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Short communication

Phylogenetic characterization of VP6 gene (inner capsid) of porcine rotavirus C collected in Japan

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ABSTRACT

Porcine rotavirus C (RVC) has been often detected in sporadic cases or outbreaks of diarrhea in suckling and weaned pigs. Previous surveillance studies using both enzyme-linked immunosorbent assays and reverse-transcription polymerase chain reaction in some countries including Japan and the United States have demonstrated a high prevalence of porcine RVCs. In order to understand the phylogenetic relatedness of RVCs, we performed genetic analysis of VP6 gene encoding inner capsid protein by using 22 porcine RVC strains collected in Japan from 2002 to 2010. Comparative analyses of the VP6 nucleotide and amino acid sequences from these porcine RVCs exhibited lower sequence identities than those from human and bovine RVCs. The phylogenetic analysis of VP6 gene of RVC indicated the presence of seven clusters (tentatively assigned I1–I7) according to host species with cut-off values of 87% at the nucleotide level, and VP6 genes of porcine RVCs were divided into five genotypes. These findings indicate that multiple porcine RVC strains with distinctive genotypes are broadly spreading and circulating among farms in Japan. Our data may provide important insights in understanding evolutionary dynamics of RVCs.

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1. Introduction

Rotaviruses, a member of the family *Reoviridae*, are one of major pathogens of gastroenteritis in young humans and animals worldwide (Estes and Kapikian, 2007). They are currently divided into eight species (A–H) on the basis of antigenic and genetic analyses (Matthijnssens et al., 2012). Their genome is composed of 11 double-stranded RNAs (dsRNAs) encoding six structural (VP1–4, VP6 and VP7) and five or six non-structural (NSP1–6) proteins. VPs comprise infectious triple-layered particles surrounding the genomic dsRNA. NSPs are primarily associated with dsRNA replication and transcription, cellular pathogenesis and virus-particle maturation (Presavento et al., 2006).

Rotavirus C (RVC) was first identified in swine as a causative agent of diarrhea in 1980s (Saif et al., 1980; Bohl et al., 1982). Subsequently, RVCs have been detected in humans, cows, ferrets and dogs (Rodger et al., 1982; Torres-Medina, 1987; Tsunemitsu et al., 1991; Chang et al., 1999; Otto et al., 1999; Mawatari et al., 2004). They have been detected in humans from various age groups in sporadic cases and/or outbreaks of diarrhea; however, they have been mainly detected in children less than 3 years old (Matsumoto et al., 1989; Caul et al., 1990; Jiang et al., 1995; Kuzuya et al., 1998; Nilsson et al., 2000). RVC infections in humans were also identified in many countries from North and South America, Asia, Africa, Europe and Oceania (Penaranda et al., 1989; Oishi et al., 1993; Hamano et al., 1999; Qiao et al., 1999; Sebeta and Steele, 1999; Kuzuya et al., 2001; Adah et al., 2002; Castello et al., 2002; Mwenda et al., 2003; Schnagl et al., 2004; Rahman et al., 2005; Yee et al., 2006). Therefore, RVC is considered an epidemiologically important emerging agent. Apart from human RVCs, several studies have shown association among porcine RVCs and enteritis in nursing, weaning and post-weaning pigs (Sigolo de San Juan et al., 1986; Saif and Jiang, 1994; Kim et al., 1999). Moreover, the epidemiological study using enzyme-linked immunosorbent assay (ELISA) in pig herds from Japan and the United States (US) showed a high prevalence of RVC antibodies in sera (93–97%) (Tsunemitsu et al., 1992). However, surveillance study by reverse-transcription polymerase chain reaction (RT-PCR) indicated 46% detection rate (3447 samples) of RVC in 7520 samples from pig herds in the US and Canada from December 2009 to October 2011 (Marthaler et al., 2013). Another epidemiological study performed by RT-PCR at several swine farms in Ohio, US for 3 years

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Table 1
The origin of 22 porcine RVC strains in fecal samples collected around Japan from 2002 to 2010.

RVC strains	GenBank ID	Prefecture, farm[a]	Date of sample collection	Age or stage[b]	VP6 genotype[c]
RVC/Pig-wt/JPN/CJ3-6/2002/GXP[X]	AB889511	Kagoshima, A	May 2002	60	I7
RVC/Pig-wt/JPN/CJ10-1/2002/GXP[X]	AB889512	Kagoshima, B	May 2002	22	I1
RVC/Pig-wt/JPN/CJ13-6/2002/GXP[X]	AB889513	Kagoshima, C	May 2002	30	I1
RVC/Pig-wt/JPN/CJ16-4/2002/GXP[X]	AB889514	Ehime, D	June 2002	7	I7
RVC/Pig-wt/JPN/CJ27-1/2002/GXP[X]	AB889515	Aomori, E	September 2002	30	I6
RVC/Pig-wt/JPN/CJ31-6/2002/GXP[X]	AB889516	Kagoshima, F	October 2002	28	I1
RVC/Pig-wt/JPN/CJ32-3/2002/GXP[X]	AB889517	Akita, G	October 2002	2	I1
RVC/Pig-wt/JPN/CJ33-5/2002/GXP[X]	AB889518	Aomori, H	November 2002	7	I7
RVC/Pig-wt/JPN/CJ49-4/2003/GXP[X]	AB889519	Aomori, I	August 2003	30	I7
RVC/Pig-wt/JPN/CJ59-32/2003/GXP[X]	AB889520	Iwate, J	October 2003	40	I7
RVC/Pig-wt/JPN/86-H3/2008/GXP[X]	AB889499	Chiba, K	July 2008	30	I7
RVC/Pig-wt/JPN/86-H5/2008/GXP[X]	AB889500	Chiba, K	July 2008	30	I7
RVC/Pig-wt/JPN/86-K5/2008/GXP[X]	AB889501	Chiba, K	July 2008	120	I7
RVC/Pig-wt/JPN/87-G2/2008/GXP[X]	AB889502	Ibaraki, L	September 2008	Suckling	I7
RVC/Pig-wt/JPN/87-I4/2008/GXP[X]	AB889503	Ibaraki, L	September 2008	60	I7
RVC/Pig-wt/JPN/91-G7/2008/GXP[X]	AB889504	Chiba, M	October 2008	Suckling	I7
RVC/Pig-wt/JPN/91-G10/2008/GXP[X]	AB889505	Chiba, M	October 2008	Suckling	I7
RVC/Pig-wt/JPN/91-H5/2008/GXP[X]	AB889506	Chiba, M	October 2008	30	I7
RVC/Pig-wt/JPN/93-H5/2008/GXP[X]	AB889507	Gifu, N	October 2008	30	I4
RVC/Pig-wt/JPN/93-Z4/2008/GXP[X]	AB889508	Gifu, N	October 2008	–	I7
RVC/Pig-wt/JPN/105-4/2009/GXP[X]	AB889509	Gifu, N	March 2009	4	I7
RVC/Pig-wt/JPN/134-9/2010/GXP[X]	AB889510	Miyazaki, O	March 2010	7	I1

[a] Different letters mean that each RVC strain originated from a different farm.
[b] The age is days. The sample with uncertain age is shown in the breeding stages: suckling, 0–34 days old.
[c] Each genotype is shown in Fig. 1. The VP6 genotypes were tentatively assigned.

showed the prevalence of RVCs at the rate of 19.5% (74/380 samples) (Amino et al., 2013). In Italy, a study also reported a detection rate (25.5–31.3%) of porcine RVCs in pig herds (Martella et al., 2007). In our previous surveillance study for porcine enteric pathogens by RT-PCR of fecal specimens collected in Japan between 2000 and 2007, porcine RVC infections was positive in 152 of 717 samples (21.2%) (Kuga et al., 2009). These data indicate that porcine RVCs are broadly distributed and transmitted in many countries, as similar to human RVCs.

The major capsid protein, VP6 is highly antigenic and immunogenic, because it accounts for more than 50% of the virion by weight; therefore, it is considered as a target protein in diagnosis and subgrouping (Gorziglia et al., 1988; Jiang et al., 1992). The VP6 gene showed the highest nucleotide identity in the comparison of whole-genome sequences among several human RVCs (Yamamoto et al., 2011). Moreover, the VP6 gene sequences of the three bovine RVCs showed relatively higher identities than those of other genes (Soma et al., 2013). In contrast, the phylogenetic analysis of partial VP6 sequences (235 bp; nucleotide position 1015–1249), including 13 Italian porcine RVC strains in several reference human, bovine and porcine RVC strains, showed that all 13 porcine RVC strains were divided into multiple branches within porcine cluster including the reference porcine RVC strains, and differentiated clearly from human and bovine clusters (Martella et al., 2007). The phylogenetic dendrogram of partial VP6 sequences (249 bp; nucleotide position 1095–1303) from several Korean porcine and bovine RVCs also exhibited the presence of multiple clusters consisting of only bovine RVC strains, and bovine and porcine RVC strains (Park et al., 2011). In a recent study, the phylogenetic classification of VP7 nucleotide sequences from a large number of porcine RVCs showed the presence of multiple porcine genotypes (Marthaler et al., 2013). However, a genetic classification of porcine RVC VP6 has not been clearly established owing to the limitation of available full-length nucleotide sequence information.

In this study, we attempted to determine the full-length sequences of VP6 genes from several porcine RVC strains collected in Japan, to investigate genetic divergence within the strains and genetic relatedness among other RVCs. Moreover, the phylogenetic analysis of the VP6 genes of porcine RVCs identified in this study were compared to those of other RVCs determined in the previous studies for better understanding of molecular evolution of RVCs.

2. Materials and methods

We obtained 717 fecal specimens collected in Japan from 2000 to 2007, and 152 RVC-positive samples were identified by RT-PCR using RVC-specific primers (Kuga et al., 2009). Several porcine RVC-positive samples were collected from the Livestock Health and Sanitation Offices existing in Japan after 2007. Out of them, we chose 22 porcine RVC strains originating from sucking and weaned pigs from 15 farms, excluding overlap of same sampling location and period (Table 1). The viral RNA was extracted from 10% fecal suspension in a minimum essential medium by using TRIsol LS reagent (Life Technologies, CA, USA) according to manufacturer's protocol. The full-length VP6 gene sequences from the 22 porcine RVCs were amplified and sequenced by RT-PCR using specific primers (forward primer: 5′-GCATTTAAAATCTCATTC-3′ and reverse primer: 5′-AGCCACATAGTTCACATT-3′). The RT-PCR reaction by using PrimeScript one step RT-PCR kit ver.2 (Takara, Shiga, Japan) was performed at 50 °C for 30 min and 94 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, 48 °C for 1 min,72 °C 2 min, and then a final extension at 72 °C for 10 min. The products were cloned into pCR2.1 TOPO vector and sequenced by BigDye Terminator v3.1 cycles sequencing kit using an automated ABI Prism 3130 Genetic Analyzer (Life Technologies, CA, USA).

The sequence data were aligned by the ClustalW method using the MegAlign program in the Lasergene software (DNASTAR, Inc., WI, USA). The phylogenetic analysis of VP6 open reading frames (ORFs) among RVCs was conducted using the Tamura-3 parameter model of maximum likelihood method with the MEGA version 5 (Tamura et al., 2011). Genetic distances were calculated using the Kimura 2-parameter correction at the nucleotide level and the Poisson correction parameter at the amino acid level. A cut-off value was calculated according to the definition recommended by the Rotavirus Classification Working Group (RCWG)

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