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Novel NSP1 genotype characterised in an African camel G8P[11] rotavirus strain

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ABSTRACT

Animal–human interspecies transmission is thought to play a significant role in influencing rotavirus strain diversity in humans. Proving this concept requires a better understanding of the complete genetic constellation of rotaviruses circulating in various animal species. However, very few whole genomes of animal rotaviruses, especially in developing countries, are available. In this study, complete genetic configuration of the first African camel rotavirus strain (RVA/Camel-wt/SDN/MRC-DPRU447/2002/G8P[11]) was assigned a unique G8-P[11]-I2-R2-C2-M2-A18-N2-T6-E2-H3 genotype constellation that has not been reported in other ruminants. It contained a novel NSP1 genotype (genotype A18). The evolutionary dynamics of the genome segments of strain MRC-DPRU447 were rather complex compared to those found in other camelids. Its genome segments 1, 3, 7–10 were closely related (>93% nucleotide identity) to those of human–animal reassortant strains like RVA/Human-tc/ITA/PA169/1988/G6P[14] and RVA/Human-wt/HUN/Hun5/1997/G6P[14], segments 4, 6 and 11 shared common ancestry (>95% nucleotide identity) with bovine rotaviruses like strains RVA/Cow-wt/CHN/DQ-75/2008/G10P[11] and RVA/Cow-wt/KOR/KJ19-2/XXXX/G6P[7], whereas segment 2 was closely related (94% nucleotide identity) to guanaco rotavirus strain RVA/Guanaco-wt/ARG/Rio_Negro/1998/G8P[1]. Its genetic backbone consisted of DS-1-like, AU-1-like, *artiodactyl*-like and a novel A18 genotype. This suggests that strain MRC-DPRU447 potentially emerged through multiple reassortment events between several mammalian rotaviruses of at least two genogroups or simply strain MRC-DPRU447 display a unique progenitor genotypes. Close relationship between some of the genome segments of strain MRC-DPRU447 to human rotaviruses suggests previous occurrence of reassortment processes combined with interspecies transmission between humans and camels. The whole genome data for strain MRC-DPRU447 adds to the much needed animal rotavirus data from Africa which is limited at the moment.

1. Introduction

Rotaviruses are the major cause of severe dehydrating diarrhoea in humans and animals worldwide (Mebus et al., 1969; Wani et al., 2003). Rotaviruses belong to the *Reoviridae* family and are composed of triple-layered capsid proteins that encase 11 genome segments of double-stranded RNA (dsRNA). With the exception of genome segment 11 that encodes two proteins; each segment encodes a single protein. Thus, there are six viral structural proteins (VP1–VP4, VP6, and VP7) and six non-structural proteins (NSP1–NSP6). Since the discovery of rotavirus, the dual classification system which uses the VP4 (P-types) and VP7 (G-type) encoding genes or the encoded outer capsid proteins, has been used to classify rotaviruses genotypes and serotypes (Estes and Kapikian, 2007). To date, 27 G- and 37 P-genotypes have been described worldwide (Abe et al., 2011; Matthijnssens et al., 2011a; Trojnar et al., 2013). This system has been recently standardized and extended to all 11 genes (Matthijnssens et al., 2008). To date, at least

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27G, 37P, 16I, 9R, 9C, 8M, 17A, 9N, 12T, 15E and 11H genotypes have been identified based on the eleven rotavirus A genes (Guo et al., 2012; Matthijnssens et al., 2011a,b; Papp et al., 2012; Trojnar et al., 2013).

Animal to human interspecies transmission has been singled out as one of the major causes of wide rotavirus strain diversity currently circulating in humans, especially in developing countries (Cunliffe et al., 2000; Esona et al., 2009; Ghosh et al., 2011b). Close proximity between animal and human dwellings is believed to accelerate interspecies transmission and/or single or multiple reassortment events. To fully validate this hypothesis, genetic characterisation of rotavirus strains circulating in animals is vital considering the vast amount of data already available from human rotaviruses. However, whole genome data for animal strains are limited from developing countries. Data from dual typing epidemiological surveys worldwide suggest that rotavirus strain diversity circulating in ruminants is complex. At least 8 VP4 (P[1], P[3], P[5], P[11], P[14], P[15], P[17] and P[21]) and 7 VP7 (G1, G3, G5, G6, G8, G10 and G18) distinct genotypes have been detected in cattle; 5 VP4 (P[1], P[7], P[11], P[12] and P[18]) and 6 VP7 (G3, G5, G8, G10, G13 and G18) genotypes in horses; whereas in sheep/goats, 7 VP4 (P[1], P[3], P[5], P[8], P[11], P[12] and P[18]) and 6 VP7 (G1, G6, G8, G9, G10 and G15) genotypes have been reported (Martella et al., 2010). Recently, data emerging from South America and the Middle-East has only characterised 3 VP4 (P[1], P[11] and P[14]) and 2 VP7 (G8 and G10) genotypes in rotaviruses circulating in camelids (Abo Hatab et al., 2008; Badaracco et al., 2013b; Matthijnssens et al., 2009; Papp et al., 2012). Unlike the genetic backbone of the camel strain RVA/Camel-wt/KUW/s21/2010/G10P[15] characterised in Kuwait (Papp et al., 2012) that has a I1-R1-C2-Mx-Ax-N2-T2-E15-H3 core genetic constellation, the guanaco rotaviruses from Argentina contains a I2-R2-C2-M2-A3/A11-N2-T6-E12-H3 genotype configuration which is typical of those frequently characterised in ruminants (Matthijnssens et al., 2011a). Recently, a I2-R2-C2-M2-Ax-N2-T6-E3-Hx genotype constellation was characterised in a wild vicuña (Vicugna vicugna; one of the four species of native camelids from South America) in Argentina which had a distinct NSP4 genotype compared to those reported in guanaco (Badaracco et al., 2013b). Previously, the VP4 and VP7 genotypes of group A rotaviruses were first detected in Sudanese camel calves in 2000 and 2002 (Ali et al., 2008, 2005). To date, there are no whole genome data for rotaviruses circulating in African camelids. We determined the whole genome constellation of a rotavirus strain with a unique G8P[11] VP4 and VP7 combination that was obtained from a camel in Sudan to understand its genetic make-up, evolutionary origin and to compare this strain with rotaviruses circulating in other host species.

2. Materials and methods

2.1. Camel faecal specimen

The stool specimen was collected in 2002 from a symptomatic 3 months old camel calf in Gedarif State, eastern Sudan, along the Sudanese and Ethiopian border. The sample was found to be group A rotavirus positive by enzyme immunoassay IDEIA™ Rotavirus kit (DAKO, UK). Due to the unusual G8 and P[11] genotype combination that were assigned to the VP7 and VP4 encoding genome segments of strain RVA/Camel-wt/SDN/MRC-DPRU447/2002/G8P[11], respectively, the nucleotide sequences of the other nine genome segments were also characterised to understand its complete genotype constellation.

2.2. Viral RNA extraction, amplification, sequencing and genotype assignment

Viral RNA was extracted from a 10% stool suspension in 2 ml of a 1:1 vertrel/water solution using an automated MagNA Pure Compact (Roche Applied Science, Indianapolis IN) with the Viral RNA isolation kit. The protocol was slightly modified, with external lysis prior to extraction. Rotavirus cDNA was synthesized and amplified from the dsRNA of all 11 genome segments using a One-Step RT-PCR kit (Qiagen, Inc., Valencia, CA) according to manufacturer's instructions. The cDNA was genotyped using previously published rotavirus multiplex PCR genotyping assays (Das et al., 1994; Gentsch et al., 1992; Iturriza-Gomara et al., 2004; Iturriza Gomara et al., 2002; Kerin et al., 2007; Matthijnssens et al., 2006a; Mijatovic-Rustempasic et al., 2011). Amplicons were analyzed by E Gel® EX Precast agarose gel system with SYBR safe stained DNA gels (Invitrogen, Carlsbad, CA, USA) for 20–30 min using blue light transilluminator. The purified PCR products were recovered from the gel and sequenced.

DNA cycle sequencing of each amplicon was performed with the same consensus primers used for RT-PCR, using a Big Dye Terminator cycle sequencing Ready kit v1.1 (Applied Biosystems, Inc., Foster City, CA). Previously published primers homologous to internal regions of each gene segment were also used (Mijatovic-Rustempasic et al., 2011). Cycle sequencing products were purified using Centri-sep spin columns (Princeton Separations, Inc., Adelphia, NJ), dried (Savant Instruments, Inc., Holbrook, NY) and reconstituted in 15 ml nuclease-free water. Automated separation and base-calling of cycle sequencing products was performed using an ABI 3130xl sequencer (Applied Biosystems, Foster City, CA). Overlapping sequence fragments were assembled and edited using Sequencher 4.8 (Gene Codes Corporation, Inc., Ann Arbor, MI).

2.3. Genotype assignment, determination of sequence homology and phylogenetic analysis

The genotypes were assigned to each genome segment of the study strain using the web-based automated rotavirus genotyping tool, RotaC (http://rotac.regatools.be; Maes et al., 2009). The selected rotavirus nucleotide sequences used to infer phylogenetic trees were retrieved from the GenBank (accession numbers listed in Supplementary data 1). Sequences were aligned using the MUSCLE program within MEGA version 5 (Tamura et al., 2011). Once aligned, the DNA Model Test program implemented in MEGA (Tamura et al., 2011) was used to identify the optimal evolutionary models that best fitted the sequence datasets. Using corrected Akaike Information Criterion (AICc) the following models; TN93+G+I (VP1, VP2, VP3, VP6, and NSP5), GTR+G+I (NSP4), T92+G+1 (NSP1 and VP4) and T92+G (NSP2 and VP7) were found to best fit the sequence data for the different genes. Using these models, maximum likelihood trees were constructed using MEGA version 5 with number of bootstrap replications of 500 for branch support. Nucleotide and amino acid sequence identities were determined using the *p*-distance algorithm of MEGA version 5 software (Tamura et al., 2011).

3. Results and discussion

3.1. Genetic constellation of strain RVA/Camel-wt/SDN/MRC-DPRU447/2002/G8P[11]

The sequences generated for all 11 genome segments of the camel strain MRC-DPRU447 were deposited in GenBank under accession numbers: KC257086–KC257096. The sizes of the nucleotide and deduced amino acid sequences are listed in Supplementary