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# Phylogenetic analyses of typical bovine rotavirus genotypes G6, G10, P[5] and P[11] circulating in Argentinean beef and dairy herds

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**Abstract**

Group A Rotavirus (RVA) is one of the main causes of neonatal calf diarrhea worldwide. RVA strains affecting Argentinean cattle mainly possess combinations of the G6, G10, P[5] and P[11] genotypes. To determine RVA diversity among Argentinean cattle, representative bovine RVA strains detected in diarrheic calves were selected from a survey conducted during 1997-2009. The survey covered the main livestock regions of the country from dairy and beef herds. Different phylogenetic approaches were used to investigate the genetic evolution of RVA strains belonging to the prevalent genotypes. The nucleotide phylogenetic tree showed that all genotypes studied could be divided into several lineages. Argentinean bovine RVA strains were distributed across multiple lineages and most of them were distinct from the lineage containing the vaccine strains. Only the aminoacid phylogenetic tree of G6 RVA strains maintained the same lineages as observed at the nucleotide level, whereas a different clustering pattern was observed for the aminoacid phylogenetic trees of G10, P[5] and P[11] suggesting that the strains are more closely related at the aminoacid level than G6 strains. Association between P[5] and G6(IV), prevalent in beef herd, and between P[11] and G6(III) or G10 (VI and V), prevalent in dairy herds, were found. In addition, Argentinean G6(III), G10, P[5] and P[11] bovine RVA strains grouped together with human strains, highlighting their potential for zoonotic transmission. Phylogenetic studies of RVA circulating in animals raised for consumption and in close contact with humans, such as cattle, contribute to a better understanding of the epidemiology of the RVA infection and evolution.

## 1. Introduction

Neonatal diarrhea represent an important sanitary problem in livestock production, causing a highly-negative economic impact related to death, veterinary treatment costs, and reduction in weight gain of affected animals (Bendali et al., 1999; Makoschey et al., 2009). In Argentina, 5% of the newborn calves die before weaning, mainly due to diarrhea and respiratory syndromes (INDEC, 2002). Group A rotaviruses (RVA) are considered the major cause of neonatal calf diarrhea, worldwide (Bellinzoni et al., 1990; Garaicoechea et al., 2006; Saif, 1994). Surveys conducted in Argentina from 1994 to 2009 indicated that bovine RVA is broadly distributed, being detected in more than 50% of the diarrhea outbreaks registered in beef and dairy herds (Badaracco et al., 2012; Bellinzoni et al., 1990; Garaicoechea et al., 2006).

RVA virions are characterized by non-enveloped triple-layered viral particles with a genome composed by 11 double-stranded RNA segments (dsRNA). Based on the genetic and antigenic variation of the two outer capsid proteins VP7 (*G*lycoprotein) and VP4 (*P*rotease sensitive protein), RVA are classified into G and P types, respectively (Estes and Kapikian, 2007).

To date, 27 G types and 37 P types have been recognized (Matthijnssens et al., 2011; Trojnar et al., 2012). Numerous RVA genotypes have been detected in calves, with at least 14 different G types (G1-G8, G10, G11, G15, G18, G21 and G24) and 11 different P types (P[1], P[3], P[5], P[7], P[10], P[11], P[14], P[17], P[21], P[29] and P[33]) reported (Abe et al., 2011; Abe et al., 2009; Estes and Kapikian, 2007; Fukai et al., 2002; Fukai et al., 2004a; Fukai et al., 2004b; Martella et al., 2010; Okada and Matsumoto, 2002; Rao et al., 2000). G6, G8, G10 associated to P[5], P[11] and, to a lesser extend with P[1] are considered epidemiologically important in cattle (Badaracco et al., 2012; Cashman et al., 2010; Falcone et al., 1999; Garaicoechea et al., 2006; Monini et al., 2008).

The current strategy to control the disease in cattle is based on vaccination of pregnant cows to protect the calves by transference of passive maternal antibodies through colostrum intake (Bellinzoni et al., 1990; Fernandez et al., 1998; Saif et al., 1987). Several inactivated vaccines are available on the market and systematic vaccination showed to be effective to reduce diarrhea morbidity (Bellinzoni et al., 1990; Cornaglia et al., 1989; Parreño et al., 2004). However, these commercial immunogens are being prepared with tissue culture (tc)-adapted bovine reference RVA strains that may not represent the currently circulating bovine RVA strains.

Phylogenetic studies of the RVA circulating in species in close contact with humans, such as cattle, contribute to a better understanding of the epidemiology of these pathogens, which results in important information to evaluate the need of vaccines update, and add comprehensive data to elucidate the mechanisms of rotavirus evolution. Previous studies have shown that the prevalent RVA strains circulating in Argentinean cattle belong to the typical bovine genotypes G6, G10, P[5] and P[11] (Badaracco et al., 2012; Garaicoechea et al., 2006). A clear differential distribution of G and P types was observed depending on the exploitation type. Thus, G6(IV)P[5] strains were most prevalent in beef herds, while similar proportions of G6(III)P[11], G6(IV)P[11] and G10P[11] were detected in dairy herds (Badaracco et al., 2012; Garaicoechea et al., 2006). The aim of this study was to conduct phylogenetic analyses using different approaches (Distance, Maximum Likelihood, and Bayesian inferences) to gain insights in the evolution and epidemiology from the most prevalent G and P types circulating in Argentinean cattle together with representative strains detected worldwide.

## Material and methods

### 2.1. Fecal samples

A total of 92 bovine RVA strains from diarrhea outbreaks which occurred from 1997 to 2009 were selected for sequence and phylogenetic analyses for the VP7 and VP4 encoding genes. All these samples were previously genotyped using multiplex RT-PCR as described previously (Badaracco et al., 2012; Garaicoechea et al., 2006). The samples included in this study belonged to a collection of 3043 fecal specimens received by the reference diagnosis service of the Virology Institute, INTA (Badaracco et al., 2012; Garaicoechea et al., 2006). Samples selected from two statistical designed surveys carried out in the Buenos Aires province to study the microbiology of calf diarrhea in beef and dairy herds and herds from the dairy area "Cuenca Lechera Mar y Sierras" were also included.

The selection of samples for phylogenetic analyses was done to cover samples collected in different years, with different G and P types within a given year, with different type of exploitation (i.e. beef, dairy and non-specified) and from different geographical locations (provinces and regions). Most of the samples belonged to herds located in Buenos Aires province (n=66). Six samples were collected from diarrhea outbreaks in Córdoba, four samples from Santa Fé, three samples from Entre Rios, two from La Pampa, one from Río Negro, one from Neuquén, and one from San Luis. The location of collection was missing in eight samples. From the 92 Argentinean RVA strains selected for this study, 37 were from beef herds, 30 from dairy herds and 25 belonged to herds of non-specified exploitation (Table 1).

### 2.2. Virus reference strains

Bovine RVA prototype strains RVA/Cow-xx/USA/INDIANA/XXXX/G6P[5] (NCDV-like, G6 lineage IV), RVA/Cow-tc/USA/B223/XXXX/G10P[11], RVA/Cow-xx/USA/NCDV-Cody_I801/XXXX/G8P[1] (kindly provided by Dr. Saif, Food Animal Health Research Program, The Ohio State University, USA) and a RVA field strain detected in a dairy calf in 1997 (RVA/Cow-wt/ARG/B61_D_BA/1997/G6(III)P[11]) (Hun4-like, lineage III) (Garaicoechea et al., 2006) were used as controls for typing assays.

### 2.3. RNA extraction, RT-PCR amplification and nucleotide sequencing

RNA was extracted from 10% fecal suspensions using TRIzol* (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. The full length of the VP7 (1062 bp), the VP8* region (876 bp) of the

VP4 encoding genes were amplified by RT-PCR using the primers and procedures previously described (Badaracco et al., 2012; Garaicoechea et al., 2006). To confirm the differences detected in the VP8* sequences, the nearly full-length sequence of 5 P[5] and 11 P[11] strains were amplified by One Step RT-PCR using the Qiagen OneStep RT-PCR Kit (Qiagen/Westburg) following the manufactures instructions, using the following primers: P5_VP4_807F (GGAAAGAAATGCAATATAATAG), P5_VP4_1640R (GGTAGCCATCGATTTTGCTGC), P5_VP4_1504F (GGAAAGRCGTTTAAATGAGTTGAG) and P5_VP4_2355R (GGTCASATCCTCCAGAAGCTG) for P[5]; and P11_VP4_1429F (5'-CCRAGTAATGATGACTACC-3'), P11_VP4_2330R (5'-GGTCACATCCTCATACAAACAGC-3'), P11_VP4_644 (5'-CGCAACAAGAGATATGTACAG-3') and P11_VP4_1543R (5'-GCTGTGACAGAGCTATATTAGC-3') for P[11].

The PCR products were run and visualized in a 1.8% molecular biology grade agarose gel, containing 4 mg/ml ethidium bromide, under UV light. Amplified products were purified from agarose gel using the QIAquick Gel Purification Kit (Qiagen, Hilden, Germany), and sequenced in both directions using the same primers used for the amplification of the PCR product. The PCR products were submitted for sequencing procedures to Macrogen Inc., Korea (http://macrogen.com).

**2.4. VP7, VP4 and VP8* phylogenetic analyses**

Phylogenetic analyses for VP7, VP4 and VP8* were performed using the sequences obtained in this study together with sequences available in the GenBank database for RVA detected in cattle as well as in other animals species including humans. Sequences were edited with the BioEdit 7.0.5.3 sequence Alignment Editor (Hall, 1999). Complete alignment was performed using ClustalX (Thompson et al., 1994). The alignments were analyzed using different phylogenetic algorithms: Distance, Maximum Likelihood and Bayesian methods. The distances analyses were performed using the Neighbor-Joining and Kimura 2-parameter as a model of nucleotide substitution and the Poisson correction parameter for aminoacid substitutions (Kimura, 1980; Saitou and Nei, 1987). The dendrograms were constructed using MEGA5 (Tamura et al. 2011). The jModeltest program (Posada, 2008) was used to infer the sequences best evolutionary model of nucleotide substitutions. Maximum Likelihood analysis were conducted using the PhyML program (Guindon and Gascuel, 2003). The Bayesian methods were performed using the MrBayes software (Huelsenbeck and Bollback, 2001; Huelsenbeck and Ronquist, 2001; Huelsenbeck et al., 2001; Ronquist and Huelsenbeck, 2003). Trees were edited and drawn with MEGA5. Bootstrap with 1000 re-sample

matrices for Distances methods and 100 re-sample matrices for Maximum Likelihood methods were performed to assess the statistical support for the identified groups (Felsenstein, 1985).

**2.5. Construction of pairwise distances frequency graphs**

To obtain suitable cut-off values to define the lineages within the G6, G10, P[5] and P[11] genotypes, the percentages of nucleotide and amino acid distances between all the sequences included in the analyses were calculated using the pairwise distances algorithm of the MEGA5. The pairwise distances frequency graphs were constructed by plotting the percentage of the distances in the abscissa (x axis) and the frequency of the pairwise distances in the ordinate (y axis), as described previously (Matthijnssens et al., 2008). The nucleotide/amino acid distances between strains belonging to the same cluster/lineage were designated as the "intra-lineages distances", while the nucleotide/amino acid distances between strains belonging to different clusters/lineages were designated as the "inter-lineage distances". The cut-off value to differentiate between lineages was defined as the percentage value that best discriminate between the intra and the inter-lineage distances (Matthijnssens et al., 2008).

**2.6. Mapping of amino acid substitutions**

Using the crystal structure obtained for the VP7 trimer (Aoki et al., 2009), the amino acid changes observed in the antigenic sites of the Argentinean G6 and G10 RVA strains were compared to the references strains WC3 and B223, respectively; and highlighted using the PyMol program (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC).

**2.7. Nucleotide sequence accession numbers**

The nucleotide sequence data reported in this paper were deposited in GenBank. The accession numbers for the G6 sequences are KC895753-KC895794, for the G10 sequences are KC895795-KC895810, for the P[5] sequences are KC895811-KC895836 and for the P[11] sequences are KC895837-KC895860.

## 3. RESULTS

To investigate the diversity and evolution of the RVAs circulating in Argentinean cattle and to compare them with RVA strains detected worldwide, phylogenetic analyses were conducted for each of the most prevalent genotypes (G6, G10, P[5] and P[11]). From the 92 RVA strains selected, 73 VP7 sequences (G6:57 and G10:16), 51 VP8* sequences (P[5]: 26 and P[11]: 25) and 16 VP4 sequences (P[5]:5 and P[11]:11) were obtained. Although samples were initially genotyped using multiplex, the lack of amplification of the entire VP7 or VP8* fragments could be due to conservation problems, low viral infectious titers, or mismatches with the sequence of the primers used in this study (Espinola et al., 2008; Garaicoechea et al., 2006).

The genes encoding the VP7, VP4 and VP8* external proteins of the Argentinean samples together with representative sequences retrieved from the GenBank database were analyzed using different phylogenetic approaches; however, prior to the construction of the phylogenetic trees, each data set was analyzed by the minimum squares method using the Tree Puzzle software (Schmidt et al., 2002), and showed to have a proper phylogenetic signal (data not shown).

### *3.1. Phylogenetic analyses of genotype G6.*

The phylogenetic analyses of the G6 genotype showed the presence of 5 lineages, as previously reported by others (Banyai et al., 2003a; Banyai et al., 2003b; Garaicoechea et al., 2006; Martella et al., 2003; Rahman et al., 2003). The lineages were supported by high bootstrap values, and Argentinean G6 samples belonged to lineages III and IV (Fig. 1A). Briefly, lineage I is composed of human G6 RVA strains in combination with P[6] and P[9]. Lineage II is formed by human G6P[14] RVA strains from different countries, one G6P[14] RVA strain detected in an antelope (RC18/08) from South Africa, two RVA strains found in goats (Cap455 from South Africa and GO34 from Bangladesh), a bovine strain from India and two porcine strains from India. Lineage III constitutes a monophyletic group and comprises human G6P[9] RVA strains from Hungary, a buffalo G6P[3] from Italy and bovine strains from Hungary and Argentina in combination with P[5] and P[11]. G6 lineage IV comprises typical P[1], P[5] and P[11] bovine RVA strains from all over the world, 3 bovine RVA P[7] strains, one equine strain from India and one porcine strain from Argentina (Parra et al., 2008). Finally, lineage V includes bovine strains and one human strain from Hungary (Fig. 1A).

A total of 33 Argentinean RVA strains belonged to G6 lineage IV (NCDV-like) and constituted a monophyletic group together with others G6(IV) strains from USA, UK, India, France, China, Venezuela, Korea, Canada and Australia, the reference strains WC3 (G6P[5]), NCDV, RF, (G6P[1]) and the strain WI79-4

included in the human vaccine Rotateq®. The genetic distance among the strains within lineage IV ranged from 0-11.7% (88.3-100% identity) on the nucleotide level (Fig. 1B). The Argentinean RVA strains belonging to G6 lineage IV were mostly associated with P[5] (29/33) and belong to beef herds (23/33) (Table 1). In addition, the combination G6P[5] was broadly distributed all over the country, being detected in the provinces of Buenos Aires, La Pampa, Córdoba, Entre Rios, Santa Fé and Río Negro. The UK, NCDV-Lincoln and INDIANA bovine strains, usually included in the vaccines available in Argentina, belong to this lineage G6(IV) but grouped in a different subcluster than the Argentinean bovine strains (Fig. 1A).

From the total of 57 Argentinean G6 strains included in the analyses, 24 strains belonged to lineage III. The maximum genetic distance among the sequences belonging to lineage G6(III) ranged from 0-7.4% (92.6-100% identity) (Fig. 1B). Most of the Argentinean G6(III) strains were associated with the P[11] type (22/24) and belong to dairy herds (17/24). Furthermore, most of the Argentinean G6(III) strains came from the Buenos Aires province, except for one strain from Entre Rios province (RVA/Cow-wt/ARG/B1190_B_ER/2000/G6P[11]) that did not group in the same cluster with the others Argentinean strains but was more closely related to European RVA strains (Fig. 1A).

The genetic distances ranged from 10.8-22% between the 5 lineages (Fig. 1B-C). Furthermore, to confirm the results obtained by distance analyses, Maximum Likelihood and Bayesian analyses were also conducted. For this data set, the best-fit nucleotide substitution model estimated by the Akaike Information Criterion was TIM3+I+G. In both analyses, a similar topology with respect to the grouping of the strains in lineages was obtained (data not shown).

According to the topology of the dendrogram and the pairwise identity frequency graph (Fig. 1A and 1C), the nucleotide cut-off value for G6 lineages was established at 13% genetic distances (87% identity). Strains with nucleotide identity higher than 87% usually belong to the same lineage while strains with lower identity usually belong to different lineage (Fig. 1B-C). The cut-off value using the deduced aminoacid sequences was established as 5% aminoacid distances (95% aa identity). Phylogenetic studies at the aminoacid level are available upon request.

The deduced aminoacid of the VP7 sequences obtained in this study together with representative strains from the proposed lineages were aligned and further studied (data not shown). Direct inspection of sequence alignment showed that the G6 (lineage III and IV) Argentinean strains possessed the 11 prolines and the 8 cysteines present in the WC3 bovine RVA reference strain. The Argentinean strains from lineage IV possess the 3 glycosylation sites (aa 69, 238 and 318) also present in other bovine strains; however, the

Argentinean strain belonging to lineage III only had two of them (aa 60 and 238). In Figure 2 the aminoacid differences between the reference strain WC3 and two Argentinean strain representing lineage IV and III were mapped in the VP7 trimer. The Argentinean strains belonging to lineage IV presented one substitution $125^{Ala\rightarrow Val}$, while the Argentinean strains from lineage III presented 7 aminoacid substitution within the antigenic regions as compared to the WC3 reference strain: $87^{Val\rightarrow Thr}$, $100^{Asp\rightarrow Asn}$, $130^{Glu\rightarrow Asp}$, $145^{Asp\rightarrow Val}$, $147^{Thr\rightarrow Ala}$, $148^{Gln\rightarrow Leu}$ and $242^{Ala\rightarrow Glu}$ (Fig. 2).

### *3.2. Phylogenetic analyses of genotype G10*

Phylogenetic analyses of G10 strains were conducted including 16 Argentinean bovine RVA strains sequenced in the present study together with G10 sequences from samples collected worldwide and present in the GenBank database. Twelve of the 16 Argentinean strains were from Buenos Aires province and were associated to the P[11] (6/12) or P[5] (6/12) genotypes. Two G10P[11] strains from San Luis and Neuquén provinces were also included in the analyses (Table 1).

Phylogenetic analyses identified six distinct G10 genotype lineages supported by high bootstrap values (Fig. 3A) as previously reported (Esona et al., 2010). Briefly, lineage I is composed of human G10 RVA strains from Africa, lineage II includes bovine and equine RVA strains from India and one human RVA strain from Thailand. Lineage III and IV contain only one strain, the human strain RVA/Human-tc/GBR/A64/1987/G10P11[14] from England and the sheep strain RVA/Sheep-tc/CHN/Lamb-NT/XXXX/G10P[15] from China, respectively. G10 lineage V is composed by a large diversity of strains, including a G10P[11] buffalo RVA strain (RVA/Buffalo-XX/ZAF/Buf1442-07SA/2007/G10P[11]), a G10P[5] porcine RVA strain P343 and bovine strains from all over the world (e.g.: India, Australia, China, USA and Thailand), including the bovine vaccine strain B223 from USA and one Argentinean strain (RVA/cow-wt/ARG/B3326_D_BA/2007/G10P[11]) from a dairy herd from Buenos Aires. Of note, this lineage comprises two reassortant human-bovine RVA strains (I321 and N155 G10P[11]) detected in children from India (Bhandari et al., 2006; Ramani et al., 2009). Finally, lineage VI presented the majority of the Argentinean bovine G10 strains (15/16), which formed a monophyletic group together with the human RVA strain (RVA/Human-xx/BRA/R239/2000-4/G10P[9]) from Brazil (Volotao et al., 2006). The genetic distances between all the lineages were 9.8-17.7% and the genetic distances between the Lineage V (including one Argentinean strain and the vaccine strain) and Lineage VI (including most of the Argentinean strains) were 13.2-16.5% (Fig. 3B).

A cut-off value of 13% nucleotide distance (87% nucleotide identity) was found most appropriate to discriminate the observed G10 lineages (Fig. 3C). The nucleotide identity of strains within each lineage ranged from 87.9-100%, while sequence variation among strains belonging to different lineages was considerably higher and ranged from 82.3-90.2% (Fig. 3B), slightly overlapping the proposed cut-off value to separate in different lineages. Maximum Likelihood and Bayesian analysis using a TPM3uf+G nucleotide substitution model estimated by the Akaike Information Criterion were also conducted and the same lineages than in Distances Methods were observed (data not shown).

The dendrogram constructed with the deduced aminoacid sequences showed that not all the lineages proposed at the nucleotide level were maintained, avoiding the possibility to establish a cut-off value. The range of aminoacid distances among the G10 strains was 0-11% (mean= 5.2%), which is lower when compared to G6 aminoacid distances (0-15.6% mean=7.2%); suggesting that all G10 strains are evolving to different lineages at the nucleotide level but they remain very closely related at the aminoacid level. Nevertheless, the Argentinean G10(VI) bovine RVA strains still form a monophyletic group together with the human RVA R239 strain from Brazil at the aminoacid level.

The complete deduced aminoacid sequences of the G10 sequences obtained in this study were determined and aligned to those of G10 sequences retrieved from the GenBank database (data not shown). Direct inspection of sequence alignment showed that the reference strain B223 used in the bovine RVA vaccines in Argentina presented 8 cysteines, 11 prolines and 2 glycosylation sites (69 and 145). The Argentinean G10(VI) strains retained all the cysteines, 10 out of the 11 prolines, and the two glycosylation sites, except for the strains B3110 and B569 that only have one of them. Figure 2B shows the aminoacid differences between the bovine vaccine strain B223 and the Argentinean G10 strains belonging to lineage VI mapped in the VP7 trimer. The Argentinean strains belonging to lineage V presented 3 aminoacid changes respect the B223 strain ($97^{Glu\rightarrow Asp}$, $100^{Ser\rightarrow Asn}$ and $213^{Arg\rightarrow Asp}$), while the Argentinean strains belonging to lineage VI present 4 aminoacid changes ($100^{Ser\rightarrow Asn}$, $213^{Arg\rightarrow Gly}$, $242^{Ala\rightarrow Thr}$ and $221^{Ala\rightarrow Asn}$) in the antigenic regions with respect to the B223 vaccine strain (Fig. 2).

### *3.3. Phylogenetic analyses of genotype P[5].*

The P[5] genotype is characteristic for bovine RVA strains and is mainly associated with G6 and to a lesser extent with the G10 genotype. To date, only a single strain bearing the P[5] genotype has been detected in humans (RVA/human-wt/USA/CJN-M/1988/G1P[5]), and only two in pigs, one from China

(RVA/Pig-xx/CHN/4S/XXXX/G3P[5]) and one (RVA/pig-xx/THA/P343/G10P7[5]) from Thailand (Burke et al., 1994; Nakagomi et al., 1994; Pongsuwanna et al., 1996). A phylogenetic tree was generated based on the P[5] VP8* fragment of the VP4 gene using the sequences of 26 Argentinean P[5] strains and available P[5] sequences retrieved from the GenBank database. Sixteen Argentinean P[5] strains were from Buenos Aires province, and these strains were associated with G6(IV) (10/16), G6(III) (1/16) and G10 (5/16); the other P[5] Argentinean strains included in the tree were from Córdoba, La Pampa, Río Negro, Entre Ríos and Santa Fé and were associated with G6(IV). From the 26 P[5] Argentinean strains, 17 belong to beef herds, 1 belong to dairy herd and 8 were from an unknown exploitation type (Table 1).

From the topology of the dendrogram, eight lineages were defined (Fig. 4A). Lineage I and II were solely composed by bovine Argentinean RVA strains from different provinces and years. Lineage I included only G6(IV)P[5] Argentinean strains belonging to beef herds, while lineage II included G6P[5] and G10P[5] Argentinean strains belonging to both beef and dairy herds. The Argentinean strains within each lineage were closely related (95.7-100% identity for lineage I and 97.1-100% identity for lineage II). The genetic distance between lineages I and II was 4.2-6.4% (93.6-95.8% identity) (Fig. 4B). Lineage III was composed by a bovine and a porcine strain from Thailand and one bovine strain from UK. Lineage IV only possessed the Chinese strain RVA/Pig-xx/CHN/4S/XXXX/G3P[5]. Lineage V and VI included the reference strains UK and WC3 respectively. Lineage VII is composed by the RVA strain RVA/Cow-tc/USA/B641/XXXX/G6P7[5]. Finally, Lineage VIII is formed by 3 bovine strains from the UK, USA and Korea, one human strain from the USA and the strain WI 79-9 included in the human vaccine Rotateq®. The cut-off value for the P[5] genotype using this data set was 4%, but a slightly overlapping was observed (Fig. 4C). The construction of the phylogenetic trees based on the nearly full-length VP4 gene sequences from representative P[5] strains of each lineages showed the same lineages that using only the VP8* region (Supplementary Data 1).

For the P[5] data set, the best-fit nucleotide substitution model estimated by the Akaike Information Criterion was TIM1+G. The Argentinean strains formed the same groups in the both nucleotide trees after Maximum Likelihood and Bayesian analyses, but the others lineages were not exactly the same (data not shown).

The alignment using the deduced aminoacid sequences of Argentinean VP8* together with other sequences obtained from the GenBank database were carried out (data not shown). The UK reference strain G6P[5] present 18 conserved prolines, while the P[5] Argentinean strains present 17 prolines and 1 cysteine. In addition, the three potential trypsin cleavage sites (69, 237 and 318) were conserved. Three substitutions

were observed between the Argentinean P[5] Lineage I and II strains (244$^{Lys→Glu}$, 247$^{Glu→Lys}$, 276 $^{Lys→Arg}$). The Argentinean P[5] strains presented four aminoacid changes in the antigenic regions of VP8* (88$^{Arg→Glu,Gly}$, 113$^{Ale→Thr}$, 193$^{Phe→Ile}$ and 194$^{Ile→Val}$) and one in the VP5* antigenic sites (394 $^{Val→Ala}$) with respect to the vaccine strain UK. In the aminoacid tree not all the lineages were maintained and low values were obtained in the bootstrap analysis (data not shown).

### *3.4. Phylogenetic analyses of genotype P[11]*

Finally, to study genotype P[11], phylogenetic analyses using 25 sequences from Argentinean strains and sequences retrieved from the GenBank database were carried out. Sixteen of these 25 P[11] strains were from Buenos Aires province and were associated with G6 (n=8), G10 (n=7) and one G15 strain (RVA/cow-wt/ARG/B383_D_BA/1998/G15P[11]) obtained from a previous study (Matthijnssens et al., 2009). Five samples were from Neuquén, Córdoba and Entre Ríos provinces and were associated to G6 and G10 (Table 1). The study of P[11] VP8* revealed that this genotype presented a high diversity and the phylogenetic tree could be divided into six lineages (Supplementary Data 2). The Argentinean P[11] strains were distributed over 4 of the 6 proposed lineages. To confirm these results the nearly full-length of the VP4 was determine for 11 Argentinean strains and analyses with other available P[11] sequences, thus having representative strains from each new lineage. The lineages II to VI were maintained as using the VP8* region, however the strains from lineage I were scattered through the phylogenetic tree in three different groups while using nucleotide sequences for the analyses (Supplementary Data 3A). Interestingly, when using aminoacid for tree reconstruction, three lineages were detected with high bootstrap values (>96), either using the VP8* region or the nearly complete VP4 gene (Fig. 5A and Supplementary Data 3B). Analyses of the aminoacid distances between the three lineages reinforces the data (Fig. 5C), and a cut-off value of 0.094 could be used to differentiate different lineages of the P[11] type. The genetic distances within and between lineages are shown (Fig. 5B).

Direct inspection of the alignment of P[11] sequences showed that the reference strain B223 present 12 prolines, 1 cysteine and 2 potential trypsin cleavage sites. In contrast, the Argentinean strains present 13 prolines (lineage III 78$^{Ser→Pro}$, lineage IV 64$^{Thr→Pro}$), except for the strains B1625, B1190, B3309, B2451 and B3035. Apart from the proline at position 78, lineage III showed 3 aminoacid substitutions (123$^{Asn→Gly}$, 167$^{Arg→Lys}$, 211$^{Asp→Gly}$) (data not shown). The Argentinean P[11] strains were conserved with respect to the

vaccine strain in the VP8* antigenic sites (8-1, 8-2, 8-3, and 8-4) and in the VP5* antigenic sites (5-1, 5-2, 5-3, 5-4 and 5-5), except for one position in 384 $^{Glu\rightarrow Val,Ala,Thr}$.

**Discussion**

RVA diarrhea in calves causes important economic losses for farmers in terms of treatment cost and reduction of weight gain in affected animals (Bartels et al., 2010; Bendali et al., 1999). In Argentina, RVA diarrhea represents a permanent problem in dairy calves reared under artificial management and causes severe outbreaks in beef herds, every calving season (Badaracco et al., 2012; Bellinzoni et al., 1989; Garaicoechea et al., 2006). Disease control is based in vaccination of pregnant cows in the last stage of pregnancy with aqueous or oil adjuvanted vaccines including bovine RVA reference strains UK or IND (G6P[5]) and B223 (G10P[11]).

The most prevalent genotypes circulating in Argentinean livestock are G6 and G10 in combination with P[5] and P[11], with G6(IV)P[5] being the most prevalent combination in beef herds, while G6(III)P[11] followed by G10P[11] are the most frequently detected strains in dairy herds (Badaracco et al., 2012; Garaicoechea et al., 2006). Although the genotypes from circulating strains match the ones present in the vaccines, bovine diarrhea caused by RVA still represents a high economic burden to our country. We sought to investigate the VP7 and VP4 diversity of the predominant RVAs circulating in Argentinean cattle to understand the consequences of continuous circulation of strains despite of the high coverage of vaccination in the cattle population.

Analyses of VP7 data confirmed that the G6 genotype could be divided into 5 lineages, as previously reported by other authors (Banyai et al., 2003a; Banyai et al., 2003b; Garaicoechea et al., 2006; Martella et al., 2003). The Argentinean bovine RVA strains belonged to Lineage III and IV. Lineage IV is typical of bovine RVA strains, while Lineage III also includes human RVA strains. The fact that G6(III) strains are highly prevalent in Argentinean cattle and have been detected in humans warrants the continuous co-surveillance of human and animal samples. The five lineages observed at the nucleotide level were also obtained in the phylogenetic tree constructed with deduced aminoacid sequences, indicating that many of the mutations in the nucleotide sequences were translated in aminoacid changes. The aminoacid differences between G6(III) and G6(IV) strains includes 7 aminoacid substitutions in known antigenic regions. Moreover, the G6(III) Argentinean strains presented 2 glycosilation sites as opposed to the G6 vaccine strains presented 3 glycosilation sites, which can have a strong influence on the antigenic properties. These findings suggest that vaccine induced antibodies could have a lower cross-neutralizing activity against strains belonging to different lineages of G6. However, *in vitro* studies have shown cross-neutralization between RVA strains belonging to

lineage V (KN4) and lineage IV (NCDV Lincoln) (Matsuda et al., 1993). In addition, protection studies performed on gnotobiotic calves demonstrated that the immunity induced by a bovine RVA strain belonging to G6(V) conferred protection against challenge with a G6(IV) strain (Chang et al., 2000). The genetic distances between lineages V and IV (16.7-20.6%) and lineages III and IV (16.2-22%) are very similar suggesting that despite of the differences observed at the nucleotide and aminoacid level, from the antigenic point of view cross-neutralization and cross-protection should exist between the two G6 lineages circulating in Argentinean cattle. Cross-neutralization assays as well as cross-protection studies in calves using G6(III) and G6(IV) strains would be needed to confirm these hypotheses based on *in silico* analyses. Whole-genome studies are under progress to evaluate the complete relationship between the G6(III)P[11] and G6(IV)P[5] field strains and the vaccines strains.

G10 is the second most prevalent RVA genotype in cattle and possess a high potential to infect other animal species including buffaloes, horses, pigs and lambs. The detection of a buffalo G10P[3] RVA strain in India, with a P type closely related to strains isolated from dogs, monkeys and goats, strongly supports the ability of G10 RVA strains to jump the species barrier and reassort (Manuja et al., 2008). This genotype also posses a strong zoonotic transmission potential to humans as previously reported (Bhandari et al., 2006; Esona et al., 2011; Ramani et al., 2009; Volotao et al., 2006). Several G10P[11] bovine–human and G10P[8] human-bovine-porcine reassortant strains have been found in symptomatic and asymptomatic children from India and Hungary, respectively (Bhandari et al., 2006; Esona et al., 2011; Ramani et al., 2009). In the present study, the fact that the Argentinean bovines G10(VI) RVA strains clustered together with an unusual G10P[9] RVA strain detected in 2-year-old hospitalized child with severe diarrhea and dehydration from Rio de Janeiro, during the seasons 2000-2004 (Volotao et al., 2006), reinforces the significant zoonotic potential of G10 RVA strains. As observed for the RVA strains belonging to the G6(III) genotype, several G10 lineages have zoonotic potential, for this reason studies of fecal samples from domestic animals and children from rural areas, could be interesting to evaluate the transmission and infection rate of these bovine RVA genotypes in human infants of our country.

Regarding vaccine efficacy in cattle it is important to remark that most of the Argentinean G10 strains grouped in a different lineage from the vaccine strain B223 (Lineage V). The nucleotide distance between these two lineages was relatively high (13.2-16.5%) and the aminoacid distances between these two lineages was 3.1-10.8%; however, the tree constructed with the deduced aminoacid sequences showed three lineages supported only by low bootstrap values. These results, together with the direct inspection of the alignments

showed that the mutations observed at the nucleotide level were translated only in 4 aminoacid changes within the antigenic sites (Fig. 2). Thus, although different lineages can be determined using nucleotide data, at the protein level the G10 strains seems to remain rather similar. The low level of amino acid mutations in G10 strains contrast with the amino acid diversity of G6 strains, which could be due to structural constraints presented by the VP7 protein from G10 strains while the one from G6 strains could allow more plasticity.

Lineages have previously been determined for several human P types, such as P[4], P[6] and P[8] (Espinola et al., 2008; Iturriza-Gomara et al., 2000; Martella et al., 2006); however, to our knowledge, these type of analyses have not been described for the bovine genotypes P[5] and P[11]. The P[5] genotype is characteristic of RVA strains affecting cattle and is broadly distributed all over the world (Alfieri et al., 2004; Alkan et al., 2010; Badaracco et al., 2012; Barreiros et al., 2004; Cashman et al., 2010; de Verdier Klingenberg and Svensson, 1998; Fukai et al., 1999; Garaicoechea et al., 2006; Martella et al., 2003; Monini et al., 2008; Parwani et al., 1993; Ramos Caruzo et al., 2010). The strong association of the P[5] genotype with G6(IV) is remarkable. Thus, out of the 12 of P[5] described in the literature, only three RVA carrying P[5] genotype were detected either in other species or associated to other G genotypes: the strains P343 (G10P[5]) and 4S (G3P[5]) were detected in pigs from Thailand and China, respectively; and the human strain CJN-M (G1P[5]) was detected in United States (Nakagomi et al., 1994).

The phylogenetic analyses using different approaches indicated that the P[5] genotype can be divided into eight lineages. Although, lineages I and II were only composed of Argentinean strains that were rather similar among each other (4.2-6.4%), and the other six lineages contained more divergent strains. The addition of more sequences to the analysis will allow a better characterization of the lineages described for this genotype using genetic distances. The phylogenetic analysis using representative strains of each lineages with nearly full length VP4 sequences showed the same number of lineages as when using the VP8* region; however, deep relationships observed among the strains were different in both trees. It is important to highlight that, at the aminoacid level, the Argentinean strains were distantly related to the UK strain included in the vaccine formulations used to prevent calf diarrhea in Argentina (Lineage I vs Lineage V, 3.2-6.9%; Lineage II vs Lineage V, 3.6-7.2% amino acid genetic distance).

Phylogenetic analyses of P[11] VP8* revealed that this type can be divided into six lineages at the nucleotide level; however, posterior analyses using the nearly full length VP4 gene fail to confirm these results. Importantly, analyses using aminoacid sequences confirmed the presence of three phylogenetic lineages among the P[11] strains. All Argentinean strains clustered within lineage III with other strains

detected worldwide, with no association between lineages and G type, exploitation type, year or location. Genotype P[11] has been found in our surveys and elsewhere in combination with multiple G types (G6, G8, G9 and G15) (Badaracco et al., 2012; Garaicoechea et al., 2006; Ghosh et al., 2008; Matthijnssens et al., 2009). Interestingly, P[11](III) strains have been detected in human samples (Bhandari et al., 2006; Ramani et al., 2009), which highlights the potential interspecies transmission of strains bearing this P type

In contrast to other countries that showed little diversity (Cashman et al., 2010), Argentinean bovine strains showed a large genetic diversity and multiple new clusters tightly related among them and distant from the bovine RVA circulating all over the world. These clusters included strains circulating for over 12 years in the Argentinean cattle. Overall, our results could be explained by the different cattle breeds present in Argentina, the two different husbandry system studies, the difference among the ecological areas sampled and the virus biology (FAO, 2005).

Overall, the present phylogenetic study describe the variability within the prevalent genotypes of the bovine RVA strains circulating in Argentinean cattle, studied their relationship with vaccine and other strains circulating worldwide and set the need of further studies to evaluate their zoonotic potential.

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**Conflict of interest statement**

None.

**Figure legends**

**Fig. 1**. Bioinformatic analyses of G6 genotype. (A) The phylogenetic tree was constructed using the Kimura 2-parameter and Neighbor-Joining methods using the program MEGA5. Bootstrap values are shown at the branch nodes. The lineages are indicated at the right hand side. The Argentinean strains isolated are shown in grey boxes and the vaccines strains are indicated with open squares. In the Argentinean strains: Name of the strain, type of exploitation and province of collection are shown. Abbreviations D/B: Dairy or Beef, BA: Buenos Aires, SF: Santa Fe, Co: Cordoba, RN; Rio Negro, ER: Entre Rios, LP: La Pampa, ♦: G6P[11], ●: G6P[5], ○: G6Px. (B) The average genetic distance and the genetic distance range of the intra (in bold) and inter lineages distances are shown. (C) The pairwise distances frequency graphs were constructed by plotting the percentage of the nucleotide distances in the abscissa (x axis) and the frequency of the pairwise distances in the ordinate (y axis). The proposed nucleotide cut-off is shown by a dotted line in the nucleotide frequency graph. The closed squares are the genetic distances intralineages and the open squares are the genetic distances interlineages.

**Fig. 2.** Changes observed in the antigenic sites of the Argentinean G6 and G10 RVA strains as compared to the references strains WC3 and B223. The VP7 crystal structure obtained for the RRV strain (Aoki et al., 2009) and the PyMol program were used to map the aminoacid substitutions.

**Fig. 3**. Bioinformatic analyses of G10 genotype. (A) The nucleotide tree was constructed using the Kimura 2-parameter and Neighbor-Joining methods using the program MEGA5. Bootstrap values are shown at the branch nodes. The lineages are indicated at the right hand side. The Argentinean strains isolated are shown in grey boxes and the vaccines strains are indicated with open squares. In the Argentinean strains: Name of the strain, type of exploitation and province of collection are shown. Abbreviations D/B: Dairy or Beef, BA: Buenos Aires, SL: San Luis, Ne: Neuquén, ■: G10P[11], ▲: G10P[5]. (B) The average genetic distance and the genetic distance range of the intra (in bold) and inter lineages distances are shown. (C) The pairwise distances frequency graphs were constructed by plotting the percentage of the nucleotide distances in the abscissa (*x-axis*) and the frequency of the pairwise distances in the ordinate (y-axis). The proposed nucleotide cut-off is shown by a dotted line in the nucleotide frequency graph. The closed squares are the genetic distances intralineages and the open squares are the genetic distances interlineages.

**Fig. 4**. Bioinformatic analyses of the P[5] genotype. (A) The nucleotide tree was constructed using the Kimura 2-parameter and Neighbor-Joining methods using the program MEGA5. Bootstrap values are shown at the branch nodes. The lineages are indicated at the right hand side. The Argentinean strains isolated are shown in grey boxes and the vaccines strains are indicated with open squares. In the Argentinean strains: Name of the strain, type of exploitation and province of collection are shown. Abbreviations D/B: Dairy or Beef, BA: Buenos Aires, SF: Santa Fe, Co: Cordoba, RN; Rio Negro, ER: Entre Rios, LP: La Pampa, ●: G6P[5], ▲: G10P[5]. (B) The average genetic distance and the genetic distance range of the intra (in bold) and inter lineages distances are shown. (C) The pairwise distances frequency graphs were constructed by plotting the percentage of the nucleotide distances in the abscissa (x axis) and the frequency of the pairwise distances in the ordinate (y axis). The proposed nucleotide cut-off is shown by a dotted line in the nucleotide frequency graph. The closed squares are the genetic distances intralineages and the open squares are the genetic distances interlineages.

**Fig. 5.** Bioinformatic analyses of the P[11] genotype. (A) The phylogenetic tree was constructed using the Poisson model and Neighbor-Joining methods using the program MEGA5. Bootstrap values are shown at the branch nodes. The lineages are indicated at the right hand side. The Argentinean strains isolated are shown in grey boxes and the vaccines strains are indicated with open squares. In the Argentinean strains: Name of the strain, type of exploitation and province of collection are shown. Abbreviations D/B: Dairy or Beef, BA: Buenos Aires, Co: Cordoba, ER: Entre Rios, Ne: Neuquén, ♦: G6P[11], ■: G10P[11], ▼ G15P[11] (B) The average genetic distance and the genetic distance range of the intra (in bold) and inter lineages distances are shown. (C). The pairwise distances frequency graphs were constructed by plotting the percentage of the aminoacid distances in the abscissa (*x-axis*) and the frequency of the pairwise distances in the ordinate (y-axis). The proposed nucleotide cut-off is shown by a dotted line in the aminoacid frequency graph. The closed squares are the distances intralineages and the open squares are the distances interlineages.

**Supplementary Data 1.** Phylogenetic tree from the P[5] genotype using the nearly full length VP4 gene. The tree was constructed using the Kimura 2-parameter and Neighbor-Joining methods using the program MEGA5. Bootstrap values are shown at the branch nodes. The lineages are indicated at the right hand side. In the Argentinean strains: The Argentinean strains isolated are shown in grey boxes and the vaccines strains are indicated with open squares. Name of the strain, type of exploitation and province of collection are shown.

Abbreviations D/B: Dairy or Beef, BA: Buenos Aires Co: Cordoba, RN; Rio Negro, ER: Entre Rios, ●: G6P[5], ▲: G10P[5].

**Supplementary Data 2.** Phylogenetic tree from the P[11] genotype using the VP8* region. Tree was constructed using the Kimura 2-paramether and Neighbor-Joining methods using the program MEGA5. Bootstrap values are shown at the branch nodes. The lineages are indicated at the right hand side. The Argentinean strains isolated are shown in grey boxes and the vaccines strains are indicated with open squares. In the Argentinean strains: Name of the strain, type of exploitation and province of collection are shown. Abbreviations D/B: Dairy or Beef, BA: Buenos Aires, Co: Cordoba, ER: Entre Rios, Ne: Neuquén, ♦: G6P[11], ■: G10P[11], ▼ G15P[11].

**Supplementary Data 3.** (A) Phylogenetic tree from the P[11] genotype using the nucleotide nearly full length VP4 gene. Tree was constructed using the Kimura 2-paramether and Neighbor-Joining methods using the program MEGA5. (B) Phylogenetic tree from the P[11] genotype using the deduced aminoacid nearly full length VP4 sequences constructed using the Poisson model and Neighbor-Joining methods using the program MEGA5. Bootstrap values are shown at the branch nodes. The lineages are indicated at the right hand side. The Argentinean strains isolated are shown in grey boxes and the vaccine strains are indicated with open squares. In the Argentinean strains: Name of the strain, type of exploitation and province of collection are shown. Abbreviations D/B: Dairy or Beef, BA: Buenos Aires, Co: Cordoba, ER: Entre Rios, Ne: Neuquén, ♦: G6P[11], ■: G10P[11], ▼ G15P[11].

**Bibliography**

Abe, M., Ito, N., Masatani, T., Nakagawa, K., Yamaoka, S., Kanamaru, Y., Suzuki, H., Shibano, K., Arashi, Y., Sugiyama, M., 2011, Whole genome characterization of new bovine rotavirus G21P[29] and G24P[33] strains provides evidence for interspecies transmission. J Gen Virol 92, 952-960.

Abe, M., Ito, N., Morikawa, S., Takasu, M., Murase, T., Kawashima, T., Kawai, Y., Kohara, J., Sugiyama, M., 2009, Molecular epidemiology of rotaviruses among healthy calves in Japan: isolation of a novel bovine rotavirus bearing new P and G genotypes. Virus Res 144, 250-257.

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.

Alkan, F., Ozkul, A., Oguzoglu, T.C., Timurkan, M.O., Caliskan, E., Martella, V., Burgu, I., 2010, Distribution of G (VP7) and P (VP4) genotypes of group A bovine rotaviruses from Turkish calves with diarrhea, 1997-2008. Vet Microbiol 141, 231-237.

Aoki, S.T., Settembre, E.C., Trask, S.D., Greenberg, H.B., Harrison, S.C., Dormitzer, P.R., 2009, Structure of rotavirus outer-layer protein VP7 bound with a neutralizing Fab. Science (New York, N.Y 324, 1444-1447.

Badaracco, A., Garaicoechea, L., Rodriguez, D., Uriarte, E.L., Odeon, A., Bilbao, G., Galarza, R., Abdala, A., Fernandez, F., Parreno, V., 2012, Bovine rotavirus strains circulating in beef and dairy herds in Argentina from 2004 to 2010. Vet Microbiol 158, 394-399.

Banyai, K., Gentsch, J.R., Glass, R.I., Szucs, G., 2003a, Detection of human rotavirus serotype G6 in Hungary. Epidemiol Infect 130, 107-112.

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

Barreiros, M.A., Alfieri, A.F., Medici, K.C., Leite, J.P., Alfieri, A.A., 2004, G and P genotypes of group A rotavirus from diarrhoeic calves born to cows vaccinated against the NCDV (P[1],G6) rotavirus strain. J Vet Med B Infect Dis Vet Public Health 51, 104-109.

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.

Bellinzoni, R.C., Blackhall, J.O., Mattion, N.M., Estes, M.K., Snodgrass, D.R., LaTorre, J.L., Scodeller, E.A., 1989, Serological characterization of bovine rotaviruses isolated from dairy and beef herds in Argentina. J Clin Microbiol 27, 2619-2623.

Bendali, F., Sanaa, M., Bichet, H., Schelcher, F., 1999, Risk factors associated with diarrhoea in newborn calves. Vet Res 30, 509-522.

Bhandari, N., Sharma, P., Glass, R.I., Ray, P., Greenberg, H., Taneja, S., Saksena, M., Rao, C.D., Gentsch, J.R., Parashar, U., Maldonado, Y., Ward, R.L., Bhan, M.K., 2006, Safety and immunogenicity of two live attenuated human rotavirus vaccine candidates, 116E and I321, in infants: results of a randomised controlled trial. Vaccine 24, 5817-5823.

Burke, B., McCrae, M.A., Desselberger, U., 1994, Sequence analysis of two porcine rotaviruses differing in growth in vitro and in pathogenicity: distinct VP4 sequences and conservation of NS53, VP6 and VP7 genes. J Gen Virol 75 ( Pt 9), 2205-2212.

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.

Chang, K.O., Parwani, A.V., Saif, L.J., 2000, Comparative sequence analysis of the VP7 genes of G6, G8 and G10 bovine group A rotarviruses and further characterization of G6 subtypes. Arch Virol 145, 725-737.

Cornaglia, E.M., Barrandeguy, 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 Lat Microbiol 31, 4.

de Verdier Klingenberg, K., Svensson, L., 1998, Group A rotavirus as a cause of neonatal calf enteritis in Sweden. Acta Vet Scand 39, 195-199.

Esona, M.D., Banyai, K., Foytich, K., Freeman, M., Mijatovic-Rustempasic, S., Hull, J., Kerin, T., Steele, A.D., Armah, G.E., Geyer, A., Page, N., Agbaya, V.A., Forbi, J.C., Aminu, M., Gautam, R., Seheri, L.M., Nyangao, J., Glass, R., Bowen, M.D., Gentsch, J.R., 2011, Genomic characterization of human

rotavirus G10 strains from the African Rotavirus Network: relationship to animal rotaviruses. Infect Genet Evol 11, 237-241.

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, S212-219.

Espinola, E.E., Parra, G.I., Russomando, G., Arbiza, J., 2008, Genetic diversity of the VP4 and VP7 genes affects the genotyping of rotaviruses: analysis of Paraguayan strains. Infect Genet Evol 8, 94-99.

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.

Falcone, E., Tarantino, M., Di Trani, L., Cordioli, P., Lavazza, A., Tollis, M., 1999, Determination of bovine rotavirus G and P serotypes in italy by PCR. J Clin Microbiol 37, 3879-3882.

FAO 2005. Livestock Sector Brief Argentina. In Livestock Sector Brief Argentina.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. In Evolution, pp. 783-791.

Fernandez, F.M., Conner, M.E., Hodgins, D.C., Parwani, A.V., Nielsen, P.R., Crawford, S.E., Estes, M.K., Saif, L.J., 1998, Passive immunity to bovine rotavirus in newborn calves fed colostrum supplements from cows immunized with recombinant SA11 rotavirus core-like particle (CLP) or virus-like particle (VLP) vaccines. Vaccine 16, 507-516.

Fukai, K., Maeda, Y., Fujimoto, K., Itou, T., Sakai, T., 2002, Changes in the prevalence of rotavirus G and P types in diarrheic calves from the Kagoshima prefecture in Japan. Vet Microbiol 86, 343-349.

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

Fukai, K., Saito, T., Inoue, K., Sato, M., 2004b, Molecular characterization of novel P[14],G8 bovine group A rotavirus, Sun9, isolated in Japan. Virus Res 105, 101-106.

Fukai, K., Sakai, T., Hirose, M., Itou, T., 1999, Prevalence of calf diarrhea caused by bovine group A rotavirus carrying G serotype 8 specificity. Vet Microbiol 66, 301-311.

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.

Ghosh, S., Samajdar, S., Sinha, M., Kobayashi, N., Taniguchi, K., Naik, T.N., 2008, Molecular characterization of rare bovine group A rotavirus G15P[11] and G15P[21] strains from eastern India: identification of simian SA11-like VP6 genes in G15P[21] strains. Virus genes 37, 241-249.

Guindon, S., Gascuel, O., 2003, A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52, 696-704.

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

Huelsenbeck, J.P., Bollback, J.P., 2001, Empirical and hierarchical Bayesian estimation of ancestral states. Syst Biol 50, 351-366.

Huelsenbeck, J.P., Ronquist, F., 2001, MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754-755.

Huelsenbeck, J.P., Ronquist, F., Nielsen, R., Bollback, J.P., 2001, Bayesian inference of phylogeny and its impact on evolutionary biology. Science (New York, N.Y 294, 2310-2314.

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., Green, J., Brown, D.W., Desselberger, U., Gray, J.J., 2000, Diversity within the VP4 gene of rotavirus P[8] strains: implications for reverse transcription-PCR genotyping. J Clin Microbiol 38, 898-901.

Kimura, M., 1980, A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16, 111-120.

Makoschey, B., Klee, W., Martella, V., Bridger, J., Smiths, D.G., Daugschies, A., Millemann, Y., Liebler-Tenorio, E., Snodgrass, D., Claerebout, E., Bendali, F., van de Ven, J., Garcia, A., Illek, J., Kaske, M., Cutler, K., Gonzalez-Martin, J.V., Carvalho, L.M., Crouch, C., Thiry, E., 2009, Neonatal health in calves--comprehensive solutions for complex enteric disorders. Berl Munch Tierarztl Wochenschr 122, 398-408.

Manuja, B.K., Prasad, M., Manuja, A., Gulati, B.R., Prasad, G., 2008, A novel genomic constellation (G10P[3]) of group A rotavirus detected from buffalo calves in northern India. Virus Res 138, 36-42.

Martella, V., Banyai, K., Ciarlet, M., Iturriza-Gomara, M., Lorusso, E., De Grazia, S., Arista, S., Decaro, N., Elia, G., Cavalli, A., Corrente, M., Lavazza, A., Baselga, R., Buonavoglia, C., 2006, Relationships

among porcine and human P[6] rotaviruses: evidence that the different human P[6] lineages have originated from multiple interspecies transmission events. Virology 344, 509-519.

Martella, V., Banyai, K., Matthijnssens, J., Buonavoglia, C., Ciarlet, M., 2010, Zoonotic aspects of rotaviruses. Vet Microbiol 140, 246-255.

Martella, V., Ciarlet, M., Pratelli, A., Arista, S., Terio, V., Elia, G., Cavalli, A., Gentile, M., Decaro, N., Greco, G., Cafiero, M.A., Tempesta, M., Buonavoglia, C., 2003, Molecular analysis of the VP7, VP4, VP6, NSP4, and NSP5/6 genes of a buffalo rotavirus strain: identification of the rare P[3] rhesus rotavirus-like VP4 gene allele. J Clin Microbiol 41, 5665-5675.

Matsuda, Y., Isegawa, Y., Woode, G.N., Zheng, S., Kaga, E., Nakagomi, T., Ueda, S., Nakagomi, O., 1993, Two-way cross-neutralization mediated by a shared P (VP4) serotype between bovine rotavirus strains with distinct G (VP7) serotypes. J Clin Microbiol 31, 354-358.

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.

Matthijnssens, J., Potgieter, C.A., Ciarlet, M., Parreno, V., Martella, V., Banyai, K., Garaicoechea, L., Palombo, E.A., Novo, L., Zeller, M., Arista, S., Gerna, G., Rahman, M., Van Ranst, M., 2009, Are human P[14] rotavirus strains the result of interspecies transmissions from sheep or other ungulates that belong to the mammalian order Artiodactyla? J Virol 83, 2917-2929.

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.

Nakagomi, O., Isegawa, Y., Ward, R.L., Knowlton, D.R., Kaga, E., Nakagomi, T., Ueda, S., 1994, Naturally occurring dual infection with human and bovine rotaviruses as suggested by the recovery of G1P8 and G1P5 rotaviruses from a single patient. Arch Virol 137, 381-388.

Okada, N., Matsumoto, Y., 2002, Bovine rotavirus G and P types and sequence analysis of the VP7 gene of two G8 bovine rotaviruses from Japan. Vet Microbiol 84, 297-305.

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.

Parreño, V., Bejar, C., Vagnozzi, A., Barrandeguy, M., Costantini, V., Craig, M.I., Yuan, L., Hodgins, D., Saif, L., Fernandez, F., 2004, Modulation by colostrum-acquired maternal antibodies of systemic and mucosal antibody responses to rotavirus in calves experimentally challenged with bovine rotavirus. Vet Immunol Immunopathol 100, 7-24.

Parwani, A.V., Hussein, H.A., Rosen, B.I., Lucchelli, A., Navarro, L., Saif, L.J., 1993, Characterization of field strains of group A bovine rotaviruses by using polymerase chain reaction-generated G and P type-specific cDNA probes. J Clin Microbiol 31, 2010-2015.

Pongsuwanna, Y., Taniguchi, K., Chiwakul, M., Urasawa, T., Wakasugi, F., Jayavasu, C., Urasawa, S., 1996, Serological and genomic characterization of porcine rotaviruses in Thailand: detection of a G10 porcine rotavirus. J Clin Microbiol 34, 1050-1057.

Posada, D., 2008, jModelTest: Phylogenetic Model Averaging. Mol Biol Evol 25, 1253-1256.

Rahman, M., De Leener, K., Goegebuer, T., Wollants, E., Van der Donck, I., Van Hoovels, L., Van Ranst, M., 2003, Genetic characterization of a novel, naturally occurring recombinant human G6P[6] rotavirus. J Clin Microbiol 41, 2088-2095.

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.

Ramos Caruzo, T.A., Diederichsen de Brito, W., Elsner, M., Munford, V., and, Rácz, M.L., 2010, Molecular characterization of G and P-types bovine rotavirus strains from Goiás, Brazil: high frequency of mixed P-type infections. Mem Inst Oswaldo Cruz, Rio de Janeiro. 105, 1040-1043.

Rao, C.D., Gowda, K., Reddy, B.S., 2000, Sequence analysis of VP4 and VP7 genes of nontypeable strains identifies a new pair of outer capsid proteins representing novel P and G genotypes in bovine rotaviruses. Virology 276, 104-113.

Ronquist, F., Huelsenbeck, J.P., 2003, MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572-1574.

Saif, L.J., Weilnau, P., Miller, K., Stitzlein, L., 1987, Isotypes of intestinal and systemic antibodies in colostrum-fed and colostrum-deprived calves challenged with rotavirus. Adv Exp Med Biol 216B, 1815-1823.

Saif, R.B., Parwani A, 1994, Animal rotaviruses, In: AZ, K. (Ed.) Virus infections of the gastrointestinal tract. Marcel-Dekker, New York, pp. 279-367.

Saitou, N., Nei, M., 1987, The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4, 406-425.

Schmidt, H.A., Strimmer, K., Vingron, M., von Haeseler, A., 2002, TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. Bioinformatics 18, 502-504.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011, MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28, 2731-2739.

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.

Trojnar, E., Sachsenroder, J., Twardziok, S., Reetz, J., Otto, P.H., Johne, R., 2012, Identification of an avian group A rotavirus containing a novel VP4 gene of close relationship to those of mammalian rotaviruses. J Gen Virol.

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.

Highlights

- We describe the RVA diversity among Argentinean cattle during a period of 13 years.
- Argentinean bovine RVA strains were distributed in phylogenetic trees across multiple lineages.
- Most of the Argentinean bovine strains RVA grouped in a distinct lineage from the vaccine strains.
- Bovine RVA strains grouped together with human strains, highlighting their potential for zoonotic transmission.

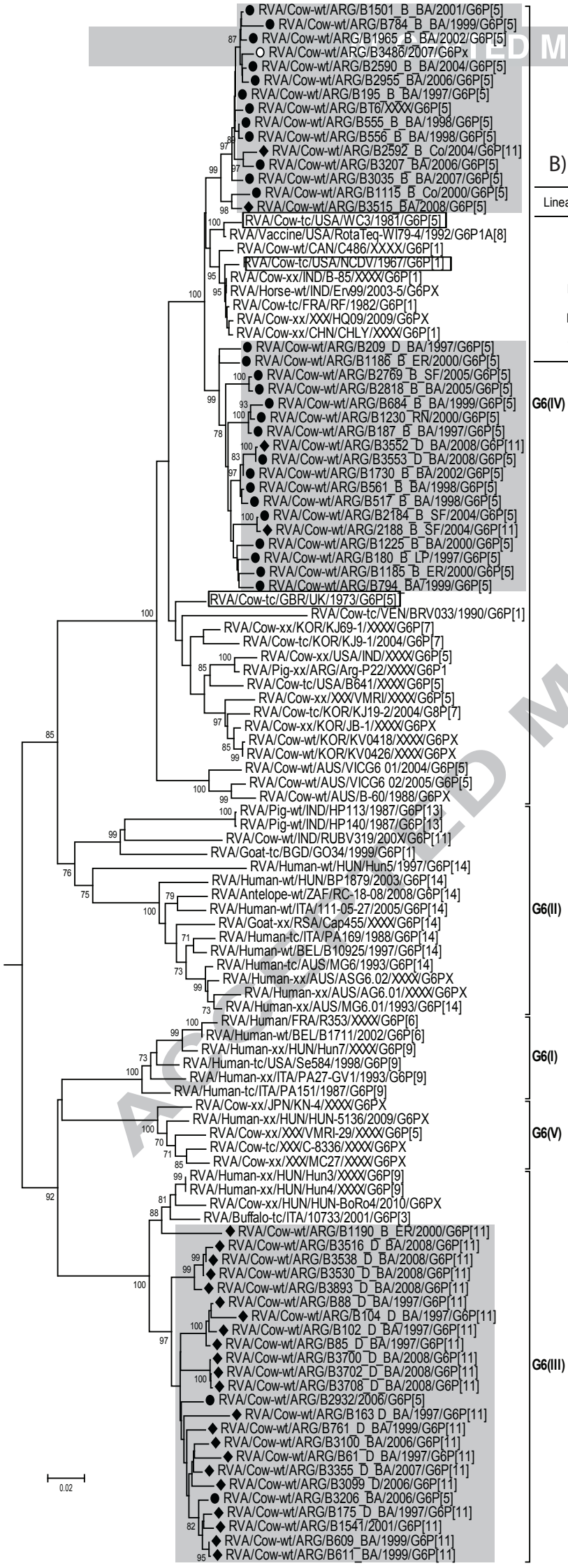
Table 1: Samples used in this study, Name, year of collection, G and P type, Province of collection and exploitation type.

| Name | Year | G-type (Lineage) | P-type (Lineage) | Province | Explotation type |
|---|---|---|---|---|---|
| RVA/Cow-wt/ARG/B61_D_BA/1997/G6P[11] | 1997 | 6(III) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B85_D_BA/1997/G6P[11] | 1997 | 6(III) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B88_D_BA/1997/G6P[11] | 1997 | 6(III) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B102_D_BA/1997/G6P[11] | 1997 | 6(III) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B104_D_BA/1997/G6P[11] | 1997 | 6(III) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B163_D_BA/1997/G6P[11] | 1997 | 6(III) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B175_D_BA/1997/G6P[11] | 1997 | 6(III) | 11(IV) | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B180_B_LP/1997/G6P[5] | 1997 | 6(IV) | 5(II) | La Pampa | Beef |
| RVA/Cow-wt/ARG/B181_B_BA/1997/G10P[11] | 1997 | 10* | 11(VI) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B187_B_BA/1997/G6P[5] | 1997 | 6(IV) | 5* | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B195_B_BA/1997/G6P[5] | 1997 | 6(IV) | 5(I) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B209_D_BA/1997/G6P[5] | 1997 | 6(IV) | 5* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B383_D_BA/1998/G15P[11] | 1998 | 15 | 11(IV) | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B517_B_BA/1998/G6P[5] | 1998 | 6(IV) | 5(II) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B555_B_BA/1998/G6P[5] | 1998 | 6(IV) | 5* | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B556_B_BA/1998/G6P[5] | 1998 | 6(IV) | 5* | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B561_B_BA/1998/G6P[5] | 1998 | 6(IV) | 5* | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B569_SL/1998/G10P[11] | 1998 | 10(VI) | 11* | San Luis | ND |
| RVA/Cow-wt/ARG/B609_BA/1999/G6P[11] | 1999 | 6(III) | 11* | Buenos Aires | ND |
| RVA/Cow-wt/ARG/B611_BA/1999/G6P[11] | 1999 | 6(III) | 11(IV) | Buenos Aires | ND |
| RVA/Cow-wt/ARG/B684_B_BA/1999/G6P[5] | 1999 | 6(IV) | 5* | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B761_D_BA/1999/G6P[11] | 1999 | 6(III) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B784_B_BA/1999/G6P[5] | 1999 | 6(IV) | 5* | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/790_BA/1999/G10P[5] | 1999 | 10(VI) | 5(II) | Buenos Aires | ND |
| RVA/Cow-wt/ARG/791_BA/1999/G10P[5] | 1999 | 10(VI) | 5(II) | Buenos Aires | ND |
| RVA/Cow-wt/ARG/792_BA/1999/G10P[5] | 1999 | 10(VI) | 5(II) | Buenos Aires | ND |
| RVA/Cow-wt/ARG/793_BA/1999/G10P[5] | 1999 | 10(VI) | 5(II) | Buenos Aires | ND |
| RVA/Cow-wt/ARG/B794_BA/1999/G6P[5] | 1999 | 6(IV) | 5* | Buenos Aires | ND |
| RVA/Cow-wt/ARG/B864_B_LP/1999/G6P[5] | 1999 | 6(IV)* | 5(I) | La Pampa | Beef |
| RVA/Cow-wt/ARG/B996_B_Co/2000/G10P[11] | 2000 | 10* | 11(V) | Córdoba | Beef |
| RVA/Cow-wt/ARG/B1036_B_Cordoba/2000/G10P[11] | 2000 | 10* | 11(IV) | Córdoba | Beef |
| RVA/Cow-wt/ARG/B1068_B_BA/2000/G10P[11] | 2000 | 10* | 11(VI) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B1115_B_Co/2000/G6P[5] | 2000 | 6(IV) | 5(I) | Córdoba | Beef |
| RVA/Cow-wt/ARG/B1185_B_ER/2000/G6P[5] | 2000 | 6(IV) | 5* | Entre Ríos | Beef |
| RVA/Cow-wt/ARG/B1186_B_ER/2000/G6P[5] | 2000 | 6(IV) | 5(I) | Entre Ríos | Beef |
| RVA/Cow-wt/ARG/B1190_B_ER/2000/G6P[11] | 2000 | 6(III) | 11(I) | Entre Ríos | Beef |
| RVA/Cow-wt/ARG/B1191_B_BA/2000/G10P[11] | 2000 | 10(VI) | 11(VI) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B1225_B_BA/2000/G6P[5] | 2000 | 6(IV) | 5* | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B1230_RN/2000/G6P[5] | 2000 | 6(IV) | 5(II) | Río Negro | ND |
| RVA/Cow-wt/ARG/B1238_B_BA/2000/G6P[11] | 2000 | 6(IV)* | 11(V) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/BT6/XXXX/G6P[5] | 2000 | 6(IV) | 5* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B1410_D_BA/2001/G6P[11] | 2001 | 6* | 11(VI) | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B1501_B_BA/2001/G6P[5] | 2001 | 6(IV) | 5(I) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B1541/2001/G6P[11] | 2001 | 6(III) | 11(IV) | ND | ND |
| RVA/Cow-wt/ARG/B1595_Ne/2001/G10P[11] | 2001 | 10(VI) | 11(V) | Neuquén | ND |
| RVA/Cow-wt/ARG/B1614_B_Co/2001/G6P[5] | 2001 | 6(IV)* | 5(I) | Córdoba | Beef |
| RVA/Cow-wt/ARG/B1625_B_BA/2001/G10P[11] | 2001 | 10* | 11(I) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B1652/2001/G6P[11] | 2001 | 6* | 11(VI) | ND | ND |
| RVA/Cow-wt/ARG/B1730_B_BA/2002/G6P[5] | 2002 | 6(IV) | 5(I) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B1813_B_Co/2002/G6P[5] | 2002 | 6(IV)* | 5(II) | Córdoba | Beef |
| RVA/Cow-wt/ARG/B1965_B_BA/2002/G6P[5] | 2002 | 6(IV) | 5(II) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B1988_BA/2002/G6P[11] | 2002 | 6(IV)* | 11(V) | Buenos Aires | ND |
| RVA/Cow-wt/ARG/B2376_D_BA/2003/G10P[5] | 2003 | 10(VI) | 5(II) | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B2184_B_SF/2004/G6P[5] | 2004 | 6(IV) | 5(I) | Santa Fe | Beef |
| RVA/Cow-wt/ARG/2188_B_SF/2004/G6P[11] | 2004 | 6(IV) | 11* | Santa Fe | Beef |
| RVA/Cow-wt/ARG/B2590_B_BA/2004/G6P[5] | 2004 | 6(IV) | 5(II) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B2592_B_Co/2004/G6P[11] | 2004 | 6(IV) | 11(VI) | Córdoba | Beef |
| RVA/Cow-wt/ARG/B2602_B_BA/2004/G6P[11] | 2004 | 6(III)* | 11(VI) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B2659_B_BA/2004/G10P[11] | 2004 | 10(VI) | 11(VI) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B2449_BA/2005/G6P[5] | 2005 | 6(III)* | 5(II) | Buenos Aires | ND |
| RVA/Cow-wt/ARG/B2451_BA/2005/G6P[11] | 2005 | 6(III)* | 11(VI) | Buenos Aires | ND |
| RVA/Cow-wt/ARG/B2769_B_SF/2005/G6P[5] | 2005 | 6(IV) | 5(I) | Santa Fe | Beef |
| RVA/Cow-wt/ARG/B2818_B_BA/2005/G6P[5] | 2005 | 6(IV) | 5(I) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B2932/2006/G6P[5] | 2006 | 6(III) | 5(I) | ND | ND |
| RVA/Cow-wt/ARG/B2934/2006/G10P[11] | 2006 | 10(VI) | 11(VI) | ND | ND |
| RVA/Cow-wt/ARG/B2955_BA/2006/G6P[5] | 2006 | 6(IV) | 5(I) | Buenos Aires | ND |
| RVA/Cow-wt/ARG/B3099_D/2006/G6P[11] | 2006 | 6(III) | 11* | ND | Dairy |
| RVA/Cow-wt/ARG/B3100_BA/2006/G6P[11] | 2006 | 6(III) | 11* | Buenos Aires | ND |
| RVA/Cow-wt/ARG/B3110_D_BA/2006/G10P[11] | 2006 | 10(VI) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B3206_BA/2006/G6P[5] | 2006 | 6(III) | 5* | Buenos Aires | ND |
| RVA/Cow-wt/ARG/B3207_BA/2006/G6P[5] | 2006 | 6(IV) | 5* | Buenos Aires | ND |

Table 1: continuation

| | | | | | |
|---|---|---|---|---|---|
| RVA/Cow-wt/ARG/B3033_B_BA/2007/G6P[5] | 2007 | 6(IV)* | 5(I) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B3035_B_BA/2007/G6P[5] | 2007 | 6(IV) | 5(I) | Buenos Aires | Beef |
| RVA/Cow-wt/ARG/B3045_D_BA/2007/G10P[11] | 2007 | 10* | 11(VI) | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B3309/2007/G10P[11] | 2007 | 10* | 11(VI) | ND | ND |
| RVA/Cow-wt/ARG/B3326_D_BA/2007/G10P[11] | 2007 | 10(V) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B3355_D_BA/2007/G6P[11] | 2007 | 6(III) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B3434/2007/G10P[11] | 2007 | 10(VI) | 11* | ND | ND |
| RVA/Cow-wt/ARG/B3486/2007/G6Px | 2007 | 6(IV) | ND | ND | ND |
| RVA/Cow-wt/ARG/B3515_BA/2008/G6P[5] | 2008 | 6(IV) | 5* | Santa Fe | ND |
| RVA/Cow-wt/ARG/B3516_D_BA/2008/G6P[11] | 2008 | 6(III) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B3530_D_BA/2008/G6P[11] | 2008 | 6(III) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B3538_D_BA/2008/G6P[11] | 2008 | 6(III) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B3552_D_BA/2008/G6P[11] | 2008 | 6(IV) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B3553_D_BA/2008/G6P[5] | 2008 | 6(IV) | 5* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B3679_D_BA/2008/G10P[11] | 2008 | 10(VI) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B3700_D_BA/2008/G6P[11] | 2008 | 6(III) | 11(VI) | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B3702_D_BA/2008/G6P[11] | 2008 | 6(III) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B3708_D_BA/2008/G6P[11] | 2008 | 6(III) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B3893_D_BA/2008/G6P[11] | 2008 | 6(III) | 11* | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/B4104_D_BA/2009/G10P[11] | 2009 | 10(VI) | 11(V) | Buenos Aires | Dairy |
| RVA/Cow-wt/ARG/4181_D_BA/2003/G10P[5] | 2009 | 10(VI) | 5* | Buenos Aires | Dairy |

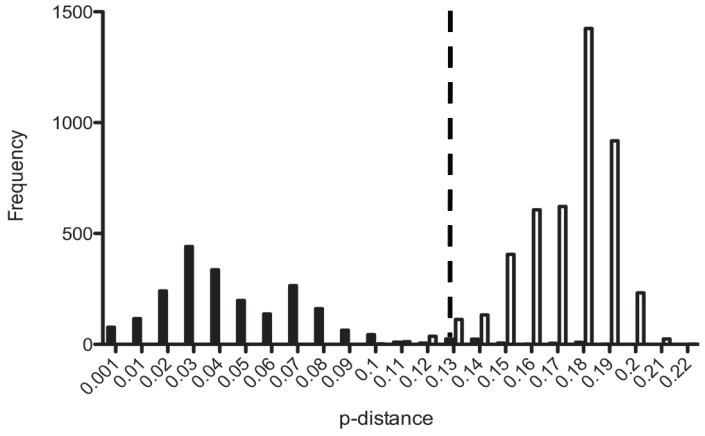
* Samples that could be amplified by Multiplex RT-PCR, but could not be sequenced.

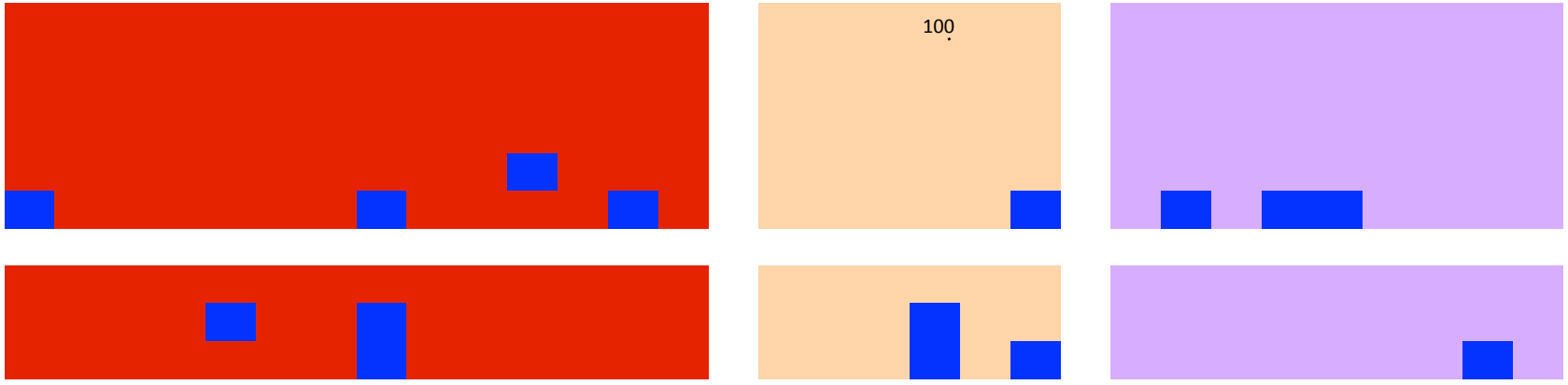



B)

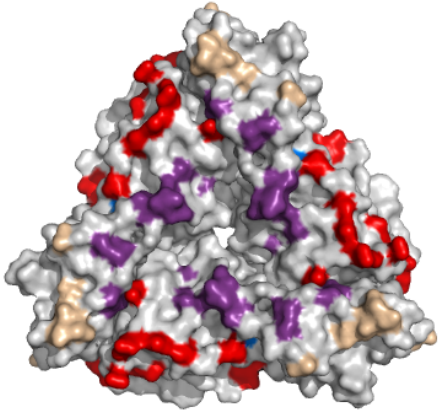
| Lineages | I | II | III | IV | V |
|---|---|---|---|---|---|
| I | **3.7 (0.8-5.5)** | | | | |
| II | 18.7 (16.6-21.8) | **9 (0.1-15.9)** | | | |
| III | 13.6 (10.8-15.7) | 19.4 (16.7-21.4) | **3.7 (0-7.4)** | | |
| IV | 17.9 (16.2-20.9) | 16.4 (14.1-19.7) | 18.8 (16.2-22) | **5.4 (0-11.7)** | |
| V | 13.6 (11.2-15.6) | 18.4 (16.8-20.5) | 14.6 (12.7-6.3) | 18.4 (16.7-20.6) | **3.5 (2.3-4.6)** |

C)

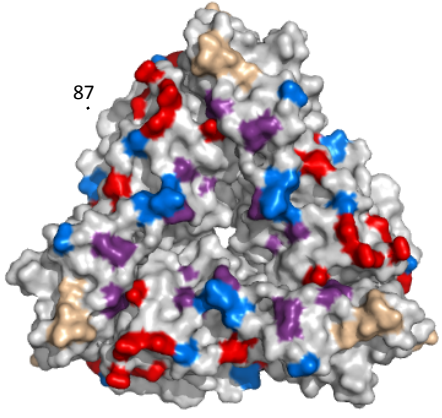




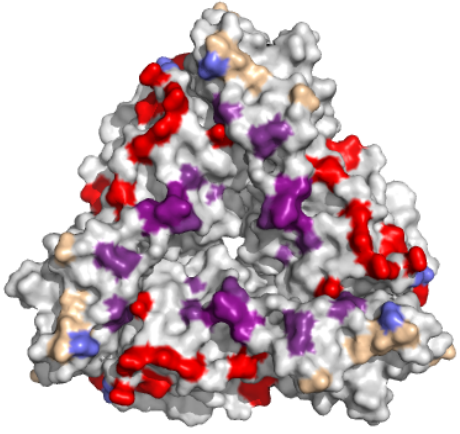

WC3 vs G6 (LIV)



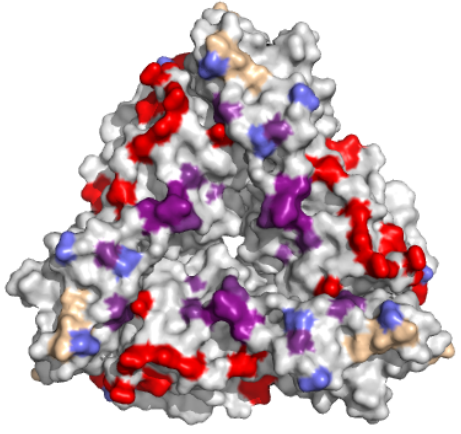
WC3 vs G6 (LIII)




B223 vs G10 (LV)



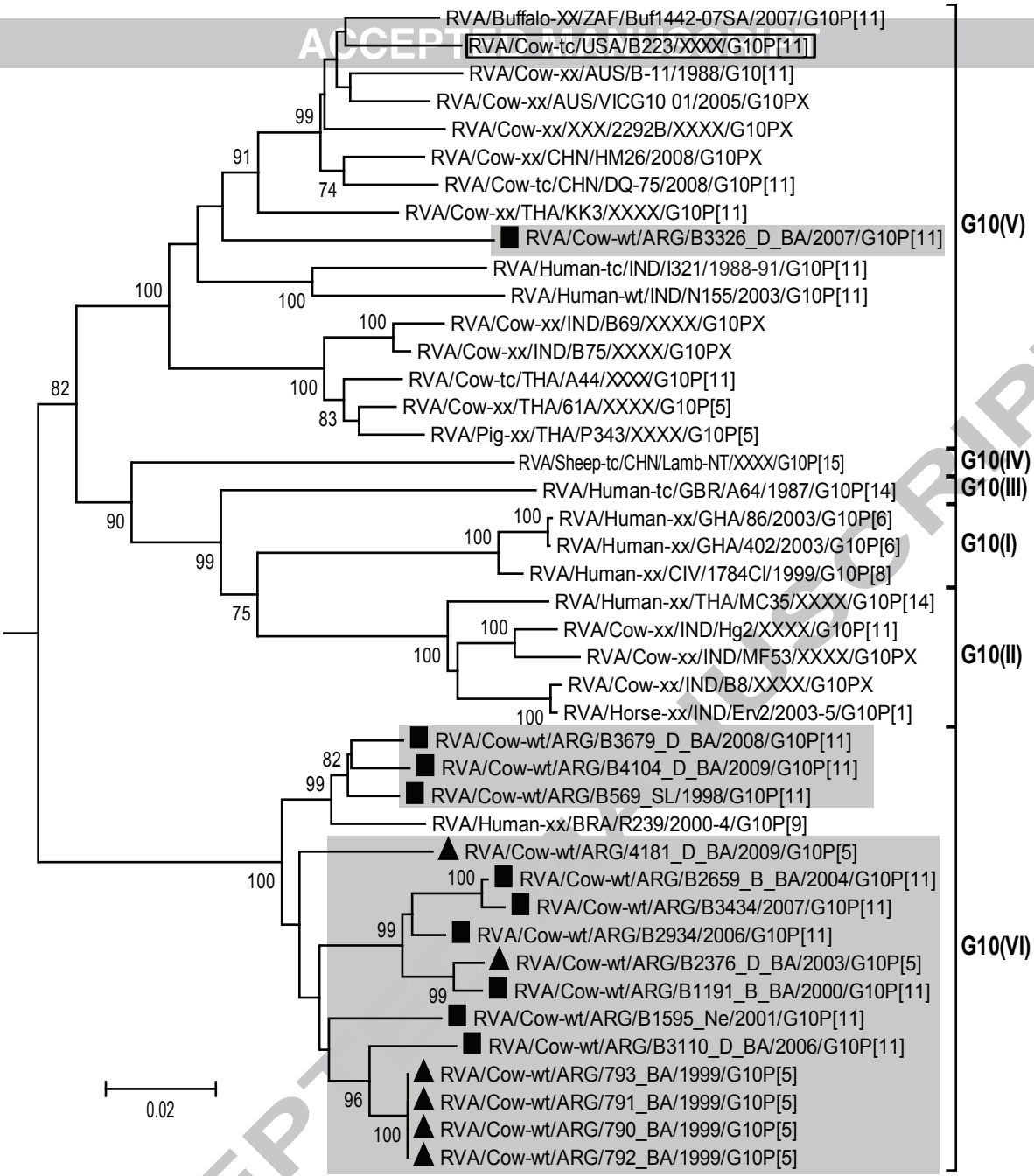
B223 vs G10 (LVI)



Ag Sites

7-1a 7-1b 7-2 Differences

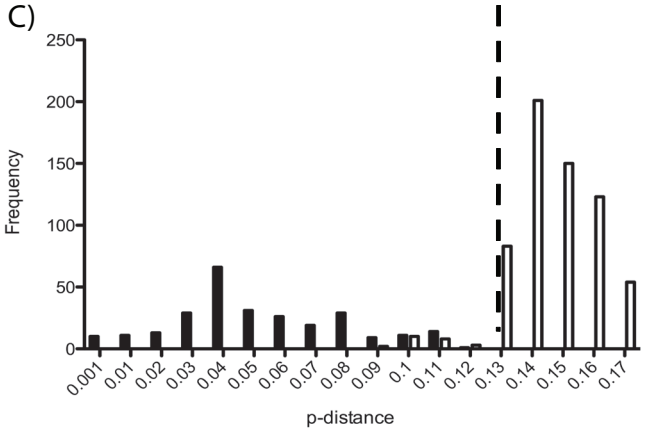
A)



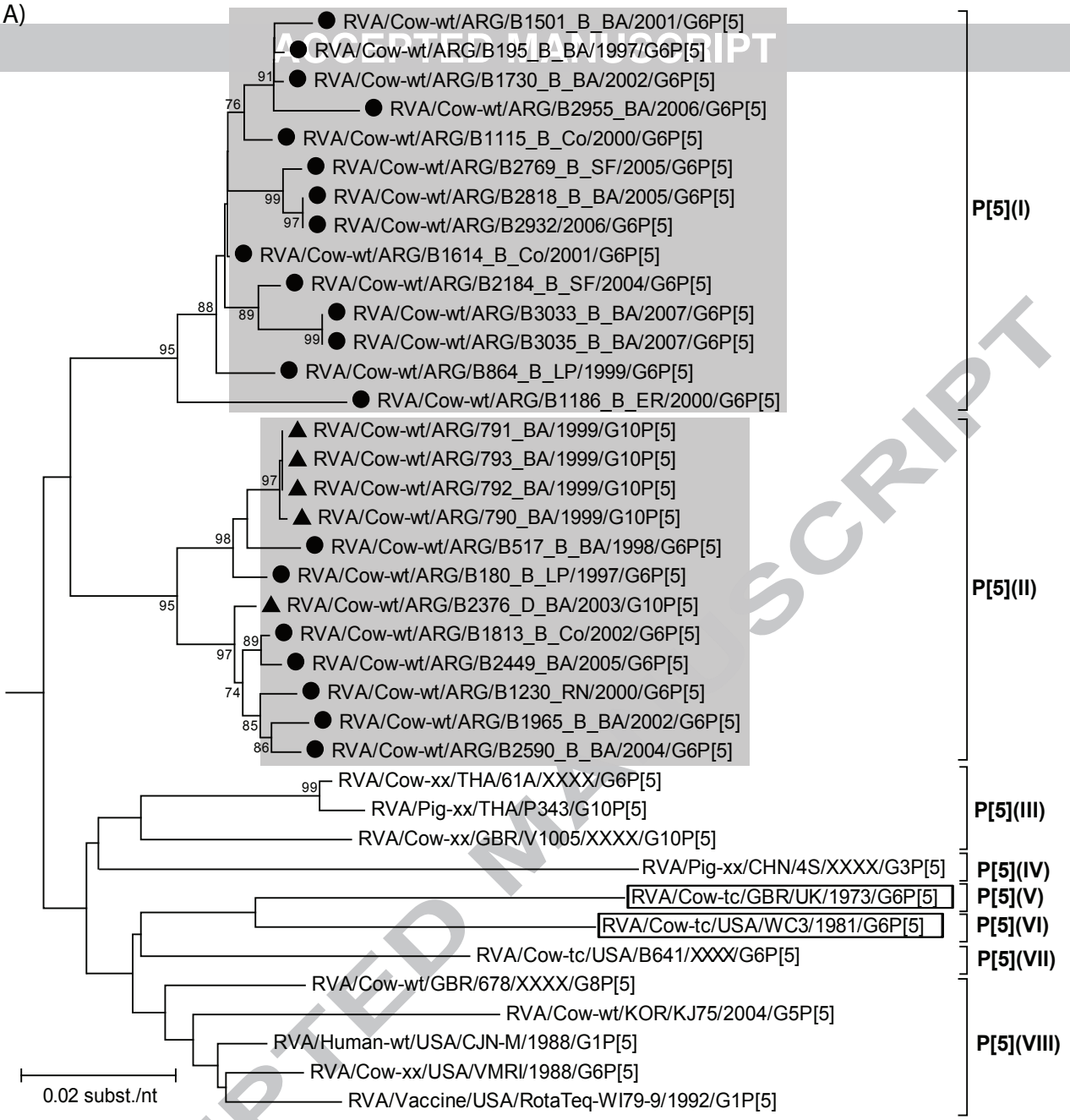

B)

| Lineages | I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|
| I | **1 (0.1-1.5)** | | | | | |
| II | 10.6 (9.8-11.3) | **3.4 (1.9-4.5)** | | | | |
| III | 12.1 (11.9-12.3) | 11.6 (11.3-12.1) | – | | | |
| IV | 15.6 (15.2-15.8) | 14.4 (13.8-15.1) | 14.5 (14.5) | – | | |
| V | 14.4 (13.1-16.2) | 15.1 (13.4-17.2) | 15 (14-16.8) | 14.9 (13.6-16.8) | **7.6 (1.3-12.1)** | |
| VI | 16.4 (15.4-17.3) | 16.8 (15.5-17.7) | 16.5 (14.6-17.1) | 15.8 (14.5-16.3) | 14.6 (13.2-16.5) | **4.5 (0-7.5)** |

C)



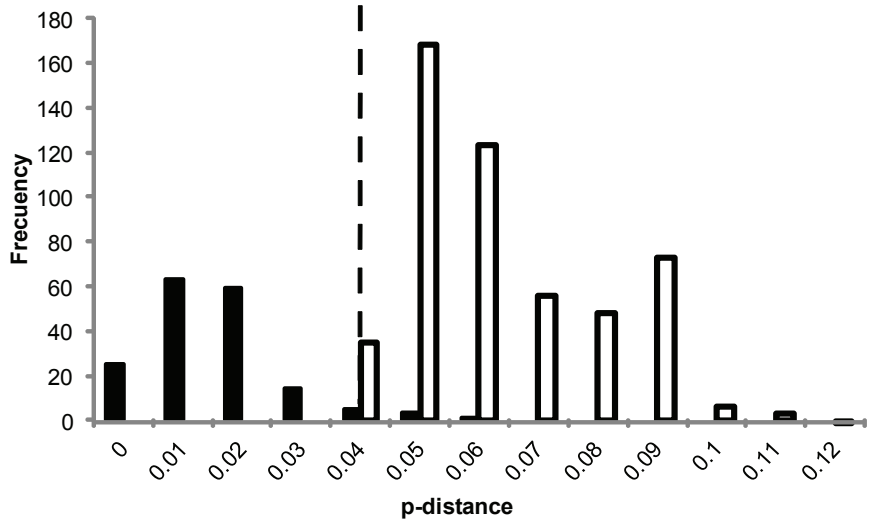
A)

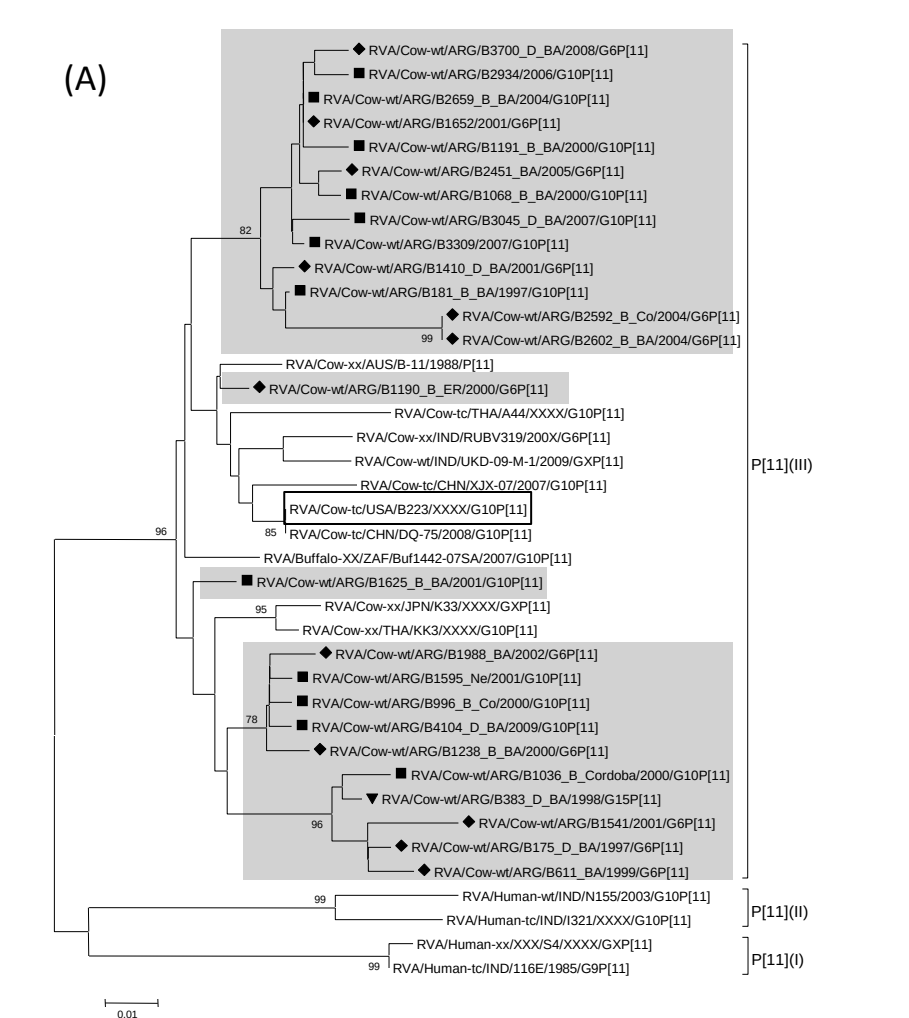



B)

| Lineages | I | II | III | IV | V | VI | VII | VIII |
|---|---|---|---|---|---|---|---|---|
| I | **1.9 (0-4.3)** | | | | | | | |
| II | 5.4 (4.2-6.4) | **1.8 (0-2.9)** | | | | | | |
| III | 6.8 (6-7.7) | 7 (6.5-7.7) | **3.7 (0.7-5.3)** | | | | | |
| IV | 9.8 (9.3-10.1) | 9.9 (9.4-10.6) | 9.3 (9.1-9.5) | - | | | | |
| V | 9.7 (9.1-10.6) | 10 (9.6-10.3) | 8.1 (7.8-8.4) | 11.3 | - | | | |
| VI | 9.7 (9.1-10.2) | 9.7 (9.5-10) | 9.4 (9.3-9.5) | 12.2 | 8.4 | - | | |
| VII | 8.2 (7.6-9) | 8.6 (8.1-9) | 8.4 (7.7-8.9) | 11 | 9.1 | 9.6 | - | |
| VIII | 6.7 (4.9-9.4) | 6.4 (5.2-8.3) | 6.6 (5.7-8.4) | 9.7 (8.9-11.1) | 9.1 (8.3-10.8) | 7.1 (5.4-9.4) | 6.8 (5.9-8.9) | **3.8 (1.7-6.1)** |

C)






(B)

| Lineages | I | II | III |
|---|---|---|---|
| I | 0.4 | | |
| II | 11.9 (11.5-12.3) | 4.3 | |
| III | 11 (9.4-13.6) | 11.8 (9.4-14) | 4.7 (0-9.4) |

(C)