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

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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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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