Evolutionary History and Global Spread of the Emerging G12 Human Rotaviruses\textsuperscript{\textdagger}\textdaggerdbl

Mustafizur Rahman,\textsuperscript{1,2} Jelle Mathijnssens,\textsuperscript{1} Xuelei Yang,\textsuperscript{1} Thomas Delbeke,\textsuperscript{1} Ingrid Arijs,\textsuperscript{1} Koki Taniguchi,\textsuperscript{3} Miren Iturriza-Gómez,\textsuperscript{4} Nadia Iftekharuddin,\textsuperscript{2} Tasnim Azim,\textsuperscript{2} and Marc Van Ranst\textsuperscript{1*}

Laboratory of Clinical and Epidemiological Virology, Department of Microbiology and Immunology, Rega Institute for Medical Research, University of Leuven, Leuven, Belgium; Laboratory of Virology, ICDDR,B, GPO Box 128, Dhaka 1000, Bangladesh; Department of Virology and Parasitology, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan; and Enteric and Respiratory Virus Laboratory, Virus Reference Division, Central Public Health Laboratory, Colindale, London NW9 5HT, United Kingdom

Received 28 July 2006/Accepted 1 December 2006

G12 rotaviruses were first detected in diarrheic children in the Philippines in 1987, but no further cases were reported until 1998. However, G12 rotaviruses have been detected all over the world in recent years. Here, we report the worldwide variations of G12 rotaviruses to investigate the evolutionary mechanisms by which they managed to spread globally in a short period of time. We sequenced the complete genomes (11 segments) of nine G12 rotaviruses isolated in Bangladesh, Belgium, Thailand, and the Philippines and compared them with the genomes of other rotavirus strains. Our genetic analyses revealed that after introduction of the VP7 gene of the rare G12 genotype into more common local strains through reassortment, a vast genetic diversity was generated and several new variants with distinct gene constellations emerged. These reassortment events most likely took place in Southeast Asian countries and spread to other parts of the world. The acquisition of gene segments from human-adapted rotaviruses might allow G12 to better propagate in humans and hence to develop into an important emerging human pathogen.

Group A rotaviruses are one of the major causes of severe gastroenteritis in young children and animals. More than 125 million infants and young children develop rotavirus diarrhea globally each year, resulting in 440,000 deaths among children less than 5 years of age, mostly in developing countries (29). This high disease burden motivated major efforts to develop rotavirus vaccines. However, the high degree of genetic and antigenic variation among rotaviruses hinders the vaccine development programs (5, 9, 16, 28, 34, 42).

The rotavirus genome contains 11 double-stranded RNA segments, ranging in size from 664 to 3,302 nucleotides, encoding six structural viral proteins (VP) and six nonstructural proteins (NSP) (8). The viral capsid is formed by three concentric layers: a central core, an inner protein layer, and an outer protein layer (31). The outer protein layer is composed of VP4 and VP7, the two major antigens of the virus, and the middle layer is composed of VP6 molecules arranged as trimers. The central core is composed mainly of VP2 and contains proteins VP7 and VP4, respectively, which are the two viral proteins that elicit neutralizing antibody responses. An 89% amino acid cutoff percentage has been used to define different G and P genotypes (11, 18). At least 15 G genotypes and 26 P genotypes have been reported to date in mammals and avian species (8, 21, 33). The segmented nature of the rotavirus genome provides an opportunity for genetic reassortment, which plays an important role in the generation of virus diversity through genetic shift as demonstrated by many investigators (1, 7, 12, 15, 22, 24, 36, 41). In addition, “genogrouping” based on overall genomic RNA homology by hybridization assays has been proposed (25, 26). Using this approach, three genogroups of human rotaviruses have been defined: Wa-like, DS-1-like, and AU-1-like. In this genogrouping system, a strain is considered to belong to a

\begin{table}
\centering
\caption{Strains sequenced and deposited in GenBank for this study}
\begin{tabular}{|c|c|c|c|c|}
\hline
Strain & Genotype & Country of origin & Yr of isolation & GenBank accession no. \\
\hline
RV176-00 & G12P[6] & Bangladesh & 2006 & DQ490551–DQ490561 \\
B4633-03 & G12P[8] & Belgium & 2003 & DQ416638–DQ416648 \\
\hline
\end{tabular}
\end{table}

† Corresponding author. Mailing address: Laboratory of Clinical and Epidemiological Virology, Department of Microbiology and Immunology, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium. Phone: 32-16-347908, Fax: 32-16-332131. E-mail: marc.vanranst@uz.kuleuven.ac.be.

\textsuperscript{2} Supplemental material for this article may be found at http://jvi.asm.org/.

\textsuperscript{3} Published ahead of print on 13 December 2006.
certain genogroup if it contains at least seven gene segments similar to those in that particular genogroup.

The first G12 strain, L26 (G12P[4]), was detected in children less than 2 years old in 1987 in the Philippines (38). More than 10 years later, G12 strains were isolated in Thailand (1998) and the United States (1999) and subsequently in several Asian countries, such as India (1999 to 2005), Bangladesh (2000 to 2005), Japan (2003), and Korea (2002 and 2003) (6, 14, 17, 30, 35, 37). In Europe, G12 strains were identified in the United Kingdom (2002 and 2006) and Belgium (2003). They were also found in Argentina (1999 to 2003) and Brazil (2004) (3, 4). Using hybridization assays, the recent G12 strains were demonstrated to be distantly related to the prototype strain L26 and belonged to the AU-1-like or DS-1-like genogroup (14, 37, 43).

In the present study, G12 rotaviruses isolated in Bangladesh (2000 to 2005) and Belgium (2003) as well as the prototype strains L26 and T152 were analyzed through comparison and phylogenetic analysis of the derived amino acid sequences of all 11 gene segments.

**MATERIALS AND METHODS**

**Sample collection.** From 1999 to 2005, a total of 441 rotavirus-positive stool specimens from patients attending the Matlab and Dhaka hospitals of ICDDR,B, Bangladesh, were genotyped, 18 of which contained G12 rotaviruses. In Belgium, three G12 rotaviruses were detected in children admitted to the Goshuhsberg hospital, Leuven, during the 2003-to-2004 rotavirus season (n = 182). The G12 strains were untypeable with our routine multiplex PCR because no G12-specific primer was used. In our routine multiplex reverse transcription-PCR (RT-PCR), six G-genotype-specific primers (G1, G2, G3, G4, G8, and G9) and five P-genotype-specific primers (P[4], P[6], P[8], P[9], and P[11]) were included. For detecting the untypeable G12 strains, the VP7 gene segments were amplified using Beg9 and End9 primers. The amplified products were sequenced in both directions with the same primers. Nucleotide sequence similarity searches were performed using the National Center for Biotechnology Information (NCBI; National Institutes of Health, Bethesda, MD) BLAST (Basic Local Alignment Search Tool) server in GenBank, release 153.0 (2). The tissue culture supernatants of the prototype G12 strain L26, isolated in the Philippines in 1987, and strain T152, isolated in Thailand in 1998, were used.

**RNA extraction.** Viral RNA was extracted using a QIAGEN viral RNA mini kit (QIAGEN/Westburg, Leusden, The Netherlands) according to the manufacturer’s instructions.

**RT-PCR.** The extracted RNA was denatured at 97°C for 5 min, and RT-PCR was carried out using a QIAGEN OneStep RT-PCR kit (QIAGEN/Westburg) as described by Gouvea and colleagues (13). The forward and reverse primers used for the amplification of different gene segments were developed based on alignments of known 5′ and 3′ sequences of the respective gene segments found in GenBank. The reaction was carried out with an initial reverse transcription step at 45°C for 30 min, followed by PCR activation at 95°C for 15 min, 40 cycles of amplification, and a final extension of 7 min at 72°C, in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems Group, Foster City, CA). The cycle conditions for the amplification of VP1, VP2, VP3, and VP4 were 30 s at 94°C, 30 s at 45°C, and 2.5 min at 72°C; for the other gene segments, the conditions were 30 s at 94°C, 30 s at 45°C, and 2.5 min at 72°C.

**RESULTS**

**Phylogenetic dendograms based on the complete amino acid (aa) sequences of the structural proteins VP1, VP2, VP3, and VP6.** Accession numbers can be found in the supplemental material. The numbers adjacent to the nodes represent the percentages of bootstrap support (of 1,000 replicates) for the clusters to the right of the nodes. Bootstrap values lower than 75% are not shown. Hu, human; La, lapine; Bo, bovine; Po, porcine; Si, simian; Eq, equine; Fe, feline; Rh, rhesus; Mu, murine; Av, avian. G12 strains analyzed in this study are in bold.
Nucleotide sequencing. The PCR products were purified with a QIAquick PCR purification kit (QIAGEN/Westburg) and sequenced using the dideoxynucleotide chain termination method with an ABI PRISM BigDye Terminator cycle sequencing reaction kit (Applied Biosystems Group) on an ABI PRISM 3100 automated sequencer (Applied Biosystems Group). The sequencing was performed with the forward and reverse primers used for the RT-PCR. Primer walking sequencing was performed to cover the complete sequences of the respective fragments on both strands.

Determination of the 5'- and 3'-terminal sequences. To obtain the complete nucleotide sequences, the 5'- and 3'-terminal sequences of the 11 gene segments were determined as previously described (23).

Nucleotide and protein sequence analysis. The chromatogram sequencing files were analyzed using Chromas 2.23 (Technelysium, Queensland, Australia), and contigs were prepared using SeqMan II (DNASTAR, Madison, WI). Multiple sequence alignments were calculated using ClustalX 1.81 (39). Sequences were manually edited by the GeneDoc version 2.6.002 alignment editor (27).

Phylogenetic analysis. The dendrograms were constructed using the neighbor-joining method with MEGA version 3.1 software (19). The similarity percentages between amino acid sequences were calculated by using the Poisson correction distance model.

Genogrouping strategy. Initially, the 11 gene segments (the VP1 to VP4, VP6, VP7, and NSP1 to NSP5 gene segments) for all G12 strains isolated in our study (n = 21) were amplified with a forward and a reverse primer described by Matthijnssens and colleagues (23). Sequencing of these RT-PCR products by using the forward primers produced a nucleotide sequence of at least 600 bp from the 5' end of each gene segment. The corresponding partial sequences of all gene segments of G12 strains were compared to each other. Based on the nucleotide similarity, at least six different gene combinations were detected among the Bangladeshi G12 strains. A single G12 strain from each unique gene constellation (strains RV161-00, RV176-00, N26-02, Dhaka25-02, Dhaka12-03, and Matlab13-03) was selected for sequencing of its entire genomic complement.

All three Belgian G12 strains were found to be nearly identical based on the partial sequences, and the complete genome of one representative Belgian G12 strain, B4633-03, was sequenced. In practical terms, each of the partial gene segments of the strains which were excluded from the analysis was virtually identical to one of the representative strains which were sequenced completely. Several gene segments of the prototype G12 strains L26 (the VP1, VP2, VP6, NSP2, NSP3, and NSP5 gene segments) and T152 (the VP1, VP2, VP3, VP6, NSP2, NSP3, NSP4, and NSP5 gene segments) which were not available in GenBank were also sequenced.

In order to establish the interrelationships among the different G12 isolates described in this study and their relationship with other human rotavirus strains, pairwise comparisons on the amino acid level were conducted between all the G12s and the human reference strains Wa, DS-1, and AU-1. In addition, phylogenetic dendrograms were constructed to compare these strains with each other and with other human and animal rotavirus strains. Both of these data sets were used to deduce and/or speculate about the possibilities that certain differences in the homologous gene sequences were due to genetic drift or due to reassortments. These deductions/speculations were made, keeping in mind that all the different rotavirus proteins, and their respective gene segments, are subjected to different selective pressures from the environment and the host immune systems, resulting in different levels of nucleotide and amino acid conservation.

Nucleotide sequence accession numbers. The nucleotide sequence data for complete genomes of rotavirus strains reported in this paper were submitted to GenBank under the accession numbers included in Table 1.

FIG. 2. Phylogenetic dendrograms based on the complete amino acid (aa) sequences of the outer capsid proteins VP4 and VP7. Accession numbers can be found in the supplemental material. The sizes of the triangles are indications of the numbers of sequences that they represent. The numbers adjacent to the nodes represent the percent-ages of bootstrap support (of 1,000 replicates) for the clusters to the right of the nodes. Bootstrap values lower than 75% are not shown. Hu, human; Po, porcine; Fe, feline. G12 strains analyzed in this study are in bold.
RESULTS

Complete nucleotide sequences for the 11 gene segments encoding VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4, and NSP5 of the representative G12 rotavirus strains isolated in our study were determined. Phylogenetic trees for each gene segment, which included the deduced amino acid sequences of the G12 strains together with the corresponding gene sequences of the rotavirus strains available in GenBank, were constructed (Fig. 1 to 4). Additionally, multiple sequence alignments for all gene segments were conducted and similarity matrices were constructed (Fig. 5 and 6).

L26. The prototype G12 strain L26 (G12P[4]) was isolated in the Philippines during 1987. Our pairwise comparisons and phylogenetic analyses revealed that seven gene segments of strain L26 (the VP1, VP2, VP4, VP6, NSP1, NSP3, and NSP4 gene segments) were very closely related to the corresponding gene segments of human DS-1-like rotavirus strains (Fig. 1 to 6). The VP3, NSP2, and NSP5 gene segments of strain L26 were closely related to the human strain Wa and recent Wa-like strains (strains RMC100 and B4633-03, etc.). It is remarkable that next to these close genetic relationships, also very high similarities (>96% for NSP5 and >98% for NSP2 [data not shown]) and a close phylogenetic clustering were found between L26 and porcine rotavirus strains OSU, YM, and RU172. These genomic characteristics indicated that the prototype G12 rotavirus L26 most likely contained an assortment of Wa- and DS-1-like gene segments in combination with a novel VP7 (G12) specificity which belonged to lineage I of the G12 branch (Fig. 2). Although there are only very few complete gene sequences available for porcine rotaviruses, the above-mentioned data indicate that porcine rotaviruses might also be involved in the reassortment events leading to the occurrence of L26.

T152. More than 10 years after the isolation of strain L26, the G12P[9] strain T152 was isolated in Thailand. Eight gene segments (the VP1 to VP4, VP6, and NSP2 to NSP4 gene segments) of strain T152 were unrelated to the prototype G12 strain L26 but were closely related to strain AU-1 (Fig. 1 to 6). The VP7 gene segment clustered in lineage II of the G12 branch (Fig. 2), and the NSP1 gene was placed in a unique branch that was not related to any other known rotavirus (Fig. 3). The NSP5 segment of strain T152 clustered with rhesus strain RRV, indicating that this gene segment might be of animal origin (Fig. 4).

RV161-00, RV176-00, and N26-02. The first generation of Bangladeshi G12 strains (n = 4), represented by strains RV161-00, RV176-00, and N26-02, was isolated between 2000 and 2002. They all clustered in the G12 lineage III branch of
the VP7 tree and possessed the P[6] (ST3-like) VP4 specificity (Fig. 2). The VP1 to VP3, VP6, NSP1, NSP3, and NSP5 gene segments of these three G12P[6] strains were very closely related to each other and to DS-1-like rotavirus strains (Fig. 1 to 5). The NSP2 gene segments of strains RV161-00 and RV176-00 were also closely related to DS-1-like rotaviruses, while the NSP2 gene of strain N26-02 was closely related to Wa-like strains (Fig. 3). The NSP4 gene segment of strain RV161-00 was closely related to Wa-like rotavirus strains, whereas the NSP4 genes of strains RV176-00 and N26-02 were distantly related to AU-1-like strains (Fig. 4). These data showed that strains RV161-00, RV176-00, and N26-02 were closely related and had a common ancestor. They were most likely generated after several reassortment events between DS-1-like strains and strains donating their VP7 (G12), VP4 (P[6]), NSP2, and NSP4 gene segments.

Dhaka25-02, Dhaka12-03, and Matlab13-03. The second generation of Bangladeshi G12 strains (n = 14), represented by strains Dhaka25-02, Dhaka12-03, and Matlab13-03, was isolated between 2002 and 2005. Their VP7 gene segments clustered very closely in the lineage III branch of the VP7 phylogenetic tree, together with the other first-generation Bangladeshi G12 strains (Fig. 2). Strains Dhaka12-03 and Matlab13-03 contained a VP4 gene segment with the P[6] (ST3-like) specificity, whereas strain Dhaka25-02 contained the P[8] (Wa-like) specificity (Fig. 2). Seven other gene segments (the VP1 to VP3, VP6, NSP2, NSP4, and NSP5 gene segments) of strains Dhaka25-02, Dhaka12-03, and Matlab13-03 were very closely related to each other and to Wa-like strains (Fig. 1 and 3 to 5). The NSP3 genes of strains Dhaka25-02 and Dhaka12-03 were also Wa-like, whereas NSP3 of strain Matlab13-03 was DS-1-like. The NSP1 gene segments of all three strains were closely related to ST3-like strains. These data suggested that strains Dhaka25-02, Dhaka12-03, and Matlab13-03 were very closely related and had a common ancestor. They were most likely generated after several reassortment events between Wa-like strains and strains donating their VP7 (G12), VP4 (P[6]), NSP1, and NSP3 gene segments.

B4633-03. For the three nearly identical Belgian G12 rotavirus strains, strain B4633-03 was sequenced completely as a representative. This strain clustered very closely with strain Dhaka25-02 and showed very high similarities, ranging from 96.5% to 100% on the amino acid level with the Wa-like Bangladeshi G12 strain Dhaka25-02 for all 11 gene segments (Fig. 1 to 6), indicating a common origin for both strains.

MV404-02. From the United Kingdom G12P[6] rotavirus strain MV404-02, isolated in 2002, only partial sequences of the VP7-, VP4-, and VP6-encoding gene segments could be determined. All three partial sequences showed close genetic relationships with the G12P[6] rotavirus strains Dhaka12-03 and Matlab13-03 (data not shown). No more genetic material was left to determine the nature of the remaining eight gene segments.

Remaining G12s. The U.S. G12P[6] strain Se585 was isolated in 1999 (14). Analysis of the VP4 and VP7 genes of strain Se585 revealed very close relationships (98.0% to 100% amino acid similarities) with the G12P[6] strains isolated in Bangladesh (strains RV161-00 and RV176-00) and India (strains ISO-1, ISO-2, and ISO-5) (Fig. 2). The NSP4 gene was closely

FIG. 4. Phylogenetic dendrograms based on the complete amino acid (aa) sequences of the nonstructural proteins NSP4 and NSP5. Accession numbers can be found in the supplemental material. For NSP4, the three established genogroups are shown. The numbers adjacent to the nodes represent the percentages of bootstrap support (of 1,000 replicates) for the clusters to the right of the nodes. Bootstrap values lower than 75% are not shown. Hu, human; La, lapine; Bo, bovine; Po, porcine; Si, simian; Eq, equine; Fe, feline; Rh, rhesus; Mu, murine; Av, avian. G12 strains analyzed in this study are in bold.

Downloaded from http://jvi.asm.org on October 31, 2017 by guest
related to Bangladeshi strain BD426 and Indian strain RMC/G66 (Fig. 4).

The Indian human G12 strains were found in combination with the P[4], P[6], and P[8] specificities (35). The VP7 gene segments of these strains were most closely related to the Bangladeshi G12 strains belonging to lineage III (Fig. 2).

The VP7 and VP4 amino acid sequences of the Japanese G12P[9] rotaviruses isolated between 2003 and 2004 (strains CP727, CP1030, and K12) (37) were almost identical to those of the Thai strain T152 and clustered together in G12 lineage II (Fig. 2).

The first nonhuman G12 strain, RU172 (G12P[7]), was iso-

related to Bangladeshi strain BD426 and Indian strain RMC/ G66 (Fig. 4).

The Indian human G12 strains were found in combination with the P[4], P[6], and P[8] specificities (35). The VP7 gene segments of these strains were most closely related to the Bangladeshi G12 strains belonging to lineage III (Fig. 2).

The VP7 and VP4 amino acid sequences of the Japanese G12P[9] rotaviruses isolated between 2003 and 2004 (strains CP727, CP1030, and K12) (37) were almost identical to those of the Thai strain T152 and clustered together in G12 lineage II (Fig. 2).

The G12P[9] strains HC91 and Buenos (named after the place of isolation, since no name was assigned) were isolated during 2003 and 2004 in Brazil and Argentina, respectively (4, 36). Both their VP7 and their VP4 gene segments were very similar to each other and to the Asian AU-1-like G12 strains in lineage II (Fig. 2).

The first nonhuman G12 strain, RU172 (G12P[7]), was iso-

related to Bangladeshi strain BD426 and Indian strain RMC/ G66 (Fig. 4).

The Indian human G12 strains were found in combination with the P[4], P[6], and P[8] specificities (35). The VP7 gene segments of these strains were most closely related to the Bangladeshi G12 strains belonging to lineage III (Fig. 2).

The VP7 and VP4 amino acid sequences of the Japanese G12P[9] rotaviruses isolated between 2003 and 2004 (strains CP727, CP1030, and K12) (37) were almost identical to those of the Thai strain T152 and clustered together in G12 lineage II (Fig. 2).

The G12P[9] strains HC91 and Buenos (named after the place of isolation, since no name was assigned) were isolated during 2003 and 2004 in Brazil and Argentina, respectively (4, 36). Both their VP7 and their VP4 gene segments were very similar to each other and to the Asian AU-1-like G12 strains in lineage II (Fig. 2).

The first nonhuman G12 strain, RU172 (G12P[7]), was iso-

related to Bangladeshi strain BD426 and Indian strain RMC/ G66 (Fig. 4).

The Indian human G12 strains were found in combination with the P[4], P[6], and P[8] specificities (35). The VP7 gene segments of these strains were most closely related to the Bangladeshi G12 strains belonging to lineage III (Fig. 2).

The VP7 and VP4 amino acid sequences of the Japanese G12P[9] rotaviruses isolated between 2003 and 2004 (strains CP727, CP1030, and K12) (37) were almost identical to those of the Thai strain T152 and clustered together in G12 lineage II (Fig. 2).

The G12P[9] strains HC91 and Buenos (named after the place of isolation, since no name was assigned) were isolated during 2003 and 2004 in Brazil and Argentina, respectively (4, 36). Both their VP7 and their VP4 gene segments were very similar to each other and to the Asian AU-1-like G12 strains in lineage II (Fig. 2).

The first nonhuman G12 strain, RU172 (G12P[7]), was iso-

related to Bangladeshi strain BD426 and Indian strain RMC/ G66 (Fig. 4).

The Indian human G12 strains were found in combination with the P[4], P[6], and P[8] specificities (35). The VP7 gene segments of these strains were most closely related to the Bangladeshi G12 strains belonging to lineage III (Fig. 2).

The VP7 and VP4 amino acid sequences of the Japanese G12P[9] rotaviruses isolated between 2003 and 2004 (strains CP727, CP1030, and K12) (37) were almost identical to those of the Thai strain T152 and clustered together in G12 lineage II (Fig. 2).

The G12P[9] strains HC91 and Buenos (named after the place of isolation, since no name was assigned) were isolated during 2003 and 2004 in Brazil and Argentina, respectively (4, 36). Both their VP7 and their VP4 gene segments were very similar to each other and to the Asian AU-1-like G12 strains in lineage II (Fig. 2).

The first nonhuman G12 strain, RU172 (G12P[7]), was iso-

related to Bangladeshi strain BD426 and Indian strain RMC/ G66 (Fig. 4).

The Indian human G12 strains were found in combination with the P[4], P[6], and P[8] specificities (35). The VP7 gene segments of these strains were most closely related to the Bangladeshi G12 strains belonging to lineage III (Fig. 2).

The VP7 and VP4 amino acid sequences of the Japanese G12P[9] rotaviruses isolated between 2003 and 2004 (strains CP727, CP1030, and K12) (37) were almost identical to those of the Thai strain T152 and clustered together in G12 lineage II (Fig. 2).

The G12P[9] strains HC91 and Buenos (named after the place of isolation, since no name was assigned) were isolated during 2003 and 2004 in Brazil and Argentina, respectively (4, 36). Both their VP7 and their VP4 gene segments were very similar to each other and to the Asian AU-1-like G12 strains in lineage II (Fig. 2).

The first nonhuman G12 strain, RU172 (G12P[7]), was iso-

related to Bangladeshi strain BD426 and Indian strain RMC/ G66 (Fig. 4).

The Indian human G12 strains were found in combination with the P[4], P[6], and P[8] specificities (35). The VP7 gene segments of these strains were most closely related to the Bangladeshi G12 strains belonging to lineage III (Fig. 2).

The VP7 and VP4 amino acid sequences of the Japanese G12P[9] rotaviruses isolated between 2003 and 2004 (strains CP727, CP1030, and K12) (37) were almost identical to those of the Thai strain T152 and clustered together in G12 lineage II (Fig. 2).

The G12P[9] strains HC91 and Buenos (named after the place of isolation, since no name was assigned) were isolated during 2003 and 2004 in Brazil and Argentina, respectively (4, 36). Both their VP7 and their VP4 gene segments were very similar to each other and to the Asian AU-1-like G12 strains in lineage II (Fig. 2).

The first nonhuman G12 strain, RU172 (G12P[7]), was iso-

related to Bangladeshi strain BD426 and Indian strain RMC/ G66 (Fig. 4).

The Indian human G12 strains were found in combination with the P[4], P[6], and P[8] specificities (35). The VP7 gene segments of these strains were most closely related to the Bangladeshi G12 strains belonging to lineage III (Fig. 2).

The VP7 and VP4 amino acid sequences of the Japanese G12P[9] rotaviruses isolated between 2003 and 2004 (strains CP727, CP1030, and K12) (37) were almost identical to those of the Thai strain T152 and clustered together in G12 lineage II (Fig. 2).

The G12P[9] strains HC91 and Buenos (named after the place of isolation, since no name was assigned) were isolated during 2003 and 2004 in Brazil and Argentina, respectively (4, 36). Both their VP7 and their VP4 gene segments were very similar to each other and to the Asian AU-1-like G12 strains in lineage II (Fig. 2).

The first nonhuman G12 strain, RU172 (G12P[7]), was iso-

related to Bangladeshi strain BD426 and Indian strain RMC/ G66 (Fig. 4).

The Indian human G12 strains were found in combination with the P[4], P[6], and P[8] specificities (35). The VP7 gene segments of these strains were most closely related to the Bangladeshi G12 strains belonging to lineage III (Fig. 2).

The VP7 and VP4 amino acid sequences of the Japanese G12P[9] rotaviruses isolated between 2003 and 2004 (strains CP727, CP1030, and K12) (37) were almost identical to those of the Thai strain T152 and clustered together in G12 lineage II (Fig. 2).

The G12P[9] strains HC91 and Buenos (named after the place of isolation, since no name was assigned) were isolated during 2003 and 2004 in Brazil and Argentina, respectively (4, 36). Both their VP7 and their VP4 gene segments were very similar to each other and to the Asian AU-1-like G12 strains in lineage II (Fig. 2).

The first nonhuman G12 strain, RU172 (G12P[7]), was iso-

related to Bangladeshi strain BD426 and Indian strain RMC/ G66 (Fig. 4).

The Indian human G12 strains were found in combination with the P[4], P[6], and P[8] specificities (35). The VP7 gene segments of these strains were most closely related to the Bangladeshi G12 strains belonging to lineage III (Fig. 2).

The VP7 and VP4 amino acid sequences of the Japanese G12P[9] rotaviruses isolated between 2003 and 2004 (strains CP727, CP1030, and K12) (37) were almost identical to those of the Thai strain T152 and clustered together in G12 lineage II (Fig. 2).

The G12P[9] strains HC91 and Buenos (named after the place of isolation, since no name was assigned) were isolated during 2003 and 2004 in Brazil and Argentina, respectively (4, 36). Both their VP7 and their VP4 gene segments were very similar to each other and to the Asian AU-1-like G12 strains in lineage II (Fig. 2).

The first nonhuman G12 strain, RU172 (G12P[7]), was iso-
lated recently from a pig in India (10). The VP7 gene of strain RU172 showed considerable sequence diversity at the amino acid level (5.4% to 7.0%) compared with the cognate genes of human G12 strains and was placed in lineage IV (Fig. 2 and 6). The VP6, NSP4, and NSP5 gene segments of this strain were shown to be closely related to the porcine rotavirus strains OSU and YM (Fig. 1 and 4).

DISCUSSION

Most rotavirus genotyping studies have focused mainly on G (VP7) and P (VP4) genotyping. Currently, there is a paucity of information regarding the other gene segments (e.g., the VP1, VP2, VP3, NSP2, and NSP3 gene segments, etc.) and their role in the pathogenicity, severity, propagation, and spread of the virus is largely unknown. All the strains discussed in this study were initially called G12 rotaviruses based on one gene segment; however, they were shown to be very different when all the 11 gene segments were considered. By characterizing several complete genomes, we have shown that rotaviruses are basically a population of reassortants where unpredictable variations in gene combinations are very common. Thus, our study underscores the need for complete genome-based “genogrouping” to illustrate the whole story regarding the diversity, evolution, and origin of viruses with segmented genomes.

G12 rotaviruses have until recently received little attention. Since 1998, a decade after their first detection in the Philippines, G12 strains have been isolated in Asia, Europe, South America, and North America, suggesting their possible emergence worldwide. The strong increase in the frequency of detection of these G12 rotavirus strains raises questions concerning their origin and evolution and how

TABLE 2. Gene-protein assignments

<table>
<thead>
<tr>
<th>G12 rotavirus strain</th>
<th>VP1</th>
<th>VP2</th>
<th>VP3</th>
<th>VP4</th>
<th>NSP1</th>
<th>VP6</th>
<th>NSP3</th>
<th>NSP2</th>
<th>VP7</th>
<th>NSP4</th>
<th>NSP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>L26</td>
<td>DS-1 (96.7)</td>
<td>DS-1 (99.0)</td>
<td>Wa (95.1)</td>
<td>DS-1 [P] (95.1)</td>
<td>DS-1 (93.6)</td>
<td>DS-1 (95.1)</td>
<td>DS-1 (99.0)</td>
<td>Wa (95.5)</td>
<td>G12-I</td>
<td>DS-1 (94.7)</td>
<td>Wa (92.0)</td>
</tr>
<tr>
<td>T152</td>
<td>AU-1 (98.3)</td>
<td>AU-1 (99.1)</td>
<td>AU-1 (92.9)</td>
<td>AU-1 [P] (95.5)</td>
<td>None</td>
<td>Au-1 (97.4)</td>
<td>Au-1 (95.4)</td>
<td>Au-1 (94.8)</td>
<td>G12-II</td>
<td>Au-1 (94.1)</td>
<td>RRV (99.0)</td>
</tr>
<tr>
<td>RV161-00</td>
<td>DS-1 (97.6)</td>
<td>DS-1 (98.5)</td>
<td>ST3 [P] (96.0)</td>
<td>DS-1 (93.0)</td>
<td>DS-1 (98.7)</td>
<td>DS-1 (97.7)</td>
<td>DS-1 (98.3)</td>
<td>G12-III</td>
<td>Wa (95.3)</td>
<td>DS-1 (95.8)</td>
<td></td>
</tr>
<tr>
<td>RV176-00</td>
<td>DS-1 (97.6)</td>
<td>DS-1 (98.5)</td>
<td>ST3 [P] (96.0)</td>
<td>DS-1 (93.0)</td>
<td>DS-1 (98.7)</td>
<td>DS-1 (97.7)</td>
<td>DS-1 (98.3)</td>
<td>G12-III</td>
<td>Wa (95.3)</td>
<td>DS-1 (95.8)</td>
<td></td>
</tr>
<tr>
<td>N26-02</td>
<td>DS-1 (97.6)</td>
<td>DS-1 (98.5)</td>
<td>ST3 [P] (96.0)</td>
<td>DS-1 (93.0)</td>
<td>DS-1 (98.7)</td>
<td>DS-1 (97.7)</td>
<td>DS-1 (98.3)</td>
<td>G12-III</td>
<td>Wa (95.3)</td>
<td>DS-1 (95.8)</td>
<td></td>
</tr>
<tr>
<td>Dhaka25-02</td>
<td>Wa (98.7)</td>
<td>Wa (97.7)</td>
<td>Wa (96.3)</td>
<td>Wa [P] (94.1)</td>
<td>ST3 (93.0)</td>
<td>Wa (97.7)</td>
<td>Wa (95.4)</td>
<td>Wa (98.0)</td>
<td>G12-III</td>
<td>Wa (95.9)</td>
<td>G12-IV</td>
</tr>
<tr>
<td>Dhaka12-03</td>
<td>Wa (98.7)</td>
<td>Wa (97.7)</td>
<td>Wa (96.3)</td>
<td>Wa [P] (94.1)</td>
<td>ST3 (93.0)</td>
<td>Wa (97.7)</td>
<td>Wa (95.4)</td>
<td>Wa (98.0)</td>
<td>G12-III</td>
<td>Wa (94.7)</td>
<td>G12-IV</td>
</tr>
<tr>
<td>Matlab13-03</td>
<td>Wa (98.7)</td>
<td>Wa (97.8)</td>
<td>Wa (96.0)</td>
<td>Wa [P] (93.6)</td>
<td>ST3 (91.0)</td>
<td>Wa (97.0)</td>
<td>Wa (96.8)</td>
<td>Wa (98.0)</td>
<td>G12-III</td>
<td>Wa (95.3)</td>
<td>G12-IV</td>
</tr>
<tr>
<td>B4633-03</td>
<td>Wa (98.7)</td>
<td>Wa (97.6)</td>
<td>Wa (96.0)</td>
<td>Wa [P] (93.6)</td>
<td>ST3 (91.0)</td>
<td>Wa (97.0)</td>
<td>Wa (96.8)</td>
<td>Wa (98.0)</td>
<td>G12-III</td>
<td>Wa (95.3)</td>
<td>G12-IV</td>
</tr>
</tbody>
</table>

a The gene-protein assignment shown here is identical to that for the SA11 rotavirus strain. Depending on the strain, the gene-protein assignments for RNA segments 7 to 9 differ; however, the RNA triplet always encodes VP7, NSP2, and NSP3.
b The NSP4 gene segments of RV176-00 and N26-02 are only distantly related to the NSP4 gene segment of AU-1.
they were able to spread all over the world. A summary of our attempts to genotype the different gene segments of G12 rotaviruses included in this study is given in Table 2, which allows the following conclusions regarding their evolution, origin, and spread.

The vast majority of G12 rotavirus strains have been isolated in Asia and, more specifically, in Southeast Asia. Our study has shown that a very large genetic diversity is present in the G12 population, caused mainly by genetic reassortments. This geographical region might be the main origin of all the different G12 strains isolated all over the world. This possibility is strengthened by the observation that from 25 G12 rotavirus strains isolated in India between 2003 and 2005, three different G- and P-genotype combinations were found (G12P[8], G12P[6], and G12P[4]) (35). Even more variation might be found when the other nine gene segments are also analyzed. A similar observation was recently made in Nepal, where 29 G12 strains showed at least five different electropherotypes, suggesting the existence of at least five different gene constellations, similar to the situation found in India and Bangladesh (40). From Southeast Asia, they might be transported across the globe by the increasing mobility of humans and animals. This might have happened to the Belgian strain B4633-03, which was nearly identical to strain Dhaka25-02 and to the United Kingdom strain MV404-02, which was closely related to strain Dhaka12-03. Although only a very limited number of sequences from G12P[9] strains isolated in Japan, Brazil, and Argentina are available, they seem to show high resemblances to the Thai AU-1-like G12 reference strain T152. Further analysis and comparison of these strains could reveal the full story of their relatedness.

The origin of the G12 moiety remains obscure, but the recent isolation of a G12 rotavirus from a pig (strain RU172) and the observation that the first G12 rotavirus (strain L26) has OSU-like NSP2 and YM/OSU-like NSP5 gene segments (Fig. 3 and 4) might be indications that G12 has an animal, more specifically porcine, origin. The further investigation of this possibility is hampered by the very small amount of sequence data available for porcine rotavirus strains, which underscores the need for more (sequence) data on animal rotaviruses.

The most successful among the current reassortant human rotaviruses is the human G9 rotavirus. G9 was first detected in 1983, and after about 12 years of being detected only very sporadically, it became one of the most predominant rotavirus strains: implications for rotavirus vaccine programs. J. Infect. Dis. 192:1182–1189.

REFERENCES


