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Research paper

Whole genome characterisation of G11P[25] and G9P[19] rotavirus A strains from adult patients with diarrhoea in Nepal

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ABSTRACT

Rotavirus A (RVA) causes acute diarrhoea in children and less frequently in adults. However, the knowledge about the genotype distribution of RVA strains circulating in adults is limited particularly in developing countries. This study aimed to characterise the RVA strains detected from adult patients with diarrhoea in Nepal. A total of 47 RVA positive stool samples from adult patients with diarrhoea in Kathmandu, Nepal during 2007–2008 were examined for the G and P genotypes by sequencing. Nearly half (49%) of the samples were genotyped as G9P[8] (n = 23), G1P[8], G2P[4] (n = 5 each), G12P[8] (n = 4), G12P[6] (n = 3), G1P[6] (n = 2), G3P[8] and G9P[6] (n = 1 each). Interestingly, two G11P[25] and one G9P[19] strains detected were further subjected to Illumina MiSeq next generation sequencing to determine their whole genome sequences. The genotype constellations of RVA/Human-wt/NPL/TK2615/2008/G11P[25] and RVA/Human-wt/NPL/TK2620/2008/G11P[25] were I12-R1-C1-M1-A1-N1-T1-E1-H1, whereas that of RVA/Human-wt/NPL/TK1797/2007/G9P[19] was I5-R1-C1-M1-A8-N1-T1-E1-H1. The 11 genes of TK2615 and TK2620 were virtually identical, and they were either porcine-like or unique except the VP2 and NSP1 genes which were of human RVA origin. The two G11P[25] strains were also very similar to KTM368, another G11P[25] isolated from a child in Nepal in 2004. On the other hand, no gene of TK1797 was likely to be of human RVA origin. The observation that porcine-like RVAs were detected from adult patients justifies further studies to explore the role of adults in the interspecies transmission of animal RVA to humans.

1. Introduction

Rotavirus A (RVA), a member of the genus *Rotavirus*, family *Reoviridae*, possesses 11 segments of double-stranded RNA as the genome (Estes and Greenberg, 2013). A binary classification system based on the two outer-capsid proteins, VP7 and VP4, which are involved in virus neutralisation and protective immunity, classifies RVA strains into G and P types, respectively (Estes and Greenberg, 2013). While numerous G and P genotypes have been reported, six G and P genotype combinations are commonly detected among human RVA strains; they are G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] (Banyai et al., 2012). To fully describe the genetic makeup of a rotavirus strain, the classification system has been expanded to include the remaining internal capsid and non-structural protein genes, and it denotes the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 genes of an RVA strain as a descriptor Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx where x indicates genotype number, respectively (Matthijnssens et al., 2008b; Matthijnssens et al., 2011). While there are currently 36 G, 51 P, 26 I, 22 R, 20 C, 20 M, 31 A, 22 N, 22 T, 27 E, and 22 H genotypes approved by the Rotavirus Classification Working Group (https://rega.kuleuven.be/cev/

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; BLAST, Basic Local Alignment Search Tool

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viralmetagenomics/virus-classification), most human RVA strains are classified into one of the three genotype constellations: *i.e.*, G1/G3/G4/G9-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1, G2 -P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2 and G3-P[9]-I3-R3-C3-M3-A3-N3-T3-E3-H3 (Heiman et al., 2008; Matthijnssens et al., 2008a; Matthijnssens et al., 2011).

Although virtually all children are infected with rotavirus by the age of 5 years and acquire immunity to rotavirus infection, they remain susceptible to rotavirus infection throughout life and sometimes get diarrhoea due to RVA (Anderson and Weber, 2004). However, there are a limited number of studies that examined the genotype distribution of RVA strains circulating in adults, particularly in developing countries. In our previous study conducted in Kathmandu, Nepal, RVA accounted for 5% of acute diarrhoea in adolescents and adults (15 years and older). The genotype distribution was similar to that of infants and young children with G1P[8] being the predominant strain (Uchida et al., 2006). However, the actual number of diarrhoeal samples tested was only 13 in that study. Thus, this study was undertaken to determine the G and P genotype distribution of RVA strains detected in the stool specimens obtained from adult-diarrhoea patients in Nepal, and to characterise any unusual strains at the whole genome level.

2. Materials and methods

2.1. Study specimens and RVA antigen detection

A 15-month cross-sectional study was conducted in Sukraraj Tropical and Infectious Disease Hospital in Kathmandu, the capital of Nepal, from March 2007 to May 2008, in order to determine the prevalence of rotaviruses A, B, and C in adult-diarrhoea patients by polyacrylamide gel electrophoresis as described previously (Alam et al., 2013). The electrophoretic migration pattern of the rotavirus RNA from the samples were previously shown to be the 4–2–3-2 pattern on the gel. The presence of RVA antigen in the samples was confirmed with an enzyme-linked immunosorbent assay (ELISA) kit (Premier Rotaclone, Meridian Bioscience, Inc., OH, USA).

2.2. G and P genotyping

Genomic RNAs were extracted from 10% suspension of RVA-positive faecal specimens in phosphate buffered saline by using the QIAamp Viral RNA Mini Kit (QIAGEN Sciences, Germantown, MD, USA) according to the manufacturer's instructions.

Complementary DNA (cDNA) was synthesized from the genomic RNA at 42 °C for 30 min using random hexamers (Invitrogen, Carlsbad, CA, USA) and the SuperScript III Reverse Transcriptase (Invitrogen). The resulting cDNAs were amplified for the VP7 gene with the Beg9 and End9 primers (Gouvea et al., 1990), and for the VP4 gene with the Con3 and Con2 primers (Gentsch et al., 1992) by carrying out 30 cycles of PCR (1 min of denaturing at 94 °C, 2 min of annealing at 42 °C and 3 min of extension at 72 °C) with a final extension cycle at 72 °C for 8 min.

PCR products were purified using Exosap-IT purification system (USB products) following the manufacturer's protocol and sequenced in both forward and reverse directions by the fluorescent dideoxy chain termination chemistry using Big Dye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems). Nucleotide sequence reads were obtained with the aid of the ABI-PRISM 3730 Genetic Analyzer (Applied Biosystems), and genotyped by using the RotaC2.0 automated genotyping tool for RVA strains (Maes et al., 2009).

2.3. Full genome sequencing using Illumina MiSeq platform

Three samples with unusual G/P types namely G11P[25] (TK2615 and TK2620), and G9P[19] (TK1797) were selected for full genome sequencing. Prior to construction of the cDNA library, the RNA concentration in each sample was quantified using the NanoDrop spectrophotometer ND–1000 (NanoDrop Technologies Inc., Wilmington, DE, USA) according to the manufacturer's instructions and the starting concentration was normalized to 100 ng. A 200 bp fragment library ligated with bar-coded adapters was prepared for the G11P[25] (TK2615 and TK2620), and G9P[19] (TK1797) strains using the NEBNext Ultra RNA library Prep Kit for Illumina v1.2 (New England Biolabs, Ipswich, MA, USA) and an NEBNext Multiplex Oligos for Illumina (New England Biolabs) following manufacturer's recommendations. The cDNA library was purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Pasadena, CA, USA) according to recommendations in the NEBNext protocol. The quantity and the quality of the purified libraries were determined on a Qubit 2.0 flourometer (Invitrogen, Carlsbad, CA, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany), respectively. Nucleotide sequencing was performed on an Illumina MiSeq sequencer (Illumina, San Diego, CA, USA) using a MiSeq Reagent Kit v3 (600 cycles) (Illumina) to generate 301 paired-end reads.

Using the default medium sensitivity parameter, both *de novo* and mapping assembly tools embedded in Geneious® software, version 11.1. (Biomatters, https://www.geneious.com/; Kearse et al., 2012) were employed to generate contigs from the Illumina MiSeq sequence data in FASTQ file format. For the mapping to reference assembly, the sequence data of study strains TK2615 and TK2620 were mapped to the genome of the reference strain RVA/Human-wt/NPL/KTM368/2004/G11P[25] (accession numbers: GU199492 – GU199502) whereas strain TK1797 was mapped to the genome of the reference strain RVA/Human-wt/THA/Mc323/1989/G9P[19] (accession numbers: JN104611-JN104618; D38052 – D38053). Number of reads for each genome segment are provided in Supplementary Table 1. The genotype of each of the genome segments was determined using the RotaC2.0 automated genotyping tool for RVA strains (Maes et al., 2009).

2.4. Phylogenetic analysis

Using the Basic Local Alignment Search Tool (BLAST), the closest sequences to the genes of the study strains were retrieved from the GenBank. In addition, representative human and porcine RVA strains possessing relevant genotypes were retrieved from the GenBank and included in the nucleotide sequence dataset for phylogenetic analyses. Multiple alignments of the nucleotide sequences were performed using the MUSCLE programme in the MEGA6 package (v6.06) (Tamura et al., 2013).

Phylogenetic trees were constructed by the Maximum Likelihood method. The selection of the best-fit evolutionary model for each gene's dataset was made based on the lowest Bayesian Information Criterion score after running the nucleotide substitution model testing implemented in MEGA6 (Tamura et al., 2013). Thus, the Tamura 3-parameter (T92) model with the discrete Gamma distribution (G) and invariant sites (I) were used for the VP6, P[8] VP4, and NSP3 genes; the T92 + G model for the VP7, NSP4, and NSP5 genes; the T92 + I model for the P[25] VP4 genes; the General Time Reversible (GTR) model with G + I for the VP1, VP3, NSP1, and NSP2 genes; the GTR + G model for the VP2 gene; the GTR + I model for the P[19] VP4 genes. The branching topology in the trees were analysed by bootstrapping with 1000 replicates.

2.5. Nucleotide sequence accession numbers

The nucleotide sequences of TK2615, TK2620, TK1797 and G9P[8] strains detected in this study were deposited in the GenBank/EMBL/DDBJ databases under accession numbers LC433791 - LC433801, LC433802 - LC433812, LC433780 - LC433790 and LC437649 - LC437662 respectively.

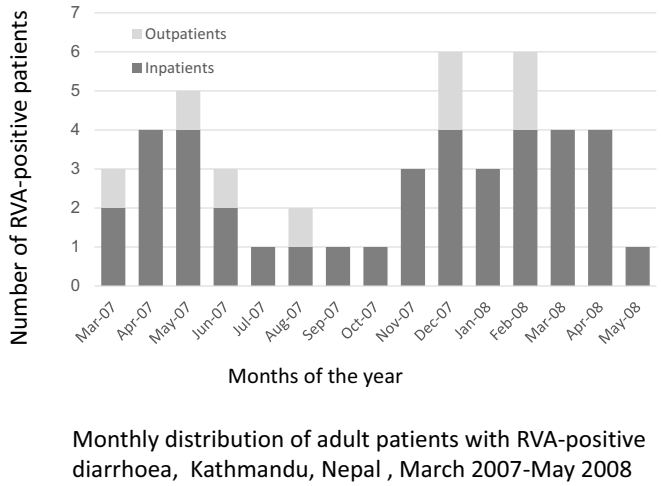

Fig. 1. Monthly distribution of adult patients with RVA-positive diarrhoea in Kathmandu, Nepal, March 2007–May 2008. Hospitalised patients are highlighted in dark grey and outpatients in light grey.

3. Results

3.1. Genotype distribution and identification of unusual strains

All the 47 specimens from adult patients with diarrhoea that contained rotaviruses possessing the 4-2-3-2 genomic RNA migration pattern typical of RVAs, were confirmed positive for RVA by Rotaclone ELISA.

Out of the 47 RVA-positive samples, nearly half were genotyped as G9P[8] (n = 23, 48.9%), which was followed by G1P[8], G2P[4] (n = 5 each, 10.6%), G12P[8] (n = 4, 8.5%), G12P[6] (n = 3, 6.4%), G1P[6] (n = 2, 4.3%), G3P[8] and G9P[6] (n = 1 each, 2.1%). In addition, there were three unusual RVA strains possessing G9P[19] (TK1797/2007) and G11P[25] (TK2615/2008 and TK2620/2008) genotypes (Fig. 1).

3.2. Phylogenetic analysis of the dominant G9P[8] strains for their VP7 and VP4 genes

The VP7 gene of the 23 G9P[8] samples showed high nucleotide sequence identities ranging from 99.4–100%, and belonged to lineage III, a lineage to which a vast majority of contemporary human G9 RVA strains belong (Fig. 2). Also, 19 of the VP4 sequences (the VP8* portion) belonged to P[8]a cluster of which 18 were > 99.5% identical. The remaining four samples belonged to P[8]b and they had an identical sequence (Fig. 3).

3.3. Genotype constellation and phylogeny of the G9P[19] strain (TK1797)

The genotype constellation of TK1797 was G9-P[19]-I5-R1-C1-M1-A8-N1-T1-E1-H1. This constellation was the most common among the eighteen P[19] strains whose full genome information was available out of the 72 P[19] strains deposited in the GenBank database. In the G9 VP7 tree, strain TK1797 belonged to lineage III, but within this lineage it was located outside of the cluster to which the vast majority of

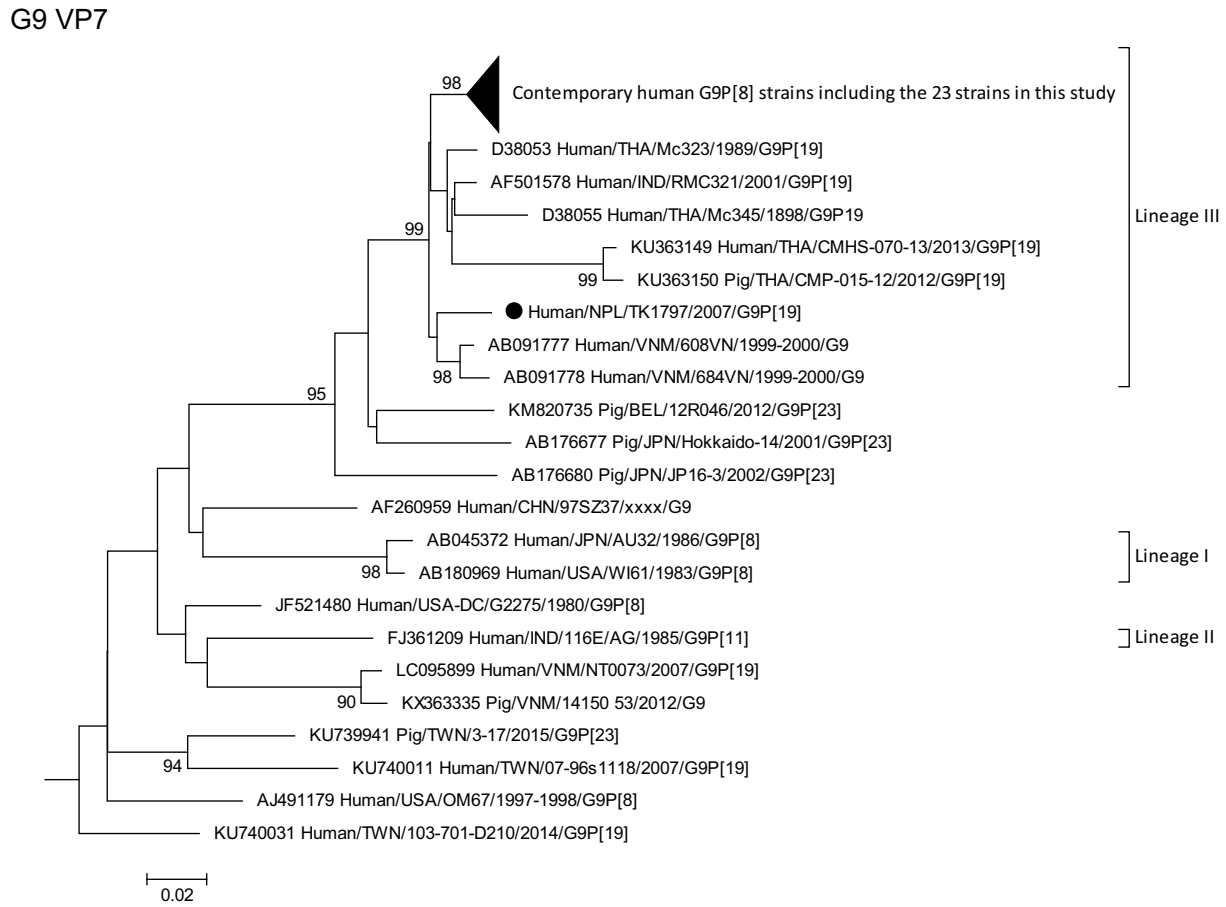

Fig. 2. A phylogenetic tree drawn for the G9 VP7 genes by the Maximum-Likelihood method. The VP7 gene of TH1797 is marked with a black dot, and those of the 23 G9P[8] strains detected in this study are enclosed within the triangle. The scale bar at the bottom indicates a genetic distance of 0.02 nucleotide substitutions per site.

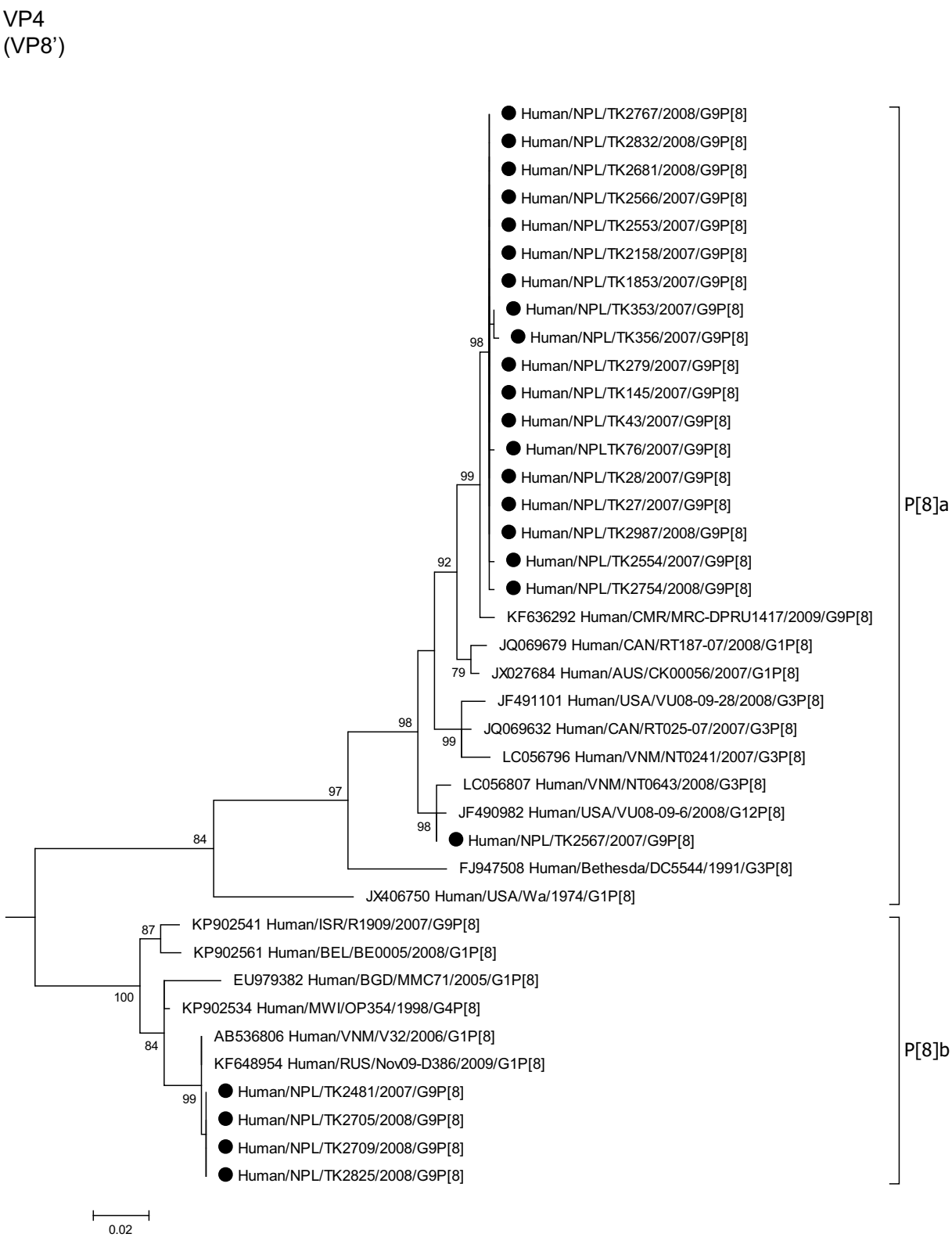

Fig. 3. A phylogenetic tree drawn for the P[8] VP4 genes by the Maximum-Likelihood method. The VP4 gene of the 23 G9P[8] strains detected in this study are marked with a black dot. Nineteen of them belonged to P[8]a, whereas four of them belonged to P[8]b. The scale bar at the bottom indicates a genetic distance of 0.02 nucleotide substitutions per site.

contemporary G9 VP7 genes of human RVA origin belonged (Fig. 2). The VP7 gene sequence identities between TK1797 and the 23 G9P[8] strains detected in this study ranged from 96.1 to 96.5%. The highest nucleotide sequence identity (97.0%) was observed between TK1797 and any of the three strains; namely, RVA/Human-wt/VNM/608VN/1999-2000/G9P[x] (Doan et al., 2003), RVA/Human-wt/IND/RMC321/1990/G9P[19] (Das et al., 2004) and Human-wt/JPN/95H115/1995/G9P[8] (Oka et al., 2000).

Table 1
Nucleotide sequence comparison of TK1797 genes with human and porcine P[19] strains.

Strain	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
TK1797	G9	P[19]	I5	R1	C1	M1	A8	N1	T1	E1	H1
Human/THA/CMP-015-12/2012/G9P[19]	93.4	95	88.5	93.4	87.7	93.6	80.2	86.7	88.5	91.2	96.3
Pig/THA/CMHS-070-13/2013/G9P[19]	93.6	94.4	88.9	93.2	87.6	86.1	82.4	86.9	88.4	90.9	96.4
Human/THA/Mc323/1989/G9P[19]	96.6	95.6	90.4	96.6	88	94.7	97.8	87.2	89.1	91.7	95.6
Human/NPL/07N1760/2007/G26P[19]	G26	96.9	I2	87.3	88.5	88.8	98	87.3	88.6	98.7	96.4
Human/VNM/NT0073/2007/G9P[19]	89.1	96.7	90	87.6	87.5	89	94.3	86.5	T7	91.3	98.6

Table 2
Nucleotide sequence identities between TK2615/TK2620 and other known G11P[25] strains for which the whole genome information is available.

Country/Year	strain	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
Nepal/2008	TK2615	G11	P[25]	I12	R1	C1	M1	A1	N1	T1	E1	H1
Nepal/2008	TK2620	100*	100	100	100	100	100	100	100	100	100	100
Bangladesh/2001	Dhaka6	99.9	99.4	**I1****	87.3	99.4	99.1	98.7	87.1	91.7	88	95.9
Nepal/2004	KTM368	100	99.8	100	99.8	99.8	99.6	99.8	99.9	99.8	99.7	99.8
India/2008	B08299	98.6	97.5	98.8	98.6	98.6	98	84.0	99.1	98.8	98.4	95.1
Korea/2012	CAU-12	98.6	98.8	98.5	86.0	98	97.9	83.9	98.9	99.2	96.8	98.8

The inner capsid and non-structural protein genes of TK1797 appeared to cluster with porcine or porcine-like human RVA strains in the phylogenetic trees (Supplementary Fig. 1). Genotypes I5 (the VP6 gene) and A8 (the NSP1 gene) are known to be carried exclusively by porcine RVA strains and human strains of porcine RVA origin.

There are three human strains and one porcine strain in the literature (Ghosh et al., 2012; Yodmeeklin et al., 2017) that possess the same genotype constellations as TK1797; they are RVA/Human-wt/THA/Mc323/1989/G9P[19], RVA/Human-wt/THA/Mc325/1989/G9P[19], RVA/Human-wt/THA/CMH-S070–13/2013/G9P[19], and RVA/pig-wt//THA/CMP-015-12/2012/G9P[19]. While none of them clustered together with TK1797 with a statistically significant bootstrap support, Mc323 showed high nucleotide sequence identities with TK1797 except for the VP2, NSP2 and NSP3 genes (Table 1). Incidentally, there were two P[19] strains detected in Nepal and Vietnam in 2007 that shared identical genotypes in nine genome segments with TK1797. They were RVA/Human-wt/NPL/07N1760/2007/G26P[19] possessing I12-R1-C1-M1-A8-N1-T1-E1-H1 (Agbemabiese et al., 2017), and RVA/Human-wt/VNM/NT0073/2007/G9P[19] possessing I5-R1-C1-M1-A8-N1-T7-E1-H1 (Do et al., 2017). While neither 07N1760 nor NT0073 clustered with TK1797 with a statistically significant bootstrap support, 07N1760 showed higher nucleotide sequence identities with TK1797 in the VP4, NSP1 and NSP4 genes than the aforementioned four strains that possess the same genotype constellation as TK1797 (Table 1). Similarly, NT0073 showed a higher nucleotide sequence identity with TK1797 in the NSP5 gene than the aforementioned four strains (Table 1).

3.4. Genotype constellation and phylogeny of G11P[25] strains (TK2615 and TK2620)

The genotype constellation of both TK2615 and TK2620 strains was G11-P[25]-I12-R1-C1-M1-A1-N1-T1-E1-H1, a rare genotype constellation that has been described for only four strains in the literature. They are RVA/Human-wt/NPL/KTM368/2004/G11P[25] (Uchida et al., 2006; Matthijnssens et al., 2010), RVA/Human-wt/IND/B08299/2008/G11P[25] (Shetty et al., 2014), RVA/Human-wt/IND/N-38/2009/G11P[25] (Mullick et al., 2013), and RVA/Human-wt/KOR/CAU12-2/2012/G11P[25] (Than et al., 2013). One more G11P[25] strain BGD/Dhaka6/2001 exists for which the whole genome sequence information is available in literature, it however possesses an I1 instead of an I12 (Matthijnssens et al., 2010).

Sequence comparison revealed that TK2615 and TK2620 had a 100% identical nucleotide sequence throughout the 11 genes; thus, they are the same strain obtained from two different patients. TK2615 and TK2620 were closely related to KTM368 with very high nucleotide sequence identities ranging from 99.6% in the VP3 gene to 100% in the VP7 and VP6 genes even though they were detected three years apart (Table 2). Phylogenetic trees of the 11 genes of the G11P[25] strains possessing the same genotype constellation as TK2615 and TK2620 (except N-38 due to insufficient sequences lengths) showed that the five G11P[25] strains belonged to the same lineage with nucleotide sequence identities ranging from 96.2% in the NSP3 gene to 98.1% in the NSP2 gene except the VP1 and NSP1 genes (Table 2). In the VP1 gene tree (Fig. 4), CAU12–2 (indicated with an arrow) belonged to a cluster consisting of mostly porcine RVAs away from the cluster to which the remaining four strains (indicated in a box) belonged. This latter cluster was located upstream of the tree with another lineage consisting of porcine RVA strains from Korea, but the nucleotide sequence identities between the two lineages were only 93–94%. In the NSP1 gene tree (Fig. 5), TK2615, TK2620 and KTM368 belonged to a lineage consisting of the vast majority of human RVA, but CAU12–2 and B08299 clustered together with N-38 in a unique, distinct lineage.

Overall, except for the VP2 and NSP1 genes of TK2615 and TK2620 which were interpreted to be of human rotavirus origin based on phylogenetic evidence, the lineages to which the five strains (TK2615 and TK2620 inclusive) belonged formed a monophyletic lineage distinct from other clusters making it very difficult to predict their host species origin.

4. Discussion

A 15-month cross-sectional study conducted in an infectious disease hospital in Kathmandu, Nepal revealed that nearly half of RVA-positive samples from adult patients possessed G9P[8] genotype. The VP7 genes

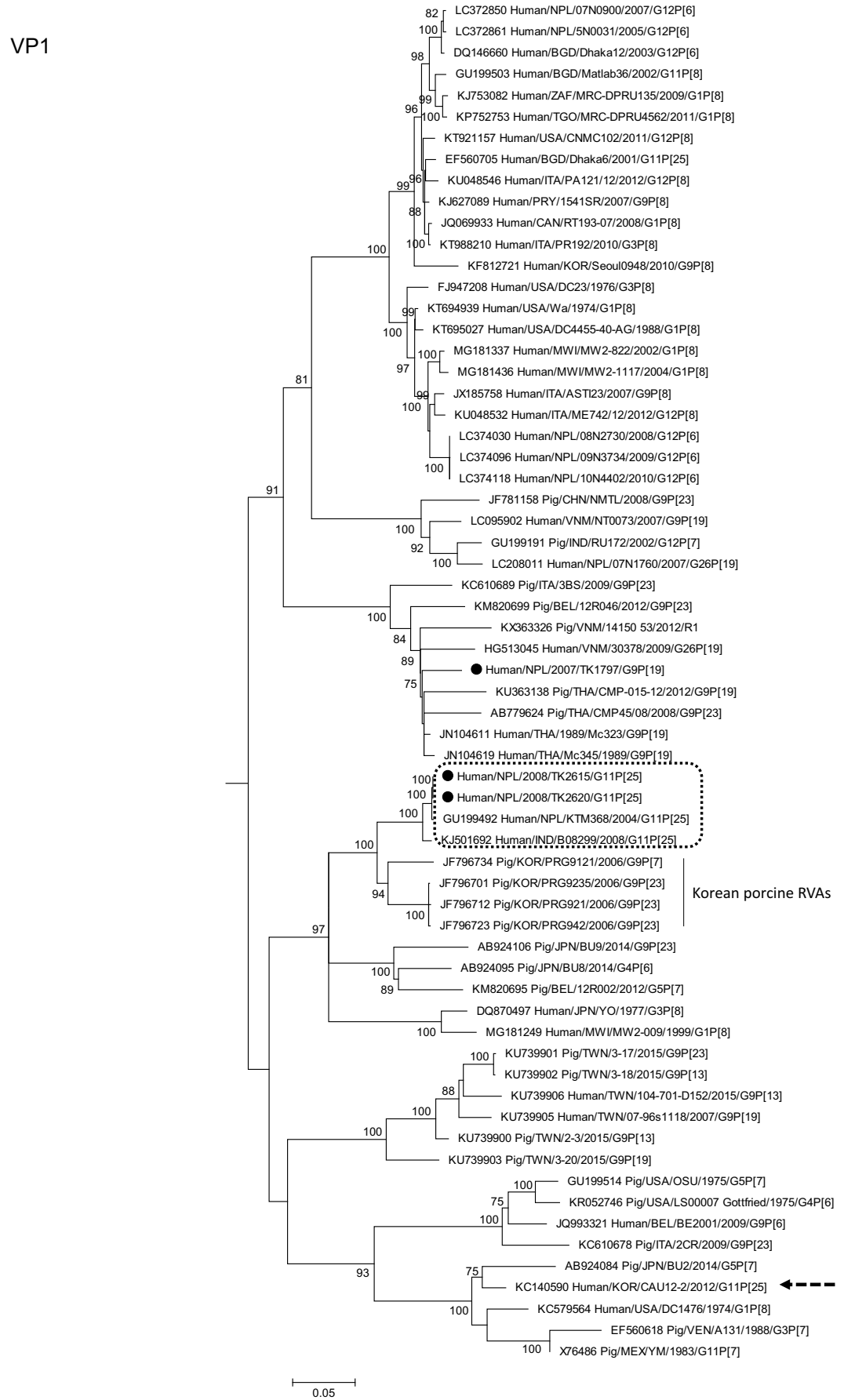

Fig. 4. A phylogenetic tree drawn for the VP1 genes by the Maximum-Likelihood method. The VP1 gene of the two G11P[25] strains (TK2016 and TK2020) detected in this study as well as one G9P[19] strain (TK1797) are indicated with a black dot. The scale bar at the bottom indicates the genetic distance of 0.05 nucleotide substitutions per site.

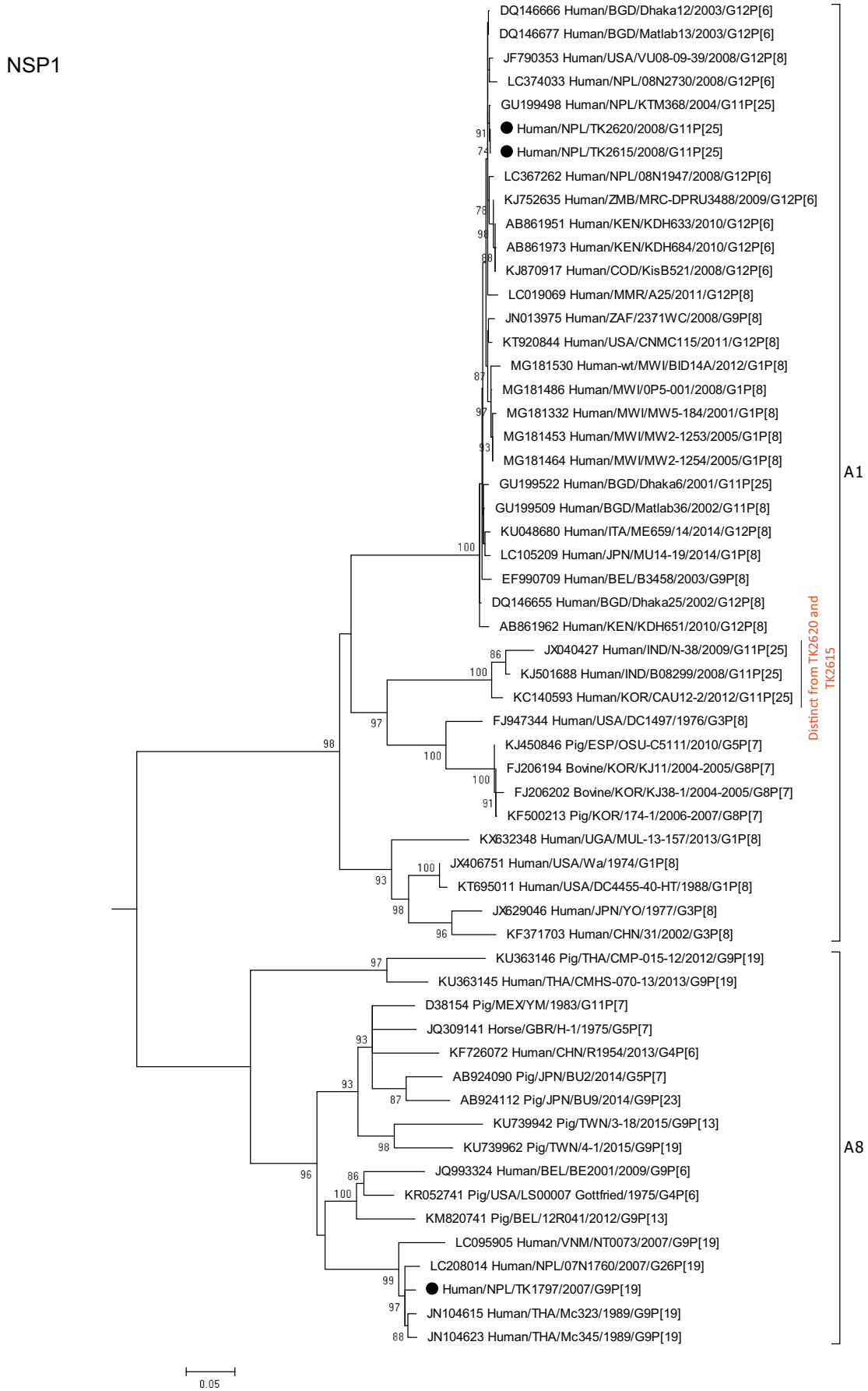

Fig. 5. A phylogenetic tree drawn for the NSP1 genes by the Maximum-Likelihood method. The VP1 gene of the two G11P[25] strains (TK2016 and TK2020) detected in this study as well as one G9P[19] strain (TK1797) are indicated with a black dot. The scale bar at the bottom indicates a genetic distance of 0.05 nucleotide substitutions per site.

of the G9P[8] strains were distinct from that of TK1797, the unusual G9P[19] strain detected in this study, although strain TK1797 also belonged to the same lineage III. On the other hand, the VP4 genes of the dominant G9P[8] strains were dichotomic with 83% belonging to P[8]a and 17% belonging to the P[8]b. The global prevalence of P[8]b, also known as OP354-like VP4, is largely unknown because, firstly, conventional P genotyping primers developed by Gentsch et al. (1992) do not detect P[8]b, and secondly, the VP4 sequence (or VP8* sequence) is needed to be able to assign the P[8]b sub-genotype. Vietnam, where the prevalence of P[8]b is thought to be highest than elsewhere in Asia, P[8]b was detected in about 25% of the P[8] VP4 genes (Nguyen et al., 2008). As to the G types that P[8]b is associated with, Zeller et al. (2015) upon analysing a global collection of P[8]b strains noted that they are often (> 75%) associated with G9 and G1 G-genotypes. They also concluded that P[8]b originated in Asia and spread to other continents within a short period of time despite the fact that P[8]b was first reported in Malawi (Cunliffe et al., 2001). The detection and prevalence of P[8]b observed in this study fit in with such recent understanding of the origin and global distribution of P[8]b. Nevertheless, to the best of our knowledge, this is the first report describing the detection of P[8]b carried by RVA from adult patients.

Of particular note in this study was the detection of an unusual genotype G9P[19] from an 18-year-old outpatient case (designated TK1797), and a very rare G11P[25] genotype from two 28-year old hospitalised patients (designated TK2615 and TK2620). A GenBank search identified only 7 and 20 human RVA strains possessing G11P[25] and G9P[19], respectively. While genotype G11 was initially identified in a porcine RVA strain detected in Mexico (Ruiz et al., 1988), genotype P[25] has thus far been detected only in human RVA strains. Upon phylogenetic analysis, most of the internal capsid protein and non-structural protein genes of G11P[25] strains were distinct from the other relevant sequences that were included in the phylogenetic analysis, making it difficult to determine their host-species origin. Nevertheless, the animal origin of G11P[25] strains is generally accepted. Similarly, the host species origin of G9P[19] rotaviruses is thought to be pigs. In this regard, Yodmeeklin et al. (2017) investigated RVA strains in pigs with diarrhoea in parallel with RVA strains in children in the same geographic area with overlapping study periods, and found two G9P[19] strains, one from a human and another from a pig, that showed 85.6%–99.0% nucleotide sequence identities across the whole genome. Such observations lent strong support to the claim that G9P[19] strains originated from pigs. On the other hand, it is noteworthy that there was only one G9P[19] strain detected out of 491 faecal specimens from piglets collected over 3 years in their study (Yodmeeklin et al., 2016), suggesting that G9P[19] is a rare genotype even among pigs. Nevertheless, the three unusual strains replicated well in the patients as suggested from the observation that TK2615, TK2620 and TK1797 contained a good amount of virus as judged from clearly-visible band density on the gel (not shown).

It may deserve mention that, to the best of the authors' knowledge, G11P[25] and G9P[19] detection in human is thus far limited to Asian countries since surveillance conducted in 14 European countries by EuroRotaNet in which they genotyped RVA strains from a large collection of > 50,000 diarrhoeal samples from children over an eight-year period reported neither G11P[25] nor G9P[19] (Hungerford and Iturriza-Gómara, 2015).

Phylogenetic analysis of all 11 genes of TK1797/G9P[19] together with those of relevant human and animal RVA strains led us to conclude that TK1797 was likely a porcine RVA strain that infected and caused diarrhoea in an adult. Although the host species origin was inconclusive in some genome segments, even in such genes there was no hint implicating that they originated from human RVA strains.

Phylogenetic analysis of all 11 genes of TK2615/G11P[25] and TK2620/G11P[25] - which were shown to be identical strains, together with those of relevant human and animal RVA strains showed that TK2615 and TK2620 possess a unique lineage constellation similar to the Nepali strain RVA/Human-wt/NPL/KTM368/2004/G11P[25] in all genes except the VP2 and NSP1 genes which were concluded to be of human RVA origin. As the G11P[25] strain is unlikely to be of completely human rotavirus origin, the presence of two genes of human rotavirus origin indicates that a strain of unknown animal rotavirus origin crossed the host species barrier. The observation that a G11P[25] strain detected in Nepal and Korea (Uchida et al., 2006; Than et al., 2013) as well as neighbouring India (Mullick et al., 2013; Shetty et al., 2014) had high nucleotide sequence identities in some genes and otherwise in some other genes suggests the occurrence of genetic reassortment over the course of evolution. However, it is challenging to determine what host species such reassortment events occurred in.

In summary, this study identified one porcine-like G9P[19] strain and two G11P[25] strains among 47 rotavirus-positive specimens collected from adults hospitalised for acute diarrhoea in Kathmandu, Nepal. These observations justify the need for further studies to explore the role of adults in the interspecies transmission of animal RVA to humans.

Conflict of interest

There is no conflict of interest for any author to declare regarding this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.02.007.

References

Agbemabiese, C.A., Nakagomi, T., Gauchan, P., Sherchand, J.B., Pandey, B.D., Cunliffe, N.A., Nakagomi, O., 2017. Whole genome characterisation of a porcine-like human reassortant G26P[19] Rotavirus A strain detected in a child hospitalised for diarrhoea in Nepal, 2007. Infect. Genet. Evol. 54, 164–169.

Alam, M.M., Pun, S.B., Gauchan, P., Yokoo, M., Doan, Y.H., Tran, T.N., Nakagomi, T., Nakagomi, O., Pandey, B.D., 2013. The first identification of rotavirus B from children and adults with acute diarrhoea in Kathmandu, Nepal. Trop. Med. Health 41, 129–134.

Anderson, E.J., Weber, S.G., 2004. Rotavirus infection in adults. Lancet Infect. Dis. 4, 91–99.

Banyai, K., Laszlo, B., Duque, J., Steele, A.D., Nelson, E.A., Gentsch, J.R., Parashar, U.D., 2012. Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: insights for understanding the impact of rotavirus vaccination programs. Vaccine 30 (Suppl. 1), A122–A130.

Cunliffe, N.A., Gondwe, J.S., Graham, S.M., Thindwa, B.D., Dove, W., Broadhead, R.L., Molyneux, M.E., Hart, C.A., 2001. Rotavirus strain diversity in Blantyre, Malawi, from 1997 to 1999. J. Clin. Microbiol. 39, 836–843.

Das, S., Varghese, V., Chaudhuri, S., Barman, P., Kojima, K., Dutta, P., Bhattacharya, S.K., Krishnan, T., Kobayashi, N., Naik, T.N., 2004. Genetic variability of human rotavirus strains isolated from Eastern and Northern India. J. Med. Virol. 72, 156–161.

Do, L.P., Kaneko, M., Nakagomi, T., Gauchan, P., Agbemabiese, C.A., Dang, A.D., Nakagomi, O., 2017. Molecular epidemiology of Rotavirus A, causing acute gastroenteritis hospitalizations among children in Nha Trang, Vietnam, 2007-2008: identification of rare G9P[19] and G10P[14] strains. J. Med. Virol. 89, 621–631.

Doan, L.T., Okitsu, S., Nishio, O., Pham, D.T., Nguyen, D.H., Ushijima, H., 2003. Epidemiological features of rotavirus infection among hospitalized children with gastroenteristis in Ho Chi Minh City, Vietnam. J. Med. Virol. 69, 588–594.

Estes, M.K., Greenberg, H.B., 2013. Rotaviruses. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Virology. Wolters Kluwer Health/Lippincott, Williams and Wilkins, Philadelphia.

Gentsch, J.R., Glass, R.I., Woods, P., Gouvea, V., Gorziglia, M., Flores, J., Das, B.K., Bhan, M.K., 1992. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J. Clin. Microbiol. 30, 1365–1373.

Ghosh, S., Urushibara, N., Taniguchi, K., Kobayashi, N., 2012. Whole genomic analysis reveals the porcine origin of human G9P[19] rotavirus strains Mc323 and Mc345. Infect. Genet. Evol. 12, 471–477.

Gouvea, V., Glass, R.I., Woods, P., Taniguchi, K., Clark, H.F., Forrester, B., Fang, Z.Y.,

1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. 28, 276–282.
Heiman, E.M., McDonald, S.M., Barro, M., Taraporewala, Z.F., Bar-Magen, T., Patton, J.T., 2008. Group A human rotavirus genomics: evidence that gene constellations are influenced by viral protein interactions. J. Virol. 82, 11106–11116.
Hungerford, D., Iturriza-Gómara, M., 2015. EuroRotaNet Annu. Rep. 2014.
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.
Maes, P., Matthijnssens, J., Rahman, M., Van Ranst, M., 2009. RotaC: a web-based tool for the complete genome classification of group A rotaviruses. BMC Microbiol. 9, 238.
Matthijnssens, J., Ciarlet, M., Heiman, E., Arijs, I., Delbeke, T., McDonald, S.M., Palombo, E.A., Iturriza-Gomara, M., Maes, P., Patton, J.T., Rahman, M., Van Ranst, M., 2008a. Full genome-based classification of rotaviruses reveals a common origin between human Wa-Like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. J. Virol. 82, 3204–3219.
Matthijnssens, J., Ciarlet, M., Rahman, M., Attoui, H., Banyai, K., Estes, M.K., Gentsch, J.R., Iturriza-Gomara, M., Kirkwood, C.D., Martella, V., Mertens, P.P., Nakagomi, O., Patton, J.T., Ruggeri, F.M., Saif, L.J., Santos, N., Steyer, A., Taniguchi, K., Desselberger, U., Van Ranst, M., 2008b. Recommendations for the classification of group a rotaviruses using all 11 genomic RNA segments. Arch. Virol. 153, 1621–1629.
Matthijnssens, J., Rahman, M., Ciarlet, M., Zeller, M., Heylen, E., Nakagomi, T., Uchida, R., Hassan, Z., Azim, T., Nakagomi, O., Van Ranst, M., 2010. Reassortment of human rotavirus gene segments into G11 rotavirus strains. Emerg. Infect. Dis. 16, 625–630.
Matthijnssens, J., Ciarlet, M., McDonald, S.M., Attoui, H., Banyai, K., Brister, J.R., Buesa, J., Esona, M.D., Estes, M.K., Gentsch, J.R., Iturriza-Gomara, M., Johne, R., Kirkwood, C.D., Martella, V., Mertens, P.P., Nakagomi, O., Parreno, V., Rahman, M., Ruggeri, F.M., Saif, L.J., Santos, N., Steyer, A., Taniguchi, K., Patton, J.T., Desselberger, U., Van Ranst, M., 2011. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). Arch. Virol. 156, 1397–1413.
Mullick, S., Mukherjee, A., Ghosh, S., Pazhani, G.P., Sur, D., Manna, B., Nataro, J.P., Levine, M.M., Ramamurthy, T., Chawla-Sarkar, M., 2013. Genomic analysis of human rotavirus strains G6P[14] and G11P[25] isolated from Kolkata in 2009 reveals interspecies transmission and complex reassortment events. Infect. Genet. Evol. 14, 15–21.
Nguyen, T.A., Hoang, L.P., Pham, L.D., Hoang, K.T., Okitsu, S., Mizuguchi, M., Ushijima, H., 2008. Use of sequence analysis of the VP4 gene to classify recent Vietnamese rotavirus isolates. Clin. Microbiol. Infect. 14, 235–241.
Oka, T., Nakagomi, T., Nakagomi, O., 2000. Apparent re-emergence of serotype G9 in 1995 among rotaviruses recovered from Japanese children hospitalized with acute gastroenteritis. Microbiol. Immunol. 44, 957–961.
Ruiz, A.M., Lopez, I.V., Lopez, S., Espejo, R.T., Arias, C.F., 1988. Molecular and antigenic characterization of porcine rotavirus YM, a possible new rotavirus serotype. J. Virol. 62, 4331–4336.
Shetty, S.A., Mathur, M., Deshpande, J.M., 2014. Complete genome analysis of a rare group a rotavirus, G11P[25], isolated from a child in Mumbai, India, reveals interspecies transmission and reassortment with human rotavirus strains. J. Med. Microbiol. 63, 1220–1227.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
Than, V.T., Park, J.H., Chung, I.S., Kim, J.B., Kim, W., 2013. Whole-genome sequence analysis of a Korean G11P[25] rotavirus strain identifies several porcine-human reassortant events. Arch. Virol. 158, 2385–2393.
Uchida, R., Pandey, B.D., Sherchand, J.B., Ahmed, K., Yokoo, M., Nakagomi, T., Cuevas, L.E., Cunliffe, N.A., Hart, C.A., Nakagomi, O., 2006. Molecular epidemiology of rotavirus diarrhea among children and adults in Nepal: detection of G12 strains with P [6] or P[8] and a G11P[25] strain. J. Clin. Microbiol. 44, 3499–3505.
Yodmeeklin, A., Khamrin, P., Chuchaona, W., Saikruang, W., Kongkaew, A., Vachirachewin, R., Kumthip, K., Okitsu, S., Ushijima, H., Maneekarn, N., 2016. Great genetic diversity of rotaviruses detected in piglets with diarrhea in Thailand. Arch. Virol. 161, 2843–2849.
Yodmeeklin, A., Khamrin, P., Chuchaona, W., Kumthip, K., Kongkaew, A., Vachirachewin, R., Okitsu, S., Ushijima, H., Maneekarn, N., 2017. Analysis of complete genome sequences of G9P[19] rotavirus strains from human and piglet with diarrhea provides evidence for whole-genome interspecies transmission of nonreassorted porcine rotavirus. Infect. Genet. Evol. 47, 99–108.
Zeller, M., Heylen, E., Damanka, S., Pietsch, C., Donato, C., Tamura, T., Kulkarni, R., Arora, R., Cunliffe, N., Maunula, L., Potgieter, C., Tamim, S., Coster, S.D., Zhirakovskaya, E., Bdour, S., O'Shea, H., Kirkwood, C.D., Seheri, M., Nyaga, M.M., Mphahlele, J., Chitambar, S.D., Dagan, R., Armah, G., Tikunova, N., Van Ranst, M., Matthijnssens, J., 2015. Emerging OP354-like P[8] rotaviruses have rapidly dispersed from Asia to other continents. Mol. Biol. Evol. 32, 2060–2071.