Veterinary Microbiology 161 (2013) 247–254

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Discovery and molecular characterization of a group A rotavirus strain detected in an Argentinean vicuña (*Vicugna vicugna*)

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ARTICLE INFO

Article history:
Received 25 May 2012
Received in revised form 16 July 2012
Accepted 19 July 2012

Keywords:
Vicuña
Vicugna vicugna
Rotavirus
G8P[14]
South American camelids

ABSTRACT

The wild vicuña (*Vicugna vicugna*) is one of the four species of native South American camelids (SACs) in addition to the wild guanaco, and their domesticated counterparts, alpaca and llama, respectively. Serological data have indicated the presence of group A rotaviruses (RVA) specific antibodies in all 4 members of the SAC, and so far, RVA has been detected from alpacas, llamas and guanacos. A total of 59 fecal samples from healthy wild newborn and juvenile vicuñas, raised in captivity in Jujuy, Argentina were collected and analyzed by ELISA to detect RVA antigen. Two samples (3%) were found to contain G8 RVA strains and one strain (RVA/Vicuña-wt/ARG/C75/2010/G8P[14]) was selected for further genome analyses, revealing the G8-P[14]-I2-R2-C2-M2-Ax-N2-T6-E3-Hx genotype constellation. Unfortunately, no sequence data could be obtained for NSP1 and NSP5. Except for the E3 NSP4 genotype, this partial genotype constellation is reminiscent to bovine RVA strains and bovine-like RVA strains isolated from sheep, guanaco, antelope and humans. This relationship was confirmed phylogenetically, providing further evidence of the widespread presence of this genotype constellation in animals belonging to the *artiodactyls*. In particular, a close phylogenetic relationship was found between C75 and guanaco RVA strain RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14] for at least 5 gene segments, suggesting a partial conservation of the genotype constellation of RVA strains infecting different species of SACs, even though nowadays their natural habitats are not overlapping. The further monitoring of the sanitary health of wild newborn and juvenile vicuñas is essential to improve the management practices applied in their sustainable exploitation.

1. Introduction

The wild species vicuña (*Vicugna vicugna*) and guanaco (*Lama guanicoe*), and the domestic species llama (*Lama glama*) and alpaca (*Vicugna pacos*) are the four species of camelids native from South America. South American camelids (SACs) used to be widely distributed all along the Andes, but at present wild vicuñas are restricted to the Northern Andean Puna (between 3200 and 4700 m above sea level) of Peru, Bolivia, Chile and Argentina, whereas wild guanacos live in the Southern Patagonia region of Argentina and Chile (Baigun et al., 2008). The vicuñas are recognized to have one of the finest fibers in the world (Wheeler et al., 2000), but the systematic killings of vicuñas to harvest their wool caused a severe reduction in

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http://dx.doi.org/10.1016/j.vetmic.2012.07.035

their population. Vicuñas have been on the verge of extinction since the latter part of the twentieth century (1975–1997) and have consequently suffered a strong reduction in their genetic diversity. After more than 20 years of effective protection laws the numbers of wild vicuñas in Argentina started to increase again from 1997. Due to the increase in the population of wild vicuñas in the Jujuy province, as well as the vicuña populations raised in captivity and distributed by the experimental station Abra Pampa of the Institucion Nacional de Tecnología Agropecuaria (INTA) to the local farmers, vicuña were re-classified under the appendix II of the Convention of International Trade of Endangered Species (CITES). At present two management practices are applied to promote the sustainable exploitation of the species, allowing the harvesting of fiber from live shorn animals raised under captivity or from wild vicuñas (Baigun et al., 2008; Vilá and Lichtenstein, 2006). For both management practices serological surveys were conducted to identify potential pathogens circulating among these wild SAC species (Marcoppido et al., 2010, 2011; Wheeler et al., 2000).

The experimental station of INTA Abra Pampa, has a population of 1400 wild vicuñas in captivity. Intensive prophylactic and sanitary programs are in place including vaccination, treatment against parasites and shaving of adult males every six months. Taking advantage of this management practice blood and feces samples are taken from both adult and juvenile animals to detect the circulation of etiological agents of infectious diseases, including agents causing infectious gastroenteritis, which is an important pathology in the young of SACs (Cebra et al., 2003; Lopez et al., 2011; Marcoppido et al., 2010; Parreño et al., 2001). A high prevalence of antibodies against group A rotavirus (RVA) has been described in all 4 SACs, suggesting the wide spread occurrence of this pathogen (Marcoppido et al., 2010, 2011; Marin et al., 2009; Parreño and Marcoppido, 2006; Puntel et al., 1999; Rivera et al., 1987). However, while RVA has been detected in feces of young guanacos, alpacas and llamas with diarrhea in Argentina, Peru and Chile, respectively (Berrios, 1988; Cebra et al., 2003; Lopez et al., 2011; Parreño et al., 2001, 2004), there have been no reports of RVA isolated from vicuñas to date.

RVAs belong to the genus *rotavirus* in the family of the *Reoviridae* and are icosahedral non-enveloped viruses, possessing a genome of 11 segments of dsRNA. The two outer capsid proteins, VP7 and VP4, independently elicit neutralizing antibodies and are used to differentiate RVA strains into G-types (*Glycoprotein*) and P-types (*Protease-sensitive*), respectively (Ciarlet and Estes, 2002). Currently, 27 G-genotypes and 35 P-genotypes are recognized (Matthijnssens et al., 2011). A uniform sequence-based genotyping system encompassing all eleven RVA gene segments was developed using the following descriptor to classify RVA strains: Gx-Px-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx (Matthijnssens et al., 2008a). This classification system is maintained and updated by the Rotavirus Classification Working Group (RCWG) (Matthijnssens et al., 2008c), which recently also developed a uniform naming scheme for rotaviruses (Matthijnssens et al., 2011).

According to this classification system the complete genomes of two guanaco RVA strains and a single bovine RVA strains isolated in Argentina were shown to possess the following genotype constellations: G8-P[1]-I2-R5-C2-M2-A13-N2-T6-E12-H3 for RVA/Guanaco-wt/ARG/Rio_-Negro/1998/G8P[1], G8-P[14]-I2-R5-C2-M2-A11-N2-T6-E12-H3 for RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14] and G15-P[11]-I2-R5-C2-M2-A13-N2-T6-E12-H3 for RVA/Cow-wt/ARG/B383/1998/G15P[11], respectively (Matthijnssens et al., 2009). No other complete RVA genome data are available for any member of the SACs. However, partial sequence data of a RVA strain (RVA/Camel-wt/KUW/21S-10/2010/G10P[15]) detected in a dromadary (*Camelus dromedarius, family Camelidae*) in Kuwait, revealed the following genotype constellation: G10-P[15]-Ix-R1-C2-Mx-Ax-N2-T6-E15-H3 (Papp et al., 2011).

In 2004, the experimental station of INTA Abra Pampa started epidemiological studies on the presence of RVA in animals housed at the station, and found positive serology in 100% of the sampled vicuñas, llamas and cattle (Marcoppido et al., 2010). The present work is a continuation of this study and represents the first detection and molecular characterization of RVA in vicuñas (*Vicugna vicugna*) from the Andean Puna, Argentina.

2. Materials and methods

2.1. Sample collection

The INTA experimental station of Abra Pampa is located in the Andean Puna, within the Jujuy province at 3500 m above sea level. The sun radiation levels are extremely high in the region and the weather is cold and dry, with average temperatures of 9–14 °C in the summer and 4 °C in the winter season. The experimental station has 1400 wild vicuñas in captivity living in a large area of 350 ha. Twice per year animals of all ages are gathered as previously described (Parreño and Marcoppido, 2006), to monitor their general heath in a prophylactic and sanitary program, which include vaccination, treatment against parasites and shaving of adult males. During this practice blood and feces samples are collected and subsequently used to study the circulation of potential pathogens associated with neonatal diarrhea, and with reproductive and respiratory syndromes (Sandra Romero, personal communication). Fecal samples from 59 juvenile vicuñas under one year of age (newborn and juveniles) and without symptoms of gastroenteritis or other diseases were collected for the analysis of viral agents.

2.2. Antigen detection

The samples were cooled on ice and sent to the Virology Institute, INTA Castelar, were they were analyzed for RVA using a double sandwich ELISA capture antigen test (KERI-INTA) (Cornaglia et al., 1989), and confirmed with two commercial immunoassays (Pathfinder, BIORAD and Megacore strip test, La Coquette, France).

2.3. Sequence analyses

Viral RNA was extracted using a QIAamp viral RNA mini kit (QIAGEN/Westburg, The Netherlands) according to the

manufacturer's instructions. The extracted RNA was denatured at 95 °C for 2 min and RT-PCR was carried out using the Qiagen OneStep RT-PCR Kit (Qiagen/Westburg). The RT-PCR reaction was carried out with an initial reverse transcription step at 50 °C for 30 min, followed by PCR activation at 95 °C for 15 min, 35 cycles of amplification, and a final extension of 10 min at 72 °C in a Biometra T3000 thermocycler (Biometra, Westburg B.V., The Netherlands). The cycle conditions for the amplification of VP1, VP2, VP3, and VP4 were 30 s at 94 °C, 30 s at 50 °C, and 4 min at 72 °C; for the other genome segments the conditions were 30 s at 94 °C, 30 s at 45 °C, and 3 min at 72 °C. The PCR products were purified with the MSB® Spin PCRapace kit (Invitek, Germany), and sequenced using the dideoxy-nucleotide chain termination method with the ABI PRISM® BigDye Terminator Cycle Sequencing Reaction kit (Applied Biosystems Group) on an ABI PRISM® 3130 automated sequencer (Applied Biosystems Group). Sequencing was performed with the forward and reverse primers used for the RT-PCR. Primer walking sequencing was performed to cover the complete sequence of the respective fragments on both strands.

2.4. Phylogenetic analysis

Phylogenetic and molecular evolutionary analyses were conducted using MEGA 5 (Tamura et al., 2011). Genetic distances were calculated using the Kimura-2 correction parameter at the nucleotide level, and the phylogenetic trees were constructed using the neighbor-joining method with 1000 bootstrap replicates.

2.5. Genotype assignment

The genotypes of each of the 11 genome segments for all the RVA strains under investigation were determined according to the genotyping recommendations of the Rotavirus Classification Working Group (RCWG) (Matthijnssens et al., 2008b) using the RotaC online classification tool (http://rotac.regatools.be/) (Maes et al., 2009).

2.6. Accession numbers

GenBank accession numbers (VP1–VP4, VP6, VP7, NSP2–NSP4) for each individual genome segment can be found in Table 1.

3. Results

Fifty-nine stool samples of captive newborn and juvenile vicuñas (below one year of age) from the experimental station of INTA Abra Pampa, Jujuy, were investigated using a double sandwich antigen capture ELISA test for RVA detection. Two samples were positive for RVA (RVA/Vicuña-wt/ARG/C69/2010/G8P[X] and RVA/Vicuña-wt/ARG/C75/2010/G8P[14]), as confirmed by two commercial strip test assays. RT-PCR amplification of the VP7 gene was strongly positive for C75, but only weakly positive for C69, and only sample C75 resulted in a RT-PCR product for the VP8* portion of VP4 protein. Sequence analyses revealed a G8 genotype for both samples and a P[14] genotype for C75. Sample C75 was selected for further molecular characterization. Attempts to amplify the complete genome of RVA strain C75 were only partially successful. The (near) complete coding region could be determined for VP7, VP6, VP4, NSP2, NSP3 and NSP4. Partial sequences (≈900 bp) could be obtained for VP1, VP2 and VP3 and unfortunately no sequence data could be obtained for NSP1 and NSP5 (Table 1). The lack of more viral RNA prevented us from performing further attempts to obtain the missing sequences. The resulting genotype constellation for C75 was G8-P[14]-I2-R2-C2-M2-Ax-N2-T6-E3-Hx. Table 2 shows the comparison of the genotype combination of RVA strain C75 with completely sequenced RVA reference strains. Except for the NSP4 E3 genotype, which has been previously detected in feline, canine, simian and some unusual human RVA strains, the genotype constellation of C75 is typical for bovine or bovine-like RVA strains isolated from guanacos, goats, antelope, buffalo, sheep and humans (Ciarlet et al., 2008; Ghosh et al., 2010; Matthijnssens et al., 2009; Bányai et al., 2009, 2010; El Sherif et al., 2011). C75 shared 8 out of the 9 known genotypes (G8-P[14]-I2-R2-C2-M2-N2-T6) with RVA strain RVA/Sheep-tc/ESP/OVR762/2002/G8P[14] isolated in Spain from a sheep and the unusual human RVA strain RVA/Human-wt/HUN/BP1062/2004/G8P[14] isolated in Hungary (Bányai et al., 2010; Matthijnssens et al., 2009). In addition, a guanaco (RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14]), an antelope (RVA/Antelope-wt/ZAF/RC-18/2008/G6P[14]) and several bovine-like human P[14] RVA strains (Bányai et al., 2009; Matthijnssens et al., 2009) share 7 out of 9 known genotypes (Table 2).

The VP7 gene segment of RVA strain C75 clusters closely with G8 strains isolated from guanacos: Chubut (P[14]) and Rio_Negro (P[1]) (97.9–98.0% identity on the nucleotide level). Other closely related G8 RVA strains were of ovine (OVR762, P[14]), simian (RVA/Rhesus-tc/USA/PTRV/1990/G8P[1]) and human (RVA/Human-tc/KEN/B12/1987/G8P[1]) origin, but all possessed typical bovine-like genotype constellation (Table 2) (Ghosh et al., 2011; Matthijnssens et al., 2009, 2010). The VP4 gene segment of C75 was again most closely related to guanaco RVA strain Chubut (94.0%), and more distantly related to a

Table 1
Length, genome position and accession numbers of sequence obtained for RVA/Vicuña-wt/ARG/C75/2010/G8P[14].

Viral proteins	Genotype	Length	Position[a]	Accession number
VP7	G8	979 bp	59–1037	JX070052
VP4	P[14]	2220 bp	1–2220	JX070050
VP6	I2	1193 bp	90–1282	JX070051
VP1	R2	908 bp	50–957	JX070047
VP2	C2	846 bp	44–889	JX070048
VP3	M2	863 bp	39–901	JX070049
NSP1	Ax	–	–	–
NSP2	N2	970 bp	44–1013	JX070053
NSP3	T6	990 bp	43–1032	JX070054
NSP4	E3	700	22–721	JX070055
NSP5	Hx	–	–	–

[a] Position with respect to RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14].

Table 2
RVA genotype constellations of the RVA/Vicuña-wt/ARG/C75/2010/G8P[14] compared to selected RVA reference strains.

RVA strain names	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/vicuña-wt/ARG/75/2010/G8P[14]	G8	P[14]	12	R2	C2	M2	Ax	N2	T6	E3	Hx
RVA/Sheep-tc/ESP/OVR762/2002/G8P[14]	G8	P[14]	12	R2	C2	M2	A11	N2	T6	E2	H3
RVA/Human-wt/HUN/BP1062/2004/G8P[14]	G8	P[14]	12	R2	C2	M2	A11	N2	T6	E2	H3
RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14]	G8	P[14]	12	R5	C2	M2	A11	N2	T6	E12	H3
RVA/Antelope-wt/ZAF/RC-18/2008/G6P[14]	G6	P[14]	12	R2	C2	M2	A11	N2	T6	E2	H3
RVA/Human-tc/ITA/PA169/1988/G6P[14]	G6	P[14]	12	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Human-wt/ITA/111-05-27/2005/G6P[14]	G6	P[14]	12	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Human-wt/BEL/B10925/1997/G6P[14]	G6	P[14]	12	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Human-wt/HUN/Hun5/1997/G6P[14]	G6	P[14]	12	R2	C2	M2	A11	N2	T6	E2	H3
RVA/Human-tc/AUS/MG6/1993/G6P[14]	G6	P[14]	12	R2	C2	M2	A11	N2	T6	E2	H3
RVA/Human-wt/HUN/BP1879/2003/G6P[14]	G6	P[14]	12	R2	C2	M2	A11	N2	T6	E2	H3
RVA/Cow-tc/KOR/KJ19-2/2004/G6P[7]	G6	P[7]	12	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Guanaco-wt/ARG/Rio_Negro/1998/G8P[1]	G8	P[1]	12	R5	C2	M2	A13	N2	T6	E12	H3
RVA/Rhesus-tc/USA/PTRV/1990/G8P[1]	G8	P[1]	12	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Human-tc/KEN/B12/1987/G8P[1]	G8	P[1]	12	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Human-tc/GBR/A64/1987/G10P11[14]	G10	P[14]	12	R2	C2	M1	A3	N2	T6	E2	H3
RVA/Cow-tc/FRA/RF/1982/G6P[1]	G6	P[1]	12	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Cow-tc/USA/NCDV/1967/G6P6[1]	G6	P[1]	12	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Goat-tc/BGD/G034/1999/G6P[1]	G6	P[1]	12	R2	C2	M2	A11	N2	T6	E2	H3
RVA/Cow-tc/USA/WC3/1981/G6P[5]	G6	P[5]	12	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Cow-tc/CHN/DQ-75/2008/G10P[11]	G10	P[11]	12	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Cow-wt/ARG/B38 3/1998/G15 P[11]	G15	P[11]	12	R5	C2	M2	A13	N2	T6	E12	H3
RVA/Human-wt/ITA/PAI58/1996/G3P[9]	G3	P[9]	12	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Cow-tc/GBR/UK/1973/G6P7[5]	G6	P[5]	12	R2	C2	M2	A3	N2	T7	E2	H3
RVA/Cow-wt/J PN/Azuk-1/2006/G21P [29]	G21	P[29]	12	R2	C2	M2	A13	N2	T9	E2	H3
RVA/Human-tc/USA/DS-1/1976/G2P1B[4]	G2	P[4]	12	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]	G2	P[4]	12	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/USA/LB2744/2005/G2P[4]	G2	P[4]	12	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Simian-tc/USA/RRV/1975/G3P[3]	G3	P[3]	12	R2	C3	M3	A9	N2	T3	E3	H6
RVA/Dog-tc/USA/CU-1/1982/G3P[3]	G3	P[3]	13	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Cat-tc/AUS/Cat97/1984/G 3P [3]	G3	P[3]	13	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Human-tc/ISR/Ro1845/1985/G3P[3]	G3	P[3]	13	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Human-tc/JPN/AU-1/1982/G3P3[9]	G3	P[9]	13	R3	C3	M3	A3	N3	T3	E3	H3
RVA/Rhesus-tc/USA/TUCH/2002/G3P[24]	G3	P[24]	19	R3	C3	M3	A9	N1	T3	E3	H6

Genotypes which are identical to the corresponding genotype of the vicuña RVA strain are gray shaded. "x" indicates that the genotype is not known, as sequencing has not been performed.

plethora of bovine-like human and animal P[14] RVA strains all found in combination with typical bovine G-genotypes: G6, G8 or G10 (Fig. 1). The VP6 gene segment of C75 clustered rather distantly to known RVA strains belonging to genotype I2 (82.7–93.6%), but again most closely to RVA strains with a bovine-like genotype constellation isolated from a variety of host species (Fig. 1 and Table 2). In the phylogenetic tree of VP1, C75 clustered with the unusual Japanese bovine RVA strain RVA/Cow-wt/JPN/Azuk-1/2006/G21P[29] (96.6%) (Abe et al., 2011) in a subcluster of the R2 genotype mainly composed of bovine-like RVA strains and simian RVA strain RVA/Simian-tc/USA/RRV/1975/G3P[3] (Fig. 1) (Matthijnssens et al., 2010). The VP2 gene segment of C75 clustered closely with the guanaco RVA strains Chubut and Rio_Negro (98.2–98.8%), and only distantly to the unusual human multi-reassortant RVA strain RVA/Human-wt/ITA/PAI58/1996/G3P[9], bovine-like simian RVA strain PTRV and bovine-like human RVA strain RVA/Human-tc/AUS/MG6/1993/G6P[14] in the C2 genotype (De Grazia et al., 2010; Matthijnssens et al., 2009, 2010). Phylogenetic analyses of the VP3 gene segment revealed again a close genetic relationship between C75 and RVA strain Chubut in the M2 genotype (98.5%), and a more distant relationship with RVA/Cow-wt/ARG/B383/1998/G15P[11] isolated in Argentina (Matthijnssens et al., 2009). The NSP2 gene segment of C75 was rather distantly related to other known RVA strains belonging to the N2 genotype (85.5–93.0%), but again most closely related to RVA strains Chubut (Fig. 2). For NSP3, C75 clustered closely (96.3–97.6%) with RVA strain Chubut and B12 in the T6 genotype, which is a typical genotype for bovine and bovine-like RVA strains (Fig. 2). The NSP4 gene segment of C75 is only distantly related to other known RVA strains with the E3 NSP4 genotype (78.3–79.8%), with nucleotide similarities below the NSP4 genotype cut-off value (85%). This sequence was sent to the RCWG for a proper genotype assignment. Based on thorough pairwise identity frequency graphs and phylogenetic analyses, RVA strains C75 was assigned as a distant member of the E3 genotype.

Overall, except for the NSP4 sequence of RVA strains C75 all other 8 obtained gene segments clustered with RVA strains with a bovine-like genotype constellation. In particular, the phylogenetic analyses of 9 gene segments of vicuña RVA strains C75 revealed a close to very close phylogenetic relationship with guanaco RVA strain Chubut for VP7, VP4, VP2, VP3 and NSP3. For VP6 and NSP2, RVA strains C75 and Chubut clustered distantly in the same genotypes, whereas they belonged to different genotypes for VP1 and NSP4 (Figs. 1 and 2).

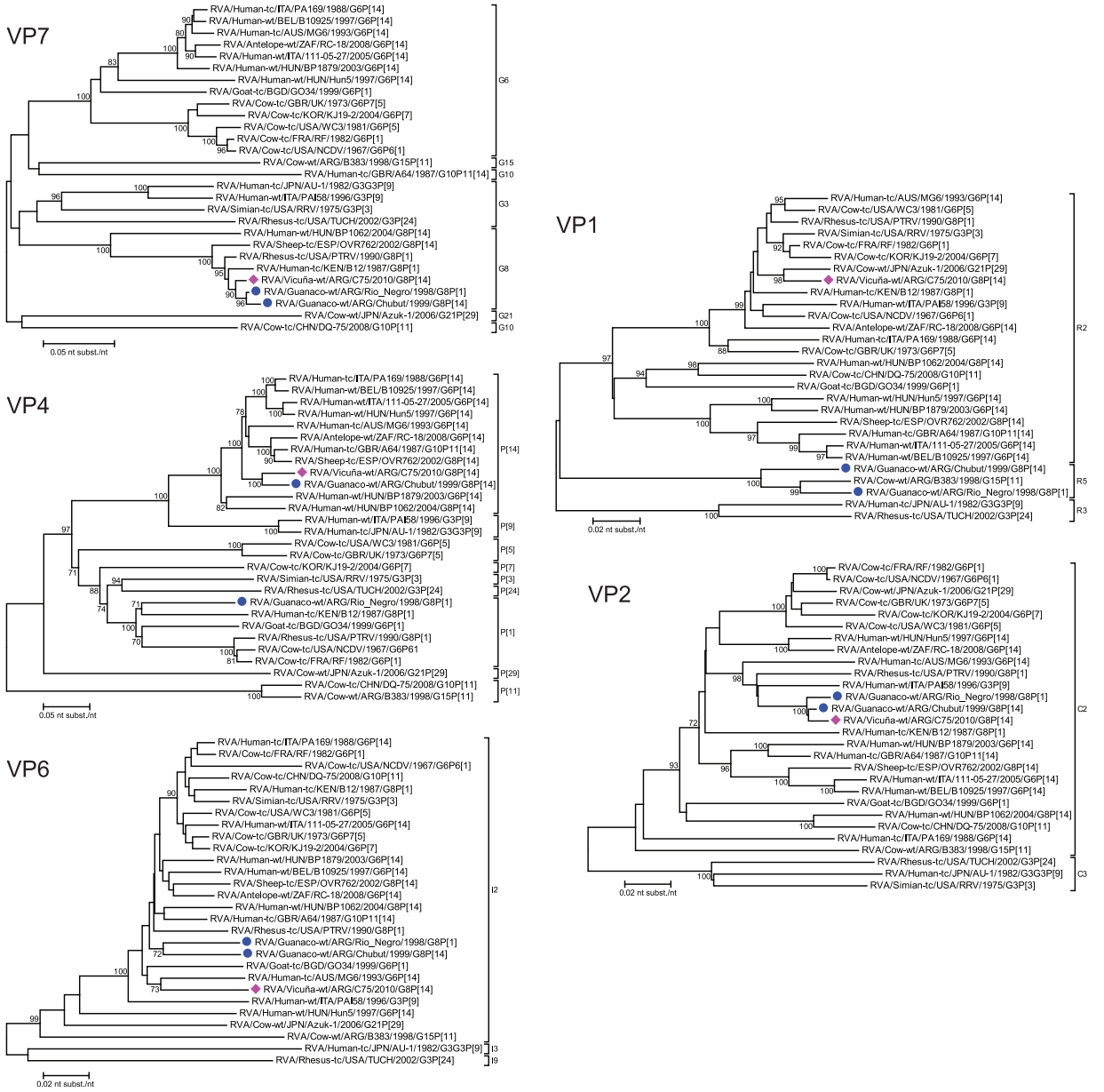

Fig. 1. Phylogenetic trees based on the partial ORF sequences of the VP7, VP4, VP6, VP1 and VP2 encoding gene segments (Table 1). Phylogenetic trees were constructed using the neighbor-joining method with the kimura-2-parameter. Bootstrap values (1000 replicates) above 70% are shown. RVA/Vicuña-wt/ARG/C75/2010/G8P[14] is indicated by diamonds, whereas the guanaco strains RVA/Guanaco-wt/ARG/Rio_Negro/1998/G8P[1] and RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14] are marked with a circle.

4. Discussion and conclusions

After the serological identification of RVA specific antibodies in serum samples from vicuñas (Marcoppido et al., 2010), to our knowledge, this paper is the first to report the detection of RVA from fecal samples from asymptomatic newborns of vicuñas (*Vicugna vicugna*) from the Andean Puna, Argentina. We have previously reported, for the first time, the detection and molecular characterization of RVA strains from guanacos from the Argentinean Patagonia region (Matthijnssens et al., 2009; Parreño et al., 2001, 2004). In addition, high seroprevalence of antibodies against RVA have been described in all 4 SACs, suggesting that RVA is a ubiquitous pathogen in all members of the SACs (Marcoppido et al., 2010, 2011; Marin et al., 2009; Parreño and Marcoppido, 2006; Puntel et al., 1999; Rivera et al., 1987).

Two out of 59 stool samples (3%) of captive vicuñas sampled at the experimental station of INTA Abra Pampa, Jujuy were found to contain RVA. Although the sampled specimens showed no apparent signs of disease, these data add further evidence that vicuñas are frequently exposed

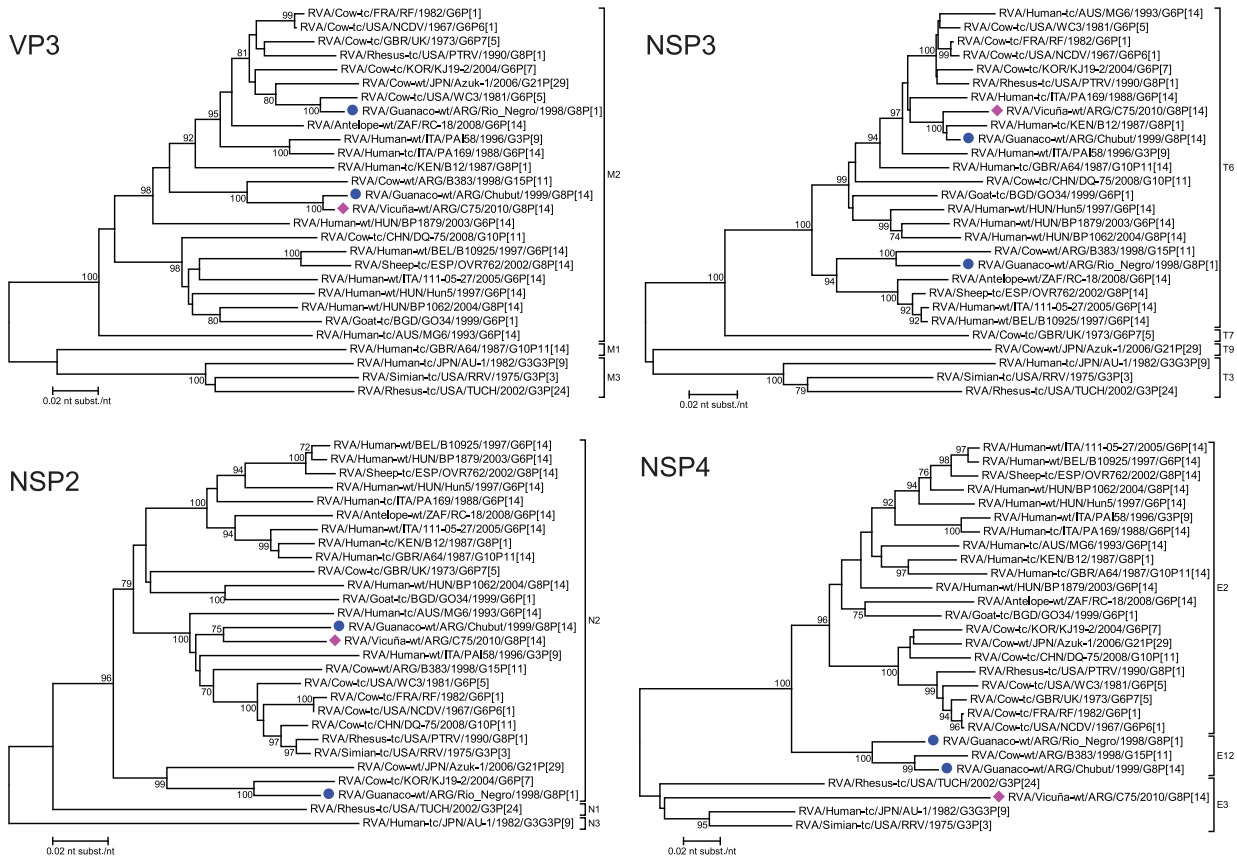

Fig. 2. Phylogenetic trees based on the partial ORF sequences of VP3 and the full ORF sequence of the NSP2, NSP3, NSP4 encoding gene segments (Table 1). Phylogenetic trees were constructed using the neighbor-joining method with the kimura-2-parameter. Bootstrap values (1000 replicates) above 70% are shown. RVA/Vicuña-wt/ARG/C75/2010/G8P[14] is indicated by diamonds, whereas the guanaco strains RVA/Guanaco-wt/ARG/Rio_Negro/1998/G8P[1] and RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14] are marked with a circle.

to RVA pathogens. However, the general severity of RVA gastroenteritis in vicuñas is unknown. From only one sample (C75) sufficient viral RNA could be obtained to attempt whole genome analysis. Sequences were obtained for 9 out of the 11 RVA genome segments. Unfortunately, no RT-PCR product or sequence data could be obtained for the NSP1 and NSP5 gene segments. Most likely these gene segments possess divergent sequences in the primer binding regions, not allowing their amplification by RT-PCR. No further experiments could be performed due to a lack of viral RNA. Genotype assignment of the obtained sequences revealed the G8-P[14]-I2-R2-C2-M2-Ax-N2-T6-E3-Ax genotype combination. Except for the E3 NSP4 genotype this partial genotype constellation is similar to bovine RVA strains and bovine-like RVA strains detected from guanacos, goats, antelope, buffalo, sheep and several human P[14] RVA strains. This finding adds further support to the hypotheses that RVA strains with closely related genotype constellations are prevalent in a large number of animal species belonging to the order of *Artiodactyla* (Matthijnssens et al., 2009). Although the NSP4 gene segment was assigned to the E3 genotype it was only distantly related to any known RVA strain (Fig. 2). Phylogenetic analyses revealed a close relationship between the gene segments of C75 and RVA strain Chubut isolated from a guanaco in Argentina for at least 5 gene segments (VP7, VP4, VP2, VP3 and NSP3). Since the guanaco is the most closely related species to the vicuña, of which RVA sequence data is currently available this may not be surprising, but suggests that RVA strains and their gene segments can be transmitted and reassort among different SACs. In addition, the close relationship between RVA strains detected in vicuñas and bovine RVA strains also raises the question whether the further introduction of domestic cattle in the Andean Puna may represent a sanitary risk for the conservation and sustainable exploitation of wild and domestic native species. In order to further elucidate this hypothesis more complete genome data from RVA strains circulating in the four species of SACs (vicuña, guanacos, alpacas and llamas) as well as from other domestic species (bovine, caprine and ovine) of the region are needed. Finally, it is important to highlight that the apparent absence of disease, in combination with the detection of high titers of antibodies in the surveyed vicuña population (Marcoppido et al., 2010) suggest that there is an equilibrium between RVA infections and immunity in the herd, and that the current management practices do

not disturb this equilibrium as reported for other situations (Parreño et al., 2001).

Monitoring the health of wildlife animals is an essential tool to make decisions to promote their conservation and sustainable exploitation, reducing the risk of neonatal mortality and the impact of man on the natural habitat of animals. Furthermore, this research may allow the development of strategies and tools for preventive health strategies, and further explore the potential transmission of pathogens between wild and domestic species living in close proximity.

Conflict of interest statement

None.

Acknowledgments

This work was financed by regional projects SALJU 05 and SALJU 320051 at the INTA PICT N 38308, MinCyt. A.B. was supported by a type II fellowship of CONICET. VP is a joined researcher, CONICET, Argentina. M.Z. was supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT Vlaanderen). J.M was supported by an FWO ('Fonds voor Wetenschappelijk Onderzoek') post-doctoral fellowship.

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