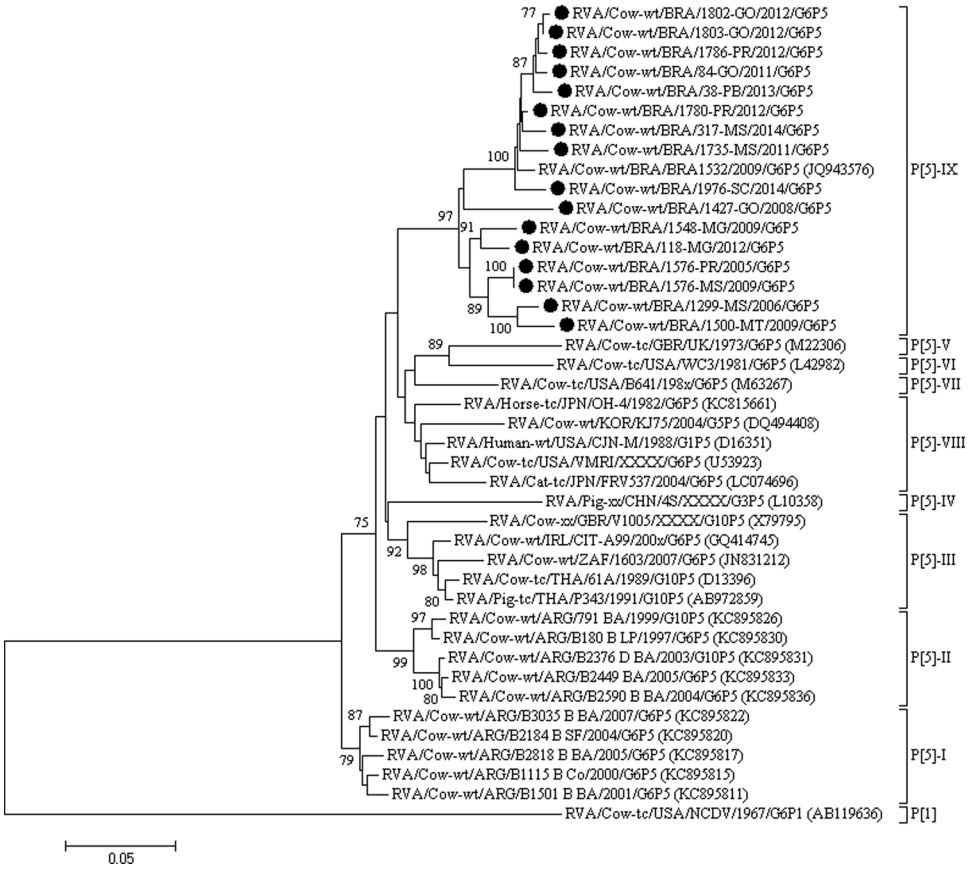

Fig. 3. The VP4 (VP8 subunit) phylogenetic tree with 632 bp amplicon (73–704 nt) of the P [5] genotype lineage assignments P[5]-I to P [5]-IX. The tree was constructed using the neighbor-joining method and the Kimura two-parameter model for nt substitution. The bootstrap values are shown at the branch nodes (values < 70% are not shown). The scale bar at the bottom of the tree represents the number of nt substitutions per site. The GenBank accession numbers of the strains are provided in parentheses. The Brazilian bovine RVA P[5] strains are indicated with a filled circle.

identified only in strains from the South and Southeast regions.

The G6P[11] genotype was not found in two years of study (2006 and 2008), and the G10P[11] genotype was only identified after 2011. Other studies previously conducted in Brazil identified these RVA genotype combinations in 1996–1999 [13]; the G6P[11] in 2011 [31], and the G10P[11] in 2003 [32].

It is likely that the genotype profile of bovine RVA strains has changed over time. The G6P[1] was not detected in any RVA strains included in this study other than the period (10 years) and number of strains (n = 155) evaluated. During 1996–1999, our research group genotyped 50 RVA strains from eight dairy and/or beef cattle herds located in the South, Southeast, and Midwest regions of Brazil. The G6P [1] (NCDV-Lincoln like) genotype was found in 12% (6/50) of the RVA strains analyzed, while the G6P[5] genotype was described in 40% (20/50). Additionally, unusual associations of the G and P genotypes, G8P [11] (n = 1) and G5P[1] (n = 1) were identified [13]. Thus, it has been observed that for over 20 years, G6P[5] has been the most frequent genotype combination found in the Brazilian wild-type bovine RVA strains identified in diarrheic calves.

G8 is a RVA genotype described in cattle [17,33,34]. However, this genotype was not found in any fecal sample analyzed in this study or in other surveys performed in Brazil [21,29,32,35] and other countries, such as Argentina [22], Tunisia [36], and Iran [37].

Considering the importance of the Brazilian cattle industry, cow vaccinations to prevent RVA infection and control neonatal diarrhea are still rare in both beef and dairy herds. However, based on data from the last decade, there is no doubt that there was a considerable increase in the use of bovine RVA commercial vaccines in Brazil, mainly containing genotype G6P[1]. In spite of this increase, reports of diarrhea in calves and even outbreaks of diarrhea have increased. This is likely due to changes in the reproductive management of beef cattle herds, as the use of fixed-time artificial insemination and 3- to 4-month breeding seasons has increased the health risk for the occurrence of neonatal calf diarrhea. At the same time, vaccine failures may occur, as diarrhea outbreaks in calves of regularly vaccinated cattle herds in Brazil, with vaccine containing RVA genotype G6P[1] have been observed [16,21].

The G6 genotype of Brazilian bovine RVA sequences were distributed more frequently in G6-III and G6-IV lineages, according to an association with the P[11] and P[5] genotypes, respectively. A similar distribution was observed in a study conducted in Argentina, where the G6-IV was detected in association with P[5] and P[11] and the G6-III with the P[11] [17]. In this study, only two G6 lineages were demonstrated. However, we observed great diversity in the G6-IV lineage with sequences grouping with different RVA strains. The G10 genotype clustered with the G10-III and G10-IV lineages, even though 10 different lineages in this genotype were described [20].

Here, we report that the P[5]-II and P[11]-VII lineages were described for the first time in the RVA strains identified in Brazilian cattle herds. The P[5] genotype of bovine RVA sequences also clustered with the P[5]-IX lineage, and had extreme diversity with other lineages described for this genotype [21,22] and the P[11] genotype sequences also clustered in the P[11]-III lineage, previously detected circulating in the Brazilian Midwest region by our research group [31].

In two RVA strains, it was not possible to characterize the G genotype, and this result was not unexpected. However, with respect to the P genotype, it was not possible to obtain an amplicon of the VP4 gene in 15 RVA strains, even performing several attempts and using three different pairs of primers [23,25,26].

The mistyping of the VP7 or VP4 genes has been documented in RVA strains of human and animal origin worldwide [13,27,35]. This can be caused by the presence of inhibitors of RT-PCR reaction in the feces, fecal sample conservation problems or viral infection with lower titers. However, this should not be the reason for the failure in the P genotype determination, since in all 15 bovine RVA strains in which P genotype was not determined was possible to determine the G genotype. This is likely due to the accumulation of point mutations or

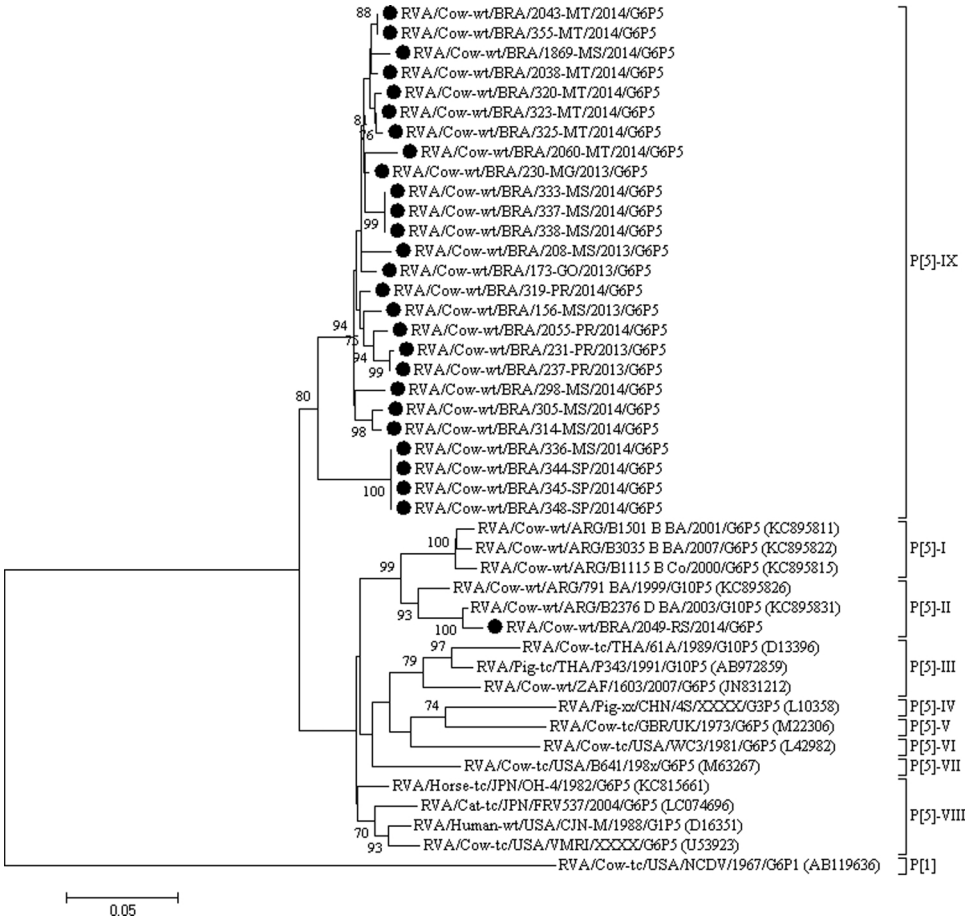

Fig. 4. The VP4 (VP5 subunit) phylogenetic tree with 694 bp amplicon (1,131-1,824 nt) of the P[5] genotype lineage assignments P[5]-I to P[5]-IX. The tree was constructed using the neighbor-joining method and the Kimura two-parameter model for nt substitution. The bootstrap values are shown at the branch nodes (values < 70% are not shown). The scale bar at the bottom of the tree represents the number of nt substitutions per site. The GenBank accession numbers of the strains are provided in parentheses. The Brazilian bovine RVA P[5] strains are indicated with a filled circle.

homologous recombination and hybridization of primers to the target sequences may have been responsible for not determining the P genotype in the 15 bovine RVA strains included in this study [27,32,38–40].

Molecular characterization of RVA strains circulating in animals is of great importance in terms of both animal and public health. Phylogenetic studies of RVA strains G6P[14], G12P[11], G10P[14] circulating in cattle has been related to diarrheal disease in children throughout the world, providing evidence for bovine to human interspecies transmission and reassortment events [41,42].

The importance of genotyping studies, mainly with retrospective design, carried out in several geographical regions and under different cattle breeding conditions (beef and dairy herds) contribute to virus evolution comprehension. The epidemiological surveillance of the RVA genotypes circulating at any given time in a given host is therefore

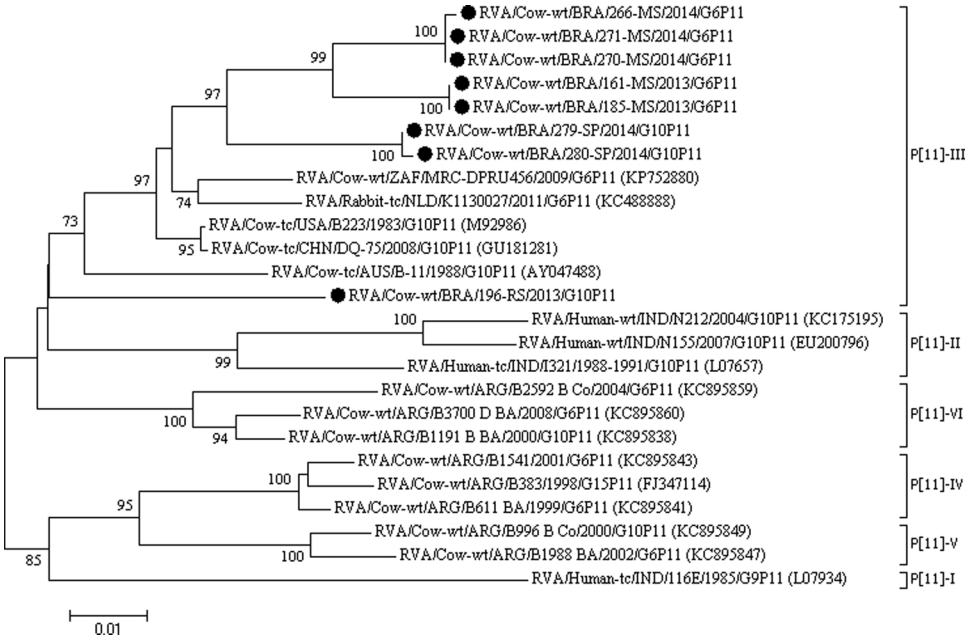

Fig. 5. The VP4 (VP5 subunit) phylogenetic tree with 760 bp amplicon (1,112-1,871 nt) of the P[11] genotype lineage assignments P[11]-I to P[11]-VI. The tree was constructed using the neighbor-joining method and the Kimura two-parameter model for nt substitution. The bootstrap values are shown at the branch nodes (values < 70% are not shown). The scale bar at the bottom of the tree represents the number of nt substitutions per site. The GenBank accession numbers of the strains are provided in parentheses. The Brazilian bovine RVA P[11] strains are indicated with a filled circle.

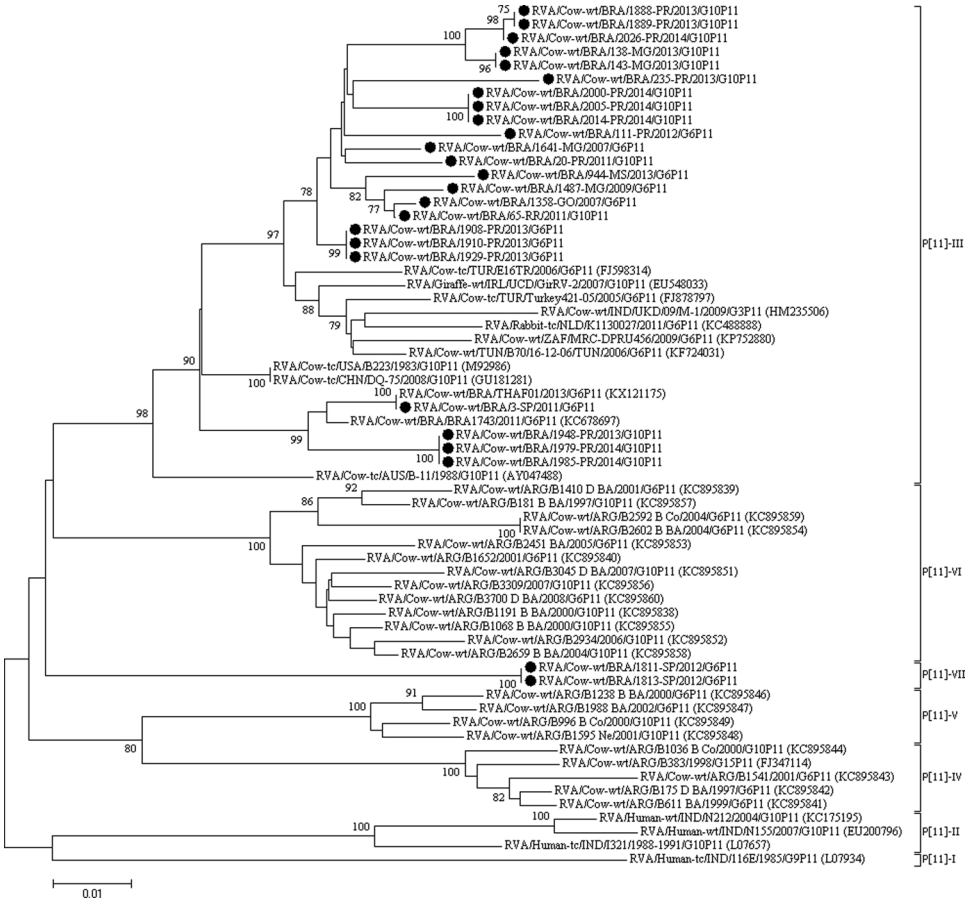

Fig. 6. The VP4 (VP8 subunit) phylogenetic tree with 697 bp amplicon (125–821 nt) of the P[11] genotype lineage assignments P[11]-I to P[11]-VII. The tree was constructed using the neighbor-joining method and the Kimura two-parameter model for nt substitution. The bootstrap values are shown at the branch nodes (values < 70% are not shown). The scale bar at the bottom of the tree represents the number of nt substitutions per site. The GenBank accession numbers of the strains are provided in parentheses. The Brazilian bovine RVA P[11] strains are indicated with a filled circle.

critical.

5. Conclusion

Compared to studies conducted in Brazil with RVA strains described in human and porcine hosts, the G and P genotype diversity in bovine RVA field strains identified in this study are relatively low. However, the diversity of G and P genotypes clearly increased after 2011.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

The study was submitted to the Ethics Committee on Animal Experiments of the Universidade Estadual de Londrina and approved under the identification 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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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cimid.2019.03.002.

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