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Short Communication

Full genomic analysis of rabbit rotavirus G3P[14] strain N5 in China: Identification of a novel VP6 genotype

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ABSTRACT

Group A rotaviruses (RVAs) are major pathogens associated with acute gastroenteritis in young children and in a wide variety of domestic animals. The full-length genome of a rabbit RVA strain, RVA/Rabbit-tc/CHN/N5/1992/G3P[14], showed a G3-P[14]-I17-R3-C3-M3-A9-N1-T1-E3-H2 genomic configuration. A novel VP6 genotype, I17, was confirmed by the Rotavirus Classification Working Group. Phylogenetic analyses revealed that strain N5 possessed VP1–3, VP7, NSP1–2 and NSP4 genes closely related to those of the simian strain TUCH, NSP3 and NSP5 genes closely related to the human strains Wa and 69M, and a VP4 gene closely related to the rabbit strain 30/96 and sheep strain OVR762. The RRV and TUCH shared their ancestry with canine/feline RVAs and showed a close relationship to the human T152/feline-like RVAs. Comparison with the genotypes of the simian strains TUCH and RRV, canine strains A79-10, CU-1, K9, feline strains Cat2 and Cat97, and human strains T152 and 69M showed that RVA/Rabbit-tc/CHN/N5/1992/G3P[14] was possibly of feline/canine origin, or was a multiple reassortment involving canine, feline and human rotaviruses. The sequencing and phylogenetic analyses of rotavirus genomes is critical to the elucidation of the patterns of virus evolution.

1. Introduction

Group A rotaviruses (RVAs) are major pathogens associated with acute gastroenteritis in young children and in a wide variety of domestic animals. Rotaviruses are members of the *Reoviridae* family and are characterized by a segmented double-stranded RNA genome (Estes and Kapikian, 2007). The rotavirus genome consists of 11 segments, encoding six viral structural proteins (VP1–VP4, VP6, and VP7) and six nonstructural proteins (NSP1–NSP6) (Estes and Kapikian, 2007).

The mature infectious rotavirus particle is a triple-layered icosahedral capsid (Estes and Kapikian, 2007). The inner layer of the rotavirus virion is composed mainly of VP2, which encases VP1, the viral RNA-dependent RNA polymerase, and VP3, the viral capping enzyme. The middle layer of the virion is composed entirely of VP6, which possesses the group and subgroup antigens. The outer layer of the virion is formed by the VP7 and VP4 proteins. They are capable of independently eliciting neutralizing antibodies, which were initially used to define rotavirus G (glycoprotein) and P (protease-sensitive) serotypes, respectively (Gentsch et al., 2005). Recently, the Rotavirus Classification Working Group (RCWG) has proposed a classification system based on nucleotide sequence identity cut-off percentages for each of the 11 RVA genome segments, which has resulted in the assignment of appropriate genotypes to each of the 11 genes of RVA strains (Maes et al., 2009; Matthijnssens et al., 2008a,b). So far, 27 G and 35 P genotypes have been identified globally, in various combinations of G and P genotypes (Abe et al., 2011; Collins et al., 2010; Esona et al., 2011; Matthijnssens et al., 2008a,b; Matthijnssens et al., 2011a).

To date, the complete open reading frame (ORF) sequences of all 11 genome segments of almost 80 rotavirus strains have been determined for viruses isolated from human, pig, cow, dog, cat, rhesus, simian, horse, rabbit, mouse, goat, sheep, turkey and chicken hosts (Arora and Chitambar, 2011; Banyai et al., 2010; Chen et al., 2008; Ciarlet et al., 2008; Esona et al., 2009; Ghosh et al., 2010; Ghosh et al., 2011; Ito et al., 2001; Matthijnssens et al., 2006; Matthijnssens et al., 2008a,b; Mukherjee et al., 2009; Rahman et al., 2010; Ramani et al., 2009; Small et al., 2007; Trojnar et al., 2009; Tsugawa and Hoshino, 2008; Wang et al., 2010). Lapine rotavirus strains have been isolated in Canada, Japan, Italy, the United States, and Hungary, and those that have been characterized are serotype G3P[14] and G3P[22] (Martella et al., 2003; Banyai et al., 2005).

In this study, we report the full genomic analysis of a rabbit rotavirus strain, RVA/Rabbit-tc/CHN/N5/1992/G3P[14], that was

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isolated in 1992 and identified in China. The novel classification system used, which is based on percentage identity cut-off values, illustrated the phylogenetic relationships of all 11 rotavirus genome segments. Phylogenetic analyses revealed that strain N5 possessed VP1–3, VP7, NSP1–2 and NSP4 genes closely related to those of the simian strain TUCH, NSP3 and NSP5 genes closely related to the human strains Wa and 69M, and a VP4 gene closely related to the rabbit strain 30/96 and sheep strain OVR762; the VP6 gene was assigned to a new genotype, I17, by the RCWG.

2. Materials and methods

2.1. Virus propagation and purification

Fecal sample N5 was collected from a 1-month-old rabbit in China in 1992 and stored at Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences. Briefly, the supernatant of the intestinal sample was treated with gentamicin and trypsin (Gibro BRL, USA), and the virus was isolated successfully by tissue culture in MA-104 cells. After five passages, rabbit rotavirus N5 showed a clear cytopathogenic effect (CPE) and was purified by plaque assay in triplicate. The rabbit rotavirus N5 was harvested, followed by extraction with an equal volume of 1,1,2-trichlorotrifluoroethane. The aqueous phase was layered in Tris-buffered saline (TBS), pH7.4 (20 mM Tris hydrochloride, 100 mM NaCl, 5 mM KCl, 0.5 mM $MgCl_2$, 5 mM $CaCl_2$), and centrifuged in an SW28 rotor at 185,000*g*, at 4 °C for 2 h. The virus pellet was suspended in TBS.

2.2. RNA extraction

Rotavirus dsRNA was extracted from the virus pellet using a QIAamp Viral RNA mini kit, according to the manufacturer's instructions (Qiagen, Netherlands). The total RNA recovered was suspended in 50 μL of RNase-free water and stored at −80 °C until needed.

2.3. RT–PCR and nucleotide sequencing

The RT–PCR primers for all genomic segments of the lapine rotavirus N5 strain are shown in Supplementary Table 1. The double-stranded RNA was denaturated by heating at 95 °C for 3 min, followed by rapid cooling in an ice-water bath. Reverse transcription and PCR were performed according to the manufacturer's instructions (Invitrogen, USA). The PCR products from each gene fragment were purified using a Gel Extraction kit (Promega, USA) according to the manufacturer's instructions. The purified PCR products were cloned into the pMD18-T vector (TaKaRa, Dalian, China) and sequenced commercially using M13 forward and M13 reverse primers in an ABI 3730 DNA Analyzer in triplicate (Sangon, Shanghai, China).

The sequences of the genome segments were assembled from the fragments of determined sequences using the SeqBuilder module of the DNASTAR software package (Lasergene, USA). The ORFs were identified and amino acid sequences were deduced from the nucleotide sequences using the same module. Searches for nucleotide and protein sequence similarity were performed using the National Center for Biotechnology Information (NCBI, National Institutes of Health, Bethesda, MD) BLAST (Basic Local Alignment Search Tool) program.

2.4. Phylogenetic analyses

Phylogenetic and molecular evolutionary analyses were conducted using the MEGA version 5.05 software package (Tamura et al., 2011). Genetic distances were calculated using the Kimura-2 correction parameter at the nucleotide level, and phylogenetic trees were constructed by using the maximum likelihood method with 1000 bootstrap replicates. The genotypes of the genome segments for N5 were assigned on the basis of the recommendations of RCWG, using the online RotaC genotyping tool (http://rotac.regatools.be/).

2.5. Nucleotide sequence accession numbers

The nucleotide sequence data reported in this paper were deposited in GenBank using the National Center for Biotechnology Information (NCBI) Bankit submission tool (http://www.ncbi.nlm.nih.gov/BankIt/) under accession numbers JQ423897–JQ423907, respectively.

Table 1
Genotype of eleven gene segments of RVA strain N5 sequenced in this study with those selected human, animal, and avian RVA strains.

Strain	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/Rabbit-tc/CHN/N5/1992/G3P[14]	G3	P[14]	I17	R3	C3	M3	A9	N1	T1	E3	H2
RVA/Rabbit-tc/ITA/30/96/1996/G3P[14]	G3	P[14]	I2	R2	C2	M3	A9	N2	T6	E5	H3
RVA/Human-wt/BEL/B4106/2000/G3P[14]	G3	P[14]	I2	R2	C2	M3	A9	N2	T6	E5	H3
RVA/Human-tc/THA/T152/1998/G12P[9]	G12	P[9]	I3	R3	C3	M3	A12	N3	T3	E3	H6
RVA/Human-wt/CHN/TB-chen/1996/G2P[4]	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Simian-tc/USA/RRV/1975/G3P[3]	G3	P[3]	I2	R2	C3	M3	A9	N2	T3	E3	H6
RVA/Rhesus-tc/USA/TUCH/2002/G3P[24]	G3	P[24]	I9	R3	C3	M3	A9	N1	T3	E3	H6
RVA/Cat-tc/AUS/Cat2/1984/G3P[9]	G3	P[9]	I3	R3	C2	M3	A3	N1	T6	E3	H3
RVA/Cat-tc/AUS/Cat97/1984/G3P[3]	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Dog-tc/USA/CU-1/1982/G3P[3]	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Dog-tc/USA/K9/1981/G3P[3]	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Dog -tc/USA/A79-10/1979/G3P[3]	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Human-tc/USA/HCR3A/1984/G3P[3]	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Human-tc/IDN/69M/1980/G8P4[10]	G8	P[10]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-tc/ISR/RO1845/1985/G3P[3]	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
RVA/Sheep-tc/CHN/Lamb-NT/xxxx/G10P[15]	G10	P[15]	110	R2	C2	M2	A11	N2	T6	E2	H3
RVA/Sheep-tc/ESP/OVR762/2002/G8P[14]	G8	P[14]	12	R2	C2	M2	A11	N2	T6	E2	H3
RVA/Cow-tc/VEN/BRV033/1990/G6P6[1]	G6	P[1]	12	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Cow-tc/FRA/RF/1982/G6P[1]	G6	P[1]	12	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Pig-tc/VEN/A131/1988/G3P9[7]	G3	P[7]	15	R1	C2	M1	A1	N1	T1	E1	H1
RVA/Pig-tc/MEX/YM/1983/G11P9[7]	G11	P[7]	15	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-tc/USA/OSU/1977/G5P9[7]	G5	P[7]	15	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Pigeon-tc/JPN/PO-13/1983/G18P[17]	G18	P[17]	I4	R4	C4	M4	A4	N4	T4	E4	H4
RVA/Chicken-tc/GER/02V0002G3/2002/G19P[30]	G19	P[30]	I11	R6	C6	M7	A16	N6	T8	E10	H8