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Molecular characterization of bovine rotavirus circulating in beef and dairy herds in Argentina during a 10-year period (1994–2003)

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Abstract

Group A bovine rotavirus (BRV) is one of the main causes of neonatal calf diarrhea. The present study reports the incidence of rotavirus diarrhea and the genotypes of BRV strains circulating in beef and dairy herds from Argentina, during a 10-year period (1994–2003). Group A BRV was detected in 62.5% (250/400) of the total studied cases of diarrhea. Positive cases were analyzed by heminested multiplex RT-PCR for P and G genotypes identification. Sixty percent of them were typed as P[5]G6, 4.4% P[11]G10, 4.4% P[11]G6 and 2.4% P[5]G10. Additionally, 9.2% of the cases were initially typed as G8 combined with P[5] or P[11], but sequence analysis revealed they belonged to genotype G6, lineage Hun4-like. Partial typing was assessed in 12.0% of the cases. One of the partially typed samples was closely related to genotype G15. BRV was detected in 71% and 58% of the outbreaks registered in beef and dairy farms, respectively. A clear differential distribution of G/P types was found according to the herd type. P[5]G6 was the prevalent strain in beef herds, while P[11] was the prevalent P-type in dairy herds (71%), associated in similar proportions with G6 and G10, These findings indicate that BRV genotypes included in the current commercially available rotavirus vaccines (G6, G10, P[5] and P[11]) should protect calves from most Argentinean field strains. Nevertheless, continuous surveillance is necessary to detect the emergence of new variants.

Keywords: Bovine rotavirus; Genotyping; VP7; VP4

1. Introduction

Neonatal calf diarrhea is a complex syndrome affecting animals within the first 4 weeks of life, causing important economic losses related to death,

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treatment costs and reduction in weight gain of affected animals. The etiology of the disease involves diverse infectious agents (virus, bacteria and protozoa) and is worsened by several factors including herd management, environment, and host nutritional and immunological conditions (Bendali et al., 1999). Group A bovine rotavirus (BRV) is considered the major cause of calf diarrhea worldwide (Kapikian, 1996). Previous studies conducted in Argentina indicate that BRV is broadly distributed, causing more than 70% of diarrhea outbreaks in beef and dairy herds (Bellinzoni et al., 1987; Costantini et al., 2002).

Rotaviruses (RV) constitute a genus within the Reoviridae family, characterized by non-enveloped triple-layered viral particles with a viral genome composed by 11 double-stranded RNA segments (dsRNA). RV are classified in seven groups (A–G), according to the antigenic variability of the inner capsid protein VP6. Group A RV are further classified into G and P types based on the genetic and antigenic variation of the two outer capsid proteins VP7 (glycoprotein) and VP4 (protease-sensitive protein), respectively. At least 15 G types and 26 P types are recognized so far (Estes, 1996; Gentsch et al., 1996; Hoshino et al., 2002; Kapikian, 1996; Liprandi et al., 2003; Martella et al., 2005a,b; McNeal et al., 2005; Rahman et al., 2005; Rao et al., 2000; Banyai et al., 2005). Although at least six P genotypes (P[1], P[5], P[11], P[14], P[17], P[21],) and eight G types (G1, G3, G5–G8, G10, and G15) have been described in RV affecting cattle, only G6, G10 and G8 combined with P[5], P[11] and P[1], are considered epidemiologically important (Alfieri et al., 2004; Barreiros et al., 2004; Blackhall et al., 1992; Chang et al., 1996; Costantini et al., 2002; Fukai et al., 1998, 1999, 2002, 2004a,b; Isegawa et al., 1994; Okada and Matsumoto, 2002; Rohwedder et al., 1995).

Continuous typing of BRV circulating strains is essential for: (i) a better understanding of the viral epidemiology within a region, (ii) improving the implemented vaccination programs by updating the vaccine strains. The objective of this study was to investigate the incidence of rotavirus diarrhea and to report the G and P types of BRV strains circulating in beef and dairy herds in Argentina, during a 10-year period (1994–2003).

2. Materials and methods

2.1. Fecal samples

The samples included in this study were selected from the collection of fecal specimens received by the Reference Diagnosis Service of the Virology Institute-INTA, from 1994 to 2003. Samples were submitted mainly from three laboratories: the Pathobiology Institute-INTA, Castelar; the Specialized Veterinary Diagnostic Service, INTA Balcarce and a private laboratory (Laboratorio Azul S.A.). Only samples from a known geographical location were selected for the study. Most samples belonged to herds located in Buenos Aires province (the main livestock region of Argentina with 80% of the cattle population). Samples from other provinces like Corrientes, Entre Rios, Santa Fe, Río Negro, Córdoba, San Luis, La Pampa, Chaco, and Neuquén were also included. Faeces were collected during episodes or outbreaks of diarrhea in calves. When several samples were submitted by a farm at different times during the same calving season, samples were considered as a unique outbreak of diarrhea for data analysis purposes. When only one sample was submitted it was considered an isolate episode or case of diarrhea. A total of 1581 stool samples corresponding to 400 cases of diarrhea were analyzed, with 929 samples corresponding to 209 cases of diarrhea registered in beef herds, and 252 samples corresponding to 65 outbreaks registered in dairy herds. Moreover, 400 samples (126 diarrhea episodes) were received from herds which did not specify the exploitation type.

2.2. Rotavirus diagnosis

All fecal samples were initially screened for the presence of BRV by an antigen capture ELISA, using the reagents and procedures previously described (Cornaglia et al., 1989). ELISA positive samples were selected for further genotyping analysis.

2.3. Virus reference strains

Bovine rotavirus prototype strains INDIANA (P[5]G6), B223 (P[11]G10), NCDV-Cody (P[1]G8) and NCDV-Lincoln (P[1]G6), (kindly provided by Dr.

Table 1
Primers used in the RT-PCR-heminested multiplex procedure

VP7 typing primers		Sequence (5′–3′)	Nucleotide (nt) position	Product length (bp)	Sense
VP7 INI	5′ end	GGC TTT AAA AGM GAG AAW TT	1–20		+
VP7 FIN	3′ end	GGT CWC ATC ATA CAA YTC T	1062–1044	1062	–
DT6	G6	CTA GTT CCT GTG TAG AAT C	499–481	499	–
HT8	G8	CGG TTC CGG ATT AGA CAC	273–256	273	–
ET10	G10	TTC AGC CGT TGC GAC TTC	714–697	714	–
VP4 typing primers					
Con 3	5′ end	TGG CTT CGC TCA TTT ATA GAC A	11–32	877	+
Con 2	3′ end	ATT TCG GAC CAT TTA TAA CC	887–868		–
P1 K	P1	ACC AA C GAA CGC GGG GGT G	264–282	624	+
P5 K	P5	RCC AGG TGT CRC ATC AGA G	336–354	552	+
pB223	P11	GGA ACG TAT TCT AAT CCG GTG	574–594	314	+

Saif, Food Animal Health Research Program, The Ohio State University, USA), were cultured in MA104 cells and used as controls for typing assays.

2.4. RNA extraction

RNA was extracted from 10% fecal suspensions using TRIzol* (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions.

2.5. Heminested multiplex RT-PCR

Ten microliters of extracted dsRNA were mixed with 1.4 μl of DMSO, 0.6 μl of 20 μM primer mix and denatured at 94 °C for 5 min. Eight microliters of RT mix [40 U of M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA), 4 μl of RT 5× buffer (250 mM Tris–HCl, 375 mM KCl, 15 mM $MgCl_2$, pH 8.3), 1.6 μl of 5 mM dNTPs and 2.2 μl of water] were added to the denatured dsRNA which was reverse transcribed for 1 h at 42 °C and incubated for 5 min at 95 °C.

For the amplification of the complete *VP7* gene, 30 μl of PCR mix [2.5 U of Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA), 5 μl of 10× PCR buffer (200 mM Tris–HCl, 500 mM KCl, pH 8.4), 1.5 μl of 50 mM $MgCl_2$, 0.4 μl of 5 mM dNTPs, 2.1 μl of DMSO, 1 μl of 20 μM primer INI-FIN mix and 19.5 μl of water] were added to each tube containing the specific cDNA and run under the following cycling profile: 95 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 47 °C for 1 min, 72 °C for 2 min and a final extension of 72 °C for 7 min. The G-typing heminested PCR assay was carried out using the consensus INI primer and a pool of G type-specific primers previously described (Table 1), (Gouvea et al., 1994a; Parreno et al., 2004). One microliter of the first round product (for first round positive samples) or 5 μl (for first PCR-negative samples) were added to a 45 μl final volume of PCR mix which contained 2.5 U of Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA), 5 μl of 10× PCR buffer (200 mM Tris–HCl, 500 mM KCl, pH 8.4), 2 μl of 50 mM $MgCl_2$, 4 μl of 5 mM dNTPs, 1 μl of DT6 20 μM, 1 μl of HT8 20 μM, 1 μl of ET10 20 μM, 1 μl of INI 20 μM and 29.5 μl of water. Amplification was performed through a first step of 2 min at 94 °C followed by 30 PCR cycles (94 °C for 30 s, 42 °C for 30 s, and 72 °C for 45 s and a final extension of 72 °C for 7 min.

Amplification of VP8 segment of gene 4 was carried out using Con2 and Con3 consensus primers obtaining an 876-bp product, as previously described (Gouvea et al., 1994b). Ten microliters of extracted dsRNA were mixed with 0.8 μl of DMSO, 0.6 μl of 20 μM Con2 primer, 0.6 μl of 20 μM Con3 primer and denatured at 94 °C for 5 min. Reverse transcription was performed as previously described for the G type.

For the PCR, 45 μl of the PCR mix [2.5 U of Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA), 4.5 μl of 10× PCR buffer (200 mM Tris–HCl, 500 mM KCl, pH 8.4), 1.6 μl of 50 mM MgCl2, 2 μl of 5 mM dNTPs, 1 μl of 20 μM Con2 primer, 1 μl of 20 μM Con3 primer and 34.4 μl of water)

were added to each tube containing the specific cDNA. The heminested PCR was performed using modified oligonucleotides based on those previously designed by Gouvea, according to the VP8 sequence of local field strains (Table 1) (Gouvea et al., 1994b). One microliter of the first round product (for first round positive samples) or 5 μl (for first PCR-negative samples) were added to a 45 μl final volume PCR mix containing 2.5 U of Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA), 5 μl of 10× PCR buffer (100 mM Tris–HCl, 500 mM ClK, pH 8.4), 2 μl of 50 mM $MgCl_2$, 4 μl of 5 mM dNTPs, 1 μl of 20 μM P1–P5–P11 primer mix, 1 μl of 20 μM Con2 primer, and 31.5 μl of water. Amplification was performed through a first step of 2 min at 94 °C followed by 30 PCR cycles (94 °C for 30 s, 47 °C for 30 s, and 72° C for 45 s) and a final extension of 72 °C for 7 min.

First and second round PCR products were run and visualized in a 1.8% molecular biology grade agarose gel, containing 4 μg/ml ethidium bromide, under UV light. G and P typing results were confirmed by sequencing VP7 and VP8 amplified products representative of each genotype detected in this study.

2.6. Nucleotide sequencing

Selected samples were sequenced in both senses using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem) in an ABI-Prism 377 DNA sequencer (Perkin-Elmer, Applied Biosystems). INI, FIN, Con2 and Con3 RT-PCR oligonucleotides were used as sequencing primers.

2.7. VP7 and VP4 sequence analysis

Sequences were aligned using default parameters of Clustal W 1.83 (Thompson et al., 1994). For maximum likelihood analyses, a best-fit model of sequence evolution was estimated by Modeltest (Posada and Crandall, 1998) used in conjunction with PAUP* (Swofford, 1998). Maximum likelihood trees were inferred with the PHYML program (Guindon and Gascuel, 2003). Model parameters were estimated maximizing the likelihood during tree searches (i.e. *phyml_linux matrix.phy 0 i 1 0 GTR e e 6 e BIONJ y y*). Maximum parsimony tree and bootstrap support (100 resampled matrixes, with replacement) were calculated using the TNT program (Goloboff et al., 2003; Hovenkamp, 2004; Giribet, 2005). Heuristics searches consisted on 1000 random addition sequences (RAS) followed by tree bisection reconnection (TBR), keeping 10 trees per replicate (mult = tbr rep 1000 hold 10), excepting for bootstrapp analyses, for which 100 RAS + TBR were performed holding one tree per replicate. All the analyses were performed on a P*IV* PC running *Fedora Core 4* linux distribution. Trees were edited and drawn with TreeDyn (http://www.treedyn.org).

3. Results

3.1. Rotavirus diarrhea incidence during a 10-year period

A total of 1581 stool samples from diarrheic calves were analyzed. The samples corresponded to 209 cases or outbreaks of diarrhea registered in beef farms, 65 cases from dairy farms and 126 cases from farms of non-specified exploitation type. Farms were mainly located in the most important Argentinean region of livestock production. Group A BRV was detected in 42.0% (663/1581) of the samples, and corresponded to 62.5% (250/400) of the total cases of diarrhea analyzed during the 10-year period. The incidence of BRV infection each year is detailed in Table 2. The incidence of BRV diarrhea was 71.3% (149/209) for beef farms and 58.5% (38/65) for dairy farms. The incidence was higher in beef than in dairy

Table 2
Incidence or bovine rotavirus during the 10-year period

Year	Number of total cases (samples)	BRV incidence cases (samples)
1994	11 (52)	100.0 (67.3)
1995	8 (27)	75.0 (55.5)
1996	18 (37)	88.9 (86.5)
1997	47 (168)	74.5 (44.6)
1998	26 (134)	84.6 (51.5)
1999	50 (204)	68.0 (46.1)
2000	50 (281)	56.0 (46.3)
2001	56 (170)	44.6 (32.3)
2002	78 (307)	29.6 (46.1)
2003	56 (201)	57.1 (39.0)
Total	400 (1581)	62.5 (41.9)

herds, but not significantly so (Fisher exact test, $p = 0.0667$). The incidence of BRV in the cases submitted from farms with non-specified exploitation was 50% (63/126).

3.2. Rotavirus typing by RT-PCT heminested multiplex and sequence analysis

The G and P types of BRV strains detected in the 250 positive cases under study are summarized in Table 3. The initial G- and P-typing analysis determined by heminested multiplex RT-PCR revealed that 60.4% (151/250) of the cases were P[5]G6 and 4.4% were P[11]G10, both considered the most common G/P associations found in bovines. Other combinations were also detected such as P[11]G6 (4.4%) and P[5]G10 (2.4%). In addition, 9.2% (23/250) of the cases were initially typed as G8 combined with P[5] or P[11]. The circulation of P[1] strains was not detected. When analyzing the distribution of G/P type combination through time, it was observed that P[5]G6 was also the prevalent strain every year. Similar G/P type combinations and distribution were observed in all the provinces under study. Partial typing was assessed in 12.0% (30/250) of the cases while circulating strains were untypable using this methodology in 4% (10/250) of the herds (Table 3). In most of these cases there was no amplified product after the RT-PCR reaction, so the VP7 and VP4 sequences of these strains could not be determined. The lack of amplification could be due to inhibitors in the fecal sample, conservation problems, low viral infectious titer or mismatches with the sequence of the primer.

Typing results were confirmed by sequence analysis of the VP7 and VP4 encoding genes of BRV strains representing each G/P combination detected. A total of 31 sequences were obtained. Sequence analysis revealed that the heminested multiplex RT-PCR properly typed all the strains except for those identified as G8. Initial BLAST analysis of those sequences showed that 3/20 were G6 NCDV-like, 16/20 cases were G6 closely related to the HRVs from Hungary, Hun3 and Hun4 (P[9]G6) and the unusual RV strain isolated in buffalos from Italy, 10733 (P[3]G6), while 1/20 was G10. Sequence analysis of the heminested PCR product derived from the samples typed as G8, using the 5′ end primer (INI),

Table 3
G and P typing of BRV strains circulating in Argentina during 1994–2003

Exploitation type		G6 NCDV like			G6 Hun 4 like[1]			G10			G?			Co-infection[2]				Mixed infection[3]				
		P5	P11	P?	P5	P11	P?	P5	P11	P?	P5	P11	P?	P[5 + 11] G6	P[5 + 11] G?	P[5 + 11] G6 Hun like		P[5] G6 P[11] G6	P[5] G6 P[11] G10	P[5 + 11]G6 P[5]G6 P[11]G6	P[5 + 11] G6 P?G10	
Beef farms		114	1	8	3	0	0	2	3	0	5	1	5	6	1	0		2	1	0	0	
Dairy farms		4	5	1	0	10	0	2	5	0	0	6	1	1	0	1		1	0	0	0	
Non-specified		33	5	4	0	1	1	2	3	1	2	2	4	1	0	0		1	0	1	1	
Total		151	11	13	3	11	1	6	11	1	7	9	10	9	1	1		4	1	1	1	

[1] Strains mistyped as G8 by the heminested multiplex RT-PCR technique, but confirmed as G6 by sequence analysis.
[2] Co-infections were considered when there were samples with nested-PCR products corresponding to more than one G or P types.
[3] Mixed infections were considered when in the same herd there were several calves, shedding a distinct BRV strain each one.

showed that DT6 primer sequence appeared in the 3′ end of the fragment, indicating that G8/G6 mistyping was originated by the miss binding of DT6 primer. Sequence alignment also showed that the accumulation of punctual mutations in the specific DT6 primer annealing site did not allow the typing of those strains as G6. In addition, the DT6 primer annealed non-specifically to a downstream location resulting in an amplified product of 271 bp.

One partially typed strain P[11]G? detected in a dairy herd was further characterized by sequence analysis as P[11]G15, a G/P combination not previously described.

Mixed RV infections were found in 6.8% of the cases. In some herds, fecal samples displaying more than one G or P type were found. For instance, stools with two bands in the VP4 typing reaction (P[5] + [11]) and one band in the G-typing assay (G6) were detected in nine herds. We considered those cases as animals suffering a co-infection. In other farms, we detected different calves shedding a distinct BRV strain each one, and they were considered farms with a mix infection (Table 3).

3.3. Differential G/P types association in beef and dairy herds

A clear differential distribution of G and P types was found between beef and dairy herds. In beef herds G6 was the predominant G type (89% of the cases) followed by G10 (9%) (Fig. 1A). In addition, P[5] was clearly prevalent circulating in 81% of the cases, followed by P[11] (3%) (Fig. 1B). Finally P[5]G6 NCDV-like was the prevalent association, detected in 75% of the cases (Fig. 1C). P[11] was the prevalent P type (71%), in dairy herds, combined in similar proportions with G6 NCDV-like, G6 Hun4-like and G10. Argentinean Hun4-like G6 strains were mostly

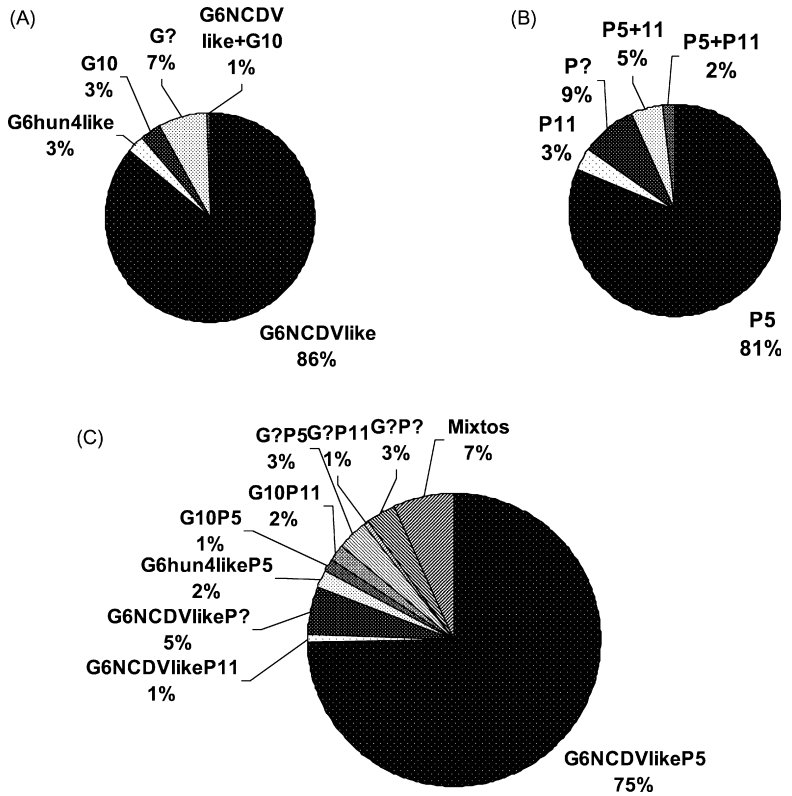

Fig. 1. Bovine RV genotypes distribution in beef herds (n = 209 beef farms). (A) G types distribution. (B) P types distribution. (C) G and P types combinations in beef herds.

detected in dairy herds associated with P[11] (Fig. 2A–C).

3.4. Argentinean G6 strains: sequence analyses

A sequence analysis of the VP7 encoding gene of the Argentinean G6 BRV strains together with others G6 RV strains available in GenBank, was conducted. Maximum Parsimony analyses resulted in two trees with a length of 3232. Maximum likelihood analysis was also conducted. For this data set, the best-fit nucleotide substitution model estimated by the Akaike Information Criterion was GTR + I + G, while the likelihood ratio test favoured TVM + I + G. The same topology was obtained in both maximum parsimony and maximum likelihood analyses. The tree obtained by maximum parsimony, is depicted in Fig. 3. All strains included, previously classified as G6 genotype, constituted a monophyletic group. Human and animals rotaviruses carrying G6 genotype showed high intra-homotypic diversity and were clustered into five distinct genetic lineages, as previously reported by other authors (Martella et al., 2003; Rahman et al., 2003; Banyai et al., 2003a,b) and were well supported by bootstrap analysis. lineage I (PA-151-like), includes P[9] HRV strains; lineage II (PA169-like) is composed by P[14] strains from humans and the caprine strain Cap455; lineage III (Hun4-like) comprises P[9] HRV strains and a buffalo P[3] strain; lineage IV (NCDV-like) is formed by P[1] and P[5] BRVs and lineage V (Mc27-like) includes exclusively P[11] BRV strains. Argentinean P[5]G6 BRVs constituted a monophyletic group within the lineage IV, together with the other P[5]G6 BRVs. The Argentinean P[11]G6 variants that failed to be typed as G6 by DT6 primers, belong to lineage III (Hun4-like).

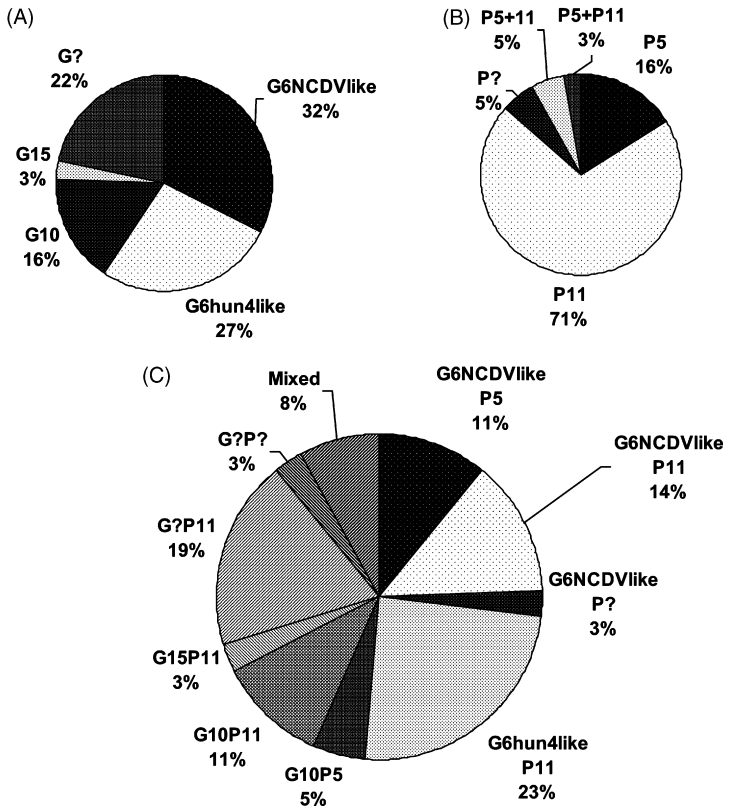

Fig. 2. Bovine RV genotypes distribution in dairy herds (n = 65 dairy farms). (A) G types distribution. (B) P types distribution. (C) G and P types combinations in dairy herds.

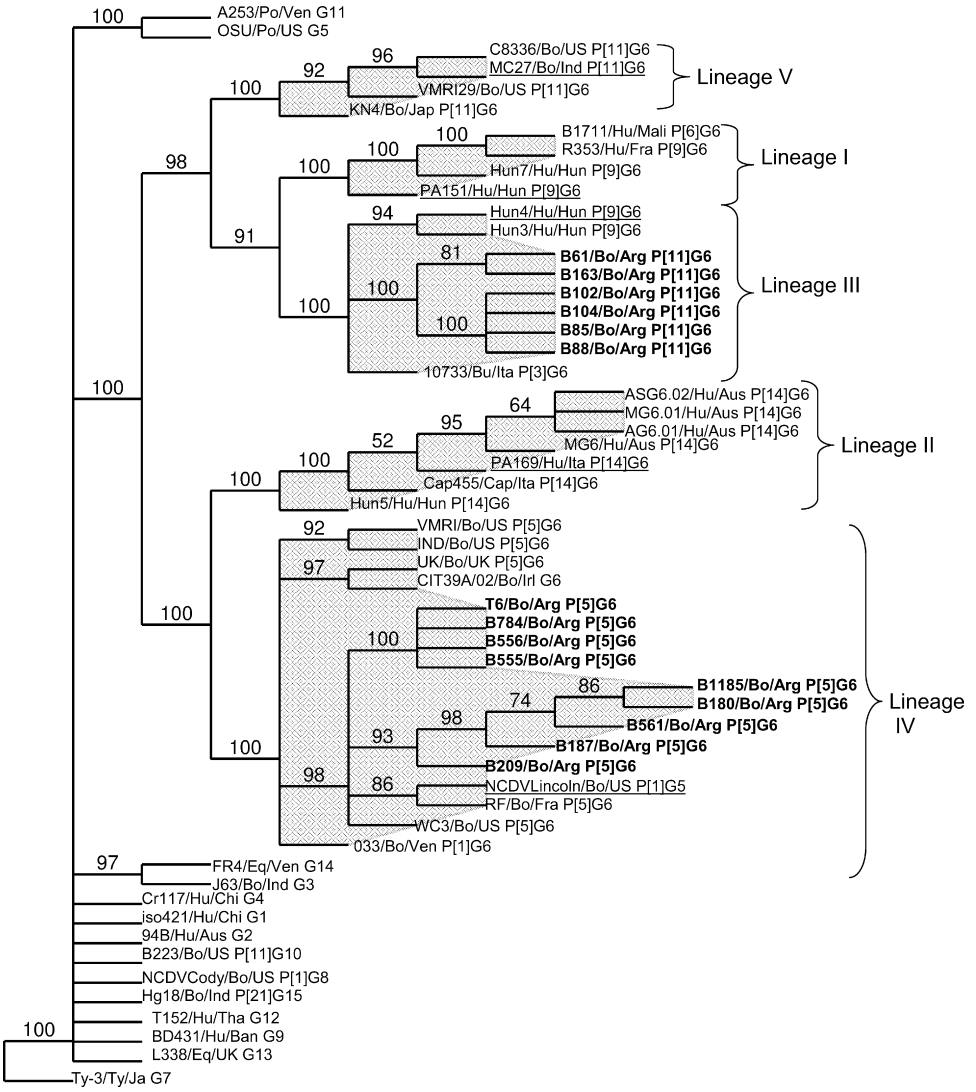

Fig. 3. Phylogenetic analysis by maximum parsimony of VP7 nucleotide sequences corresponding to genotype G6 strains (Argentinean strains are indicated in bold type). Percentage bootstrap values are indicated on branches. Argentinean strains (accession numbers): B61/Bo/Arg (DQ631812); B85/Bo/Arg (DQ631813); B88/Bo/Arg (DQ63814); B102/Bo/Arg (DQ631815); B104/Bo/Arg (DQ631816); B163/Bo/Arg (DQ631817); B180/Bo/Arg (DQ631818); B187/Bo/Arg (DQ631819); B209/Bo/Arg (DQ640025); B555/Bo/Arg (DQ631820); B556/Bo/Arg (DQ631821); B561/Bo/Arg (DQ631822); B784/Bo/Arg (DQ631823); B1185/Bo/Arg (DQ631824); BT6/Bo/Arg (DQ631825). The following VP7 sequences were taken from the Gen Bank data base (accession numbers): A253 (AF317122); OSU (X06722); C-833 (U14997); MC27 (AF162435); VMRI-29 (U50332); KN-4 (D12710); B1711 (AF532202); R353 (DQ122400); Hun7 (AJ488134); PA 151 (L20881); Hun4 (AJ487833); Hun3 (AJ487831); 10733 (AY281360); ASG6.02 (AF421183); MG6.01 (AF207062); AG6.01 (AF207063); MG6 (U22011); PA169 (L20880); cap455 (AY128708); Hun5 (AJ487832); VMRI (U53924); IND (U15000); UK (X00896); CIT39A.02 (AY629556); NCDV Lincoln (M12394); RF (X65940); WC3 (AY050272); 033 (U62154); FR4 (U05348); J63 (AF386914); cr117 (AF450294); iso 421 (D16326); 94B (U73952); B223 (X52650); NCDV Cody (U14999), Hg18 (AF237666); T152 (AB071404); BD431 (AJ250542); L338 (D13549); Ty-3 (AB080737). Po: porcine, Bo: bovine, Hu: human; Cap: caprine; Eq: equine, Ty: Turkey. Ven: Venezuela, US: United States, Ind: India, Jap: Japan, Fra: France, Hun: Hungary, Arg: Argentina, Aus: Australia, Ita: Italy; UK: United Kingdom; Irl: Ireland, Chi: China, Tha: Thailand, Ban: Bangladesh.

4. Discussion

In Argentina, BRV is the major cause of diarrhea in calves, followed by *E. coli*, and *Cryptosporidium* (Bellinzoni et al., 1987, 1989; Costantini et al., 2002). In the present study, group A BRV was detected in 42% of the samples and 62.5% of the total cases/outbreaks of diarrhea received during the 10-year period under analysis (1994–2003). Percentage of positive cases detected each year ranged from 100% to 29.6%. Herds affected by RV diarrhea were found in all provinces studied, indicating the broad circulation of BRV among Argentinean livestock. These findings are in concordance with previous surveys (Bellinzoni et al., 1987, 1990; Costantini et al., 2002).

In this study most of the BRV strains were typed as P[5] and P[11] associated to G6 and G10. A strain carrying G15 was also detected. This is the first survey reporting the P type of BRV circulating in Argentinean cattle. Regarding the G type, the results add information to previous typifications based on antigenic methods (Bellinzoni et al., 1989; Costantini et al., 2002). Antigenic characterizations previously conducted by our group using monoclonal antibodies found 21 samples serotyped as G1 (Costantini et al., 2002). However, VP7 sequence analysis of those strains revealed that all of them belonged to genotype G6 and evidenced that G1 RV has not been detected in Argentinean calves during the period under study. Rotavirus P[5]G6 was the prevalent combination detected in Argentinean herds from 1994 to 2003. This result is in agreement with previous studies conducted in Italy (Falcone et al., 1999); Brazil (Alfieri et al., 2004); USA (Parwani et al., 1993) and Japan (Okada and Matsumoto, 2002) and support the hypothesis that P[5]G6 combination is prevalent in cattle, worldwide. In addition, evidences of mixed and co-infections were found, suggesting an increased probability of natural reassortment in the field.

When studying the epidemiology of the disease, the strong association between herd type and G/P types distribution became evident. While P[5]G6 (NCDV-like) was the prevalent strain in beef herds, similar proportions of G6 NCDV-like, G6 Hun4-like, and G10 combined mainly with P[11], were detected in dairy herds. The predominance of G6 in beef herds and G10 in dairy herds was observed in previous surveys in Sweden (de Verdier Klingenberg et al., 1999) and Argentina (Bellinzoni et al., 1989). This behaviour is difficult to explain and could be due to different animal management, reduced inter-farm contact and different bovine biotypes. In Argentinean beef herds under extensive management; calving season occurs once a year during the winter time, suggesting that only highly infectious BRV strains shed in high titers would be able to infect susceptible hosts a year later. Rotavirus P[5]G6 seems to be the strain with the best fitness. In contrast, calves are born all year long in dairy farms, allowing a continuous availability of susceptible hosts where strains with a lower fitness could circulate, thus generating a higher diversity.

Genotype G8 is considered the third G type of epidemiological importance in cattle. It was previously detected in bovines in Japan (Fukai et al., 1999, 2002); Sweden (de Verdier Klingenberg et al., 1999) and Italy (Falcone et al., 1999). In this study the cases initially typed as G8, were classified a G6, lineage III (Hun4-like), thus G8 genotype has not been detected in Argentinean cattle so far. However, it was recently found in guanacos of the Patagonia, associated to P[1] and [14] (Parreno et al., 2004). The G8/G6 Hun4-like mistyping evidences the need of a continuous updating of the G type-specific primers according to the variants circulating in each geographical region.

The phylogenetic tree obtained by maximum parsimony was highly consistent with the trees obtained by maximum likehood, distance methods (data not shown) and with those previously reported (Martella et al., 2003; Rahman et al., 2003). Likelihood analyses indicated that the best-fit models for the VP7 data set were TVM + I + G and GTR + I + G. These models are more complex than Kimura's two parameters (Kimura, 1980), which were usually applied prior to distance-based analysis.

Argentinean P[5]G6 strains belong to lineage IV supporting previous hypothesis about the association of this lineage with P[5] genotype (Martella et al., 2003). In contrast, P[11]G6 Argentinean strains that failed to be typed as G6 by DT6 primer, were more closely related to lineage III (Hun4-like) instead of falling within lineage V (together with other P[11] BRVs). Previous studies showed *in vitro* cross neutralization and *in vivo* protection between BRVs of two different G6 lineages (Matsuda et al., 1993; Chang et al., 2000). This evidence suggests that although Argentinean G6 strains fall within two

different lineages, it would not be necessary to add a G6 Hun4-like strain to the current vaccine.

A BRV strain from a dairy herd typed as P[11] and carrying a VP7 closely related to a BRV isolated in India in 1999, assigned as G15 genotype (Rao et al., 2000), was detected. Both strains clustered together by phylogenetic analysis sharing an 87% nucleotide similarity (data not shown). This is the first report of a G15 BRV in America and the first report of a P[11]G15 combination, worldwide. Further analysis of this strain is currently under progress.

Overall, our results demonstrate that the G and P genotypes included in the current BRV vaccine (G6, G10, P[5] and P[11]) should protect calves from Argentinean rotavirus field strains. However, continuous genotyping of BRV field strains is necessary in order to keep the vaccine strains updated.

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