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Short communication

Identification of two novel Rotavirus A genotypes, G35 and P[50], from Peruvian alpaca faeces

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A B S T R A C T

Rotaviruses (RV) are non-enveloped double-stranded RNA viruses in the Reoviridae family and in the Rotavirus genus, and are classified into eight species (A-H) and two candidate species (I and J) (Matthijnssens et al., 2012; Mihalov-Kovács et al., 2015; Bányai et al., 2017). Species A rotavirus (RVA) is a major cause of dehydrating diarrhea in humans and animals worldwide (Estes and Greenberg, 2013). The RVA genome consists of 11 segments of double-stranded RNA (dsRNA) encoding six structural proteins (VP1–4, VP6, and VP7) and five or six nonstructural proteins (NSP1–NSP5/6) depending on the strain (Estes and Greenberg, 2013). The RVA genomic classification nomenclature is based on all 11 segments of dsRNA (Matthijnssens et al., 2008, 2011). Currently, there are 35 VP7 (G), 50 VP4 (P), 26 VP6 (M), 30 NSP1 (A), 21 NSP2 (N), 21 NSP3 (T), 27 NSP4 (E), and 21 NSP5/6 (H) genotypes (Rotavirus Classification Workgroup (RCWG), 2016). In this study, we described two new RVA VP4 and VP7 genotypes in strain RVA/Alpaca-wt/PER/Alp11B/2010 from a Peruvian alpaca.

During the first week of February 2010, a diarrhea outbreak occurred that resulted in high rates of morbidity and mortality among neonatal alpacas in a community in Silli, Peru (Rojas et al., 2016a). This community is located in the southern highlands of Peru (14°24′45.3″S, 71°11′32.6″W; ~4000 m above sea level) in the province of Canchis in the state of Cusco. The animals were subjected to postmortem examinations at the Laboratory of Histology, Embryology and Veterinary Pathology, Universidad Nacional Mayor de San Marcos, Peru.

Intestinal lavage samples were obtained during necropsy by thoroughly washing the intestines with warm water, and then these samples were analyzed for E. coli, Clostridium spp., Eimeria spp., Cryptosporidium spp., coronavirus and RVA. The sample Alp11b was positive only for RVA (Rojas et al., 2016a). The importation of these alpaca specimens was approved by the Brazilian Institute of Environment (IBAMA; Brasília, DF, Brazil; license 14BR012948/DF 02/20/2014).

For RVA detection the lavage samples were diluted in 10% v/v using sterile phosphate-buffered saline and clarified by low speed centrifugation at 2500g for 5 min. Total RNA was extracted from 300 μl of the supernatant using the Totally RNA® Kit, according to the manufacturer’s instructions (Applied Biosystems/Ambion, Austin, USA). RVA detection was performed by RT-PCR with primers that ampliﬁed a small conserved portion of the VP6 gene (Rojas et al., 2016b). Once RVA was detected, the sample was subjected to additional RT-PCR ampliﬁcations to identify the VP4, VP7, VP6, NSP4, and NSP5 genotypes using speciﬁc primers (Appendix) that were previously published or designed based on RVA sequences available in GenBank. Overlapping sequences were assembled and edited using SeqMan, EditSeq, and MegAlign in the Lasergene software package (DNASTAR, Madison, WI).

Phylogenetic analysis was performed with MEGA software version 7.0.14 (Kumar et al., 2016). Dendograms were constructed using the Maximum Likelihood method based on the Kimura two-parameter model. The Kimura two-parameter was chosen using the Find Best Phylogenetic analysis was performed with MEGA software version 7.0.14 (Kumar et al., 2016). Dendograms were constructed using the Maximum Likelihood method based on the Kimura two-parameter model. The Kimura two-parameter was chosen using the Find Best DNA/Protein Model tool on the MEGA software. Statistical significance
was estimated by bootstrap analysis with 1000 pseudoreplicates. The sequences of our strain were compared to the sequences of the RVA strains obtained from GenBank. Genotypes were assigned to each genome segment by the web-based automated rotavirus genotyping tool RotaC (Maes et al., 2009). Sequences of Alp11B strain were aligned and compared to that of each corresponding gene of RVA strains obtained from GenBank, by using MegAlign, which are available in the Lasergene software package (DNASTAR, Madison, WI). Multiple alignments were done by using the complete open reading frame (ORF) for each gene segment. Nucleotide and amino acid identities were determined with the MegAlign p-distance algorithm. Sequences generated in this study were deposited into GenBank under accession numbers KM276820, KM276822, KY971955, KY971977 and KY972004.

The genotypes of the VP6, NSP4, and NSP5 genes in Alp11B were identified as I13, E16 and H6, respectively. The VP6 gene was closely related to the human strain Ecu534 (88.5% nucleotide identity). The NSP4 gene was closely related to the vicuña strain C75 (91.1% nucleotide identity), and the NSP5 gene was 100% identical to strain SA44, which is also from a Peruvian alpaca, and closely related to the
The nucleotide sequences of the VP4 and VP7 genes from Alp11B were not related to any RVA strain available in GenBank (Fig. 2). The highest nucleotide identity found for the VP4 gene was 76.7% with the human strain Ecu534, and the highest nucleotide identity for the VP7 sequence was 79.8% also with strain Ecu534. Both of these identity values were below the 80% cutoff value proposed by the RCWG (Matthijnssens et al., 2008). The VP4 and VP7 nucleotide sequences were submitted to the RCWG for further analysis and were assigned novel P[50] and G35 genotypes, respectively. However, the VP7 sequence was a borderline new genotype, and it would be possible that there might be some cross reactivity at the serological level with G20.

Few South American camelid (SAC) RVA strains have been characterized, but those that have showed great genotype diversity. The vicuña strain RVA/vicuña-wt/ARG/C75/2010/G8P[14] (Badaracco et al., 2013) and the guanaco strains RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14] and RVA/Guanaco-wt/ARG/RioNegro/1998/G8P[1] possess a bovine-like genome constellation, G8-P[1/14]-I2 (Matthijnssens et al., 2009). The guanaco strains also possess E12 and H3 NSP4 and NSP5 genotypes, respectively. The vicuña strain possesses the unique NSP4-E16 genotype, but the NSP5 gene was not characterized. In contrast, the VP4 and VP7 genes of the alpaca strains RVA/Alpaca-wt/PER/Alp11B/2010/G35P[50] and known human and animal rotavirus strains obtained from GenBank. Bootstrap values above 75% are given at branch nodes. The distance scale is in substitutions/site. Black triangles indicate Alp11B genes.

Strain Alp11B and strain SA44 were detected from the same location in Peru, and Alp11B also has a unique genetic constellation, G35-P[50]-I2.
E16-H6 with high identity to camelid, bat, and human-like RVA strains. The NSP4-E16 genotype was also found in vicuña strain C75 from a camelid in Argentina, thus it could be a common genotype in camels. The NSP5-H6 genotype of Alp11B was related to alpaca strain SA44 and bat strain 4754, but VP6-I13 is a rare genotype that has only been found in the human strain Ecu534, which was detected in Ecuador in 2006 (Solberg et al., 2009). The VP4 and VP7 genes Alp11B represent two new genotypes with the highest identities to the Ecu534 strain. Because of limited sample, the entire genome constellation of Alp11B could not be characterized and the origin of strain Alp11B remains unclear. Moreover, because the sequences were obtained directly from the clinical sample we could not exclude the possibility of mixed infection with two or more RVA strains in the sample.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.meegid.2017.08.019.

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