Veterinary Microbiology 191 (2016) 52–59

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Short communication

The first detection and whole genome characterization of the G6P[15] group A rotavirus strain from roe deer

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ARTICLE INFO

Article history:
Received 1 April 2016
Received in revised form 25 May 2016
Accepted 26 May 2016

Keywords:
Rotavirus
G6P[15]
Roe deer
Phylogenetic analysis
Next generation sequencing

ABSTRACT

Although rotaviruses have been detected in a variety of host species, there are only limited records of their occurrence in deer, where their role is unknown. In this study, group A rotavirus was identified in roe deer during a study of enteric viruses in game animals. 102 samples of intestinal content were collected from roe deer (56), wild boars (29), chamois (10), red deer (6) and mouflon (1), but only one sample from roe deer was positive. Following whole genome sequence analysis, the rotavirus strain D38/14 was characterized by next generation sequencing. The genotype constellation, comprising 11 genome segments, was G6-P[15]-I2-R2-C2-M2-A3-N2-T6-E2-H3. Phylogenetic analysis of the VP7 genome segment showed that the D38/14 rotavirus strain is closely related to the various G6 zoonotic rotavirus strains of bovine-like origin frequently detected in humans. In the VP4 segment, this strain showed high variation compared to that in the P[15] strain found in sheep and in a goat. This finding suggests that rotaviruses from deer are similar to those in other DS-1 rotavirus groups and could constitute a source of zoonotically transmitted rotaviruses. The epidemiological status of group A rotaviruses in deer should be further investigated.

1. Introduction

Rotaviruses are members of the *Reoviridae* family that are characterized by a segmented dsRNA genome enclosed in a triple layer capsid. Eleven segments of the genome generate six structural proteins (VP1-4, VP6 and VP7) and six non-structural proteins (NSP1-6) (Estes and Greenberg, 2013). The classification of rotaviruses into at least eight groups, from A to H, is based on the antigenic properties of the VP6 proteins (Estes and Greenberg, 2013; Matthijnssens et al., 2012). From a medical and veterinary perspective, the rotavirus group A (RVA) is the most important member of the genus, and is associated with acute gastroenteritis in children and in young domestic and wild animals (Estes and Greenberg, 2013; Martella et al., 2010; Matthijnssens et al., 2010). The genetic characteristics of the VP7 and VP4 rotavirus genes are of significant interest for rotavirus molecular epidemiology, since they determine the dual genotype classification (Gentsch et al., 1992; Gouvea et al., 1990). To date, 29G and 41P genotypes have been described, with more than sixty G–P combinations. The Rotavirus Classification Working Group (RCWG) has presented a whole genome based genotyping system, based on the nucleotide sequence identity cut-off percentages for each of the 11 RVA gene segments (Matthijnssens et al., 2011).

Despite the differences between genotypes, there have been several reports of interspecies transmission between animals and humans, as well as between various animal species (Bányai et al., 2010; Matthijnssens et al., 2010; Steyer et al., 2008; Trojnar et al., 2013). It is a matter of concern that wildlife, as well as domestic animals may act as a source of infection for humans (Martella et al., 2010). There is only one study, from 1979, in which a deer rotavirus was detected using gel electrophoresis (Smith and Tzipori, 1979; Tzipori et al., 1976). Nevertheless, the genome of deer rotaviruses, knowledge of which would provide limited insights into the evolutionary patterns of these viruses, has not been analysed in detail (Ghosh and Kobayashi, 2014).

A positive sample from roe deer, containing RVA, was detected during a survey throughout Slovenia designed to screen some of

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http://dx.doi.org/10.1016/j.vetmic.2016.05.019
0378-1135/

the game animals as a potential source of enteric viruses and to evaluate the possible zoonotic transmission. The aim of the present study was to analyse the whole genome of the novel rotavirus strain detected in a roe deer. This first reported whole genome analysis provides additional information about rotavirus circulation in nature and its evolutionary pattern.

2. Materials and methods

2.1. Sample collection and molecular detection of RVA

In 2014 and 2015 a survey throughout Slovenia was performed to screen certain game animals as a potential source of rotaviruses. In total, 102 samples of game animal intestinal content were collected from five different wildlife species, comprising 56 samples from roe deer (*Capreolus capreolus*), 29 from wild boars (*Sus scrofa*), 10 from chamois (*Rupicapra rupicapra*), 6 from red deer (*Cervus elaphus*) and 1 from mouflon (*Ovis musimon*). The age of game animals was from 5 months to 10 years and were culled in 5 Slovenian hunting families between July 2014 and March 2015. The RVA positive sample D38/14 was collected in September 2014 in Sv. Ožbolt in hunting family Škofja Loka, in the area where there is no livestock pasture or farmlands. A sample was collected from female roe deer aged 5 months having the appropriate weight and no specific clinical signs. Samples of intestinal content of each animal were collected by hunters after culling. The lower part of each intestine was placed in a sterile plastic bag. Samples were stored at −20 °C and, as soon as possible, sent to the Veterinary Faculty where they were stored below − 60 °C until used. 10% suspensions of samples were prepared in RPMI 1640 (Thermo Fisher Scientific, Carlsbad, CA, USA) and centrifuged for 10 min at 1000*g*. The supernatant was used for nucleic acid (NA) extraction using the QIAamp viral RNA mini kit, according to the manufacturer's instructions (Qiagen, Germany). Each sample was tested with a real-time RT-PCR assay using VP2-specific primers, as described (Gutiérrez-Aguirre et al., 2008). Each sample was also tested with RT-PCR for the detection and amplification of a short VP7 segment (Machnowska et al., 2014) followed by direct sequencing of the amplicon for confirmation.

2.2. Enrichment of viral particles

Rotavirus enrichment, with separation from background NA, was achieved for positive sample using ion exchange chromatography, with methacrylate monolithic supports in a manner similar to that described by Steyer et al. (2013) with minor modifications. Two mL of 10% suspension of intestinal content was diluted in 18 ml of loading buffer (50 mM HEPES, pH 7) and centrifuged at 2000*g* for 2 min. In order to prevent clogging of the monolithic chromatography column, the supernatant was filtered through a 0.45 μm cellulose acetate filter (Sartorius, Göttingen, Germany). Filtrated sample was loaded on to a CIM QA disk monolithic column (BIA separations, Ajdovščina, Slovenia) at a flow rate of 3 ml/min, using an AKTA purifier chromatographic system (GE Healthcare, Uppsala, Sweden). After loading, non-bound material was washed off with 12 ml of loading buffer. Bound material was then eluted with a linear gradient from 0 to 1 M NaCl in 50 mM HEPES, pH 7 in 64 column volumes; 26 fractions of 1 ml were collected.

2.3. RNA extraction of selected chromatography fractions and evaluation of enrichment

Total RNA was extracted from fractions 3 to 26 (250 μl) using the Trizol® method (Invitrogen, GB) and phase lock gel tubes (5 PRIME, Germany) according to the manufacturer's instructions. The RNA extracted from each fraction was tested with real-time RT-PCR, as described by Gutiérrez-Aguirre et al. (2008), to determine the theoretical viral RNA concentration in units/μl. To obtain the fraction with the highest virus/background NA ratio, the total NA concentrations in fractions from 11 to 17 were measured, using a Qubit® fluorometer (Invitrogen, Carlsbad, CA, USA) with a Qubit® dsDNA HS assay kit and a Qubit® RNA HS assay kit, following the manufacturer's instructions (Invitrogen, CA, USA). Accordingly, fraction 14 was used for next generation sequencing (NGS).

2.4. NGS and analysis of sequence reads

The RNA library was prepared using the Ion Total RNA Sequencing Kit v2 (Thermo Fisher Scientific – Ion Torrent, Carlsbad, CA, USA) according to the manufacturer's protocol (4476286, revision D). Emulsion PCR and enrichment were carried out using the Ion PGM™ Template OT2 200 Kit (Thermo Fisher Scientific—Ion Torrent, Carlsbad, CA, USA) according to the manufacturer's protocol (MAN0007221, revision A.0). The concentrations of library DNA fragments and their size distribution were determined with a LabChip GX instrument using the DNA High-Sensitivity Assay (Perkin Elmer, Waltham, MA, USA). The amplified library was sequenced on the Ion PGM platform using the Ion PGM HiQ Sequencing Kit and Ion 314 Chip v2 (Thermo Fisher Scientific—Ion Torrent, Carlsbad, CA, USA). Sequenced Ion Torrent reads were checked for quality and trimmed using Ion Torrent Suite v5.0.2. Reads from the NGS analysis were mapped to the concatenated reference genome from PA169 strain obtained from GenBank (accession numbers EF554126–EF554136) using Geneious software with default parameters (Biomatters Ltd., Auckland, New Zealand). The consensus sequence was constructed by manual examination of polymorphisms and indels compared to the reference sequence.

Genome segments were aligned with the most identical strains deposited in GenBank using the BLAST search for each of the genome segments. The genome segment genotype was assigned according to the genotype cut-offs determined previously with the recommendations for rotavirus classification proposed by the RCWG (Matthijnssens et al., 2008).

2.5. Phylogenetic analysis of the genome segments

Nucleotide alignment was carried out for the 11 genome segments, using the ClustalW algorithm. Sequences were aligned with the rotavirus genomes deposited in GenBank. Maximum Likelihood phylogenetic trees were constructed, based on the Kimura-2 parameter model and using MEGA 6.06 software (Tamura et al., 2013). Branch statistics were calculated with bootstrap analysis of 1000 replicates.

3. Results

Of the 102 samples tested, only one sample of intestinal content from roe deer (D38/14) collected in September 2014 in the hunting family Škofja Loka, Slovenia was positive for rotavirus RNA with real-time RT-PCR as well as with RT-PCR. The RT-PCR amplification product from the VP7 region of rotavirus was sequenced and identified as an RVA of genotype G6, most closely identical to the bovine-like human PA169 strain with G6P[14] genotype combination.

3.1. NGS analysis and construction of the complete genome sequence

According to the short VP7 nucleotide sequence the whole genome sequence of the PA169 strain was used as the reference sequence for constructing the whole genome sequence of RVA