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Whole genome characterisation of a porcine-like human reassortant G26P[19] *Rotavirus A* strain detected in a child hospitalised for diarrhoea in Nepal, 2007

Running head: G26P[19] human *Rotavirus A* strain in Nepal

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Abbreviations:

RVA: *Rotavirus A*

I: **I**ntermediate capsid shell

R: **R**NA polymerase

C: **C**ore shell

M: RNA-capping **M**ethyltransferase

A: interferon **A**ntagonist

N: octameric **N**TPase

T: **T**ranslation regulation

E: **E**nterotoxin

H: p**H**osphoprotein

VP: viral protein

NSP: non-structural protein

MEGA: Molecular Evolutionary Genetics Analysis

ViPR: **Vi**rus **P**athogen **R**esource

Abstract

A rare G26 *Rotavirus A* strain RVA/Human-wt/NPL/07N1760/2007/G26P[19] was detected in a child hospitalised for acute diarrhoea in Kathmandu, Nepal. The complete genome of 07N1760 was determined in order to explore its evolutionary history as well as examine its relationship to a Vietnamese strain RVA/Human-wt/VNM/30378/2009/G26P[19], the only G26 strain whose complete genotype constellation is known. The genotype constellation of 07N1760 was G26-P[19]-I12-R1-C1-M1-A8-N1-T1-E1-H1, a unique constellation identical to that of the Vietnamese 30378 except the VP6 gene. Phylogenetic analysis revealed that both strains were unrelated at the lineage level despite their similar genotype constellation. The I12 VP6 gene of 07N1760 was highly divergent from the six currently deposited I12 sequences in the GenBank. Except for its NSP2 gene, the remaining genes of 07N1760 shared lineages with porcine and porcine-like human RVA genes. The NSP2 gene belonged to a human RVA N1 lineage which was distinct from typical porcine and porcine-like human lineages. In conclusion, the Nepali G26P[19] strain 07N1760 was a porcine RVA strain which derived an NSP2 gene from a human Wa-like RVA strain by intra-genotype reassortment probably after transmission to the human host.

1. Introduction

Rotavirus A (RVA), a species within the genus *Rotavirus* and family *Reoviridae*, is a major cause of acute gastroenteritis in infants and young children as well as the young of many animal species (Bishop et al., 1973; Estes and Greenberg, 2013). The virion has a triple-layered capsid which encloses a genome of 11 segments of double-stranded RNA. The genome encodes six structural viral proteins (VP1-VP4, VP6, VP7) and six non-structural proteins (NSP1-NSP6) (Estes and Greenberg, 2013).

RVA strains are classified into G and P genotypes based on the nucleotide sequence diversity of the two outermost capsid proteins VP7 and VP4, respectively. Currently, there are 32 G-types and 47 P-types (Matthijnssens et al., 2008; https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/rcwg). In addition, a complete genome based classification system developed by Matthijnssens et al. (2008a) denotes the whole genome VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 of RVA strains by the descriptor Gx-Px-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx (where x represents the genotype number). In this regard, human RVA strains were 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) genotype constellations (Matthijnssens et al., 2008a; Matthijnssens et al., 2008b).

The whole genome classification system further revealed that human Wa-like and porcine RVA strains share a common evolutionary origin. Porcine rotaviruses usually possess G3, G4, G9 and G11 in association with P[6] or P[7] whereas G1, G2, G6, G10, G12 and G26 in combination with P[5], P[8], P[11], P[13], P[14], P[19], P[26], P[27] and P[32] are sporadically detected (Papp et al., 2013; Silva et al., 2015; Silva et al.,

2016; Theuns et al., 2015). At the whole genome level, porcine RVA strains typically possess the genotype constellation G3/4/5/9/11-P[6]/[7]/[13]/[19]/[23]-I5-R1-C1-M1-A8-N1-T1/7-E1-H1 (Kim et al., 2012; Martel-Paradis et al., 2013; Matthijnssens et al., 2008a; Monini et al., 2014; Silva et al., 2016; Theuns et al., 2015). A recent comprehensive phylogenetic analysis of the whole genome sequences of genotype 1 genes of RVA strains revealed that, typical modern human Wa-like strains belonged to a separate cluster from that of typical modern porcine RVA strains (Silva et al., 2016).

RVA strains with the G26 genotype were reported in pigs in Japan (Miyazaki et al., 2011) Kenya (Amimo et al., 2015) and Brazil (Lorenzetti et al., 2016). Sporadic cases of G26P[19] strains were also reported in children and a sewage sample in recent years (My et al., 2014; Ruggeri et al., 2015; Theamboonlers et al., 2008). A GenBank database search conducted on 19th October, 2016 revealed a total of 18 G26 strains but only one detected in a child in Vietnam in 2009 had its whole genome sequenced (My et al., 2014).

During a rotavirus surveillance study in the 2007-2008 season in Kathmandu, Nepal, a few specimens were non-typeable (Sherchand et al., 2011). One of such specimens registered as RVA/Human-wt/NPL/07N1760/2007/G26P[19] (hereafter referred to as 07N1760), possessed a unique electropherotype upon polyacrylamide gel electrophoresis. Sequence analysis showed that the VP7 and VP4 genes of 07N1760 respectively possessed porcine RVA genotypes G26 and P[19]. The scarcity of whole genome information of G26 strains prompted us to determine the full genotype constellation of 07N1760 in order to examine its relationship to the Vietnamese G26P[19] strain and other rotavirus strains at the whole genome level and also explore evidence suggestive of animal rotavirus origin of 07N1760.

2. Materials and Methods

2.1 Rotavirus strain

The study strain RVA/Human-wt/NPL/07N1760/2007/G26P[19] was detected in the diarrhoea stool specimen of an 11-month-old boy hospitalised in Kanti Children's Hospital, Kathmandu, Nepal in November 2007. This strain was one of the 11 (11%) non-typeable RVA strains reported by Sherchand et al. (2011).

2.2 Genome amplification and sequencing

Viral RNA was extracted from 140 µL of supernatant obtained from 10% stool suspension (w/v) using the QIAamp Viral RNA Mini Kit (Qiagen Sciences, Germantown, MD, USA) according to the manufacturer's protocol. Complementary DNA (cDNA) was generated using the SuperScript™ III first-strand synthesis system for reverse-transcription (RT)-PCR (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

The structural protein genes VP1, VP2, VP3, VP4, VP6, and VP7 and the non-structural protein genes NSP1 and NSP2 were amplified by PCR from 2 µL of the cDNA with gene specific primers (primers can be obtained upon request) and the PrimeSTAR GXL DNA Polymerase (Takara Bio, Inc., Shiga, Japan) (Fujii et al., 2012). Amplicons for the remaining non-structural protein genes, NSP3, NSP4 and NSP5 were generated using gene-specific end primer pairs (Matthijnssens et al., 2008) (primers can be obtained upon request) and the AccessQuick™ RT-PCR system (Promega Corporation, Madison, WI, USA). The amplicons were purified using Exosap-IT™ purification system (USB products, Cleveland, OH, USA) following the manufacturer's instructions.

The amplicons generated from the 11 genes of 07N1760 were completely sequenced by first using the end PCR primers and the Big Dye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems) which is based on the fluorescent dideoxy chain termination chemistry. The primer walking technique was used to complete the internal portions of the larger genes as well as the 5' and 3' ends of the amplicons generated from the 11 genes.

2. 3 Sequence and phylogenetic analyses

Sequence contigs were assembled from the sequence data of the 11 genome segments using the SeqMan program in Lasergene core suite software version 14 (DNAstar, Inc. Madison, WI, USA). Genotypes of the genes were determined using the RotaC v.2.0 automated online genotyping tool for RVA and the Virus Pathogen Resource (ViPR) (Maes et al., 2009; Pickett et al., 2012).

For sequence comparison and phylogenetic analysis, sequences were retrieved from the GenBank database using the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) with sequences of 07N1760 being the query. Multiple sequence alignment files were constructed with the online version of Multiple Alignment using Fast Fourier Transform (MAFFT version 7) (Katoh and Standley, 2013). Nucleotide sequence similarities were calculated for the genome segments using the p-distance algorithm in MEGA 6 (Tamura et al., 2013). With the best fit substitution models bearing the lowest Bayesian Information Criterion scores (Schwarz, 1978) as follows: T92 + I (VP7, VP4); T92 + G (VP6, NSP2, NSP3, NSP4, NSP5) GTR + G (VP1); GTR + G + I (VP3, NSP1) and T93 + G + I (VP2), maximum likelihood phylogenetic trees were constructed using 1000 bootstrap replicates.

2.4 Nucleotide sequence accession numbers

Nucleotide sequences were submitted to the GenBank/DDBJ/EMBL under the accession numbers LC208008-LC208018.

3. Results

3.1 Genotype constellation of 07N1760

The nearly-full length of the 11 genes was sequenced for 07N1760 and the genotype constellation as determined by RotaC and ViPR was G26-P[19]-I12-R1-C1-M1-A8-N1-T1-E1-H1. This constellation even though was similar to genotype constellations typically found in pig RVA strains, it has never been described in literature. Our study strain also shared the same genotype in all but the VP6 gene with RVA/Human-wt/VNM/30378/2009/G26P[19], the only G26 strain whose complete genotype constellation i.e. G26-P[19]-I5-R1-C1-M1-A8-N1-T1-E1-H1 is thus far reported in literature by My et al. (2014).

3.2 Sequence and phylogenetic analysis

The VP7 gene of 07N1760 was closest to that of a G26P[19] strain NA11-144 detected in sewage in Italy in 2011 with a nucleotide sequence identity of 98.7% (Table 1). Phylogenetic analysis of the G26 VP7 gene revealed four distinct lineages namely: the Indian G26 cluster, TJ4-1 cluster, 30378 cluster and the 07N1760 cluster (Fig. 1a). 07N1760 formed a cluster with the Italian strain NA11-144 (Fig. 1a). A Kenyan porcine strain Ke-003 was the only G26 strain with a verified host species origin which was >95% identical to 07N1760. The remaining G26 VP7 genes detected in pigs and

179 humans in other parts of the world including the Vietnamese 30378 were <95% similar
180 to 07N1760 and they belonged to the other three lineages (Fig. 1a).
181 The P[19] VP4 genotype of 07N1760 has so far been detected in porcine and
182 porcine-like human RVA strains with a single Italian strain detected in sewage in Italy
183 (Fig. 1b). The nucleotide sequence identities of 07N1760 to the other P[19] strains
184 ranged from 92.7% to 96.8%. At the lineage level, 07N1760 belonged to a separate
185 cluster from that of the Vietnamese strain 30378 (Fig. 1b).
186 The VP6 gene of 07N1760 was typed as I12 by RotaC. While this genotype is
187 believed to be of porcine RVA origin, RotaC commented that the 07N1760 was a
188 borderline case. Upon BLAST interrogation, the VP6 of 07N1760 was closest to a
189 G5P[6] porcine-like human strain LL4260 from China (Li et al., 2008) (Table 1).
190 Nucleotide sequence comparison and phylogenetic analysis of the only six I12
191 sequences in the GenBank showed that 07N1760 was less similar to the previously
192 reported I12 sequences (range: 81.1 to 92.0%). Nevertheless, 07N1760 formed a cluster
193 with the porcine-like human RVA strain LL4260 and porcine RVA strain TA-1-1 with a
194 high bootstrap probability value (Fig. 1c).
195 The NSP1 gene of 07N1760 belonged to genotype A8 which is often detected in
196 porcine and porcine-like human RVA strains. In the NSP1 gene tree (Fig. 1d), 07N1760
197 formed a cluster with porcine and porcine-like human RVA strains with strains Mc345
198 and Mc323 detected in Thailand by Ghosh et al. (2012) being the closest with high
199 nucleotide sequence identities of 97.4-97.5% (Table1). The Vietnamese 30378 strain
200 shared the same cluster with 07N1760 but was less identical (94%) to 07N1760 (Fig.
201 1d).

The VP1, VP2, VP3, NSP3, NSP4 and NSP5 of 07N1760 belonged to genotype 1 and they formed clusters with genes of previously published porcine and porcine-like human RVA strains (Chitambar et al., 2009; Do et al., 2016; Ghosh et al., 2006; Komoto et al., 2013; Zhou et al., 2015) (Supplementary Fig. 1). The nucleotide sequence identities of these genes to the Vietnamese 30378 strain ranged from 86.2% (VP3 gene) to 95.9% (NSP5 gene) and their sequences belonged to different lineages (Supplementary Fig.1).

The NSP2 gene of 07N1760 was considered of human RVA origin since the lineage to which it belonged contained only human Wa-like RVA sequences detected globally since the late 1990s (1999-2014). This lineage was distinct from typical archival or modern porcine N1 lineages, lineages with intermingled N1 sequences of both porcine and human RVA origin, and archival human N1 lineages (Fig. 1e). The closest NSP2 gene was from a Brazilian human G9P[9] RVA strain (R138) with a nucleotide sequence identity of 98.9% (Table 1). By contrast, the Vietnamese 30378 strain belonged to a lineage that contained both porcine and porcine-like human RVA sequences (Fig. 1e) and all these sequences were <91% identical to 07N1760.

4. Discussion

In this study, we provided molecular evidence that a rare G26P[19] strain (07N1760) detected in a child with diarrhoea in Nepal was a porcine RVA strain which obtained an NSP2 gene from human RVA through an intra-genotype reassortment event. Previously, My et al. (2015) reported four G26P[19] strains in Vietnamese children with diarrhoea and noted that a Thai G3P[19] strain described previously by Theamboonlers et al. (2008) was rather a G26P[19] strain. Based on whole genome

226 analysis, My et al. (2015) speculated that their G26P[19] strain originated from porcine
227 and porcine-like human RVA. Another study by Ruggeri et al. (2014) detected G26 and
228 P[19] genotypes in a sewage sample in Italy and speculated that these were probably
229 from porcine RVA in animal faeces disposed into the sewer system by nearby swine
230 farms or slaughterhouses.
231 While G26 and P[19] genotypes are of putative porcine rotavirus origin
232 (Maneekarn et al., 2006; Miyazaki et al., 2011; My et al., 2014; Papp et al., 2013),
233 strains with both genotypes together i.e. G26P[19], have never been detected in pigs
234 (Table 2, Table 3). The G26 genotype have so far been detected in pigs in association
235 with P[6], P[7] or P[23] but not P[19] (Amimo et al., 2015; Lorenzetti et al., 2016;
236 Miyazaki et al., 2011); nevertheless, it is likely that G26 strains acquire a capacity to
237 infect humans when they combine with the P[19] VP4 gene.
238 Previously, Liu et al. (2012) grouped together genotypes P[4], P[6], P[8] and
239 P[19] into P genogroup (GG) P[II] based on the phylogenetic similarity of their VP8*
240 protein. It was shown that the recombinant VP8* molecule of GG P[II] binds to both the
241 H type 1 and Lewis b antigens of humans, enabling RVA strains carrying the GG P[II]
242 to infect humans. Furthermore, a comprehensive GenBank search by Do et al. (2016)
243 revealed that the most frequent G and P genotype combination in porcine-like human
244 RVA strains such as G9P[19] was not necessarily the most common G and P genotype
245 combination among porcine RVA strains. By referring to the hypothesis by Liu et al.
246 (2012), Do et al. (2016) attributed the discrepancy to the VP4 spike protein which plays
247 an important role during the initial attachment of the virus to the host cells. The
248 observation in this study as well as those previously published on G26P[19] strains are

thus consistent with Liu et al.'s hypothesis about the VP4 factor in interspecies transmission of RVA strains.

Six I12 VP6 sequences have so far been reported (Li et al., 2008; Matthijnssens 2010; Mullick et al., 2013; Shetty et al., 2014). Four of them were porcine-like human G11P[25] strains with the prototype strain KTM368 detected in Nepal. The remaining include a porcine-like human G5P[6] strain (LL4260) and a porcine G9P[x] strain. LL4260 was the closest to 07N1760 and further phylogenetic investigation revealed close relationship of its VP7, NSP1, NSP2, NSP3 and NSP5 genes to porcine RVA strains. Also, clustering together with 07N1760 and LL4260 in the phylogenetic tree was a porcine G9 strain TA-1-1 (GenBank data, unpublished). These evidences support the probable porcine RVA origin of the I12 VP6 gene of 07N1760.

Phylogenetic comparison of 07N1760 to the only G26P[19] strain with a known full genotype constellation (30378) revealed that despite the similarity in genotype constellation as well as their porcine rotavirus origin, both strains evolved independently in the porcine population based on the following evidences. Firstly, the two strains possessed different porcine-RVA specific VP6 genotypes: I12 for 07N1760 and I5 for 30378. Secondly, the NSP2 genes of both strains were N1 genotypes but that of 07N1760 was of human RVA origin while that of 30378 was of porcine RVA origin. Thirdly, for the genes in which both strains shared the same genotype, their sequences belonged to clearly distinguishable lineages with low nucleotide sequence identities ranging from 86.2% (VP3 gene) to 96.0% (NSP5 gene).

The human RVA origin of the NSP2 gene of 07N1760 is well supported by the phylogenetic evidence; however, the host species in which the reassortment event occurred could only be speculated. Two alternative scenarios may be plausible. First, a

G26P[19] porcine RVA strain infected a human host habouring a Wa-like human RVA strain. A reassortment event occurred and the porcine RVA strain acquired the NSP2 gene from the human RVA. The mono-reassortant strain infected another child and this was the child from whom 07N1760 was detected. Second, a pig harbouring a G26P[19] porcine was infected by a Wa-like human RVA strain. A mono-reassortant strain generated in the pig crossed the host-species barrier to infect a child; this was the child from whom 07N1760 was detected. The former scenario may be more likely as it presupposes the minimum number of interspecies transmission events.

In conclusion, the Nepali G26P[19] strain 07N1760 derived its NSP2 gene from human RVA by intra-genotype reassortment probably after transmission of a porcine rotavirus to a human host. As this study provides evidence for the role of pig RVA strains in the diversification of human RVA genomes, there is the need to examine the whole genome of the G26 RVA strains detected from pig populations to gain insight into how they relate to those detected in humans.

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Conflict of interest

296 The authors declare that they have no conflict of interest regarding this study.

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436 **Legends for figures**

437 **Fig. 1:** Phylogenetic trees of the nucleotide sequences of **(a)** G26 VP7 **(b)** P[19] VP8*
438 region **(c)** I12 and I5 VP6 (**d)** A8 NSP1 **(e)** N1 NSP2 and representative strains bearing
439 the same or similar genotypes with 07N1760 selected from the GenBank database. The
440 strain 07N1760 characterised in this study is in red font and indicated with a red dot
441 while the Vietnamese G26P[19] strain - 30378 whose whole genome information is
442 available for comparison is in blue font and indicated with a blue dot. Maximum
443 likelihood phylogenetic analyses were performed using the best fit models in MEGA6
444 software package. The trees presented here are rooted trees. Significant bootstrap values
445 (1000 replicates) are indicated at each node. The scale bar at the bottom of the trees
446 indicates genetic distance expressed as nucleotide substitutions per site.

447

448 **Supplementary Fig. 1:** Phylogenetic trees of the nucleotide sequences of **(a)** R1 VP1
449 **(b)** C1 VP2 **(c)** M1 VP3 (**d)** T1 NSP3 **(e)** E1 NSP4 **(f)** H1 NSP5 and representative
450 strains bearing the same genotypes with 07N1760 selected from the GenBank database.
451 The strain 07N1760 characterised in this study is in red font and indicated with a red dot
452 while the Vietnamese G26P[19] strain - 30378 whose whole genome information is
453 available for comparison is in blue font and indicated with a blue dot. Maximum likeli-
454 hood phylogenetic analyses were performed using the best fit models in MEGA6 soft-
455 ware package. The trees presented here are rooted trees. Significant bootstrap values
456 (1000 replicates) are indicated at each node. The scale bar at the bottom of the trees in-
457 dicates genetic distance expressed as nucleotide substitutions per site.

Fig. 1a VP7: G26

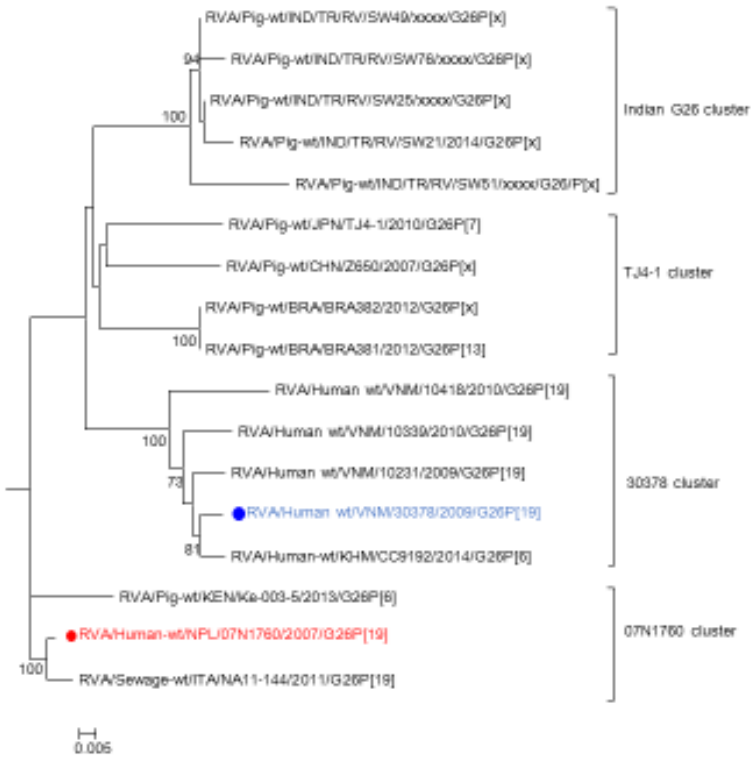

Fig. 1b VP4 gene: P[19]

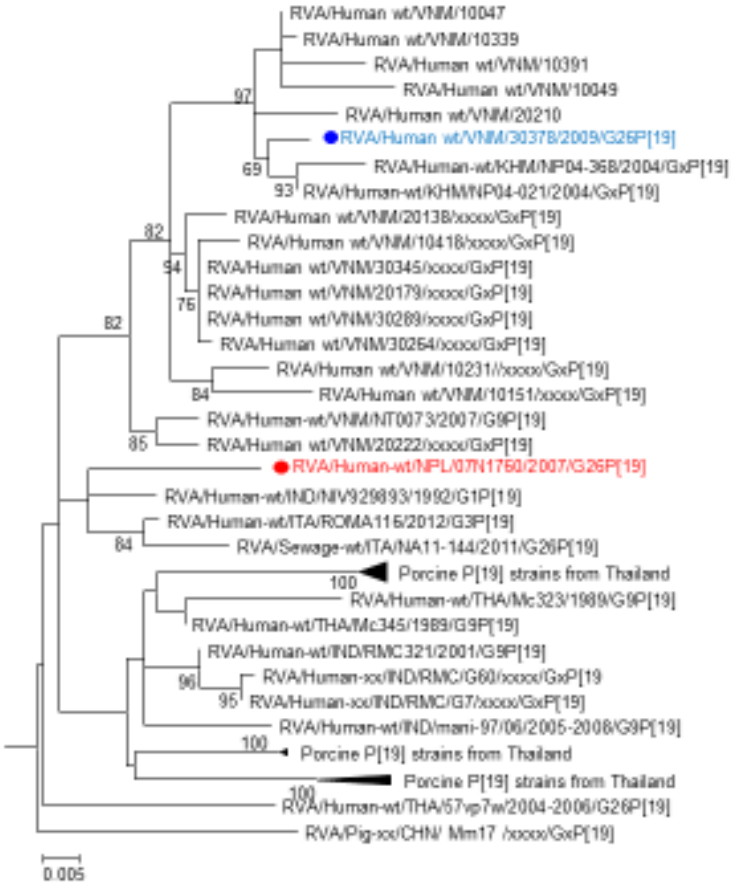

Fig. 1c VP6 gene

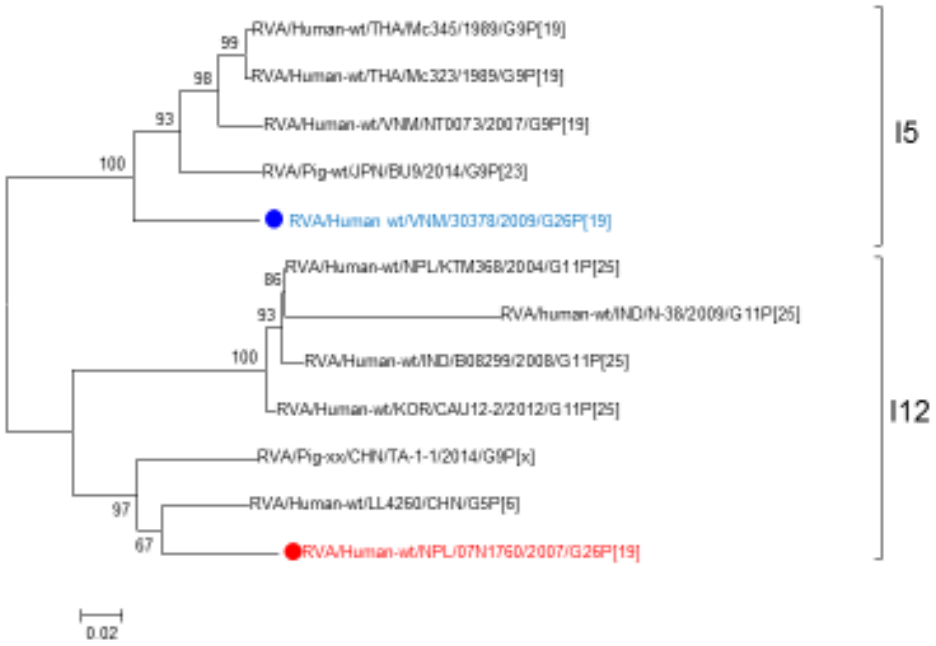

Fig. 1d NSP1: A8

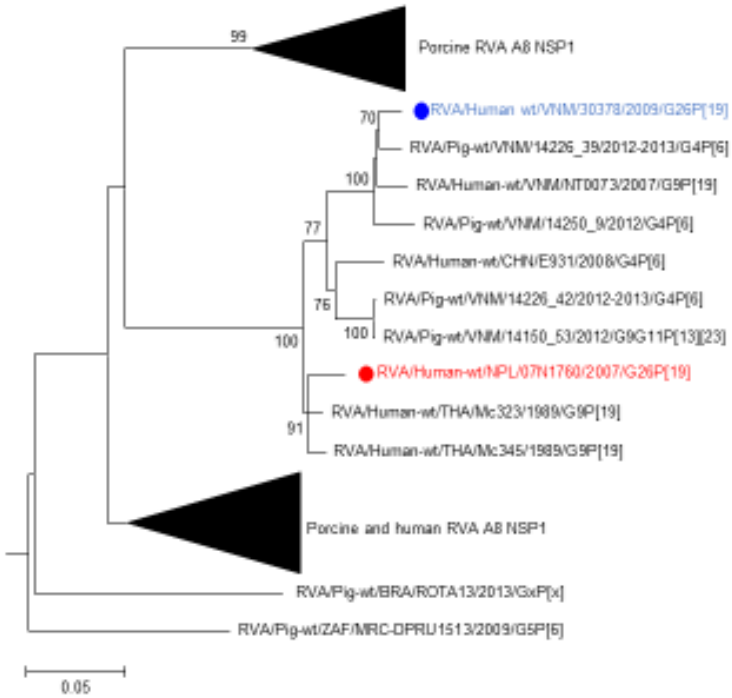

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Fig. 1e NSP2: N1

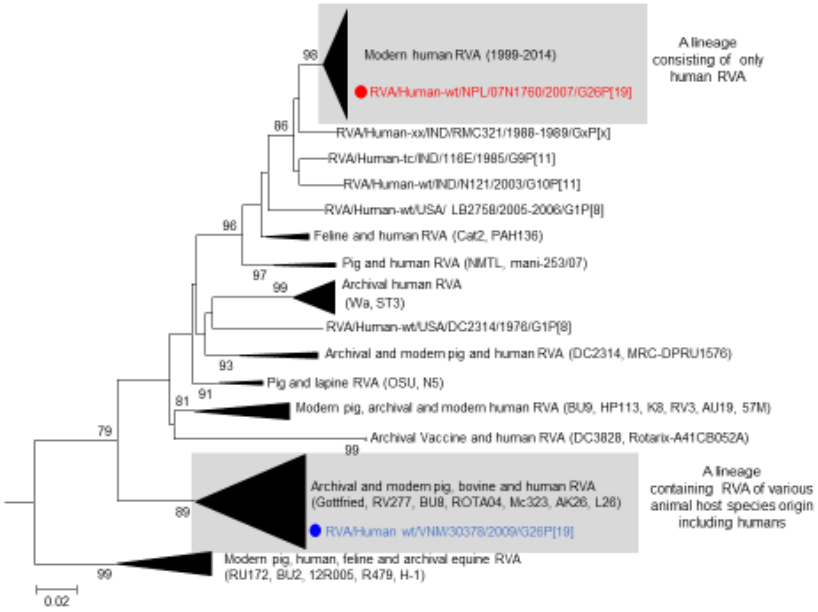

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Table 1: Strains closest 07N1760 in the 11 genome segments and the possible host species origin of the genes of 07N1760

Gene	Genotype	Possible host origin	Sequence clustering with	Closest strain	Nucleotide sequence identity to the closest strain (%)	Reference
VP7	G26	Porcine	Sewage and pig strains	RVA/Sewage-wt/ITA/NA11-144/2011/G26P[19]	98.7	Ruggeri et al., 2015
				RVA/Pig-wt/KEN/Ke-003-5/2013/G26P[6]	97.0	Amimo et al., 2015
VP4	P[19]	Porcine	Porcine-like human strains	RVA/Human-wt/IND/NIV929893/1992/G1P[19]	96.8	Chitambar et al., 2009
VP6	I12	Porcine	Porcine-like human and porcine strains	RVA/Human-wt/LL4260/CHN/G5P[6]	92.1	Li et al., 2008
VP1	R1	Porcine	Porcine strains	RVA/Pig/IDN/RU172/xxxx/G12P[7]	96.7	Ghosh et al., 2006
VP2	C1	Porcine	Porcine-like human and porcine strains	RVA/Human-wt/CHN/E931/2008/G4P[6]	95.2	Zhou et al., 2015
VP3	M1	Porcine	Porcine-like human and porcine strains	RVA/Human-wt/VNM/NT0073/2007/G9P[19]	96.9	Do et al., 2016
NSP1	A8	Porcine	Porcine-like human and porcine strains	RVA/Human-wt/THA/Mc345/1989/G9P[19]	97.5	Ghosh et al., 2012
NSP2	N1	Human	Human strains	RVA/Human-tc/BRA/R138/1998/G9P[9]	98.9	Tsugawa et al., 2015
NSP3	T1	Porcine	Porcine strain	RVA/Pig/IDN/RU172/xxxx/G12P[7]	97.2	Ghosh et al., 2006
NSP4	E1	Porcine	Porcine and porcine-like human strains	RVA/Human-xx/IND/RMC/G7/xxxx/GxP[x]	98.6	GenBank data (2005), Varghese and Naik (Unpublished)
				RVA/Pig-wt/IVRI-UP/IND/Por-174/2015/GxP[x]	98.4	
				RVA/Human-wt/IND/NIV929893/1992/G1P[19]	98.1	Chitambar et al., 2009
NSP5	H1	Porcine	Human and pig strains	RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6]	98.8	Komoto et al., 2013

Table 2: Published G26 RVA strains and their host species origin

Nucleotide accession number	Strain	Whole genome sequence	Reference
LC208008	RVA/Human-wt/NPL/07N1760/2007/G26P[19]	Determined	This study
HG513053	RVA/Human-wt/VNM/30378/2009/G26P[19]	Determined	My et al., 2014
HG513056	RVA/Human-wt/VNM/10231/2009/G26P[19]	ND	My et al., 2014
HG513057	RVA/Human-wt/VNM/10339/2010/G26P[19]	ND	My et al., 2014
HG513058	RVA/Human-wt/VNM/10418/2010/G26P[19]	ND	My et al., 2014
DQ674932	RVA/Human-wt/THA/57vp7w/2004-2006/G26P[19]*	ND	Theamboonlers et al., 2008
KF414613	RVA/Sewage-wt/ITA/NA11-144/2011/G26P[19]	ND	Ruggeri et al., 2014
AB605258	RVA/Pig-wt/JPN/TJ4-1/2010/G26P[7]	ND	Miyazaki et al., 2011
KT310239	RVA/Pig-wt/BRA/BRA382/2012/G26P[x]	ND	Lorenzetti et al., 2016
KT310238	RVA/Pig-wt/BRA/BRA381/2012/G26/P[13]	ND	Lorenzetti et al., 2016
KP057834	RVA/Pig-wt/KEN/Ke-003-5/2013/G26P[6]	ND	Amimo et al., 2015

ND: Not determined *Described as a G3 in the original publication

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Table 3: Distribution of the G-types of P[19] RVA strains in different host species*

		G genotype					
		G1	**G3**	**G9**	**G26**	**Gx**	**Total**
Host species	**Human**	1	1	19	5	3	29
	Pig	0	13	1	0	3	17
	Sewage	0	0	0	1	0	1
	Total	1	14	20	6	6	**47**

**Information compiled from sequences available in the GenBank database and Virus Pathogen Resource*

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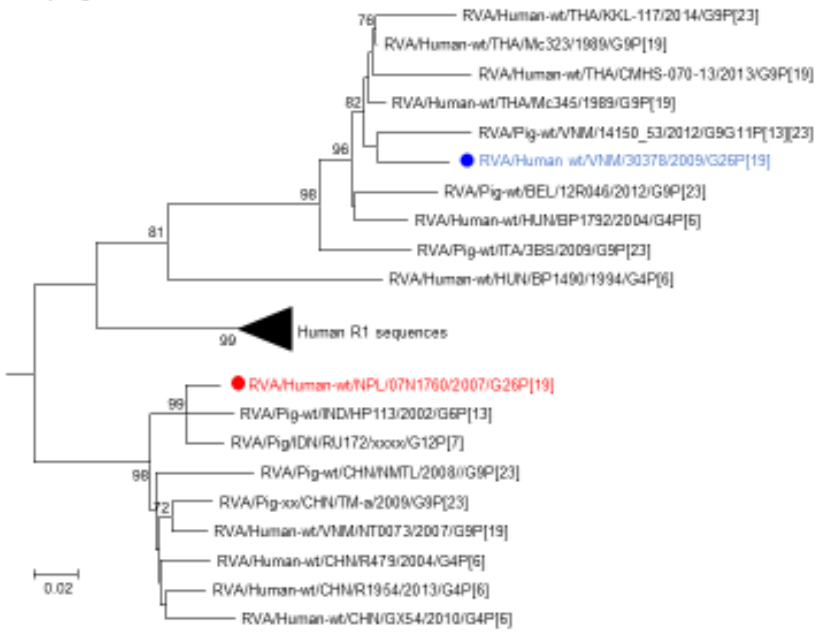
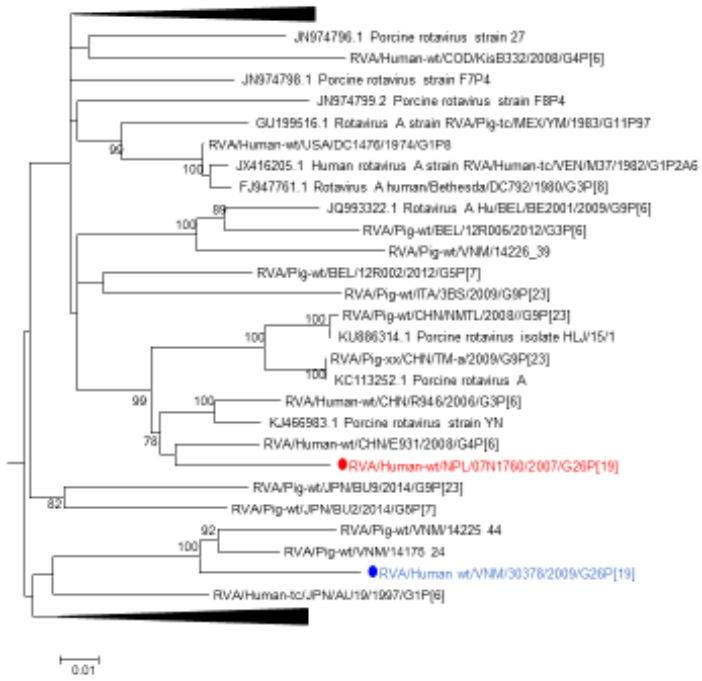
Supplementary Fig. 1a VP1: R1



Supplementary Fig. 1b VP2: C1



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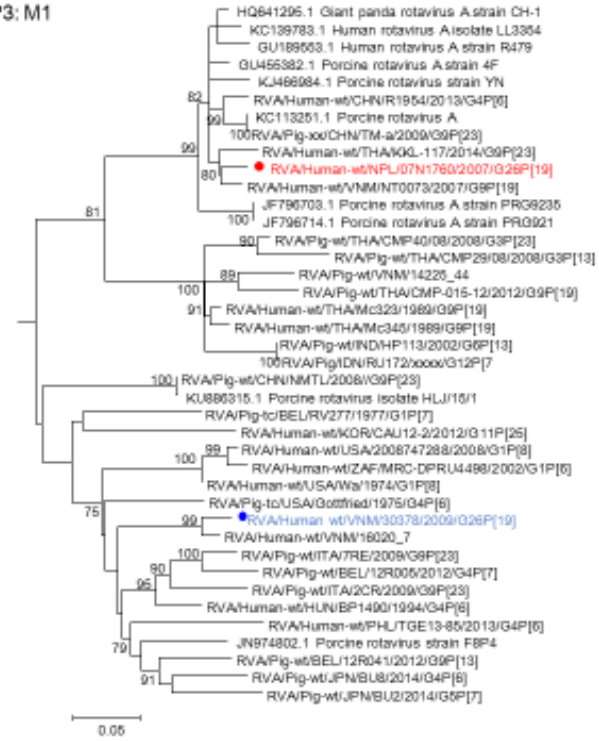
Supplementary Fig. 1c VP3: M1



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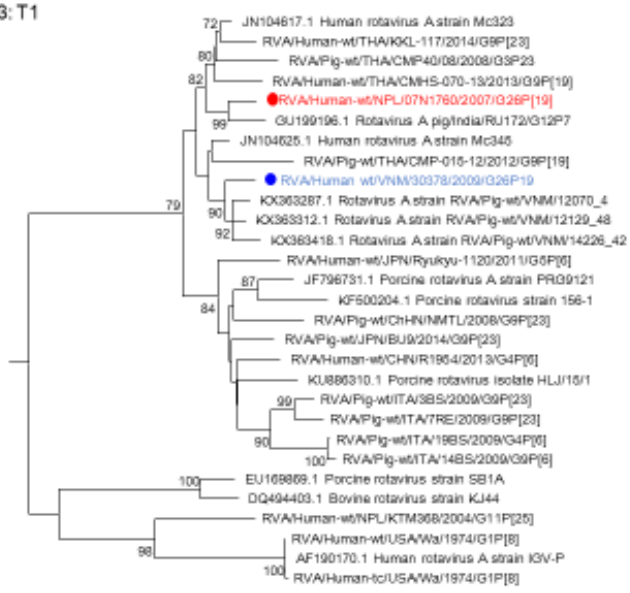
Supplementary Fig. 1d NSP3: T1



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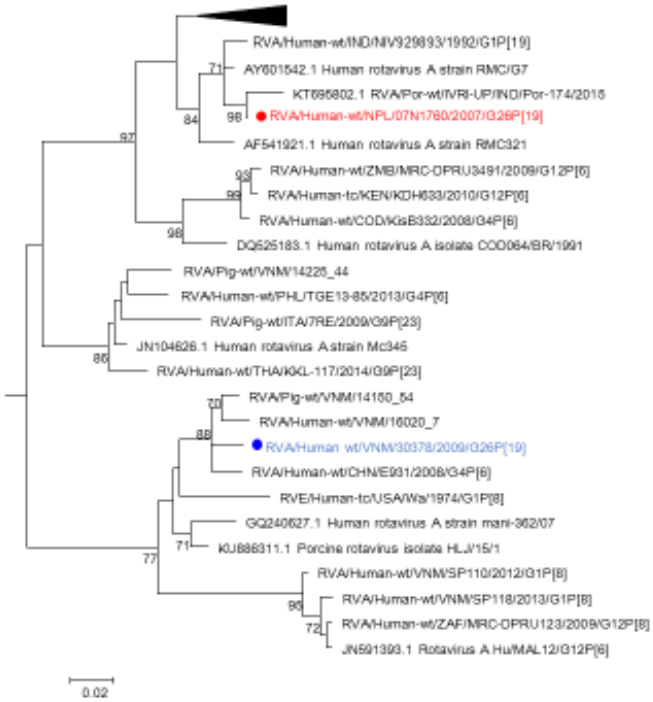
Supplementary Fig. 1e NSP4: E1



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Supplementary Fig. 1f NSP5: H1