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BRIEF REPORT

Rotavirus infections with multiple emerging genotypes in Sri Lanka

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Abstract Rotavirus diarrhea is an important cause of child mortality in developing countries, but studies on this diarrhea are scarce in Sri Lanka. A prospective study conducted in Sri Lanka on rotavirus infection among children in a hospital setting ($n = 611$) versus children residing in tsunami camps ($n = 52$) showed that prevalence of rotavirus infection was comparable, 21.9 and 20%, respectively. The hospital and camps were located in different districts. Analysis of the genotypes of 122 rotaviruses from the hospital and 12 from the camps indicated that G9P[8] was associated with 35 and 33%; G12P[8/nt] with 14.7 and 33%; G3P[8/4/nt] with 17 and 8% and G1P[8/4] with 6.5 and 16.7%. Rotaviruses with G2P[8/4/6] and G4P[8/4] were hospital-associated only, and some rotaviruses (9 and 8% from the hospital and the camps, respectively) were G- and P-nontypable. We conclude from the present study that multiple emerging genotypes were prevalent in Sri Lanka, and children in camps were at risk of developing diarrhea due to rotaviruses.

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Globally, 611,000 children die each year due to rotavirus infection, and 85% of these deaths occur in developing countries [9]. A significant number of children suffer from diarrhea in Sri Lanka; however, studies on rotaviruses are scarce in this country [7, 8]. Sri Lanka was devastated by the Asian tsunami; many people lost their homes and sheltered in camps. We were concerned to understand whether overcrowding and adverse conditions in camps favor the spread of rotaviruses that are different from strains prevalent among hospitalized children. Therefore, a prospective study was done to (a) estimate the burden of rotavirus infections among diarrhoeic children admitted to a tertiary care hospital and those residing in tsunami camps and (b) to genotype these strains at the molecular level for G and P types from their faecal specimens.

The study was approved by the ethical review boards of the Faculty of Medicine, University of Colombo, and Sri Lankan College of Pediatrics. A single stool sample was collected from each subject with acute diarrhea, transported on ice and stored at −70°C until use. Acute diarrhea was defined by the passing of three or more loose stools within the preceding 24 h. Rotavirus was detected using the commercially available ELISA kit, Rotaclone (Meridian Bioscience Inc., Cincinnati, OH), according

to manufacturer's instructions. Genomic dsRNA was extracted and electropherotype was determined by polyacrylamide gel electrophoresis (PAGE) [6]. The G and P genotypes were determined by reverse transcription (RT)-PCR [3–5, 13]. The nucleotide sequence of the VP7 gene was determined using a BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster city, CA, USA) according to the instructions of the manufacturer, and the product was examined using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Sequence identity determination and construction of phylogenetic trees were done as described previously [2].

Table 1 Number of strains in each electropherotype identified in Sri Lanka

Electropherotype	Number of strains
E1	7
E2	1
E3	1
E4	1
E5	1
E6	3
E7	7
E8	1
E9	1
E10	3
E11	1
E12	31
E13	3
E14	3
E15	2
E16	3
E17	4
E18	1
Total	74

From April 2005 through October 2006, 611 stool samples were collected from children (43.1% female, 56.9% male) admitted at Colombo North Teaching Hospital (CNTH), Gampaha. Gampaha is 18 km north of the capital and was unaffected by the tsunami. The patients were one to 144 months (median 13 months) old. Rotavirus was detected in 20% of the patients; their median age was 16.5 months. In the 0–59-month age group, the prevalence of rotavirus infection was 21.9% and similar in both sexes.

From March 2005 through November 2005, 52 stool samples were collected from children (40.4% female and 59.6% male) residing at camps (Peraliya Temple, Magalla, Wellabada, Sambodi, IDH watta, Senanyake, Thalaramba and Kumbalgama) in Galle district. Galle is 116 km south of the capital Colombo and was severely affected by the tsunami. About 30,355 people took refuge in 83 camps in Galle. Our patients were 0.4–20 months (median 18 months) old. Rotavirus was detected in 12 (23.1%) samples from six males and six females. These patients were 7–120 months old (median 18 months). In the 0–59-month age group, the prevalence of rotavirus infection was 20%.

Segments of the rotavirus genome were visible in 96 and 9 of 122 and 12 samples from CNTH and the camps, respectively. Of these, in 31 samples (27 and 4 samples from CNTH and the camps, respectively), electropherotypes could not be determined because the staining of RNA segments was not clear enough to permit their assignment to a specific electropherotype, and they thus remained untypable. All 11 segments were visible in 69 and 5

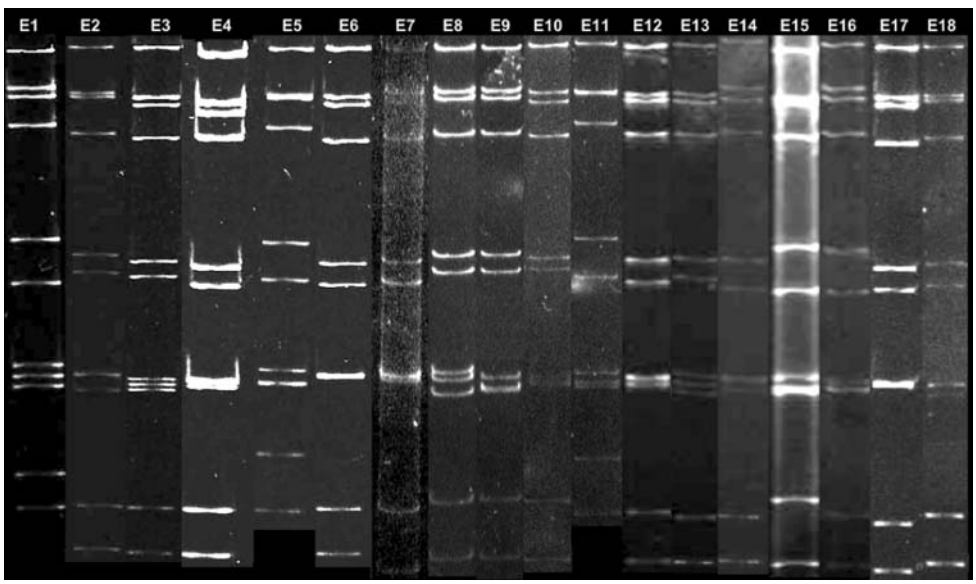

Fig. 1 The electropherotypes of rotavirus strains identified in Sri Lanka. Electropherotype E12 was dominant, and E1 and E7 were co-dominant during the study period. Electropherotype E1, E5 and E11 contained G2P[4] rotaviruses with a short pattern. Electropherotype E2 contained one G2P[8] with a long pattern

Table 2 Numbers and percentages of rotaviruses with different G and P genotype combinations detected in samples from Colombo North Teaching Hospital and camps. Gnt and P[nt] indicate non-typable G and P genotype for some rotaviruses

Type	Colombo North Teaching Hospital	Camps
G1P[8]	7	2
G1P[4]	1	
Subtotal G1	8 (6.5%)	2 (16.7%)
G9P[8]	43	4
Subtotal G9	43 (35.2%)	4 (33.3%)
G2P[8]	6	
G2P[4]	9	
G2P[6]	1	
G2P[8] & P[4]	1	
Subtotal G2	17 (13.9%)	
G3P[8]	19	
G3P[nt]	2	
G3P[4]		1
Subtotal G3	21 (17.2%)	1 (8.3%)
G4P[8]	3	
G4P[4]	1	
Subtotal G4	4 (3.3%)	
G12P[8]	17	4
G12P[nt]	1	
Subtotal G12	18 (14.7%)	4 (33.3%)
GntP[8]	7	1
GntP[6]	1	
GntP[4] & [8]	1	
GntP[nt]	2	
Subtotal Gnt	11 (9.0%)	1 (8.3%)
Total	122	12

samples from CNTH and the camps, respectively. Thus, in a total of 74 samples, 18 electropherotypes were identified (Table 1 and Fig. 1).

E10, containing G9P[8] rotaviruses, consisted of one sample from a camp and two samples from CNTH collected in June and July of 2005 and August 2005, respectively. E17, containing G12P[8] rotaviruses, consisted of three samples from the camps and one sample from CNTH collected in April and June of 2005 and April 2005, respectively.

In CNTH, G9 was dominant, followed by G3, G12, G2, G1 and G4 (Table 2). In the camps, G9 and G12 were equally dominant, followed by G1 and G3. Comparison of genotype distribution showed that G3 and G12 were dominant in the hospital and the camps, respectively. G9, G1 and G nontypable had a similar distribution in both settings. G2 and G4 were found in the hospital only.

Phylogenetic trees were constructed with the VP7 amino acid sequences of representative G9, G12 and G3 strains

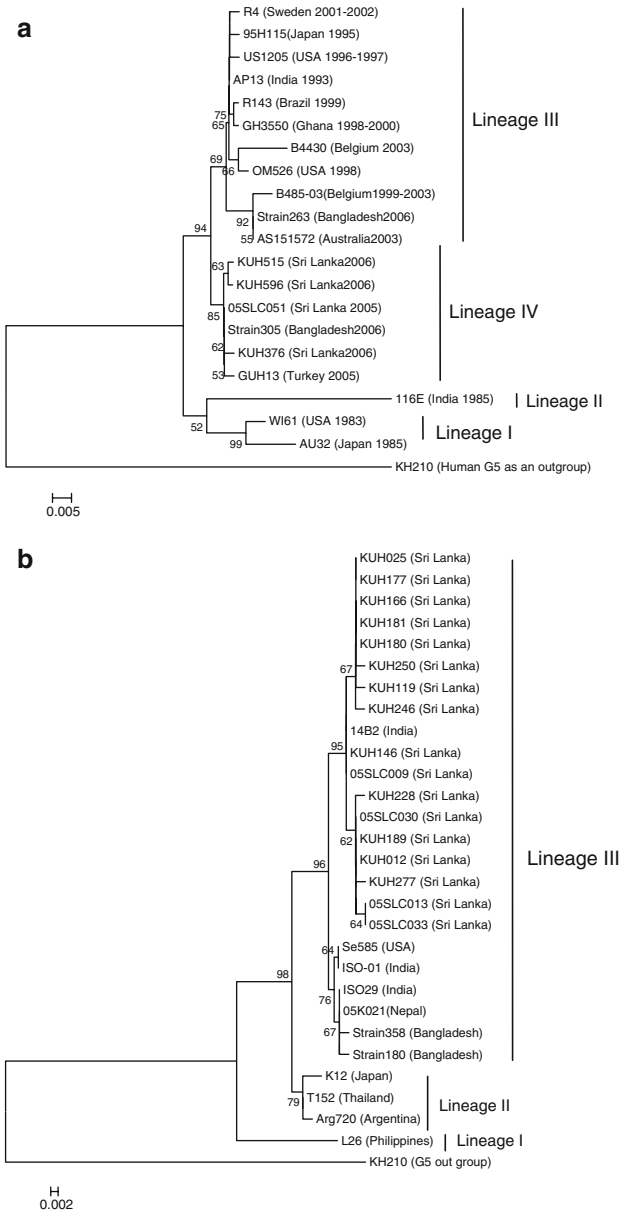

Fig. 2 Phylogenetic tree constructed with the VP7 amino acid sequences of (**a**) G9 and (**b**) G12 strains from Sri Lanka and other countries. The number adjacent to the nodes represents the percentage of bootstrap support (of 1,000 replicates) for the clusters to the right of the node. Bootstrap values lower than 50% are not shown

from several countries. All Sri Lankan G9 strains belonged to the newly identified [2] lineage IV together with strains from Turkey and Bangladesh (Fig. 2a). The VP7 of Sri Lankan G9 showed 96-100% amino acid identity with strains from lineages III and IV. All Sri Lankan G12 strains belonged to lineage III together with one Indian strain (Fig. 2b). The VP7 of Sri Lankan G12 showed 98–100% amino acid identity among them and other strains of same lineage. Sri Lankan G3 strains belonged to lineage I (Fig. 3), clustered with strains from China, Vietnam,

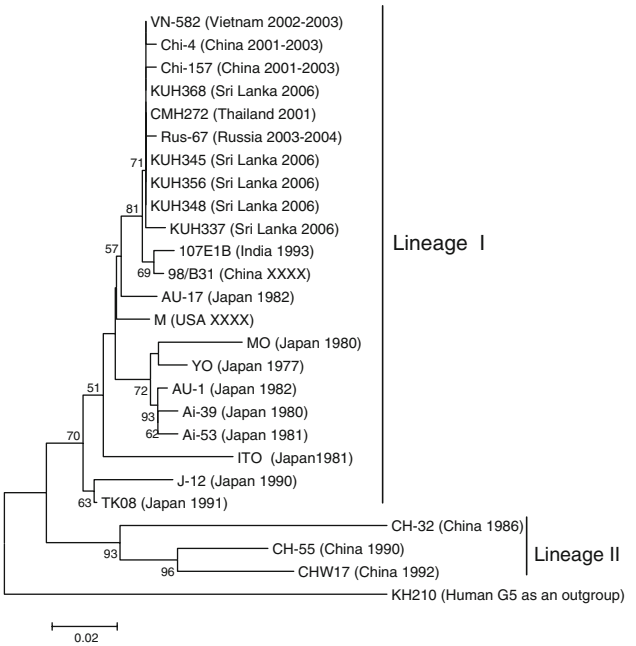

Fig. 3 Phylogenetic tree constructed with the VP7 amino acid sequences of G3 strains from Sri Lanka and other countries. The number adjacent to the nodes represents the percentage of bootstrap support (of 1,000 replicates) for the clusters to the right of the node. Bootstrap values lower than 50% are not shown. XXXX followed by the country name indicates that the year of sample collection is unknown

Russia and Thailand [12]. The VP7 of Sri Lankan G3 showed 99-100% amino acid identity among them and with those strains.

The burden of rotavirus diarrhea among Sri Lankan children found in this study was similar to what was reported before [7] and is comparable with that of neighboring India and Nepal [1, 13]. In the present study, none of the samples showed evidence of mixed infections by PAGE. However, diverse strains were detected, such as G2 with a long pattern and various combinations of G and P types. It is not known why the camp situation favored the spread of G12 and not G2 and G3 rotaviruses in the population. The impact of demographic variations or a pattern of adaptation to selective constraints of the camp situation, such as overcrowding and malnutrition, might have affected the type distribution.

Sri Lanka is the latest addition to the list of countries with emerging G12 rotaviruses in the Southeast Asian region [10, 11, 13]. Closer association of Sri Lankan and Indian G12 rotaviruses in the phylogenetic tree indicated a possible common ancestor from which it spread in that region. However, subcontinental G12s are frequently associated with P[6], whereas all Sri Lankan G12 and also G9 strains were associated with P[8], raising the possibility that a particular clone is spreading in Sri Lanka.

Phylogenetically, all Sri Lankan G9 strains belonged to lineage IV, together with strains from Turkey and Bangladesh. Although this is the first published report of G9 from Sri Lanka, it is possible that it has been present there for a long time, since neighboring countries reported it several years back. The introduction of lineage IV may be responsible for the present emergence of G9 in Sri Lanka. Circulating Sri Lankan G3s were closely related to the emerging G3s of China, Russia, Thailand and Vietnam. It is possible that these G3s are now emerging in different parts of the world.

The present study demonstrated that multiple (G9, G3 and G12) emerging genotypes were prevalent in Sri Lanka, and children in camps were at particular risk of developing diarrhea due rotaviruses. PAGE results and phylogenetic analysis of VP7 genes provided evidence that rotaviruses identified in camps were already in circulation in Sri Lanka. Further studies are needed to understand the long-term consequences of the camp situation for the evolution of rotavirus.

The DNA Data Bank of Japan/European Molecular Biology Laboratory/GenBank accession nos. of the VP7 gene sequence of the strains of this study are: AB306264, AB306268–AB306271, AB527006–AB527046, AB530266–AB530274.

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