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Research paper

Human-porcine reassortant rotavirus generated by multiple reassortment events in a Sri Lankan child with diarrhea

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ABSTRACT

A human-porcine reassortant rotavirus, strain R1207, was identified from 74 group A rotaviruses detected in 197 (37.6%) stool samples collected from patients who attended a tertiary care hospital in Ragama, Sri Lanka. This is the first report of a human-porcine reassortant rotavirus in Sri Lanka. The patient was a 12-month-old boy who had been hospitalized with fever and acute diarrhea with a duration of 6 days. *The family had pigs at home before the birth of this boy. However, the neighbors still practice pig farming.* The genotype constellation of R1207 was G4-P[6]-I1-R1-C1-M1-A1-N1-T1-E1-H1. This is based on the assignment of all the eleven gene segments a full genome-based genotyping system. R1207 showed a 4-2-3-2 genomic electrophoretic migration pattern, which is characteristic of group A rotaviruses. Our analyses revealed that five (NSP2, NSP4, VP1, VP2, and VP7) of the 11 genes were closely related to the respective genes of porcine strains. Although the remaining six genes (NSP1, NSP3, NSP5, VP3, VP4, and VP6) were related to human strains, with the exception of the gene sequence of NSP1, all of these human strains were human-porcine reassortants. With a genogroup 1 genetic backbone, this strain was possibly formed via multiple genetic reassortments. We do not know whether this strain is circulating in pigs, as no data are available on porcine rotaviruses in Sri Lanka. Surveillance should be strengthened to determine the epidemiology of this genotype of rotavirus in Sri Lanka and to assess whether the infection was limited or sustained by ongoing human-to-human transmission.

1. Introduction

Rotavirus is one of the important causes of gastroenteritis in children and is responsible for over 200,000 deaths annually in children under five years of age (Tate et al. 2016), over 90% of these deaths occur in developing countries. The virus is divided into ten groups A-J (Banyai et al. 2017), with group A being most common in human. Rotavirus has a double-stranded RNA genome which are divided into 11 segments, those encode six structural (VP1, VP2, VP3, VP4, VP6, and VP7) and six non-structural proteins (NSP 1–6) (Matthijnssens et al. 2008b). Based on the nucleotide sequences of the genes for two capsid proteins, VP7 and VP4, a binary classification system, G and P genotypes, is used to classify different strains of rotaviruses. Till now 35 G genotypes and 50 P genotypes have been reported (Agbemabiese et al. 2017). Among numerous possible G and P genotype combinations, only a limited number of genotype combinations are found commonly in human infections, they are G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8].

Some of the rotavirus genotypes that have emerged and spread in humans worldwide during the last decade are thought to have originated by zoonotic transmission from different animals and gene reassortment (Martella et al. 2010).Although rotaviruses infect particular host species preferentially (for which they have been defined as the homologous strains), heterologous rotavirus infections may occur (Martella et al. 2010; Midgley et al. 2012). Evidence of interspecies transmission and genetic reassortment between human and animal ro-

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Table. 1
G and P consensus, type-specific and gene-specific primers used for RT-PCR amplification.

Primer name	5′- sequence −3’	Position	Product size (bp)
VP7			
Beg9	GGCTTTAAAAGAGAGAATTTCCGTCTGG	1–28	1062
End9	GGTCACATCATACAATTCTAATCTAAG	1062–1036	
G-typing			
VP7-R	AAC TTG CCA CCA TTT TTT CC	914–932	
aBT1	CAAGTACTCAAATCAATGATGG	314–335	618
aCT2	CAATGATATTAACACATTTTCTGTG	411–435	521
aDT4	CGTTTCTGGTGAGGAGTTG	480–498	452
G3	ACG AAC TCA ACA CGA GAG G	250–269	682
G9	CTT GAT GTG ACT AYA AAT AC	757–776	179
VP4			
Con3	TGGCTTCGCCATTTTATAGACA	11–32	876
Con2	ATTTCGGACCATTTATAACC	868–887	
P-typing			
HumCom5	CTC TCG ATG GTC CAT ATC AAC C	200–221	
P[8]P1A	TGT ACG TCT ATT ATA AAA TTC ATT T	456–480	280
P[4]P1B	ATA TAT TGC CTA TTT GTT TGA C	347–368	168
P[6]P2	GTA TTA CAG TTT CTA CTT CAG A	592–613	413
P[9]P3	CGT CGC TCC TTG ATA CCA GT	533–552	352
Gene-specific primers			
VP1 F	GGCTATTAAAGCTRTACAATGG	1–22	3299
VP1 R	CACATCTAAGCACTCTAATCTTG	3277–3299	
VP2 F	GGCTATTAAAGGCTCAATGG	1–20	2696
VP2 R	TTGGCGTTTACARTTCGTTCA	2676–2696	
VP3 F	TGCGTTTTACCTCTGATGGTG	24–44	2566
VP3 R	TCACATCATGACYAGTGTGTTAAG	2566–2589	
VP4 F	GGCTATAAAATGGCTTCGCT	1–20	2362
VP4 R	GGGGGTCACATCCTC	2348–2359 (+3)	
VP6 F	GGCTTTWAAACGAAGTCTTC	1–20	1356
VP6 R	GGTCACATCCTCTCACT	1340–1356	
VP7 F	GGCTTTAAAAGMGAGAATTTCC	1–22	1062
VP7 R	GGGGGTCACATCATACAATTCT	1041–1059 (+3)	
NSP1 F	GGCTTTTTTTATGAAAAGTCTTGTG	1–25	1547
NSP1 R	CTAGGCGCTACTCTAGT	1531–1547	
NSP2 F	GGCTTTTAAAGCGTCTCAGTC	1–21	1058
NSP2 R	GGTCACATAAGCGCTTTCTATTC	1036–1058	
NSP3 F	GGCTTTTAATGCTTTTCAGTGGTTG	1–25	1050
NSP3 R	GGTCACATAACGCCCCTATAG	1030–1050	
NSP4 F	CTTTTAAAAGTTCTGTTCCGAGAG	3–26	739
NSP4 R	AAGACCATTCCTTCCATTAAC	721–741	
NSP5 F	GGCTTTTAAAGCGCTACAGT	1–20	663
NSP5 R	GGTCACAAAACGGGAGTGGGGA	642–663	

Reference: Gouvea et al. JCM 1990.Vol.28.No2.276–282
Iturriza-Gómara M et al. J Clin Virol. 2004 Dec;31(4):259–65.
Fujii Y et al. Microbiol Immunol. 2012 Sep;56(9):630–8. doi: https://doi.org/10.1111/j.1348-0421.2012.00479.x.

taviruses has been accumulating continuously in the literature. Several studies have shown that G3 and G6 are reassortant of human-canine and human-bovine strains, respectively (Martella et al. 2010). Even the widely circulating G9 and G12 strains are reassortants of human-porcine strains (Matthijnssens et al. 2010). Reassortant rotaviruses bearing a mixed genome constellation from human and animal rotaviruses seem to be more successful compared with completely heterologous rotaviruses (Martella et al. 2010; Nakagomi and Nakagomi 2002). A whole genome based rotavirus classification system denotes the genome of rotavirus as Gx-Px-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx where x represents the genotype number (Matthijnssens et al. 2008a). In this classification system human rotavirus strains are grouped into the Wa-like (G1/G3/G4-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1), DS-1-like (G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2), and AU-1-like (G3-P[9]-I3-R3-C3-M3-A3-N3-T3-E3-H3)(Matthijnssens et al. 2008a; Matthijnssens et al. 2008b). This classification system revealed that human Wa-like and porcine rotavirus strains share a common ancestor. Porcine rotavirus strains possess the following genomic constellation (G3/4/5/9-P[6]/[7]/[13]/[19]/[23]-I5-R1-C1-M1-A8-N1-T1/7-E1-H1)(Agbemabiese et al. 2017; Matthijnssens et al. 2008a; Monini et al. 2014; Silva et al. 2016; Theuns et al. 2015).

Rotavirus infection is a burden for the Sri Lankan health care system, as a considerable number of children suffer and die from rotavirus-associated diarrhea each year (Ahmed et al. 2010b; Chandrasena et al. 2009). Studies of rotavirus diarrhea in Sri Lanka are scarce, and our study performed during 2005–2006 showed that a diversity of rotavirus strains were circulating among children, with G9 as the dominant genotype, followed by G3, G12, G2,G1, and G4(Ahmed et al. 2010b). In that study, no strain of animal origin was detected, although the genotype could not be determined for 9% of the strains. During 2008–2009, we initiated another study for the surveillance of rotavirus diarrhea in the same locality and detected an animal-like rotavirus of genotype G4P[6] in a child with diarrhea. Genetic analyses of animal-like rotaviruses are important for the assessment of whether

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