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Short communication

Cloning and nucleotide sequence analyses of 11 genome segments of two American and one British equine rotavirus strains

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ABSTRACT

Group A equine rotavirus (ERV) is the main cause of diarrhea in foals and causes severe economic loss due to morbidity and mortality on stud farming worldwide. Molecular evolution of equine rotaviruses remains understudies. In this study, whole-genomic analysis of 2 group A ERV, FI-14 (G3P[12]), H-2 (G3P[12]) isolated from American, and FI23 (G14P[12]) from British was carried out and genotype constellations were determined as G3-P[12]-I6-R2-C2-M3-A10-N2-T3-E2-H7 for FI-14; G14-P[12]-I2-R2-C2-M3-A10-N2-T3-E2-H7 for FI23; and G3-P[12]-I6-R2-C2-M3-A10-N2-T3-E2-H7 for H-2, respectively. With the exception of the VP7 and VP6 gene, 2 G3P[12] strains (FI-14 and H-2) and one G14P[12] strain (FI23) were highly related genetically. Of note, the VP6 genotype of H-2 strain was previously reported to be I2, however, sequence and phylogenetic analyses demonstrated that it was I6. Therefore, it showed that G3P[12] ERV strains and G14P[12] ERV strains bore a distinct VP6 genotype: I6 for G3P[12] strains and I2 for G14P[12] strains. Moreover, it demonstrated that T-cell epitope 299P–300P/Q residues (PP/Q) of VP6 may be considered as I2 ERV typical molecular marker, which facilitates the analysis of the molecular evolution of equine rotaviruses.

1. Introduction

Rotaviruses form a genus in the *Reoviridae* family and are classified into 7 distinct groups (A to G) or a tentative H group according to VP6 gene sequences. Group A rotaviruses (RVA) are major pathogens associated with acute gastroenteritis in humans and animals (Kapikian et al., 2001).

Rotavirus genome consists of 11 segmented double-strand RNA (dsRNA), being enclosed in a triple-layered capsid. The VP7 and VP4 genes, encoding outer capsid, are used to classify rotaviruses into G and P genotypes, respectively (Kapikian et al., 2001). Group A equine rotavirus (ERV) is the major cause of diarrhea in foals up to 3 months of age and the acute dehydration can incur severe economic burden due to morbidity and mortality in studs (Bailey et al., 2013; Ghosh et al., 2013; Papp et al.,

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2013). The G3P[12] and G14P[12] are the most prevalent equine rotavirus strains worldwide (Ghosh et al., 2013; Papp et al., 2013).

ERV FI-14 strain (RVA/Horse-tc/USA/FI-14/1981/G3P[12]) was identified to be G3P[12] genotype and was characterized to bear both subgroup I and subgroup II specificities. However, the whole genome sequence of ERV FI-14 strain was unavailable except segments 4, 5, 6 and 10 (Hoshino et al., 1987). Strain FI23 (RVA/Horse-tc/USA/FI23/1981/G14P[12]) was originally isolated from a diarrheic foal and only segments 4, 5, 6, 9 and 10 were sequenced (Taniguchi et al., 1994). H-2 strain (RVA/Horse-tc/GBR/H-2/1976/G3P[12]) was isolated from a Prezewalski foal and identified as a G3P[12] strain and sequences of segments 4, 5, 6 and 10 were determined (Ciarlet et al., 2000). Until now, the unavailability of whole-genome sequences of equine rotaviruses of epidemiologic importance impedes us to analyze the characteristics, evolution or possible interspecies transmission.

In 2009, a novel nomenclature system was proposed by a Rotavirus Classification Working Group (RCWG) based on complete nucleotide sequences of all 11 genomic RNA segments (Matthijnssens et al., 2008a,b, 2011). Here we report the complete genome classification of 2 American strains, FI-14 and FI23 and one British strain H-2ERV based on all the 11 segment sequence data by using RotaC v2.0 (Maes et al., 2009).

2. Materials and methods

RNA samples of ERV FI-14, FI23 and H-2 were provided by Yasutaka Hoshino (NIH) and polyadenylated using A-plus poly(*A*) Tailing Kits (Epicentre Biotechnologies, USA) as described previously (Meis, 2006) and the cDNAs were synthesized using oligodT primer with the SuperScript II RT kit (Invitrogen) as described previously (Cook and McCrae, 2004). PCR specific primers were designed according to GenBank database or published data (Tsugawa and Hoshino, 2008).

The sequencing of rotavirus segments was performed with the BigDye terminator v3.1 cycle sequencing reaction kit (Applied Biosystems Group) on an ABI 3730 DNA analyzer (Applied Biosystems Group). Sequence data were analyzed using Sequencher 4.7 (Gene Codes Corporation) or BLAST (NCBI) program. Phylogenetic analysis was performed using MEGA6 software (Tamura et al., 2011, 2013). The nomenclature of the 3 ERV strains were determined using the RotaC 2.0 (http://rotac.regatools.be/) (Maes et al., 2009). The RT-PCR and sequencing primers are listed in Supplementary Table S1.

GenBank accession numbers of three ERVs: The VP1 to NSP5 of ERV strain FI14 are KM454481–KM454491; H-2 VP1 KM454492; H-2 VP2 KM454493; H-2 VP3 KM454494; H-2 VP4 KM454495; The VP1 to NSP5 of ERV strain H-2 VP1–NSP5 are KM454492–KM454502; The VP1 to NSP5 of ERV strain FI23 are KM454503–KM454513.

3. Results

According to all the 11 genomic RNA segment sequences, a novel nomenclature for the 3 ERV strains were genotyped with RotaC v2.0b tool (http://rotac.regatools.be/) (Maes et al., 2009) and the complete genotype constellations were showed in Table 1. The genome configuration of FI23 strain was distinct from the other 2 G3P[12] strains, with G14 VP7 genotype and I2 VP6 genotype (Table 1). Similar to all other reported ERV strains, the 3 ERV strains shared the P[12], C2, M3, A10, N2, T3 and H7 genotypes. The I6 genotype was associated with G3 genotype and the I2 genotype was associated with G14 genotype (Table 1). This finding was conflicted with a previous study where I6 genotype of the G3P[12] strain H-2 was reported as I2 (Matthijnssens et al., 2012).

The VP7 sequences of FI-14 and H-2 formed a phylogenetic genotype cluster for sharing higher than 98% identity (99%) and formed a sub-genotype with other 7 Japanese ERV strains. Obviously, the G3 genotype divided into Japanese sub-lineage (G3a) and Argentine sub-lineage (G3b). FI23 was separated to a single phylogenetic genotype cluster because it shared less than 84% identity (82.4–83.4%) with FI-14 and H-2 strains (Fig. 1A). The G14 genotype strains were divided into 2 sub-lineages, Argentine sub-lineage (G3a) and Japanese sub-lineage (G14b). The results suggested that G3P[12] and G14P[12] ERV strains were the dominant epidemic strains in equine.

The VP4 nucleotides of FI-14, FI23 and H-2 shared higher than 98.8% identity (98.8–99.7%) and formed a phylogenetic genotype cluster with most of Japanese ERV strains and the 3 Argentine ERV strains also formed a single P[12] sub-genotyped. The other 4 ERV strains,

Table 1
The genotyping of the three equine rotavirus strains in this study and compared with selected sequenced ERV group A reference strains.

ERV strains	Genotypes
*RVA/Horse-wt/ARG/E30/1993/G3P[12]	G3-P[12]-I6-R2-C2-M3-A10-N2-T3-E12-H7
*RVA/Horse-wt/IRL/03V04954/2003/G3P[12]	G3-P[12]-I6-R2-C2-M3-A10-N2-T3-E2-H7
RVA/Horse-tc/GBR/H-2/1976/G3P[12]	**G3-P[12]-I6-R2-C2-M3-A10-N2-T3-E2-H7**
RVA/Horse-tc/USA/FI-14/1981/G3P[12]	**G3-P[12]-I6-R2-C2-M3-A10-N2-T3-E2-H7**
RVA/Horse-tc/USA/FI23/1981/G14P[12]	**G14-P[12]-I2-R2-C2-M3-A10-N2-T3-E2-H7**
*RVA/Horse-wt/ARG/E403/2006/G14P[12]	G14-P[12]-I2-R2-C2-M3-A10-N2-T3-E12-H7
*RVA/Horse-wt/ARG/E4040/2008/G14P[12]	G14-P[12]-I2-R2-C2-M3-A10-N2-T3-E12-H7
* RVA/Horse-wt/IRL/04V2024/2004/G14P[12]	G14-P[12]-I2-R2-C2-M3-A10-N2-T3-E2-H7
* RVA/Horse-wt/ZAF/EqRV-SA1/2006/G14P[12]	G14-P[12]-I2-R2-C2-M3-A10-N2-T3-E2-H7

* Reference data from Matthijnssens et al. (2012).