Veterinary Microbiology 196 (2016) 27–35

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Whole-genome characterization of a Peruvian alpaca rotavirus isolate expressing a novel VP4 genotype

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ARTICLE INFO

Article history:
Received 16 March 2016
Received in revised form 5 October 2016
Accepted 8 October 2016

Keywords:
Rotavirus
Alpaca
Camelid
Genotyping

ABSTRACT

The SA44 isolate of *Rotavirus A* (RVA) was identified from a neonatal Peruvian alpaca presenting with diarrhea, and the full-length genome sequence of the isolate (designated RVA/Alpaca-tc/PER/SA44/2014/G3P[40]) was determined. Phylogenetic analyses showed that the isolate possessed the genotype constellation G3-P[40]-I8-R3-C3-M3-A9-N3-T3-E3-H6, which differs considerably from those of RVA strains isolated from other species of the order Artiodactyla. Overall, the genetic constellation of the SA44 strain was quite similar to those of RVA strains isolated from a bat in Asia (MSLH14 and MYAS33). Nonetheless, phylogenetic analyses of each genome segment identified a distinct combination of genes. Several sequences were closely related to corresponding gene sequences in RVA strains from other species, including human (VP1, VP2, NSP1, and NSP2), simian (VP3 and NSP5), bat (VP6 and NSP4), and equine (NSP3). The VP7 gene sequence was closely related to RVA strains from a Peruvian alpaca (K'ayra/3368-10; 99.0% nucleotide and 99.7% amino acid identity) and from humans (RCH272; 95% nucleotide and 99.0% amino acid identity). The nucleotide sequence of the VP4 gene was distantly related to other VP4 sequences and was designated as the reference strain for the new P[40] genotype. This unique genetic makeup suggests that the SA44 strain emerged from multiple reassortment events between bat-, equine-, and human-like RVA strains.

1. Introduction

Among the South American camelids (SACs), alpacas (*Vicugna pacu*) and llamas (*Llama glama*) are domesticated species, whereas vicuñas (*Vicugna vicugna*) and guanacos (*Llama guanicoe*) are wild species. A cornerstone of the Andean highland economy is raising alpacas for meat and fiber. Peru has the largest alpaca herd in the world (4–4.5 million alpacas) (Tuckwell, 1994), producing an average of approximately 3.5 million kilograms of alpaca fiber annually (~90% of the total worldwide production) (Rosadio et al., 2012). Infections, particularly diarrheal infections, caused by various pathogens are a major cause of neonatal death for SACs (Cebra et al., 2003; Whitehead, 2009; Lopez et al., 2011). Previous studies have demonstrated the importance of rotavirus infections among these animals (Parreño et al., 2001, 2004; Lopez et al., 2011; Papp et al., 2012; Rosadio et al., 2012; Badaracco et al., 2013; Garmendia et al., 2015).

Whole-genome rotavirus sequences in SACs are available for only two strains isolated from a guanaco (Matthijnssens et al., 2009). Partial genome characterization is available for one strain from a vicuña and two strains from alpacas; however, only one or two of the 11 genome segments have been sequenced for the alpaca *Rotavirus A* (RVA) isolates (Badaracco et al., 2013; Garmendia et al., 2015). Here, we report the complete genome sequence of rotavirus strain RVA/Alpaca-tc/PER/SA44/2014/G3P[40] and describe a novel VP4 genotype.

Rotaviruses are members of the *Rotavirus* genus of the *Reoviridae* family, and are classified into eight species (A-H) (Matthijnssens et al., 2012a). RVA is a major cause of dehydrating diarrhea in humans and animals worldwide (Santos and Hoshino,

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http://dx.doi.org/10.1016/j.vetmic.2016.10.005
0378-1135/

2005). The RVA genome consists of 11 segments of double-stranded RNA (dsRNA) encoding six structural proteins (VP1-4, VP6, and VP7) and six nonstructural proteins (NSP1-6) (Estes and Greenberg, 2013). The RVA genomic classification nomenclature is based on all 11 segments of dsRNA. The notation Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx is used to represent genotypes of the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6–encoding gene segments, respectively, with x indicating the number of the genotype (Matthijnssens et al., 2008, 2011a). Currently, there are 28 G, 39 P, 21 I, 14 R, 14C, 13 M, 24 A, 14 N, 16 T, 21 E, and 16 H genotypes (https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/minutes-of-the-7th-rcwg-meeting).

2. Materials and methods

2.1. Stool samples and RT-PCR detection of RV

The RVA strain described in this study (SA44) was isolated from a stool sample collected in February 2014 from a neonatal diarrheic alpaca belonging to the herd of Silli. This community is located in the southern highlands of Peru (14°24′45.3″s, 71°11′32.6″ W; ~4000 m ASL) in the province of Canchis in the state of Cusco. The importation of alpacas stool samples was approved by the Brazilian Institute of Environment (IBAMA; Brasília, DF, Brazil) license in 14BR012948/DF 02/20/2014.

The stool sample was initially analyzed by RT-PCR for detection of RVA. Viral dsRNA was extracted by using the Totally RNA® Kit, according to the manufacturer's instructions (Applied Biosystems/Ambion, Austin, TX, USA). Next, dsRNA was subjected to reverse transcription followed by PCR amplification of the VP6 and NSP4 genes. The cDNA of the VP6-encoding gene was synthesized by RT-PCR by using the primers VP6F-Deg 3′CTACDTGGTATTTYAAYCCAGT5′ described in this study and VP6R 3′ GTCCAATTCATNCCTGGTGG5′ previously described (Iturriza-Gómara et al., 2002). Primers NSP41a 3′GCCTTTTAAAAGTTCTGTTCC5′ and NSP42b 3′ GGTCACATTAAGACCGTTCC5′ were used for the NSP4-encoding gene (Kudo et al., 2001). PCR products were analyzed by 1.2% agarose gel electrophoresis and visualized by staining with ethidium bromide. Expected lengths of the amplicons were 370 bp for VP6 and 750 bp for NSP4. Products were subjected to a nested-PCR reaction with the VP6 primers VP6F.1-Deg 3′TKAGACCACCAAAYATGACRCCMG5′ (designed in this study) and VP6R (Iturriza-Gómara et al., 2002).

Primers used for nested-PCR amplification of NSP4 were NSP4.F.M 3′GAGAGAGCGC GTGCGGAAAGATGG5′ and NSP4.2b (Kudo et al., 2001). PCR conditions were as follows: 10 min at 95 °C followed by 40 cycles of 40 s at 94 °C, 1 min at 50 °C, and 1 min 30 s at 72 °C, and a final extension for 10 min at 72 °C. The amplicon generated was 729 bp. The PCR products were visualized as described above.

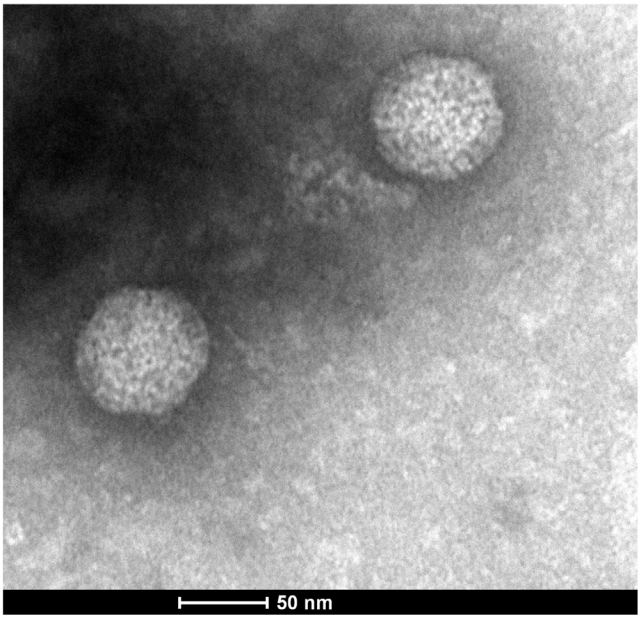

Fig. 1. Electron Micrograph of strain RVA/Alpaca-tc/PER/SA44/2014/G3P[40].

2.2. Virus isolation

The stool sample was suspended in 10% (w/v) Eagle's minimum essential medium (MEM-Eagle, Invitrogen, Carlsbad, CA) and then centrifuged (2,500 × *g* for 5 min). The supernatant was passed through a 0.22-μm syringe filter (Merck Millipore, Darmstadt, Germany), treated with 10 μg/ml trypsin (Sigma-Aldrich, St. Louis, MO) at 37 °C/30 min, and cultured with African green monkey cells (MA-104) in MEM-Eagle media supplemented with 1 μg/ml trypsin at 37 °C in an atmosphere of 5% CO_2. Control cells were cultured in the presence of trypsin (1 μg/ml). The culture was examined daily and harvested when the cytopathic effect was observed. When approximately 75% of the cells showed signs of CPE, the cultures were frozen and thawed once, and the lysate was treated with trypsin and inoculate onto MA-104 as described

Table 1
Nucleotide and amino acid percentage identity of complete genome segments of strain RVA/Alpaca-tc/PER/SA44/2014/G3P[40] compared to cognate gene sequence of the closer strains from GenBank database.

Gene	Cut-off values%[a]	Reference strain	Nucleotide%	Amino acid%	Genotype	Accession number
VP1	80	RVA/Human-wt/US/2012841174/2012/G8P[14]	92.9	98.0	R3	KJ411432
VP2	80	RVA/Human-wt/AUS/RCH272/2012/G3P[14]	88.9	97.8	C3	KF690126
VP3	85	RVA/Rhesus-tc/USA/TUCH/2002/G3P[24]	87.5	94.1	M3	EF583012
VP4	83	RVA/Simian-tc/USA/RRV/1975/G3P[3]	75.7	88.5	P[40][b]	EU636927
VP6	84	RVA/Bat-tc/CHN/MSLH14/2012/G3P[3]	89.2	99.2	I8	KC960623
VP7	81	RVA/Alpaca-tc/Per/K'ayra/3368-10/2011/G3P[11]	99.0	99.7	G3	KT250942
NSP1	79	RVA/Human-wt/AUS/RCH272/2012/G3P[14]	86.4	89.1	A9	KF690131
NSP2	85	RVA/Human-wt/US/09US7118/2009/G14P[24]	89.3	98.6	N3	KF541288
NSP3	85	RVA/Horse-wt/ARG/E403/2006/G14P[12]	94.8	96.1	T3	JF712585
NSP4	85	RVA/Bat-tc/CHN/MYAS33/2013/G3P[10]	90.3	95.4	E3	KJ020890
NSP5	91	RVA/Rhesus-tc/USA/TUCH/2002/G3P[24]	97.3	98.5	H6	FJ816617

[a] Nucleotide percentage cut-off values defined by the Rotavirus Classification Working Group (RCWG) (40).
[b] New VP4 genotype assigned by the RCWG.