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Short communication

Evidence for the porcine origin of equine rotavirus strain H-1

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ABSTRACT

Equine group A rotavirus (RVA) strain H-1 (RVA/Horse-tc/GBR/H-1/1975/G5P9[7]) was found to have VP4, VP6-7, NSP1 and NSP4 genes of porcine origin. In order to obtain conclusive information on the exact origin and evolution of this unusual equine strain, the remaining six genes (VP1–3, NSP2–3 and NSP5 genes) of strain H-1 were analyzed in the present study. By whole genomic analysis, strain H-1 exhibited a porcine RVA-like genotype constellation (G5-P[7]-I5-R1-C1-M1-A8-N1-T1-E1-H1), different from those of typical equine RVA strains. The VP2–3 and NSP2–3 genes of strain H-1 were found to originate from porcine RVAs. On the other hand, it was difficult to pinpoint the exact origin of the VP1 and NSP5 genes of strain H-1, though phylogenetically, these genes appeared to be possibly derived from porcine or Wa-like human strains. Taken together, at least nine (VP2–4, VP6–7 and NSP1–4 genes) of the 11 gene segments of strain H-1 were found to be of porcine origin, revealing a porcine RVA-like genetic backbone. Therefore, strain H-1 is likely a porcine RVA strain that was transmitted to horses.

1. Introduction

Equine group A rotavirus (RVA) is a major cause of severe diarrhea in foals (Browning and Begg, 1996; Collins et al., 2008; Imagawa et al., 1991; Saif et al., 1994). To date, RVAs are classified into at least 27 G and 35 P genotypes on the basis of differences in the nucleotide sequences of their outer capsid VP7 and VP4 genes, respectively (Matthijnssens et al., 2011a). Among them, G3P[12] and G14P[12] are regarded as the common equine RVA strains (Ciarlet et al., 1994; Collins et al., 2008; Elschner et al., 2005; Garaicoechea et al., 2011; Nemoto et al., 2011; Ntafis et al., 2010; Tsunemitsu et al., 2001). On the other hand, unusual RVA strains, such as G3P[3], G5P[7], G6P[1], G8P[1], G10P[1], G10P[11], G13P[18] strains, have also been detected in horses (Browning et al., 1991; Ciarlet et al., 2001; Garaicoechea et al., 2011; Imagawa et al., 1991, 1993; Iša et al., 1996; Taniguchi et al., 1994). The G5 and P[7] RVA genotypes have been reported widely in pigs (Collins et al., 2010; Martella et al., 2010).

Equine RVA strain H-1 (RVA/Horse-tc/GBR/H-1/1975/G5P9[7]) was detected in a diarrheic stool sample collected from a young foal at a racing stable in United Kingdom in 1975 (Flewett et al., 1975), and isolated in AGMK and MA-104 cells by Hoshino et al., 1983. Nucleotide sequencing of the partial genome (VP4, VP6–7, NSP1 and NSP4 genes), RNA–RNA hybridization and serological studies pointed towards the porcine origin of strain H-1 (Ciarlet et al., 2000, 2001; Flewett et al., 1975; Hoshino et al., 1983; Iša and Snodgrass, 1994; Kojima et al., 1996; Matthijnssens et al., 2008; Taniguchi et al., 1994; Wu et al., 1993). However, whole genomic analysis of a RVA strain is essential to obtain conclusive information on its origin and evolution (Ghosh and Kobayashi, 2011; Matthijnssens et al., 2008, 2011a). Therefore, the nearly full-length nucleotide sequences (full-length sequences excluding the 5′- and 3′- end primer binding regions) of the remaining six gene segments (VP1–3, NSP2–3 and NSP5 genes) of strain H-1 were analyzed in the present study. Moreover, the previously reported nucleotide sequence of the NSP1 gene of strain H-1 (GenBank accession no. U23728, Kojima et al.,

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1996) was found to lack three nucleotides in its putative open reading frame (ORF), resulting in a NSP1 protein that was one amino acid shorter than those of other RVA strains. To confirm this observation, we repeated nucleotide sequencing of the NSP1 gene of strain H-1.

2. Materials and methods

The source of the tissue culture adapted isolate of strain H-1 analyzed in the present study has been described previously (Wu et al., 1993). For RT-PCR, viral RNA was extracted from the tissue culture fluid of strain H-1 using the QIAamp Viral RNA Mini kit (Qiagen Sciences, MD, USA). The VP1–3, NSP1–3 and NSP5 genes of strain H-1 were amplified using primers reported previously (Ghosh et al., 2010a,b, 2011; Wang et al., 2010). Nucleotide sequences were obtained using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, CA, USA) on an automated DNA sequencer (ABI PRISM 3100). Sequence comparisons were carried out as described previously (Ghosh et al., 2010a,b). Phylogenetic trees were constructed by the Neighbor-Joining method (Saitou and Nei, 1987) using MEGA (v5.01) software. The trees were statistically supported by bootstrapping with 1000 replicates, and phylogenetic distances measured by the Kimura two-parameter model. The GenBank accession numbers for the nucleotide sequences of the VP1–3, NSP1–3 and NSP5 genes of strain H-1 are JQ309138–JQ309144, respectively.

3. Results and discussion

Whole genomic analyses of RVA strains derived from interspecies transmission and/or genetic reassortment events are important to gain a proper understanding of the complex genetic diversity of RVAs (Ghosh and Kobayashi, 2011; Martella et al., 2010). Moreover, novel RVA strains arising from these events may have the ability to efficiently infect and spread among the population of the new host, presenting a challenge to the efficacy of the existing RVA vaccines (Palombo, 2003).

The VP4, VP6–7, NSP1 and NSP4 genes of equine RVA strain H-1 were assigned to the P[7], I5, G5, A8 and E1 genotypes, respectively (Matthijnssens et al., 2008). In the present study, the VP1–3, NSP2–3 and NSP5 genes of strain H-1 were assigned to the R1, C1, M1, N1, T1 and H1 genotypes, respectively (Table 1). Taken together, strain H-1 was found to share 10 out of the 11 genotypes with those of typical porcine RVA strains RVA/Pig-tc/USA/OSU/1975/G5P9[7], RVA/Pig-xx/KOR/ PRG9121/2006/G9P[7] and RVA/Pig-tc/MEX/YM/1983/G11P9[7] (Table 1). Therefore, the overall genotype constellation of strain H-1 was found to be porcine-like, different from those of other equine RVA strains (Table 1).

In previous studies, the VP4, VP6–7, NSP1 and NSP4 genes of equine RVA strain H-1 were shown to be derived from porcine strains (Ciarlet et al., 2000, 2001; Kojima et al., 1996; Matthijnssens et al., 2008; Taniguchi et al., 1994). Among the remaining six gene segments of strain H-1 analyzed in this study, the H-1 VP1 gene shared low nucleotide sequence identities (<90%) with those of other RVA strains, and phylogenetically, clustered separately, near those of human G3P[8] strain RVA/Human-tc/JPN/ YO/1977/G3P1A[8], porcine-derived human strain RVA/Human-wt/NPL/KTM368/2004/G11P[25] (Matthijnssens et al., 2010) and porcine strains PRG9121, RVA/Pig-xx/KOR/PRG942/2006/G9P[23] and RVA/Pig-xx/KOR/

Table 1
Genotype nature of the eleven gene segments of group A rotavirus (RVA) strain H-1 with those of selected RVA strains with known genomic constellations.

Strain	Genotypes										
	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/**Horse**-tc/GBR/H-1/1975/G5P9[7]	G5	P[7]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-tc/USA/Wa/1974/G1P1A[8]	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/BGD/MMC71/2005/G1P[8]	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/USA/DS-1/1976/G2P1B[4]	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/**Horse**-wt/ARG/E30/1993/G3P[12]	G3	P[12]	I6	R2	C2	M3	A10	N2	T3	E2	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]	I2	–	–	–	A10	–	–	E2	–
RVA/**Horse**-tc/USA/FI-14/1981/G3P[12]	G3	P[12]	I6	–	–	–	A10	–	–	E2	–
RVA/Pig-tc/USA/Gottfried/1975/G4P[6]	G4	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-tc/USA/SB1A/xxxx/G4P9[7]	G4	P[7]	–	–	–	–	–	N1	T1	E1	H1
RVA/Pig-tc/USA/OSU/1975/G5P9[7]	G5	P[7]	I5	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/BRA/IAL28/1992/G5P[8]	G5	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Pig-xx/KOR/PRG9121/2006/G9P[7]	G9	P[7]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-xx/KOR/PRG9235/2006/G9P[23]	G9	P[23]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/**Horse**-tc/JPN/R-22/1984/G10P[11]	G10	P[11]	I2	–	–	–	–	–	–	–	–
RVA/Pig-tc/MEX/YM/1983/G11P9[7]	G11	P[7]	I5	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-wt/NPL/KTM368/2004/G11P[25]	G11	P[25]	I12	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Pig-wt/IND/RU172/2002/G12P[7]	G12	P[7]	I5	R1	C1	M1	A1	N1	T1	E1	H1
RVA/**Horse**-tc/GBR/L338/1991/G13P[18]	G13	P[18]	I6	R9	C9	M6	A6	N9	T12	E14	H11
RVA/**Horse**-wt/ARG/E403/2006/G14P[12]	G14	P[12]	I2	R2	C2	M3	A10	N2	T3	E2	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
RVA/**Horse**-tc/USA/FI23/1981/G14P[12]	G14	P[12]	I2	–	–	–	A10	–	–	E2	–

Grey indicates the gene segments with a genotype identical to that of strain H-1. "–" indicates that no sequence data were available in the Gen Bank database.
The species of origin of equine RVA strains are highlighted in bold.