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Short communication

Bovine rotavirus strains circulating in beef and dairy herds in Argentina from 2004 to 2010

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ABSTRACT

Bovine Group A Rotavirus (RVA) is one of the main causes of neonatal calf diarrhea worldwide. The present study reports the genotyping of bovine RVA strains circulating in Argentinean cattle from 2004 to 2010. Additionally, a new set of typing primers was designed and tested to differentiate between G8 and G6 (lineage III and IV) RVA strains.

Bovine RVA was detected in 30% (435/1462) of the tested samples, corresponding to 49% (207/423) of the studied outbreaks with a similar detection rates in beef (53%; 67/127) and dairy herds (52%; 65/126). The RVA strains circulating in Argentinean cattle belonged to the common bovine genotypes G6 (lineages III and IV), G8, G10, P[5] and P[11]. A different RVA G/P-genotype distribution was found between the exploitation types, with the combination G6(IV)P[5] being by fare the most prevalent RVA strain in beef herds (58%), whereas a more even distribution of G6(III)P[11] (15%), G10P[11] (17%), G6(IV)P[5] (14%), and G6(IV)P[11] (6%) RVA strains was detected in dairy herds. G8 RVA strains were found in two dairy farms in calves co-infected with G8+G6(III)P[11]. A high percentage of co-infections and co-circulation of RVA strains with different genotypes during the same outbreak were registered in both exploitation types (20% of the outbreaks from beef herds and 23% from dairy herds), indicating a potential environment for reassortment. This finding is significant because G10P[11] and G6(III)P[11] strains may possess zoonotic potential. Continuous surveillance of the RVA strains circulating in livestock provides valuable information for a better understanding of rotavirus ecology and epidemiology.

1. Introduction

Neonatal diarrhea represents an important health problem in livestock production, causing a highly negative economic impact in terms of veterinary treatment costs and reduction in weight gain of affected animals, death. In Argentina 5% of the newborn calves die before weaning, mainly due to diarrhea and respiratory syndromes (INDEC, 2002). Bovine Group A rotavirus (RVA) are considered the major cause of neonatal calf diarrhea worldwide (Saif and Parwani, 1994). Previous studies conducted in Argentina indicated that bovine RVA strains are broadly distributed and are associated with more than 70% of the diarrhea outbreaks in beef farms and more than 50% of the outbreaks detected in dairy farms (Bellinzoni et al., 1990; Garaicoechea et al., 2006).

Rotaviruses constitute a genus within the *Reoviridae* family and are characterized by non-enveloped triple-layered viral particles with a viral genome composed by 11 double-stranded RNA segments (dsRNA). RVA strains are

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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 (Estes and Kapikian, 2007; Matthijnssens et al., 2008). Numerous rotavirus genotypes have been detected in calves, with at least 10 different G types (G1, G3, G6–G8, G10, G11, G15, G18 and G21) and 7 different P types (P[1], P[5], P[11], P[14], P[17], P[21], P[29]) (Matthijnssens et al., 2011) were reported. The G-types G6, G8 and G10 were associated with P[5], P[11] and to a lesser extent with P[1] and are therefore considered epidemiologically important worldwide (Alfieri et al., 2004; Cashman et al., 2010; Monini et al., 2008).

In a previous survey of Argentinean bovine RVA strains from 1994 to 2003, the circulation of genotype G6 lineage III and IV was described (Garaicoechea et al., 2006). That study reported that the G6 specific DT6 typing primer caused incorrect typing of G6 strains belonging to lineage III, as G8. The mistyping was caused by two events: (i) the accumulation of mutations in the nucleotide sequence corresponding to the DT6 binding region that avoided the generation of the PCR product of the size expected for a G6 genotype; and (ii) unspecific annealing of the primer in a downstream region producing a PCR product of similar size to that of G8 specific HT8 typing primer (Garaicoechea et al., 2006). To overcome this problem, in the present study new primers were designed based on the alignments of local strains and they were used to analyze field samples collected from 2004 to 2010.

2. Materials and methods

2.1. Fecal samples

The present study used a total of 1462 individual samples from diarrheic calves received by the Diagnosis Service of the Virology Institute, INTA, between 2004 and 2010. The samples were collected from 127 registered outbreaks of diarrhea in beef herds, 126 outbreaks in dairy herds and 170 outbreaks from herds of non-specified exploitation type.

2.2. Rotavirus diagnosis

Fecal samples were initially screened for RVA using an antigen capture ELISA (Cornaglia et al., 1989) or a commercial EIA kit (Panthfinder, BioRad®; Marnes, La Coquette, France). The percentages of RVA detection between beef and dairy herds were compared by Fisher exact test, $p < 0.05$, (Medcalc Statistical Software, version 11.1.1.0, 2003–2009, Mariakerke, Belgium).

2.3. Virus reference strains

Controls for the typing assays were bovine RVA prototype strains INDIANA, G6(IV)P[5]; B223, G10P[11]; NCDV-Cody I801, G8P[1] (provided by Dr. Saif, Food Animal Health Research Program, The Ohio State University, USA) and a field strain detected in a dairy calf in 1997 (B61/Bo/Arg) typed as G6(III)P[11] (Garaicoechea et al., 2006).

2.4. G and P genotyping by heminested multiplex RT-PCR

Bovine RVA dsRNA was extracted from 10% fecal suspensions using TRIzol* (Invitrogen, Carlsbad, CA, USA). The full length of the VP7 encoding gene (1062 bp) and the VP8* region (876 bp) of VP4 encoding genes were amplified by RT-PCR using the primers detailed in Table 1 and previously described procedures (Garaicoechea et al., 2006). The G-typing heminested multiplex assay for G8 and G10 was carried out using previously described forward Gra-5 (Chang et al., 1996) and reverse HT8 and ET10 primers (Gouvea et al., 1994a) respectively (Table 1). To properly discriminate G6 and G8 bovine RVA strains and to further characterize local field strains as G6 lineage III (G6(III)) or G6 lineage IV (G6(IV)), new reverse primers (N168, H500 and H168) were designed based on the alignment of local field G6(III) and G6(IV) strains and sequences from GenBank (Table 1). The final heminested multiplex reaction was carried out as follows: 1 μl of a 1/10 dilution of the first round RT-PCR product was added to a 50 μl final volume of PCR mix which contained 2.5 U of Taq DNA Polymerase (Promega Corporation, Madison, USA), 10 μl of 5× Green Go Taq Reaction Buffer (1.5 mM $MgCl_2$), 0.5 μl of 50 mM $MgCl_2$, 4 μl of 5 mM dNTPs, 1 μl of N168 20 mM, 1 μl of H168 20 mM, 1 μl of H500 20 mM, 1 μl of HT8 20 mM, 1 μl of ET10 20 mM, 1 μl of Gra-5 20 mM and 29 μl of molecular grade water. The amplification was performed through a first step of 2 min at 94 °C followed by 30 PCR cycles (94 °C for 30 s, 45 °C for 30 s, and 72 °C for 45 s) and a final extension of 72 °C for 7 min. The performance of the new heminested multiplex was confirmed by sequence analysis. The P-typing heminested multiplex assay was performed using the primers and procedures described previously (Garaicoechea et al., 2006; Gouvea et al., 1994b) (Table 1).

2.5. Nucleotide sequencing and sequence analysis

To confirm the G and P-typing results, VP7 and VP8* amplified products representative for each genotype were purified and submitted for sequencing to Macrogen Inc., Korea (http://macrogen.com). Gra-5, Gra-3, Con-2 and Con-3 primers were used as sequencing primers. The sequencing of the nested PCR products of two co-infections, was performed with primers HT8 and Gra-5 for the band representing the G8 genotype, and with the primers H500 and Gra-5 for the band representing the G6(III) genotype. Sequences were edited with the BioEdit 7.0.5.3 sequence Alignment Editor (Hall, 1999). Alignment was performed using ClustalX (Thompson et al., 1994). The phylogenetic analysis was conducted by Maximum Parsimony. The phylogenetic tree and bootstrap support (500 re-sampled matrices with replacement) were calculated using the TNT program (Goloboff et al., 2003). The tree was edited and drawn with TreeDyn (http://www.treedy.org).

3. Results

Bovine RVA was detected in 30% (435/1462) of the tested samples, corresponding to 49% (207/423) of the diarrhea outbreaks under study. Rotavirus was detected in

Table 1
Primers used in this study.

Primers		Sequence (5′ to 3′)	Position	Sense	Reference
VP7 primers					
VP7 Gra-5	5′ end	GGC TTT AAA AGC GAG AAT TT	1–20	+	Chang et al. (1996)
VP7 Gra-3	3′ end	GGT CAC ATC ATA CAA CTC TA	1062–1044	–	Chang et al. (1996)
N168	3′ end	TAT AAG CAG AAA GCT GTA A	168–150	–	In the present study
H168	3′ end	AAC GAA TAA AAA TCT GTA A	168–150	–	
H500	3′ end	TCC AAT TCC AAC GTT GAA A	500–482	–	
HT8	3′ end	CGG TTC CGG ATT AGA CAC	273–256	–	Gouvea et al. (1994a)
ET10	3′ end	TTC AGC CGT TGC GAC TTC	714–697	–	Gouvea et al. (1994a)
VP8* primers					
VP8* Con-3	5′ end	TGG CTT CGC TCA TTT ATA GAC A	11–32	+	Gouvea et al. (1994b)
VP8* Con-2	3′ end	ATT TCG GAC CAT TTA TAA CC	887–868	–	Gouvea et al. (1994b)
P1K	5′ end	ACC AA C GAA CGC GGG GGT G	266–284	+	Gouvea et al. (1994b), Garaicoechea et al. (2006)
P5K	5′ end	RCC AGG TGT CRC ATC AGA G	333–351	+	Gouvea et al. (1994b), Garaicoechea et al. (2006)
pB223	5′ end	GGA ACG TAT TCT AAT CCG GTG	574–594	+	Gouvea et al. (1994b)

53% (67/127) of outbreaks recorded in beef farms, 52% (65/126) in dairy farms and 47% (75/170) in farms of non-specified exploitation type. The detection rate of diarrhea associated with bovine RVA was similar between beef and dairy herds (Fisher exact test, $p = 0.9001$). From the total outbreaks studied in each province, RVA was detected as follows: 72/213 from Buenos Aires; 16/28 from Santa Fé, 8/8 from Córdoba; 1/4 from Corrientes, 1/2 from La Pampa, 1/1 from Santiago del Estero, while no RVA was detected in the three outbreaks from Entre Ríos.

Regarding the characterization of the detected RV strains, the reaction conducted with Gra-5 forward and N168/H168 reverse primers was able to detect G6(IV) and G6(III) strains without generating any unspecific results with G8 and G10 genotypes. Both N168 and H168 recognized lineages G6(III) and G6(IV), but the use of the N168 primer resulted in a stronger band for G6(IV) RVA strains while H168 resulted in a stronger band for G6(III) RVA strains. Additionally, to distinguish between G6(III) and G6(IV) strains, a new primer (H500), specific for G6(III), was added to the reaction. Therefore, the genotyping of G6(III) RVA strains produced two bands, the one corresponding to G6 (168 bp) and an extra band of 500 bp (Fig. 1A and B). The results of the new heminested multiplex were confirmed by sequence analysis. Fig. 1C shows the alignment of the new typing primers (N168, H168 and H500) compared with the old DT6 primer and their reactivity with local and reference RVA strains representative for each genotype.

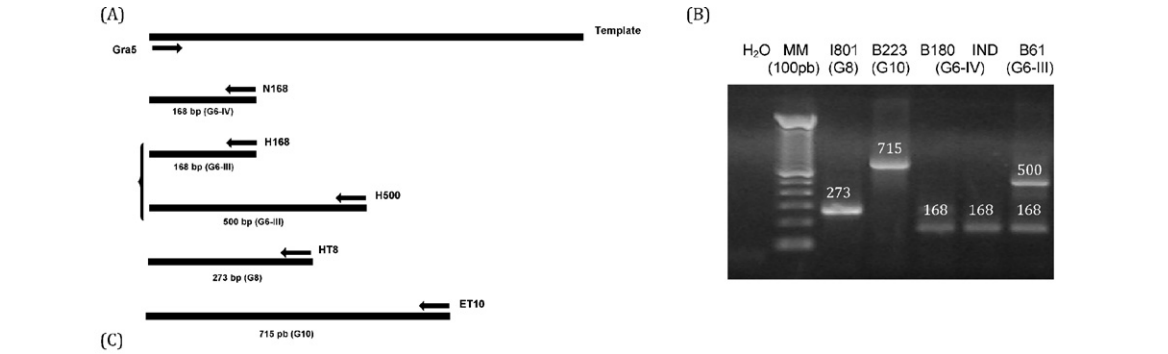
The most prevalent genotype in beef herds G6(IV)P[5] was present in 58% of the studied outbreaks, while similar percentages of a wider variety of bovine RVA strains were circulating in dairy herds: G10P[11], 17%; G6(III)P[11], 15%; G6(IV)P[5], 14%; and G6(IV)P[11], 6%. In the herds with a non-specified exploitation type, G6(IV)P[5] was the predominant combination with 61% (Table 2). High percentages of co-infections and co-circulation of bovine RVA strains with different genotypes during the same outbreak were detected in both exploitation systems (20% in beef and 23% in dairy). When two or more G or P types were detected by nested PCR in the same fecal sample, the outbreak was considered a co-infection. Co-circulation was considered when in the same outbreak there were different calves shedding a distinct bovine GARV strain each one; farms with co-circulation also included calves shedding a unique strain together with calves suffering a co-infection (Table 3).

In this survey two calves co-infected with bovine RVA strains G8 + G6(III) P[11] were detected in two dairy herds located in two distant locations within the Buenos Aires province. The presence of both genotypes in these samples was confirmed by sequencing the heminested multiplex PCR products of 273 bp expected for G8 and 500 bp expected for G6(III), respectively. The sequences obtained with the HT8 primer was confirmed phylogenetically to belong to genotype G8, while the sequence generated with the H500 primer was confirmed to belong to a phylogenetic cluster containing previously reported G6(III) Argentinean RVA strain (Fig. 2).

4. Discussion

Rotavirus diarrhea in calves is a high morbidity syndrome causing important economic losses for the farmers in terms of treatment cost and reduction of weight gain in affected animals (Bartels et al., 2010). In the present study, bovine RVA was detected in 48% of the diarrhea outbreaks registered in 6 out of 7 surveyed provinces. The survey covered five ecological regions (Humid and Dry Pampa, Mesopotamia, Patagonia and Cuyo) with 57% of the Argentinean cattle population living in the Pampean region (INDEC, 2002; SAGPyA, 2004). Similar results were obtained in a recent survey conducted in several states covering four geographical regions of Brazil (Alfieri et al., 2006). The Brazilian study revealed the presence of bovine RVA in all the regions analyzed, however the incidence of RVA diarrhea in terms of positive samples was lower (19.4%) (Alfieri et al., 2006) compared with the incidence found in Argentina (30%). These differences could be related to differential weather, husbandry systems, rearing conditions and the biotype of cattle breed in each country.

It is a disadvantage of the heminested multiplex PCR technique for the typing of RVA samples that a percentage of the samples is partially typed, cannot be typed or is



(C)

G type	Strains	DT6 (G6 primer)* 481–499 GATTCTACACAGGAACTAG	N168 (G6IV primer)* 150–168 TTACAGCTTTCTGCTTATA	H168 (G6-III primer)* 150–168 TTACAGATTTTTATTCGTT	H500 (G6-III primer)* 482–500 TTTCAACGTTGGAATTGGA	Reactivity with N1: DT6	68	H168	H500	HT8
G6 III	Hun4/Hu/G6P[9]/Hun	.C...A..GTT....T.G.	...T..A...T..T..G.T	......C...C.GC.TA.A	C.....	nd	nd	nd	nd	nd
	B88/Bo/G6P[11]/Arg	.T...A..GTT....T.G.	......A...T.AT.CG.T	.....	.....	*	+	+	+	-
	B163/Bo/G6P[11]/Arg	.T...A..GTT....T.G.	......A...T.AT.CG.T	.....	.....	*	+	+	+	-
	B3530/Bo/G6P[11]/Arg	.T...A..GTT....T.G.	......A...T.AT.CGCT	.....C.	.....	*	+	+	+	-
G6 IV	UK/Bo/G6P[5]/UK	.....	......A.....	.....C.GC.TA.A	A...T..ACA....C.A..	+	+	+	-	-
	IND/Bo/G6P[5]/USA	.....	......A.....	.....C.GC.TA.A	A...T..ACA....C.A..	+	+	+	-	-
	B1185/Bo/G6P[5]/Arg	..C..C.....A.....	......A.....	.....C.GC.TA.A	AC..C..ACAA...C.A..	+	+	+	-	-
	B1730/Bo/G6P[5]/Arg	..C..C.....A.....	......A.....	.....C.GC.TA.A	AC..C..ACAA...C.A..	+	+	+	-	-
G8	Tokushima9503 Bo/G8/Japan	A..G...ATTCA...T...	C..TC.T...T.A...T.T	C..TC.T.....C.TT..	A.G.T.AT.CA.....A..	nd	nd	nd	nd	nd
	BRV16/Bo/G8/Japan	A..G...GTTCA...T...	C..TC.T...T.....T.T	C..TC.T.....GC.TT..	A.G.T.GT.CA.....A..	nd	nd	nd	nd	nd
	Chubut/Gua/G8P[14]Arg	A..G...ATTCA...T...	C..TC.T...T.....T.T	C..TC.T.....GC.TT..	A.G.T.AT.CA.....A..	-	-	-	-	+
	RioNegro/Gua/G8P[1]Arg	A..G...ATTCA...T...	C..TC.T...T.....T.T	C..TC.T.....GC.TT..	A.G.T.AT.CA.....A..	-	-	-	-	+
G10	B223/Bo/G10P[11]/USA	A....G.GC.TA.....T.	.....AA...T.....	.....A.....GC.TA.A	A...G.GCC.A...C.T..	-	-	-	-	-

[a] Reverse complement sequences
nd: not determined
* The use of primer DT6 resulted in mistyping of G6 (III) RVA strains, since a PCR product was generated with the size expected for genotype G8

Fig. 1. (A) Design of RT-PCR heminested multiplex to type G6, G10 and G8 bovine RVA strains and to discriminate between G6 lineages III and IV. (B) RT-PCR heminested multiplex results. (C) Nucleotide alignment of G6, G8 and G10 RVA strains compared to DT6, N168, H168 and H500 primer sequences, used for genotyping.

typed incorrectly. The mistyping of RVA strains has been well documented for human RVA strains, and it is mainly caused by the accumulation of point mutations or homologous recombination (Iturriza-Gomara et al., 2004; Parra and Espinola, 2006). Mistyping of bovine G6 RVA strains as G8 was reported for the G6(III) bovine RVA strains from Argentina (Garaicoechea et al., 2006) and Ireland (Cashman et al., 2010) and signals the need to continuously update the type-specific primers. In the present study, the replacement of the DT6 primer by the new set of primers (N168, H168 and H500), not only overcame the mistyping of G6 (III) strains, but also allowed to easily differentiate between bovine G6(III) and G6(IV) RVA strains. It is important to note that the region of the VP7 gene selected for the design of this new set of primers, has been successfully used by other authors to discriminate human RVA strains from different lineages of G6 (Banyai et al., 2003).

The results of this study, together with the previous survey (Garaicoechea et al., 2006) allow us to state that the differential epidemiology for the distribution of G and P-types according to the exploitation type was maintained over time, with P[5] as the most prevalent genotype in beef herds and P[11] most prevalent in dairy herds. This finding is not easily explained but it could be speculated that this is the effect of different rearing conditions of beef and dairy herds. In beef herds the calving season is scheduled once a year, resulting in a long period without newborns. The G6(IV)P[5] RVA strains may be more resistant to the environment, or they may circulate asymptomatically for a longer period in the adult cattle population. The yearly bottleneck without susceptible newborns could explain the lower genetic RVA diversity observed in beef herds. Conversely, in dairy farms calves are born all year around and hence there are always susceptible hosts, allowing for a larger variety of different G/P-genotypes to co-circulate

Table 2
G and P-genotyping of bovine RVA strains circulating in Argentinean calves from 2004 to 2010.

Exploitation type	No. of farms											
	G6(IV)P[5]	G6(IV)P[11]	G6(IV)P[?]	G6(III)P[11]	G6(III)P[?]	G10P[5]	G10P[11]	G?P[5]	G?P[11]	G?P?	Mixed[a]	Total
Beef	39	1	2	2			3	5	1	1	13	67
Dairy	9	4		10	2		11		4	10	15	65
Non specified	46	3	2	3		1	3		2	3	12	75
Total	94	8	4	15	2	1	17	5	7	14	40	207

[a] Column assigned as "Mixed" includes co-infections (calves shedding bovine RVA with nested-PCR products corresponding to more than one G or P-genotypes) and co-circulation (different calves in the same herd shedding a distinct bovine RVA strain each one). See Table 3 for details.

Table 3
Outbreaks with co-circulation/co-infections with different bovine RVA strains (2004–2010).

Genotypes	Beef	Dairy	Non-specified	Total
G6IV P[5]P[11]	2	1	1	4
G6IV G10P[5]P[11]	3	3	5	11
G6IV G10P[5]	4		2	6
G6IV G10P[11]	1	1	2	4
G6IV G10P?		1		1
G6IV G6III P[5]P[11]	2			2
G6IV G6III P[5]			1	1
G6IV G6III P[11]		2		2
G6III P[5]P[11]	1	1		2
G6III G10P[11]		4		4
G8G6III P[11]		2		2
G? P[5]P[11]			1	1
Total	13	15	12	40

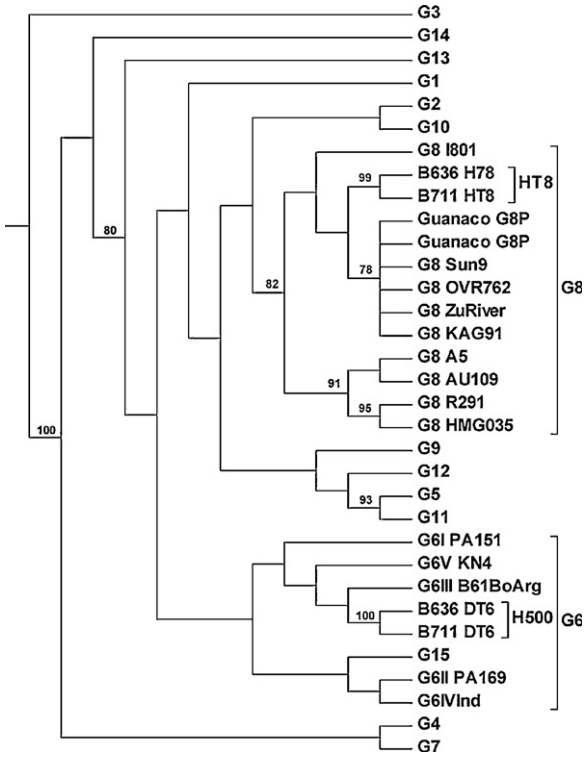


Fig. 2. Maximum Parsimony analyses of fragments of VP7 corresponding to nucleotides 1–273 of G8 and G6(III) RVA strains detected in the two co-infections. Bootstrap values are indicated. Branches with bootstrap values below 50 are not shown. The following reference strains from GenBank were included: Accession numbers: *G6*: KN-4 (D12710); B61/Bo/Arg (DQ631812); PA-169 (L20880); NCDV Lincoln (M12394); PA 151 (L20881); *G8*: R291 (58197890); AU109 (126635340); HMG035 (14549672); OVR762 (156118459); Sun9 (49659446); U35-96 (2766547); KAG91 (18150185); A5 (222527); NCDV Cody (U14999); GuaP[1]G8 (33333745); GuaP[14]G8 (33333747); *G14*: ArgE400; *G3*: ArgE30; *G4*: D151 (26190403); *G1*: T449 (333827); G2: 94B (U73952); *G10*: B223 (X52650); *G12*: T152 (AB071404); *G9*: US1071 (6465807); *G13*: L338 (D13549); *G7*: Ty-3 (AB080737); *G5*: OSU (X06722); *G11*: A253 (AF317122). Po, porcine; Bo, bovine; Hu, human; Gua, guanaco; Ov, ovine; Nig, Nigeria; Bra, Brazil; US, United States; Jap, Japan; Sui, Switzerland; Esp, Spain; Hun, Hungary; Arg, Argentine; Aus, Australia; Ita, Italy.

in dairy herds. The lineage G6(III) circulating in dairy farms, has been detected in children with diarrhea in Hungary, while G10 was found in children from South America, Europe, Asia and Africa (Banyai et al., 2003; Coluchi et al., 2002; Esona et al., 2010; Ramani et al., 2009; Volotao et al., 2006; Zuccotti et al., 2010). These findings suggest the need of conducting surveys including domestic and food animals as well as human infants from rural areas to evaluate the zoonotic potential of these RVA strains.

G8 RVA strains are considered the third most important RVA genotype affecting cattle (Fukai et al., 2004; Monini et al., 2008). During the present survey, two calves shedding G8 + G6(III)P[11] bovine RVA strains were found in two dairy farms in Buenos Aires. This result represents the first detection the bovine G8 genotype in Argentinean cattle. However G8 was detected in two co-infections and no single infection with G8 bovine RVA strains has as yet been detected. The circulation of G8 RVA strain was not an unexpected result given that it has been reported in non-diarrheic pigs (Parra et al., 2008) and two species of south American camelids, guanacos (Parreno et al., 2004) and vicugnas (Badarraco et al., unpublished results) in Argentina.

The results of this study highlight the importance of conducting a continuous surveillance of RVA genotypes circulating in cattle as well as in humans and other animal species. A better understanding of the epidemiology of RVA infection within the region is important to design better strategies to control this enteric disease.

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References

Alfieri, A.A., Parazzi, M.E., Takiuchi, E., Medici, K.C., Alfieri, A.F., 2006. Frequency of group A rotavirus in diarrhoeic calves in Brazilian cattle herds, 1998–2002. Trop. Anim. Health Prod. 38, 521–526.

Alfieri, A.F., Alfieri, A.A., Barreiros, M.A., Leite, J.P., Richtzenhain, L.J., 2004. G and P genotypes of group A rotavirus strains circulating in calves in Brazil, 1996–1999. Vet. Microbiol. 99, 167–173.

Banyai, K., Gentsch, J.R., Griffin, D.D., Holmes, J.L., Glass, R.I., Szucs, G., 2003. Genetic variability among serotype G6 human rotaviruses: identification of a novel lineage isolated in Hungary. J. Med. Virol. 71, 124–134.

Bartels, C.J., Holzhauer, M., Jorritsma, R., Swart, W.A., Lam, T.J., 2010. Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves. Prev. Vet. Med. 93, 162–169.

Bellinzoni, R.C., Blackhall, J., Terzolo, H.R., Moreira, A.R., Auza, N., Mattion, N., Micheo, G.L., La Torre, J.L., Scodeller, E.A., 1990. Microbiology of diarrhoea in young beef and dairy calves in Argentina. Rev. Argent. Microbiol. 22, 130–136.

Cashman, O., Lennon, G., Sleator, R.D., Power, E., Fanning, S., O'Shea, H., 2010. Changing profile of the bovine rotavirus G6 population in the south of Ireland from 2002 to 2009. Vet. Microbiol. 146, 238–244.

Coluchi, N., Munford, V., Manzur, J., Vazquez, C., Escobar, M., Weber, E., Marmol, P., Racz, M.L., 2002. Detection, subgroup specificity, and genotype diversity of rotavirus strains in children with acute diarrhea in Paraguay. J. Clin. Microbiol. 40, 1709–1714.

Cornaglia, E.M.B.M., Fitjman, N., Schudel, A.A., 1989. Enzyme linked immunosorbent assay, immunofluorescent test and electrophoresis analysis of rotaviral RNA in the diagnosis and characterization of the bovine rotavirus. Rev. Latinoam. Microbiol. 31, 4.

Chang, K.O., Parwani, A.V., Saif, L.J., 1996. The characterization of VP7 (G type) and VP4 (P type) genes of bovine group A rotaviruses from field samples using RT-PCR and RFLP analysis. Arch. Virol. 141, 1727–1739.

Esona, M.D., Page, N.A., Akran, V.A., Armah, G.E., Steele, A.D., 2010. Characterization of 2 human genotype G10 rotavirus strains, 3008CM and 1784/CI/1999, isolated in Cameroon and Cote d'Ivoire during the 1999–2000 rotavirus season. J. Infect. Dis. 202 (Suppl.), S212–S219.

Estes, M.K., Kapikian, A.Z., 2007. Rotaviruses. In: Knipe, D.M., Howley, P.M. (Eds.), Field's Virology. Lippincott Williams & Wilkins, Philadelphia, pp. 1917–1974.

Fukai, K., Onoda, H., Itou, T., Sato, M., Miura, Y., Sakai, T., 2004. Genetic and serological characterization of novel serotype G8 bovine group A rotavirus strains isolated in Japan. J. Vet. Med. Sci. 66, 1413–1416.

Garaicoechea, L., Bok, K., Jones, L.R., Combessies, G., Odeon, A., Fernandez, F., Parreno, V., 2006. Molecular characterization of bovine rotavirus circulating in beef and dairy herds in Argentina during a 10-year period (1994–2003). Vet. Microbiol. 118, 1–11.

Goloboff, P.A., Farris, J.S., Nixon, K., 2003. Tree analysis using new technology. <http://www.cladistics.com/aboutTNT.html>

Gouvea, V., Santos, N., Timenetsky Mdo, C., 1994a. Identification of bovine and porcine rotavirus G types by PCR. J. Clin. Microbiol. 32, 1338–1340.

Gouvea, V., Santos, N., Timenetsky Mdo, C., 1994b. VP4 typing of bovine and porcine group A rotaviruses by PCR. J. Clin. Microbiol. 32, 1333–1337.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 95–98.

INDEC, 2002. Encuesta Nacional Agropecuaria (ENA 2001) (Buenos Aires, Intituto Nacional de Estadísticas y Censos. (INDEC) – Secretaria de Política Económica – Ministerio de Economía).

Iturriza-Gomara, M., Kang, G., Gray, J., 2004. Rotavirus genotyping: keeping up with an evolving population of human rotaviruses. J. Clin. Virol. 31, 259–265.

Matthijnssens, J., Ciarlet, M., Heiman, E., Arijs, I., Delbeke, T., McDonald, S.M., Palombo, E.A., Iturriza-Gomara, M., Maes, P., Patton, J.T., Rahman, M., Van Ranst, M., 2008. Full genome-based classification of rotaviruses reveals a common origin between human Wa-Like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. J. Virol. 82, 3204–3219.

Matthijnssens, J., Ciarlet, M., McDonald, S.M., Attoui, H., Banyai, K., Brister, J.R., Buesa, J., Esona, M.D., Estes, M.K., Gentsch, J.R., Iturriza-Gomara, M., Johne, R., Kirkwood, C.D., Martella, V., Mertens, P.P., Nakagomi, O., Parreno, V., Rahman, M., Ruggeri, F.M., Saif, L.J., Santos, N., Steyer, A., Taniguchi, K., Patton, J.T., Desselberger, U., Van Ranst, M., 2011. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). Arch. Virol. 156, 1397–1413.

Monini, M., Cappuccini, F., Battista, P., Falcone, E., Lavazza, A., Ruggeri, F.M., 2008. Molecular characterization of bovine rotavirus strains circulating in northern Italy, 2003–2005. Vet. Microbiol. 129, 384–389.

Parra, G.I., Espinola, E.E., 2006. Nucleotide mismatches between the VP7 gene and the primer are associated with genotyping failure of a specific lineage from G1 rotavirus strains. Virol. J. 3, 35.

Parra, G.I., Vidales, G., Gomez, J.A., Fernandez, F.M., Parreno, V., Bok, K., 2008. Phylogenetic analysis of porcine rotavirus in Argentina: increasing diversity of G4 strains and evidence of interspecies transmission. Vet. Microbiol. 126, 243–250.

Parreno, V., Bok, K., Fernandez, F., Gomez, J., 2004. Molecular characterization of the first isolation of rotavirus in guanacos (*Lama guanicoe*). Arch. Virol. 149, 2465–2471.

Ramani, S., Iturriza-Gomara, M., Jana, A.K., Kuruvilla, K.A., Gray, J.J., Brown, D.W., Kang, G., 2009. Whole genome characterization of reassortant G10P[11] strain (N155) from a neonate with symptomatic rotavirus infection: identification of genes of human and animal rotavirus origin. J. Clin. Virol. 45, 237–244.

SAGPyA, 2004. Principales Indicadores Pecuarios.

Saif, L.R.B., Parwani, A., 1994. Animal rotaviruses. In: Az, K. (Ed.), Virus Infections of the Gastrointestinal Tract. Marcel-Dekker, New York, pp. 279–367.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Volotao, E.M., Soares, C.C., Maranhao, A.G., Rocha, L.N., Hoshino, Y., Santos, N., 2006. Rotavirus surveillance in the city of Rio de Janeiro-Brazil during 2000–2004: detection of unusual strains with G8P[4] or G10P[9] specificities. J. Med. Virol. 78, 263–272.

Zuccotti, G., Meneghin, F., Dilillo, D., Romano, L., Bottone, R., Mantegazza, C., Giacchino, R., Besana, R., Ricciardi, G., Sterpa, A., Altamura, N., Andreotti, M., Montrasio, G., Macchi, L., Pavan, A., Paladini, S., Zanetti, A., Radaelli, G., 2010. Epidemiological and clinical features of rotavirus among children younger than 5 years of age hospitalized with acute gastroenteritis in Northern Italy. BMC Infect. Dis. 10, 218.