[7, 9, 46]. We found 17 (25%) out of 68 strains that closely matched the 95/96-US strain (Fig. 2). These results suggested that the 95/96-US-like strain was also an important cause of sporadic cases of gastroenteritis. Further epidemiological studies may help determine whether strains from GII/4 including 95/96-US-like strains continue to be dominant in this region.

We showed that six strains from GII/3 were in fact Arg320-like strains, based on the partial RdRp and capsid sequences (Figs. 2 and 3). These six Arg320-like strains were detected between January 2000 and March 2000 but not after this period. Interestingly, a similar study of sporadic gastroenteritis conducted in Japan between April 1996 and March 2000 reported that Arg320-like strains suddenly appeared and spread between October 1999 and February 2000 [14]. Likewise, we found that 95/96-US-like strains suddenly appeared in October and November 2000 in Ho Chi Minh City. This sudden appearance and disappearance of strains may indicate that the population developed immunity. However, several studies have also found that dominant strains can persist in one region over a number of years, which may suggest that some strains, such as the 95/96-US strain, could be more virulent [39, 46].

Other NoV strains belonging to GI/4, GI/8, GII/1, and GII/7 were co-circulating, but these were infrequent. We also identified three recently published genotypes (Fig. 2), one in the GI (GI/11 genotype) and two in the GII (GII/10 and GII/14 genotypes). Recently, NoV GI and GII strains were predicted to consist of at least 14 and 17 genetic genotypes, respectively [21], but this number is expected to increase with improved detection techniques and increased surveillance [20].

Two NoV GII strains (026 and 0703) were shown to be almost the same virus as Mc37 strain, a recombinant NoV (Figs. 2 and 3). In 1999, Jiang et al. [18] first reported a naturally occurring recombinant NoV, and later several other strains were described as recombinants [24, 30, 45]. They discovered a region of genetic recombination between the RdRp and capsid genes. Our sequence analysis was comparable to these recombinant NoV studies. Genomic sequence analysis of 026 with other recombinant NoVs showed the region of genetic recombination was between 5,033 and 5,100 nucleotides (with reference to the 026 sequence) (Fig. 4B). We further analyzed 026 by expressing the VP1 of 026 and 9912-02F in a baculovirus expression system. Hyperimmune sera against the VLPs indicated that GII/12 and GII/10 are distinct antigenic types, though a considerable level of cross-reactivity was found between them. A similar cross-reactivity was also reported when the antigenicity was examined by antibody ELISA [27].

Co-circulation of two potential parental strains may allow a recombination event when their nucleic acid sequences come within physical contact in infected cells, e.g., during copy choice recombination. If 026, 0703, and Mc37 represented NoV "strain A", and 9912-02F and Saitama U1 represented NoV "strain B", at least two possible scenarios of genetic recombination are suggested. Scenario one: both "strain A" and "strain B" are recombinant NoVs and the parent strain(s) have not yet being identified. Scenario two: "strain A" was a parent of "strain B", or

vice versa. There is no direct evidence to support either scenario at the moment. Further extensive studies by sequence analysis of ORF1 and ORF2 using other strains is needed.

SaV infection causes gastroenteritis in all age groups, though it occurs predominantly in infants and young children [5]. Our study detected SaV in only one of the 448 children hospitalized with non-rotavirus gastroenteritis in Ho Chi Minh City. Several reports have noted that SaV detection was usually much lower than NoV detection [3, 25, 42, 47]. In one of these studies, Pang et al. found NoV in 10% of hospitalized children with gastroenteritis and 3% with SaV, while Kirkwood et al. found only 0.6% with SaV. In addition, several reports found SaV gastroenteritis is milder in symptoms as compared with NoV, therefore often not requiring hospitalization [25, 42, 44]. On the other hand, we performed a similar epidemiological study among hospitalized infants with gastroenteritis in Thailand and found SaV in 3.8% (4/105 of single infection) of the stool specimens [11]. Comparisons of the Thailand and Vietnam studies showed that this dissimilarity of the SaV detection rates was significant (Fisher Exact $P < 0.005$), whereas the dissimilarity of the NoV detection rates was not significant (8/105 were NoV positive in Thailand of single infection; Fisher Exact $P = 0.1$). The same primers and conditions were used in both studies, which suggested that SaV was an uncommon etiological agent of gastroenteritis in Ho Chi Minh City. Climatic and environmental conditions as well as cultural differences, including eating habits and hygiene practices, may be important factors that accounted for these differences in the SaV detection between these two countries [33]. Further epidemiological investigations of SaV in these two countries may help determine why SaV detection was significantly different and help ascertain the possible routes of SaV infections.

In many countries, NoV infection is prevalent in the winter months [12, 29, 36], though several studies showed no seasonal distribution [37, 40]. In our study, NoV infections prevailed at the end of the rainy season and the first half of the dry season, which was statistically significant. During this period the average temperature is cooler than the rainy season, which suggests a winter-like prevalence.

In conclusion, this study has shown that NoV was an important cause of sporadic gastroenteritis in Ho Chi Minh City. NoV strains belonging to the GII/4 genotype represented the dominant NoV strain, though several other NoV strains were also found to be co-circulating. SaV was detected in only one specimen, suggesting that SaV infection was an uncommon cause of gastroenteritis in Ho Chi Minh City.

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References

1. Atmar RL, Estes MK (2001) Diagnosis of noncultivatable gastroenteritis viruses, the human caliciviruses. Clin Microbiol Rev 14: 15–37
2. Bertolotti-Ciarlet A, Crawford SE, Hutson AM, Estes MK (2003) The 3′ end of norwalk virus mRNA contains determinants that regulate the expression and stability of the viral capsid protein VP1: a novel function for the VP2 protein. J Virol 77: 11603–11615
3. Buesa J, Collado B, Lopez-Andujar P, Abu-Mallouh R, Rodriguez Diaz J, Garcia Diaz A, Prat J, Guix S, Llovet T, Prats G, Bosch A (2002) Molecular epidemiology of caliciviruses causing outbreaks and sporadic cases of acute gastroenteritis in Spain. J Clin Microbiol 40: 2854–2859
4. Chiba S, Sakuma Y, Kogasaka R, Akihara M, Horino K, Nakao T, Fukui S (1979) An outbreak of gastroenteritis associated with calicivirus in an infant home. J Med Virol 4: 249–254
5. Chiba S, Nakata S, Numata-Kinoshita K, Honma S (2000) Sapporo virus: history and recent findings. J Infect Dis 181 [Suppl 2]: S303–S308
6. Doan LP, Okitsu S, Nishio O, Pham DT, Nguyen DH, Ushijima H (2003) Epidemiological features of rotavirus infection among hospitalized children with gastroenteritis in Ho Chi Minh City, Vietnam. J Med Virol 69: 588–594
7. Fankhauser RL, Noel JS, Monroe SS, Ando T, Glass RI (1998) Molecular epidemiology of "Norwalk-like viruses" in outbreaks of gastroenteritis in the United States. J Infect Dis 178: 1571–1578
8. Foley B, O'Mahony J, Morgan SM, Hill C, Morgan JG (2000) Detection of sporadic cases of Norwalk-like virus (NLV) and astrovirus infection in a single Irish hospital from 1996 to 1998. J Clin Virol 17: 109–117
9. Foley B, O'Mahony J, Hill C, Morgan JG (2001) Molecular detection and sequencing of "Norwalk-like viruses" in outbreaks and sporadic cases of gastroenteritis in Ireland. J Med Virol 65: 388–394
10. Gray JJ, Jiang X, Morgan-Capner P, Desselberger U, Estes MK (1993) Prevalence of antibodies to Norwalk virus in England: detection by enzyme-linked immunosorbent assay using baculovirus-expressed Norwalk virus capsid antigen. J Clin Microbiol 31: 1022–1025
11. Hansman GS, Katayama K, Maneekarn N, Peerakome S, Khamrin P, Tonusin S, Okitsu S, Nishio O, Takeda N, Ushijima H (2004) Genetic diversity of norovirus and sapovirus in hospitalized infants with sporadic cases of gastroenteritis in Chiang Mai, Thailand. J Clin Microbiol 42: 1305–1307
12. Hedlund KO, Rubilar-Abreu E, Svensson L (2000) Epidemiology of calicivirus infections in Sweden, 1994–1998. J Infect Dis 181 [Suppl 2]: S275–S280
13. Inouye S, Yamashita K, Yamadera S, Yoshikawa M, Kato N, Okabe N (2000) Surveillance of viral gastroenteritis in Japan: pediatric cases and outbreak incidents. J Infect Dis 181 [Suppl 2]: S270–S274
14. Iritani N, Seto Y, Kubo H, Murakami T, Haruki K, Ayata M, Ogura H (2003) Prevalence of Norwalk-like virus infections in cases of viral gastroenteritis among children in Osaka City, Japan. J Clin Microbiol 41: 1756–1759
15. Jiang X, Wang M, Graham DY, Estes MK (1992) Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. J Virol 66: 6527–6532
16. Jiang X, Matson DO, Ruiz-Palacios GM, Hu J, Treanor J, Pickering LK (1995) Expression, self-assembly, and antigenicity of a snow mountain agent-like calicivirus capsid protein. J Clin Microbiol 33: 1452–1555
17. Jiang X, Matson DO, Velazquez FR, Calva JJ, Zhong WM, Hu J, Ruiz-Palacios GM, Pickering LK (1995) Study of Norwalk-related viruses in Mexican children. J Med Virol 47: 309–316

18. Jiang X, Espul C, Zhong WM, Cuello H, Matson DO (1999) Characterization of a novel human calicivirus that may be a naturally occurring recombinant. Arch Virol 144: 2377–2387
19. Jiang X, Zhong WM, Farkas T, Huang PW, Wilton N, Barrett E, Fulton D, Morrow R, Matson DO (2002) Baculovirus expression and antigenic characterization of the capsid proteins of three Norwalk-like viruses. Arch Virol 147: 119–130
20. Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushi S, Hoshino FB, Takeda N, Katayama K (2003) Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J Clin Microbiol 41: 1548–1557
21. Kageyama T, Shinohara M, Uchida K, Fukushi S, Hoshino F, Kojima S, Takai R, Oka T, Takeda N, Katayama K (2004) Co-existence of multiple genotypes, including newly identified genotypes, in outbreaks of norovirus gastroenteritis. J Clin Microbiol (in press)
22. Kapikian AZ, Wyatt RG, Dolin R, Thornhill TS, Kalica AR, Chanock RM (1972) Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. J Virol 10: 1075–1081
23. Karst SM, Wobus CE, Lay M, Davidson J, Virgin HWt (2003) STAT1-dependent innate immunity to a Norwalk-like virus. Science 299: 1575–1578
24. Katayama K, Shirato-Horikoshi H, Kojima S, Kageyama T, Oka T, Hoshino F, Fukushi S, Shinohara M, Uchida K, Suzuki Y, Gojobori T, Takeda N (2002) Phylogenetic analysis of the complete genome of 18 Norwalk-like viruses. Virology 299: 225–239
25. Kirkwood CD, Bishop RF (2001) Molecular detection of human calicivirus in young children hospitalized with acute gastroenteritis in Melbourne, Australia, during 1999. J Clin Microbiol 39: 2722–2724
26. Kobayashi S, Sakae K, Suzuki Y, Ishiko H, Kamata K, Suzuki K, Natori K, Miyamura T, Takeda N (2000) Expression of recombinant capsid proteins of chitta virus, a genogroup II Norwalk virus, and development of an ELISA to detect the viral antigen. Microbiol Immunol 44: 687–693
27. Kobayashi S, Sakae K, Suzuki Y, Shinozaki K, Okada M, Ishiko H, Kamata K, Suzuki K, Natori K, Miyamura T, Takeda N (2000) Molecular cloning, expression, and antigenicity of Seto virus belonging to genogroup I Norwalk-like viruses. J Clin Microbiol 38: 3492–3494
28. Kojima S, Kageyama T, Fukushi S, Hoshino FB, Shinohara M, Uchida K, Natori K, Takeda N, Katayama K (2002) Genogroup-specific PCR primers for detection of Norwalk-like viruses. J Virol Methods 100: 107–114
29. Koopmans M, Vinje J, de Wit M, Leenen I, van der Poel W, van Duynhoven Y (2000) Molecular epidemiology of human enteric caliciviruses in The Netherlands. J Infect Dis 181 [Suppl 2]: S262–S269
30. Lochridge VP, Hardy ME (2003) Snow Mountain virus genome sequence and virus-like particle assembly. Virus Genes 26: 71–82
31. Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, Ingersoll R, Sheppard HW, Ray SC (1999) Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J Virol 73: 152–160
32. Lopman BA, Brown DW, Koopmans M (2002) Human caliciviruses in Europe. J Clin Virol 24: 137–160
33. Matsui SM, Greenberg HB (2000) Immunity to calicivirus infection. J Infect Dis 181 [Suppl 2]: S331–S335
34. McEvoy M, Blake W, Brown D, Green J, Cartwright R (1996) An outbreak of viral gastroenteritis on a cruise ship. Commun Dis Rep CDR Rev 6: R188–R192

35. McIntyre L, Vallaster L, Kurzac C, Fung J, McNabb A, Lee MK, Daly P, Petric M, Isaac-Renton J (2002) Gastrointestinal outbreaks associated with Norwalk virus in restaurants in Vancouver, British Columbia. Can Commun Dis Rep 28: 197–203
36. Mounts AW, Ando T, Koopmans M, Bresee JS, Noel J, Glass RI (2000) Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. J Infect Dis 181 [Suppl 2]: S284–S287
37. Nakata S, Honma S, Numata K, Kogawa K, Ukae S, Adachi N, Jiang X, Estes MK, Gatheru Z, Tukei PM, Chiba S (1998) Prevalence of human calicivirus infections in Kenya as determined by enzyme immunoassays for three genogroups of the virus. J Clin Microbiol 36: 3160–3163
38. Noel JS, Liu BL, Humphrey CD, Rodriguez EM, Lambden PR, Clarke IN, Dwyer DM, Ando T, Glass RI, Monroe SS (1997) Parkville virus: a novel genetic variant of human calicivirus in the Sapporo virus clade, associated with an outbreak of gastroenteritis in adults. J Med Virol 52: 173–178
39. Noel JS, Fankhauser RL, Ando T, Monroe SS, Glass RI (1999) Identification of a distinct common strain of "Norwalk-like viruses" having a global distribution. J Infect Dis 179: 1334–1344
40. O'Ryan ML, Mamani N, Gaggero A, Avendano LF, Pena A, Jiang X, Matson DO (2000) Human caliciviruses are a significant pathogen of acute sporadic diarrhea in children of Santiago, Chile. J Infect Dis 182: 1519–1522
41. Okada M, Shinozaki K, Ogawa T, Kaiho I (2002) Molecular epidemiology and phylogenetic analysis of Sapporo-like viruses. Arch Virol 147: 1445–1451
42. Pang XL, Honma S, Nakata S, Vesikari T (2000) Human caliciviruses in acute gastroenteritis of young children in the community. J Infect Dis 181 [Suppl 2]: S288–S294
43. Russo PL, Spelman DW, Harrington GA, Jenney AW, Gunesekere IC, Wright PJ, Doultree JC, Marshall JA (1997) Hospital outbreak of Norwalk-like virus. Infect Control Hosp Epidemiol 18: 576–579
44. Sakai Y, Nakata S, Honma S, Tatsumi M, Numata-Kinoshita K, Chiba S (2001) Clinical severity of Norwalk virus and Sapporo virus gastroenteritis in children in Hokkaido, Japan. Pediatr Infect Dis J 20: 849–853
45. Vinje J, Green J, Lewis DC, Gallimore CI, Brown DW, Koopmans MP (2000) Genetic polymorphism across regions of the three open reading frames of "Norwalk-like viruses". Arch Virol 145: 223–241
46. White PA, Hansman GS, Li A, Dable J, Isaacs M, Ferson M, McIver CJ, Rawlinson WD (2002) Norwalk-like virus 95/96-US strain is a major cause of gastroenteritis outbreaks in Australia. J Med Virol 68: 113–118
47. Wolfaardt M, Taylor MB, Booysen HF, Engelbrecht L, Grabow WO, Jiang X (1997) Incidence of human calicivirus and rotavirus infection in patients with gastroenteritis in South Africa. J Med Virol 51: 290–296

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Human Astrovirus, Norovirus (GI, GII), and Sapovirus Infections in Pakistani Children With Diarrhea

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Fecal specimens from 517 infants and young children admitted to the Civil Karachi Hospital, Dow Medical College, Karachi city, Pakistan with acute gastroenteritis from 1990 to 1994 were collected and screened by RT-PCR for human astrovirus (AstV), norovirus (NV), and sapovirus (SV). The specific epidemiological data for illness caused by these viruses in Pakistan are not available. AstV, NV, and SV were detected in 58, 51, and 17 of 517 fecal specimens, and this represented 11.2, 9.9, and 3.2%, respectively. An outbreak of gastroenteritis attributable to AstV serotype 1 was identified during September and October 1990. Moreover, one specimen with a triple mixed infection between AstV (serotypes 1 and 3) and NV GII was found. NV and SV were subjected to molecular analysis by sequencing. One of the sequenced specimens positive for SV turned out to be similar to a strain tentatively called a genogroup IV. The result underscores the importance of these viruses in association with acute gastroenteritis in Karachi city, Pakistan. ***J. Med. Virol. 73:256–261, 2004.***

KEY WORDS: multiplex PCR; serotype; genotype; enteropathogen

INTRODUCTION

Viral gastroenteritis is a common disease with a high morbidity reported worldwide especially in infants and the elderly. The mortality among children due to gastroenteritis is greater in developing than in the developed countries. Acute gastroenteritis ranks consistently as one of the principal six causes of all deaths. Apart from group A rotavirus as the most common cause of gastroenteritis, norovirus (NV), and sapovirus (SV) are considered to be significant global enteropathogens. These viruses also are associated with sporadic outbreaks of gastroenteritis in such settings as kindergartens, schools, and nursing homes for the elderly and among military recruits [Carter and Willcocks, 1996; Bon et al., 1999].

Astrovirus (AstV), so called to describe the distinctive five-or six-pointed star visible on some particles when viewed under the electron microscope (EM), is a small, 28-nm-diameter, nonenveloped, single capsid layered viruses with a positive-sense single-stranded RNA genome. This virus has three open reading frames (ORFs)—ORF1a, ORF1b, and ORF2. Moreover, ORF1a and ORF1b at the 5, end of the genome encode the viral protease and polymerase, respectively, whereas ORF2 at the 3, end of the genome encodes the capsid protein precursor [Schnagl et al., 2002]. AstV is classified currently into eight serotypes [Sakamoto et al., 2000].

NV and SV contain a positive sense single-strand RNA genome surrounded by an icosahedral capsid. The NV genome contains three ORFs (ORF1, 2, and 3); ORF 2 encodes the capsid proteins [Glass et al., 2000]. In the two SV ORFs (ORF 1 and 2), however, it is ORF 1 that encodes the non-structural as well as the capsid proteins. Based on the sequence analysis of the capsid gene, both human NV and human SV are divided into two genogroups (I, II) [Schuffenecker et al., 2001].

The objectives of this study were: to describe the prevalence of AstV, NV (GI, GII), and SV in fecal

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specimens from children with acute gastroenteritis in Karachi, Pakistan; to determine the age-related distribution, geographical distribution, and seasonal patterns of AstV, NV (GI, GII), and SV infections; and to characterize the detected viruses according to type.

MATERIALS AND METHODS

Fecal Specimens

Fecal specimens were collected from 517 infants and young children admitted to the Civil Karachi Hospital, Dow Medical College, Karachi city, Pakistan with acute gastroenteritis from 1990 to 1994. All fecal specimens were determined previously to be negative for rotavirus and adenovirus [Nishio et al., 2000]. These specimens were diluted with Eagle's minimum essential medium to 10% suspensions, and clarified by centrifugation at 5,000*g* for 20 min. Supernatants were stored at −30°C until use.

Extraction of Viral RNA

Viral RNA was extracted from 140 μl of a 10% fecal suspension by the use of a spin column technique according to the instructions in the QIAamp® Viral RNA Mini Kit Handbook.

Viral Detection and RT-PCR

AstV, NV (GI, GII), and SV were detected by RT-PCR analysis of extracted viral RNA. Four pairs of specific primers published were mixed [Yan et al., 2003] for the multiplex PCR.

Nucleotide Sequencing and Phylogenetic Analysis

All PCR products (DNA) positive for NV and SV were purified and then sequenced. Sequence analysis was performed using E-CLUSTAL W and the neighbor-joining method was used for the construction of the phylogenetic tree [Lui et al., 1995; Katayama et al., 2002]. The nucleotide sequence data for the capsid region from strains Karachi/1001/1990 and Karachi/730/1992 has been submitted to the DDBJ DNA database and has been assigned accession numbers AB126940 and AB126249, respectively. Reference strains and accession numbers used in this study are as follows: Birmingham (AJ277612), Southampton (L07418), Musgrove (AJ277614), Chiba (AB022679), NV68 (M87661), WUG1 (AB081723), SzUG1 (AB039775), Stav (AF145709), Hawaii (U07611), Girllington (AJ277606), Melksham (X81879), Chitta (AB032758), Wortley (AJ277618), Hillington (AJ277607), Alphatron (AF195847), Toronto (U02030), Seacroft (AJ277620), Leeds (AJ277608), Lordsdale (X86557), Manchester (X86560), London/92 (U95645), Stockholm (AF194182), Sapporo/82/JP (U65427), Plymouth (X86559), Houston/86 (U95643), Potsdam (AF294739), Lyon/30388/98/F (AJ251991), Lyon/598/97/F (AJ271056), Chiba/00067T/ 1999 (AJ412805), Chiba/010604F/2001 (AJ412826).

Human AstV Serotyping

Human AstV serotyping was carried out using RT-PCR analysis of extracted viral RNA with serotype specific primers (1–8) described by Sakamoto et al. [2000].

RESULTS

Epidemiology of Viral Infections

Of 517 fecal specimens negative for rotavirus and adenovirus, 168 were collected in 1990; 86 in 1991; 76 in 1992; 99 in 1993; and 88 in 1994. In each of the 5 years, the number of male was higher than that of female.

Among all children with acute gastroenteritis, 95.5% were aged less than 35 months. AstV, NV (GI, GII), and SV were detected mostly in the under 35 month age range. The viral infectious rate was highest in the 6–11 months old group (29.3%) and lowest in children over 35 months of age (13.0%). Surprisingly, infants under 6 months of age had a rather high rate of viral infections (24.1%) (Table I).

Viral enteropathogens were detected in 122 (23.6%) children with acute gastroenteritis, of these, 69 (56.6%) were boys. AstV was identified in 58 (47.5%) fecal specimens. NV was detected in 51 specimens (41.8%) of which NV GII was responsible for 39 cases (76.5%) and NV GI for 12 cases (23.5%). SV was found in 17 specimens (13.9%).

The percentages of monoinfections were 44.3% (54 specimens), 40.2% (49), and 12.3% (15) for AstV, NV, and SV, respectively. Two (1.6%) of all the viral positives were mixed infections between AstV and NV GII, and two more (1.6%) were mixed infections between AstV and SV.

In this study, the majority of AstV (81.1%) was detected in September and October 1990 (Fig. 1). This type of seasonality is indicative of an outbreak of AstV. All the Pakistani children in this presumed outbreak were under 3 years old. The highest incidence group was the 6–11 month old group (40%), and the second highest group was the 12–23 month old group (30%). We detected only one AstV in 2-year-old children (3.3%), and no case was found in children over 2 years old. Infants aged less than 6 months had a rather high rate of AstV infection (26.7%). Unlike the yearly distribution of AstV, there was no large difference between NV and SV infection rates from 1990 to 1994. The NV incidence was higher than those of AstV and SV in each of the 5 years except for 1990 (Table II).

TABLE I. Age-Related Gastroenteritis and Viral Infections Among Children in Karachi City, Pakistan

Age (month)	Number of tested specimens (%)	Number of positive specimens (%)
<6	87 (16.8)	21 (24.1)
6–11	164 (31.7)	48 (29.3)
12–23	179 (34.6)	38 (21.2)
24–35	64 (12.4)	12 (18.8)
>35	23 (4.5)	3 (13.0)
Total	517	122

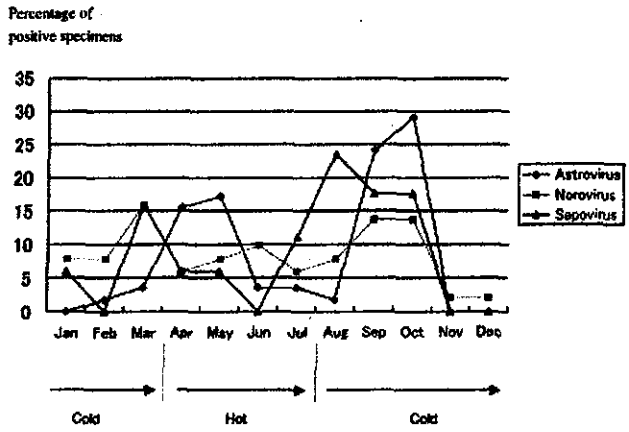

Fig. 1. Monthly distributions of astrovirus (AstV), norovirus (NV) (GI, GII), and sapovirus (SV) infections identified in children during the 5-year period, showing the cold season and hot season in Karachi, Pakistan.

Human AstV Serotypes

In our study, all 58 AstV (100%) detected were serotypes 1. Interestingly, we identified one possible infection with mixed serotypes 1 and 3. More remarkably, this same sample, Karachi/831/1991, contained AstV and NV GII. Apparently, this sample represented a triple mixed infection. Other AstV serotypes could not be found.

NV Nucleotide Sequencing and Phylogenetic Analysis

The total of 51 NV nucleotide sequences including 12 NV GI and 39 NV GII were analyzed by phylogenetics and grouped using the recent NV capsid region classification scheme of Katayama et al. [2002]. The NV GI sequences clustered into three distinct GI genotypes. Most of the NV sequences (66.7%) belonged to NV GI genotype 7 (NV GI/7) (typified by the Chiba virus cluster). Three NV sequences (25%) clustered into NV GI/1 (known as the NOR89JB virus cluster). Only one NV sequence (8.3%) was classified into NV GI/6 (known as the Musgrove virus cluster) (Fig. 2).

All 39 NV GII sequences were classified into five distinct GII genotypes. Sixteen (41.0%) of these NV sequences were similar to the genotype 1 (known as the Lordsdale virus). Twelve (30.8%) NV sequences belonged to the Hawaii virus cluster (the genotype 7), six (15.4%) to the genotype 2 (typified by the Toronto), two to the genotype 4 (known as the Melksham), and two more to the genotype 8 (known as the Seacroft). In addition to the five distinct GII genotypes, we also identified one sequence, isolate Karachi/1001/1990, representing one new NV GII genotype (Fig. 3).

SV Nucleotide Sequencing and Phylogenetic Analysis

The phylogenetic analysis indicated that SV group I (typified by the Manchester) was a more common genogroup (70.6%) than SV group II (23.5%) (London virus). Interestingly, one strain named Karachi/730/1992 was found and one strain, Karachi/730/1992, was shown to be different from the other SV strains; this was classified into the group Chiba/000671T/1999 and tentatively called genogroup IV by Okada et al. [2002] (Fig. 4). The nucleotide identity between Karachi/730/1992 and Chiba/000671T/1999 was 95%, and the amino acid identity was 96%.

DISCUSSION

AstV is a leading cause of infantile viral gastroenteritis worldwide [Koci et al., 2003]. Outbreaks of gastroenteritis resulting from AstV infection in Brazil [Silva et al., 2001], and in France [Belliot et al., 1997] have been reported, but this is the first similar report from Karachi, Pakistan. In this outbreak, the highest incidence of cases fell into the 6–11 month old age group, and the rate of incidence decreased with increasing age over 12 months. Quite possibly, children aged from 6 to 11 months might lack antibody protection to AstV, whereas by the time children have reached 11 months they have begun to acquire viral immunity.

Our findings showed that in infants less than 6 months old the prevalence of viral infections was high. Perhaps because of limited breastfeeding or for other reasons, these infants might not have enough maternally acquired passive antibody against viral enteropathogens. Studies conducted in Pakistan show a decline in breastfeeding since 1966, especially in urban areas [Lambert, 1988]. Many women perceive childbirth as being a major physical stress, and consider early initiation of breastfeeding as being just an added stress. In several urban squatter settlements, human breastmilk was regarded as a potential menace [Mull, 1992].

TABLE II. Incidence of Norovirus (NV) (GI, GII) Compared to That of Astrovirus (AstV), and Sapovirus (SV) in Children With Gastroenteritis in Karachi, Pakistan

Year	Number of specimens tested	Number (%) of AstV positive	Number (%) of NV positive	Number (%) of SV positive
1990	168	37 (22.0)	18 (10.7)	4 (2.4)
1991	86	7 (8.1)	8 (9.3)	5 (5.8)
1992	76	4 (5.3)	8 (10.5)	4 (5.3)
1993	99	6 (6.1)	7 (7.1)	1 (1.0)
1994	88	4 (4.5)	10 (11.4)	3 (3.4)

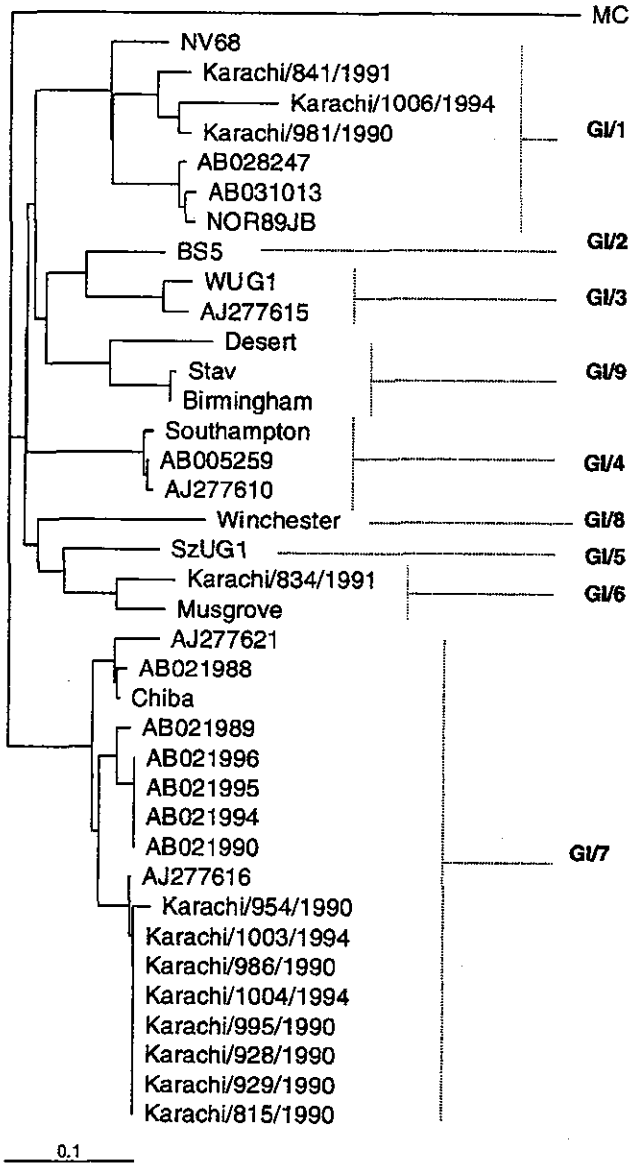

Fig. 2. Phylogenetic tree of NV GI (NV GI). The tree was reconstructed for partial sequences of the capsid region of NV GI found in Karachi city, Pakistan from 1990 to 1994. Reference strains of NV GI were registered in DDBJ/GenBank under the accession number indicated in the text. In the phylogenetic tree, NV GI was classified into nine distinct serotypes from 1 to 9. Manchester (MC) strain was used as an out-group for phylogenetic analysis.

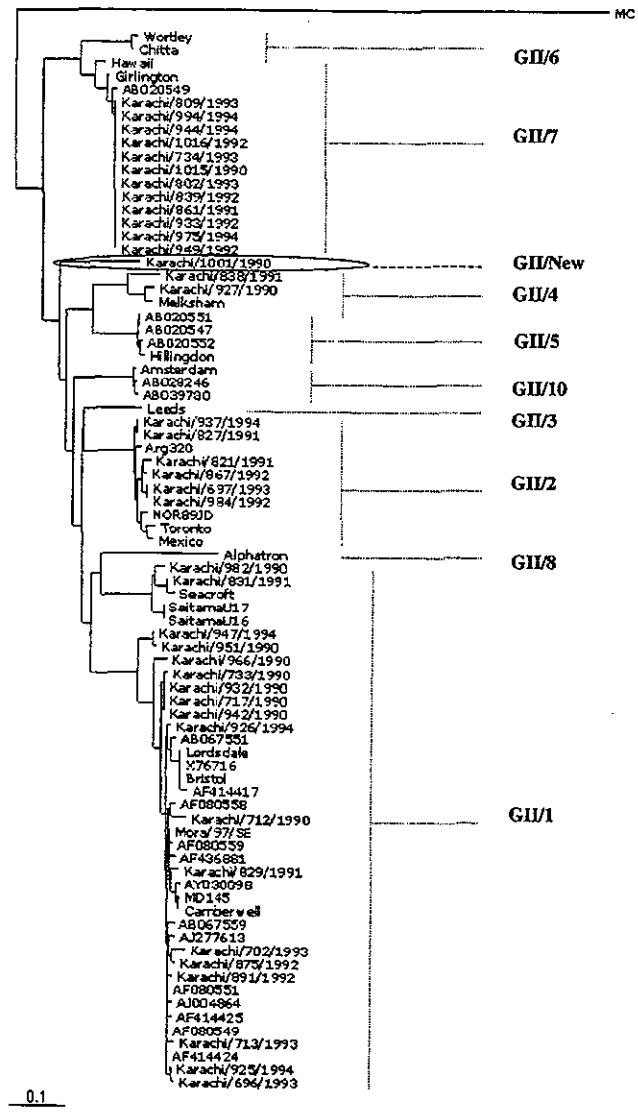

Fig. 3. Phylogenetic tree of NV GII (NV GII). The tree was reconstructed for partial sequences of the capsid region of NV GII identified in Karachi city, Pakistan during the 5-year period (1990–1994). Reference strains of NV GII were registered in DDBJ/GenBank under the accession number indicated in the text. In the phylogenetic tree, NV GII was classified into ten distinct serotypes from 1 to 10. The new serotype was signified in the oval circle. Manchester virus (MC) was used as an out-group strain for phylogenetic analysis.

Some hospitals in Pakistan separated newborns from their mothers, so that infants would not have a chance to initiate early breastfeeding. In practice, health practitioners did not inform the mothers of the importance of breastfeeding, and did not instruct them on how to establish and sustain breastfeeding. Further, many mothers preferred to give their babies formula instead of breastfeeding because of fear of insufficient milk supply due to their workload [Lambert, 1988]. Infants commonly drink unsafe water during the first 6 months after birth in Pakistan, and this may increase the risk of gastroenteritis [Shah et al., 2003]. Moreover, as substitutes for breastfeeding, the majority of infants were given honey as part of a quasi-religious ritual along with other foods intended for "cleansing of the stomach" [Badruddin et al., 1997].

The climate in Karachi city, Pakistan is distinctively seasonal. The summer lasts from April to July, and the hot temperature may reach over 37°C. The cold season characterized by less rain begins in August and ends in March. The coldest month is January when the temperature may dip as low as 5°C. In this study, AstV

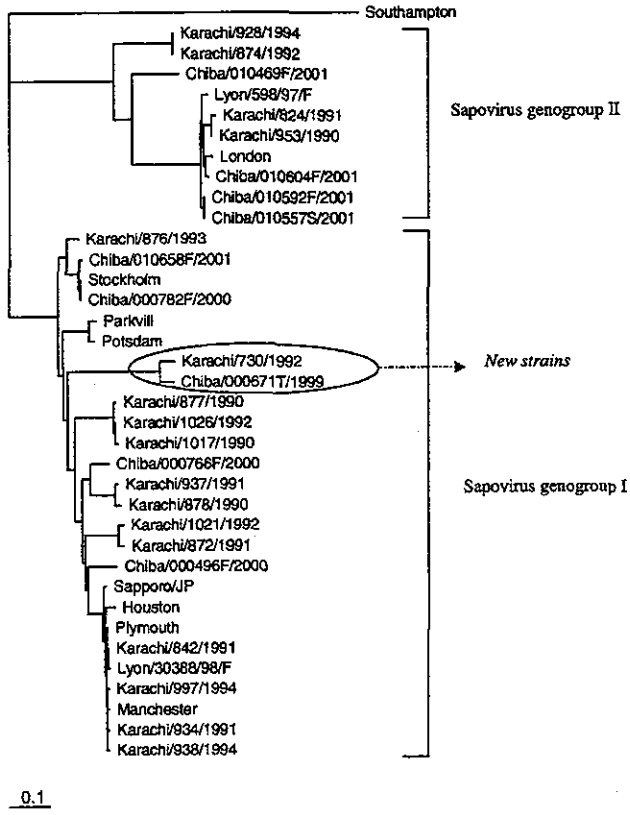

Fig. 4. Phylogenetic tree of SV. The tree was reconstructed for partial sequences of the capsid region of SV detected in Karachi city, Pakistan from 1990 to 1994. Reference strains of SV were registered in DDBJ/GenBank under the accession number indicated in the text. In the phylogenetic tree, SV was classified into genogroup I and II. The new strains were highlighted in the oval circle. Southampton strain was used as an out-group for phylogenetic analysis.

was observed to prevail in the hot summer months except for the outbreak in September and October. In many countries, AstV is prevalent in the rainy and cold season, and several studies did not find a seasonal correlation.

The NV detection rate was highest in March and secondly high in September as well as October. Moreover, almost all SV were found in August, September and October. These results suggest that these two major groups of human caliciviruses are prevailing during the cold season.

AstV was divided into eight serotypes in which serotype 1 was the most frequently detected. The mixed infections among these serotypes are uncommon [Palombo and Bishop, 1996; Sakamoto et al., 2000; Silva et al., 2001]. An outbreak of gastroenteritis associated with AstV serotype 1 occurred during September and October 1990 in Karachi, Pakistan. The outbreak of gastroenteritis attributable to AstV serotype 1 was also reported in Brazil [Silva et al., 2001]. Interestingly, we found one sample with a triple mixed infection between AstV (serotypes 1 and 3) and NV GII. Another less likely possibility, however, was that this AstV had undergone genetic recombination between serotypes 1 and 3. Although interesting, this mixed infection caused by different serotypes, in different AstV, and the extent of infection with different serotypes of the same virus were not addressed in this study.

NV belonging to the Lordsdale cluster represent the highest detection rate in sporadic gastroenteritis among children in Karachi, Pakistan. Other strains including one new NV GII genotype were also identified as being co-circulating.

The results indicated that SV group I (typified by the Manchester virus, MC) was a dominant genogroup. Interestingly, one strain named Karachi/730/1992 was similar to the group of Chiba/000671T/1999 tentatively called as genogroup IV by Okada et al. [2002]. These two viruses had a high identity on the nucleotide as well as the amino acid. However, the phylogenetic tree of SV showed that Karachi/730/1992 was a part of a genogroup I.

This is the first report from Karachi city, Pakistan of acute gastroenteritis associated with human AstV, NV (GI, GII), and SV, and the first indication of an outbreak attributable to AstV serotype 1. Our findings confirm the presence of these viruses in acute gastroenteritis among infants and young children in Karachi city, Pakistan.

REFERENCES

Badruddin SH, Inam SN, Ramzanali S, Hendricks K. 1997. Constraints to adoption of appropriate breast-feeding practices in a squatter settlement in Karachi, Pakistan. J Pak Med Assoc 47:63–68.

Belliot G, Laveran H, Monroe SS. 1997. Outbreak of gastroenteritis in military recruits associated with serotype 3 astrovirus infection. J Med Virol 5:101–106.

Bon F, Fascia P, Dauvergne M, Tenenbaum D, Planson H, Petion AM, Pothier P, Kohli E. 1999. Prevalence of group A rotavirus, human calicivirus, astrovirus, and adenovirus type 40 and 41 infections among children with acute gastroenteritis in Dijon, France. J Clin Microbiol 37:3055–3058.

Carter MJ, Willcocks MM. 1996. The molecular biology of astroviruses. Arch Virol 12:277–285.

Glass PJ, White LJ, Ball JM, Leparc GI, Hardy ME, Estes MK. 2000. Norwark virus open reading frame 3 encodes a minor structural protein. J Virol 74:6581–6591.

Katayama K, Horikoshi HS, Kojima S, Kageyama T, Oka T, Hoshino FB, Fukushi S, Shinohara M, Uchida K, Suzuki Y, Gojobori T, Takeda N. 2002. Phylogenetic analysis of the complete genome of 18 Norwalk-like viruses. Virology 299:225–239.

Koci MD, Moser LA, Kelley LA, Larsen D, Brown CC, Schultz-Cherry S. 2003. Astrovirus induces diarrhea in the absence of inflammation and cell death. J Virol 77:798–808.

Lambert J. 1988. Pakistan: Update on breastfeeding. Mothers Child 7:5–6.

Lui BL, Clarke IN, Caul EO, Lambden PR. 1995. Human enteric caliciviruses have a unique genome structure and are distinct from the Norwalk-like viruses. Arch Virol 140:1345–1356.

Mull DS. 1992. Mother's milk and pseudoscientific breastmilk testing in Pakistan. Soc Sci Med 34:1277–1299.

Nishio O, Matsui K, Oka T, Ushijima H, Mubina A, Dure-Samin A, Isomura S. 2000. Rotavirus infection among infants with diarrhea in Pakistan. Pediatr Int 42:425–427.

Okada M, Shinozaki K, Ogawa T, Kaiho I. 2002. Molecular epidemiology and phylogenetic analysis of Sapporo-like viruses. Arch Virol 147:1445–1451.

Palombo EA, Bishop RF. 1996. Annual incidence, serotype distribution, and genetic diversity of human astrovirus isolates from hospitalized children in Melbourne, Australia. J Clin Microbiol 34:1750–1753.

Sakamoto T, Negishi H, Wang QH, Akihara S, Kim B, Nishimura S, Kaneshi K, Nakaya S, Ueda Y, Sugita K, Motohiro T, Nishimura T, Ushijima H. 2000. Molecular epidemiology of astroviruses in

Japan from 1995 to 1998 by reverse transcription-polymerase chain reaction with serotype-specific primers (1 to 8). J Med Virol 61:326–331.

Schnagl RD, Belfrage K, Farrington R, Hutchinson K, Lewis V, Erlich J, Morey F. 2002. Incidence of human astrovirus in Central Australia (1995 to 1998) and comparison of deduced serotypes detected from 1981 to 1998. J Clin Microbiol 40:4114–4120.

Schuffenecker I, Ando T, Thouvenot D, Lina B, Aymard M. 2001. Genetic classification of "Sapporo-like viruses". Arch Virol 146: 2115–2132.

Shah SM, Yousafzai M, Lakhani NB, Chotani RA, Nowshad G. 2003. Prevalence and correlates of diarrhea. Indian J Pediatr 70:207–211.

Silva AMV, Leite EG, Assis RMS, Majerowicz S, Leite JPG. 2001. An outbreak of gastroenteritis associated with astrovirus serotype 1 in a day care Center, in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz 96:1069–1073.

Yan H, Yagyu F, Okitsu S, Nishio O, Ushijima H. 2003. Detection of norovirus (GI, GII), sapovirus and astrovirus in fecal samples using reverse transcription single-round multiplex PCR. J Virol Methods 14:37–44.

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Feature Article

Molecular epidemiology of viral gastroenteritis in Asia

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Abstract Acute gastroenteritis is one of the most common diseases in humans, and continues to be a significant cause of mortality and morbidity worldwide. Recently the estimates of mortality associated with diarrhea declined, however the majority of deaths still occur in developing countries and thus urgent intervention is needed for the prevention of these diseases. In Asian countries it is very important to study the distribution, transmission and characteristics of prevalent viruses in order to produce viral vaccines. The viruses which cause gastroenteritis are primarily from four distinct families – group A rotaviruses, caliciviruses, enteric adenoviruses and astroviruses. Rotavirus is a common virus that causes severe gastroenteritis in children <5 years of age. The reassortant viruses with animal virus, or directly animal viruses are isolated in humans. The future development of a safe and effective vaccine against rotavirus, along with the expansion of understanding of the distribution of types in Asia and an availability of rapid diagnostic tests, could reduce mortality and might be able to prevent severe gastroenteritis. Calicivirus is a causative virus of acute gastroenteritis in children and has been known to contaminate food causing viral outbreaks affecting people of all ages. Recently, the understanding of calicivirus and the improvement of detection techniques has increased the total frequencies of diarrheal viruses. For the future control and prevention of diarrheal diseases it is necessary to examine the molecular epidemiology of caliciviruses as well as rotaviruses.

Key words Asia, infants, gastroenteritis, norovirus, rotavirus.

Acute gastroenteritis is one of the most common illnesses in humans worldwide and it has a great impact on people, particularly children. More than 20 viruses have been recognized as important causes of this illness. The major symptoms are vomiting and diarrhea, and severe cases of the disease can lead to dehydration that in some cases is fatal. Annual mortality associated with diarrhea was estimated to be 2.1 million in 2000, worldwide.[1] This estimate shows a decrease in annual mortality when compared with estimates between 1992 and 2000.[2] Deaths from diarreal diseases in children have essentially declined; however, the fact that the majority of deaths occur in developing countries has not changed. It was further estimated that children under 5 years of age have several episodes of diarreal diseases each year in developing countries, in contrast to industrialised countries where people of all ages have approximately one episode of gastroenteritis each year, and the percentage of hospitalization is high but only few patient's die.[1]

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Until 1972 the pathogens of the diarrheal episodes were unknown; the viruses were discovered by the use of electron microscopy. By studying the feces of diarrheal patients, researchers discovered Norwalk virus in 1972,[3] rotavirus in 1973,[4] enteric adenovirus in 1975[5,6] and astrovirus in 1975.[7] The characteristics of these viruses are shown in Table 1. Recent scientific advances have lead to the expansion of our understanding of the roles that the viruses play in diarrhea, and have helped in our efforts to prevent and control the viral diseases. Here, we describe the molecular epidemiology of viral gastroenteritis in Asian countries.

Rotaviruses

Rotaviruses belong to the family *Reoviridae*, and the molecular biology and biochemistry of the virus are well characterized.[8] The viruses are icosahedral particles composed of multiple protein layers enclosing the genome that consists of 11 segments of double-stranded (ds) RNA. Each segment codes for one protein, with the exception of segment 11 which codes for two proteins. Out of a total of 12 proteins, six proteins are structural proteins and six are non-structural proteins. The outer capsid layer is made by VP7

Table 1 Characteristics of four etiologic viruses of acute gastroenteritis in children

Virus	Family	Diameter (nm)	Nucleic acid	Genome size	Types infected with humans	Cultivation	Diagnosis
rotavirus	*Rotaviridae*	70	dsRNA	18.5kb	group A, B, C group A: subgroup I. II G serotypes (1,12) (1,2,3,4,5,6,8,9,10,12)	+	EM, IC, ELISA, RT-PCR, PAGE latex agglatination
norovirus	*Caliciviridae*	28–35	ss(+)RNA	7.5–7.7kb	genogroup I, II	–	EM, ELISA, RT-PCR
sapovirus	*Caliciviridae*	28–35	ss(+)RNA	7.6kb	genogroup I, II	–	EM, ELISA, RT-PCR
enteric adenovirus	*Adenoviridae*	80	dsDNA	35.0kb	mainly subgroup 40, 41	+	EM, IC, ELISA, PCR-RFLP
astrovirus	*Astroviridae*	28–35	ss(+)RNA	7.2kb	1–8 subtypes	+	EM, ELISA, RT-PCR

ELISA, enzyme-linked immunosorbent assay; EM, electron microscopy; IC, immunochromatography; PCR, polymerase chain reaction; PCR-RFLP, PCR-restriction enzyme length polymorphism.

and VP4; their proteins are associated with attachment and entry of virus into the cells. The intermediat layer is composed of VP6, which distinguishes group specific antigens A to G. Most human rotaviruses belong to group A. However, group B rotaviruses have caused outbreaks of severe diarrhea among adults in China and India, and the viruses are therefore known as adult diarrhea rotaviruses. During 2000–2001, group B rotavirus caused severe diarrhea among adults and children in Bangladesh.[9] Group C rotaviruses have been shown to cause sporadic diarrhea outbreaks. In Japan, outbreaks of diarrhea caused by group C rotavirus have been reported during April to June almost every year.[10]

The two proteins VP7 and VP4 carry distinct epitopes, which specifically are determined by neutralizing antibody responses, and thus determine serotypes: VP7-specific serotypes are termed G types (G stands for glycoprotein) and VP4-specific serotypes are termed P types (P stands for protease-sensitive protein). The genes coding for VP4 and VP7 have also been used for genotypic classification of strains. For G types, serotypes have been fully coincident with genotype, and RT-PCR for G serotype classification is conducted by analyzing virus RNA. Among A group rotaviruses, 14 different G serotypes and 20 different P genotypes are identified, and there is cocirculation of different G and P serotypes/genotypes of rotaviruses. Initially almost all episodes were found to be caused by G serotypes 1, 2, 3 or 4, however recently the occurrence of G 9 serotype increased worldwide and unusual G serotypes such as G5 and G8 serotypes which were previously known only as animal rotaviruses have been found in humans.

The surveillance and diagnosis of rotavirus infections are conducted by detection of viral genes or viral antigens in feces of patients with diarrhea. Viral antigens can be detected by enzyme-linked immunosorbent assay (ELISA) or latex agglutination test. Monoclonal or polyclonal antibodies using these methods were induced by immunization with rotavirus, which could be cultivated and propagated *in vitro*. Group-specific antibodies are used for screening test of rotaviruses. Recently, immunochromatography methods (IC) have been developed to be used for rapid diagnosis of outpatients in clinics. RT-PCR techniques are more sensitive than immunoassay methods and are useful for reconfirmation of the other techniques. This molecular technique has been developed to design specific primers for rotavirus classification due to G serotypes, P serotypes and NSP4 types, and specific primers for group B and C rotaviruses are also available. Thus RT-PCR has become the general method for detecting viral genes. Another technique to differentiate strains is the separation of the 11 segments of RNA by polyacrylamide gel electrophoresis (PAGE). The migration pattern (electropherotype) can classify and differentiate rotavirus isolates. Using these techniques molecular epidemiology of rotavirus gastroenteritis clarifies the transmission, prevalence, and evolution of rotaviruses.

During 1986–2000, approximately 111 million dirreal episodes due to rotaviruses occurred annually worldwide, including children who were cared for at home, 25 million outpatients and 2 million hospitalizations. The mortality has been estimated to be 352,000–592 000 in children <5 years of age.[1] Every child aged <5 years has been infected with rotavirus. One out of five outpatients and one out of 293 deaths are due to rotavirus infection. Eighty-two percent of deaths due to rotavirus occur in children in developing countries. Therefore, rotavirus vaccine administration is necessary to decrease the number of deaths in the developing countries. In order to develop a safe and highly effective vaccine, it is necessary to investigate the prevalence of serotypes and genotypes of rotavirus in Asian countries. Some epidemiological studies of rotavirus diarrhea in Asia (especially Bangladesh, China, India, Japan, Korea, Thailand, and Vietnam) in the 1990s are described in Table 2.[11–27] G1 was found to be the most predominant serotype in most published reports; however, in some reports G2 or G4 was most predominant. The percentage of G3 was low except China from 1994 to 1996 and from 1998 to 1999.[12,13] In contrast, G9 emergence was remarkable. Human G9 serotype

Table 2 Distribution of serotype G of rotavirus in Asian countries

Country	Year	Total no. tested	No. rotavirus positive feces investigated	G1 (%)	G2 (%)	G3 (%)	G4 (%)	G9 (%)	Mixed (%)	Nontypeable (%)	References
Bangladesh	1992–1997		351	55 (15.7)	72 (20.5)	0 (0.0)	122 (34.8)	56 (16.0)	26 (7.4)	20 (5.7)	11
China	1994–1996		95	56 (58.9)	15 (15.7)	16 (16.8)	6 (6.3)	0 (0.0)	1 (1.1)		12[†]
China	1996–1999		283	184 (65.0)	84 (29.7)	14 (5.0)	6 (2.1)		8 (2.8)	16 (5.7)	13
China	1998–1999	3177	1307	949 (72.6)	158 (12.1)	185 (14.2)	32 (2.5)	12 (0.9)	40 (3.1)	9 (0.7)	14
India	1993	458	63	7 (11.1)	14 (22.2)	7 (11.1)	6 (9.5)	15 (23.8)	7 (11.1)	7 (11.1)	15
India	1996–1998		313	51 (16.3)	99 (31.6)	2 (0.6)	32 (10.2)	50 (16.0)	31 (9.9)	22 (7.0)	16
India	1995–1999		126	50 (39.7)	24 (19.0)	1 (0.8)	30 (23.8)	5 (4.0)	10 (7.9)	25 (19.8)	17
India	1999–2000		150	49 (32.7)	27 (18.0)	0 (0.0)	30 (20.2)		18 (12.0)		18
Japan	1995–1997	1027	396	326 (82.3)	2 (0.5)	9 (2.3)	2 (0.5)	1 (0.3)	14 (3.5)	44 (11.1)	19
Japan	1996–2000	2218	642	520 (81.0)	37 (5.8)	15 (2.3)	6 (0.9)	57 (8.9)	3 (0.5)	4 (0.6)	20
Korea	1998–1999		97	23 (23.7)	13 (13.4)	0 (0.0)	56 (57.7)		3 (3.1)	2 (2.1)	21
Korea	1999–2000	348	205	58 (28.3)	3 (1.5)	6 (2.9)	84 (40.9)	0 (0.0)	28 (14.2)	26 (12.7)	22
Pakistan	1990–1997	818	112	30 (26.8)	10 (8.9)	0 (0.0)	30 (26.8)			42 (37.5)	23
Thailand	1995–1997	164	100	47 (47.0)	40 (40.0)	0 (0.0)	3 (3.0)	6 (6.0)	0 (0.0)	4 (4.0)	19
Vietnam	1994–1996	158	79	23 (29.1)	13 (16.5)	0 (0.0)	21 (26.6)			22 (27.8)	24
Vietnam	1998–1999		224	29 (13.0)	119 (53.0)	8 (3.6)	38 (17.0)	3 (1.3)	20 (9.0)	5 (2.1)	25
Vietnam	1999–2000	1355	889	610 (68.6)	109 (12.3)	6 (0.7)	137 (15.4)	5 (0.6)	17 (1.9)	5 (0.6)	26
Vietnam	2000	123	49	25 (51.0)[‡]	19 (38.8)	0 (0.0)	3 (6.1)	0 (0.0)	2 (4.1)	0 (0.0)	27

[†]1 sample was identified as serotype 8. [‡]23 out of 25 G1 serotype strains were variant.

strains were first detected in the USA in 1983 and the strains have emerged globally since the mid-1990s. In Asian countries they were continually detected: G9 serotype was the predominant strain in Bangladesh in the year 1996–97 and in India in the year 1996–98 (Table 2).[11,16] In Japan, G9 strains were first reported to detect in 1985–1986.[28,29] Reports from Japan from 1996 to 2000 showed that the G9 serotype virus spread quickly (see Table 3).[19,20] Studies comparing G9 V7 seen in recently prevalent strains with prototype strains, reveal that prototype strains in the 1980s have not reemerged in Asia.[19,20,30,31]

Other unusual serotypes have been detected in developing countries, such as G5, G8, and G10, which have been commonly found in animals. The trend towards an increase in diversity of cocirculating viruses is worldwide. The detections of G8 in India and G12 in Thailand are examples of this (Table 3).[17,32]

G/P combination due to G type for VP7 and P type for VP4 are able to distinguish rotaviruses. The most common four combinations are G1P[8], G2P[4], G3P[8] and G4P[8]. It was described that these four type of viruses in most temperate climate constitute over 90% of cocirculating strains,[33] however in other regions of the world viruses of other G types in combination with various P types have been found (Table 3). P[6] type is known as a classical neonatal type, and it has been detected among children with diarrhea. Surveillance in China from 1998 to 1999 demonstrated that four common combinations remained predominant (75.6%), 14 different G/P combinations including neonatal serotypes and uncommon combinations were found, as shown in Tables 3 and 4.[14] In India between April and December 1993, 10 different strains were detected.[15] The percentage of common worldwide type strains was 33%, classical neonatal strains (P[6]) were common (43%) and the most prevalent type was G9P[6]. Genomic reassortant can occur easily in nature; it isolates after double infections of different strains.[34]

Caliciviruses (noroviruses and sapoviruses)

There are four genera in the family *Calidiviridae*, in which two genera, norovirus (known as Norwalk-like virus) and sapovirus (known as Sapporo-like virus), infects humans. Norovirus and sapovirus cause acute gastroenteritis with diarrhea and vomiting. Novovirus is the pathogen not only in infantile diarrhea but also in food poisoning including outbreaks. Sapovirus is also frequently found in feces of infantile diarrhea, however it is less common than norovirus. In Japan, surveillance on pediatric cases and outbreaks of viral gastroenteritis between 1994 and 1999 showed that the most predominant pathogen of acute diarrhea in the infants <3 years of age was rotavirus, and norovirus was the main agent in the children >3 years of age.[35] Furthermore, rotavirus-induced

Table 3 Summary of prevalence of G9 serotypes and their combinations with P types

G/P combination	% in rotavirus-positive samples	Country	Year round	Reference
G9P[8]	10/243 (4.1%)	Bangladesh	1995–1996	11†
G9P[6]	37/243 (15.2%)	Bangladesh	1995–1996	11†
G9P[6]	11/327 (3.3%)	China	1998–1999	14
G9P[8]	1/327 (0.3%)	China	1998–1999	14
G9P[11]	1/63 (1.6%)	India	1985	33
G9P[6]	15/63 (23.8%)	India	1993	15
G9P[6]	4/126 (3.1%)	India	1995–1999	17‡
G9P[8]	14/287 (4.9%)	India	1996–1998	16
G9P[6]	26/287 (9.1%)	India	1996–1998	16
G9P[8]	1/148 (0.7%)	Japan	1996–1997	19
G9P[8]	23/199 (11.6%)	Japan	1998–1999	20
G9P[8]	32/133 (15.2%)	Japan	1999–2000	20
G9P[4]	1/133 (0.8%)	Japan	1999–2000	20
G9P[8] + G9P[4]	1/133 (0.8%)	Japan	1999–2000	20
G9P[6]	5/37 (13.5%)	Thailand	1996–1997	19
G9P[4]	1/37 (2.7%)	Thailand	1996–1997	19
non-typeable G9	9% in 224	Vietnam	1998–1999	25
G9P[8]	3/886 (0.3%)	Vietnam	1999–2000	26
G9P[4]	2/886 (0.2%)	Vietnam	1999–2000	26

†P type of 9 strains were unidentified. ‡P type of 1 strain was unidentified.

Table 4 Uncommon G/P combination of rotaviruses in Asian countries

G/P combination	Country	Year round	Reference
G1P[4], G1P[6], G2P[8], G2P[6], G4P[4], G4P[6]	Bangladesh	1992–1997	11
G1P[6], G2P[6], G3P[6], G4P[6]	India	1993	15
G4P[11]	India	1993	15
G2P[6], G3P[6], G4P[6], G2P[8], G4P[11]	India	1996–1998	16
G8P[8]	India	1997–1998	17
G9P[6], G1P[6], G3P[6]	China	1998–1999	14
G12P[9]	Thailand	1998–1999	32
G1P[9], G1P[4], G3P[4], G4P[4], G2P[8]	China	1998–1999	14
G3P[6], G4P[6]	Vietnam	1998–1999	24
G1P[4], G1P[6], G2P[6], G4P[4]	Korea	1998–2000	22
G1P[4], G1P6], G2P[6], G4P[4]	Korea	1998–2000	22
G1P[4], G1P[6]	Vietnam	1999–2000	26

diarrhea usually occurs after January and the main peak of norovirus infection is between November and December. More than 60% of norovirus infection outbreaks were food borne.[35]

Virus genomes in the family *Calisiviridae* are single-stranded, positive-sense RNA approximately 7300–7800 nucleotides in length, possessed poly A structure at the 3′ terminus. Norovirus genome consists of three open reading frames (ORFs). The 5′ region of genome (ORF1) encodes large non-structural proteins such as NTPase, 3 A-like protein, VPg, protease and RNA dependent RNA polymerase (RdRp), ORF2 encodes the structural protein of the capsid, and ORF3 encodes for a small protein. The function of the small protein is unknown, although it seems to be associated with the stable expression of capsid proteins by the study of recombinant virus-like particles (VLPs) using baculoviruses.[36,37] Sapovirus genome consists of ORF1, which encodes non-structural proteins and capsid protein, and ORF2, which encodes for a small protein. Both norovirus and sapovirus are genetically divided into two distinct groups: genogroup I (GI) and genogroup II (GII). Recently, at least three other genogroups have been identified. Two genogroups (GI and GII) can be further divided into genetic clusters.[38–40] It was indicated that the clustering based on capsid N-teminal/Shell domain (capsid N/S) successfully distinguished noroviruses as well as the grouping based on the antigenicity.[41]

Norovirus and sapovirus were detected through electron microscopy. In the absence of an *in vitro* culture system for these viruses, virus isolation methods using adequate cultured cells have not yet been established. It is difficult to apply the antigen-antibody system to calicivirus classification because of the epidemiology for their antigenic diversity.

Table 5 Distribution of four categories of diarrheal viruses in China and Japan

	Total no. samples investigated	No. virus-positive samples	Rotavirus (%)	Calicivirus (%) (NV + SV)	Enteric adenovirus (%)	Astrovirus (%)	Mixed (%) Infection	Not detected (%)	Other viruses (%)	References
China 1994–1999	186		104/186 (55.9)	9/118 (7.6)	3/118 (2.5)	10/116 (8.5)	–	–	Group C rotavirus 1/118 (0.8)	12
Japan 1995–1998	1382	975	504 (51.7)	297 (30.5)	150 (15.4)	82 (8.4)	58 (5.9)	407 (41.7)	–	53
Japan 1996–2000	669	316†	189 (59.8)†	105 (33.2)†	17 (5.4)†	1 (0.3)†	4 (1.1)	353 (52.8)	Enterovirus: 6 (1.9)†	50

†Numbers are represented as number of virus strains.

Although it has been attempted to establish VLP-based antigenic typing ELISAs for noroviruses, they are not yet available for all genotypes that could be detected by RT-PCR.[42,43]

At present, RT-PCR is the most reliable method for calicivirus detection. The conserved RdRp gene region was used as target regions for genotyping studies and many primers and probes were designed, however the molecular typing is required using some primer pairs due to great diversity of virus genes. Recently the improved primer pairs based on the highly conserved N′-terminal region of capsid gene for GI and GII were reported by Kojima *et al.* who indicated that new primer pairs were capable of amplifying the clinical specimens more efficiently than primer pairs based on RdRp genes.[43] In addition, the real-time quantitative RT-PCR assay methods using primer pairs based on ORF1-ORF2 junction region were described to be highly sensitive and broadly reactive.[45]

As previously mentioned, there are few epidemiological studies of caliciviruses in Asian countries except in Japan. Because the incidents of rotavirus infections are high in developing countries, the studies and prevention of rotavirus infection is considered an important issue. In addition the epidemiological studies of caliciviruses are difficult and the clinical severity of calicivirus gastroenteritis is considered to be lower than that of rotavirus.[46] In contrast, children in developing countries have been described as experiencing many more episodes of diarrhea due to calicivirus infection each year in the first 5 years of life than children in developed countries.[47] A more sensitive method is required to detect the viruses in specimens from Asian countries. Distributions of diarrheal viruses in Japan and China are shown in Table 5. Two Japanese studies represented that following rotavirus, the incidents of calicivirus infections were high (>30%).

In developing countries, caliciviruses are also present, but bacterial and parasitic transmissions are more important for the children's health. The prevalence of anticaliciviruses antibodies was studied in China, Japan and South-east Asian countries.[48,49] Detecting antibodies against Norwalk virus of genogroup I and Mexico virus of genogroup II using VLPs were found in >80% of healthy adults in Japan and South-east Asia. In China, positive percentage of infants (7–11 months of age) was 36–41% and children (4 months to 3 years of age) was <10%.[49] These results indicate that the viruses were common in these areas. Epidemiological surveillance of caliciviruses is required in Asian countries.

There are few analyses of distribution of genotypes in noroviruses. The prevalence of causative noroviruses infections in children with acute gastroenteritis were studied by RT-PCR following southern hybridization.[50,51] The predominant genotype of norovirus in Osaka of Japan from 1996 to 2000 was Lordsdale virus-like virus in genogroup II. The Lordsdale-

like viruses were reported to spread worldwide from 1995 to 1996.[52] In the Netherlands it was reported that Lordsdale-like viruses were the most common virus in caliciviruses in 1997–1999, but they declined in 1999.[40] They also indicated that the related strains of caliciviruses in human were highly prevalent in animals. In Japan it is suggested that viruses in infantile acute gastroenteritis are related to outbreaks due to food poisoning in adults and children. The occurrence of food poisoning also crossed national borders, so it is necessary to identify the genotypes and to investigate the molecular epidemiology of calicivirus in food contamination and acute gastroenteritis in infants in Asian countries.

Human astroviruses and enteric adenoviruses

Astroviruses are positive-stranded RNA viruses and are composed of three ORFs. ORF1a and ORF1b encode viral protease and polymerase, respectively, and ORF2 encode capsid protein precursor. Capsid protein structure is not clear, it seems that a precursor protein with 87 kDa cleavage to the structural proteins with 32 kDa, 29 kDa and 26 kDa. The studies by antibodies against human astroviruses suggest that 29 kDa or 26 kDa proteins express the important epitopes that are reactive with antibodies. Human astroviruses are classified into eight serotypes based on the reactivity of the antibodies, and prevalent serotypes are 1–5 and 6–8 serotypes are rare. The epidemiological study in Japan during 1995–1996 reported that prevalence of serotypes 1, 3 and 4 were 66%, 14% and 2%, respectively (Table 5).[53]

Human adenoviruses belong to the family *Adenoviridae*. They are DNA viruses without envelope, and are 70 nm in diameter. The protein capsid is composed of 252 capsomers that has 240 hexones and 12 pentons. The pentons consist of fiber proteins with penton bases. The fiber contains the receptor attachment site. Serotypes 40 and 41 adenoviruses belong to subgenus F adenoviruses and are most frequently associated with gastroenteritis. Other serotypes adenoviruses, serotypes 12, 18 and 31 of subgenous A and serotypes 1, 2, 5 and 6 of subgenus C have been found in the diarrheal specimens. Early surveillance had shown that the incidence of occurrence of serotypes 40 and 41 was approximately equal, however after 1986, serotype 41 infection was predominant worldwide. Table 5 shows the distribution of four categories of diarrheal viruses including enteric adenovirus and astrovirus in Japan and China.

Conclusion

We have described details of epidemiological studies of rotavirus infection and a few studies of calicivirus infection in Asia. Few etiological studies have examined four viral agents (in Table 1) in diarrhea among infants worldwide. The conventional RT-PCR assays are potentially expensive and resource intensive, would be very time consuming. Recently the multiplex RT-PCR assays for simultaneous detection of plural numbers of diarrheal viruses were developed and reported.[54–56]

Reports from Germany and the United Kingdom have investigated the prevalence of rotaviruses, enteric adenoviruses, astroviruses and noroviruses in acute gastroenteritis among young children.[57,58] The total frequencies of four categories of viruses accounted for 59% and 60.3%, respectively. No etiologic agents were found in over a third of the specimens investigated. These results indicate that there are gaps between the etiological studies and diagnoses. Further studies are required for optimizing sampling methods of specimens, increasing of sensitivity for the assay methods, selecting of primers for RT-PCR assay and identifying other infectious agents.

References

1 Parashar UD, Hummerlman EG, Bresee JS, Milleeer MA, Glass RI. Global illness and deaths caused by rotavirus disease in Children. *Emerg. Infect. Dis.* 2003; **9**: 565–72.
2 Kosek M, Bern C, Guerrant RL. The global burden of dirrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull. World Health Organ.* 2003; **81**: 197–204.
3 Kapikian AZ, Wyatt RG, Dolin R, Thornhill TS, Kalica AR, Chanock RM. Visualization by immune electron microscopy of a 27 nm particle associated with acute infectious nonbacterial gastroenteritis. *J. Virol.* 1972; **10**: 1075–81.
4 Bishop RF, Davidson GP, Holmes IH, Ruck BJ. Virus particles in epithelial cells of duodenal mucosa from children with non-acute gastroenteritis. *Lancet* 1973; **ii**: 1281–3.
5 Wadell G, Allard A, Johansson M, Svensson L, Uhnoo I. Enteric adenoviruses. In: Bock G, Whelan J (eds). *Novel Diarrhea Viruses (Ciba Foundation Symposium 128)*. Wiley, Chichester, 1987; 63–91.
6 Madeley CR, Cosgrove BP. 28 nm particles in faeces in infantile gastroenteritis. *Lancet* 1975; **ii**: 451–2.
7 Appleton H, Buckley M, Thom BT, Cotton JL, Henderson S. Virus-like particles in winter vomiting disease. *Lancet* 1975; **i**: 1297.
8 Estes MK. Rotavirus and replication. In: Fields BN, Knipe DM, Howley PM *et al.* (eds). *Virology*. Raven Press, New York, 1996; 1009–34.
9 Sanekata T, Ahmaed MU, Kadar A, Taniguchi K, Kobayashi N. Human group B rotavirus infections cause severe diarrhea in children and adults in Bangladesh. *J. Clin. Microbiol.* 2003; **41**: 2187–90.
10 Kuzuya M, Fujii R, Hamano M, Ogura H. Outbreak of acute gastroenteritis caused by human group C rotavirus in a youth educational center in Okayama Prefecture. *Kansenshougaku Zasshi* 2003; **77**: 53–9.
11 Unicomb LE, Podder G, Gentsch JR *et al.* Evidence of high-frequency genomic reassortment of group A rotavirus strains in Bangladesh: Emergence of type G9 in 1995. *J. Clin. Microbiol.* 1999; **37**: 1885–91.

12 Qiao H, Nilsson M, Abreu ER *et al.* Viral diarrhea in children in Beijing, China. *J. Med. Virol.* 1999; **57**: 390–6.
13 Fang ZY, Yang H, Zhang J *et al.* Child rotavirus infection in association with acute gastroenteritis in two Chinese sentinel hospitals. *Pediatr. Int.* 2000; **42**: 401–5.
14 Fang Z-Y, Yang H, Qi J *et al.* Diversity of rotavirus strains among children with acute diarrhea in China 1998–2000 surveillance study. *J. Clin. Microbiol.* 2002; **40**: 1875–8.
15 Ramachandran M, Das BK, Vij A *et al.* Unusual diversity of human rotavirus G and P genotypes in India. *J. Clin. Microbiol.* 1996; **34**: 436–9.
16 Jain V, Das BK, Bhan MK, Glass RI, Gentsch JR; Indian strain surveillance collaborating laboratories. Great diversity of group A strains and high prevalence of mixed rotavirus infections in India. *J. Clin. Microbiol.* 2001; **39**: 3524–9.
17 Kang G, Green J, Gallimore CI, Brown DWG. Molecular epidemiology of rotavirus infection in south Indian children with acute diarrhea from 1995–1996 to 1998–1999. *J. Med. Viol.* 2002; **67**: 101–5.
18 Khetawat D, Dutta P, Bhattacharya SK, Chakrabarti S. Distribution of rotavirus VP7 genotypes among children suffering from watery diarrhea in Kolkata, India. *Virus Res.* 2002; **87**: 31–40.
19 Zhou Y, Supawadee J, Khamwan C *et al.* Characterization of human rotavirus serotype G9 isolated in Japan and Thailand from 1995 to 1997. *J. Med. Virol.* 2001; **65**: 619–28.
20 Zhou Y, Li L, Okitsu S, Maneekarn N, Ushijima H. Distribution of human rotaviruses, especially G9 strains, in Japan from 1996 to 2000. *Microbiol. Immunol.* 2003; **47**: 591–9.
21 Seo JK, Sim AJ. Overview of rotavirus infections in Korea. *Pediatr. Int.* 2000; **42**: 406–10.
22 Song M-O, Kim K-J, Lim I, Kang SY, An C-N, Kim W. Distribution of human group A rotavirus VP7 and VP4 types circulating in Seoul, Korea between 1998 and 2000. *J. Med. Virol.* 2003; **70**: 324–8.
23 Nishio O, Matsui K, Oka T *et al.* Rotavirus infection among infants with diarrhea in Pakistan. *Pediatr. Int.* 2000; **42**: 425–7.
24 Nishio O, Matsui K, Doan TPL, Ushijima H, Isomura S. Rotavirus infection among infants with diarrhea in Vietnam. *Pediatr. Int.* 2000; **42**: 422–4.
25 Nguyen MV, Nguyen TV, Huynh LP *et al.* The epidemiology and disease burden of rotavirus in Vietnam: Sentinel surveillance at 6 hospitals. *J. Infect. Dis.* 2001; **183**: 1707–12.
26 Doan LTP, Okitsu S, Nishio O, Phan DT, Nguyen DH, Ushijima H. Epidemiological features of rotavirus infection among hospitalized children with gastroenteritis in Ho Chi Minh City, Vietnam. *J. Med. Virol.* 2003; **69**: 588–94.
27 Landaeta ME, Dove W, Vinh HA *et al.* Characterization of rotaviruses causing diarrhoea in Vietnamese children. *Ann. Trop. Med. Parasitol.* 2003; **97**: 53–9.
28 Green KY, Hoshino Y, Ikegami N. Sequence analysis of the gene encoding the serotype-specific glycoprotein (VP7) of two new human serotypes. *Viorology* 1989; **168**: 429–33.
29 Nakagomi T, Ohshima A, Akatani K, Ikegami N, Katsushima N, Nakagomi O. Isolation and molecular characterization of a serotype 9 human rotavirus strain. *Microbiol. Immunol.* 1990; **34**: 77–82.
30 Ramachandran M, Kirkwood CD, Unicomb L *et al.* Molecular characterization of serotype G9 rotavirus strains from a global collection. *Virology* 2000; **278**: 436–44.
31 Nakagomi T, Nakagomi O. Genogroup characterization of reemerging serotype G9 human rotavirus strain 95H115 in comparison with earlier G9 and other human prototype strains. *Microbiol. Immunol.* 2002; **46**: 575–8.
32 Pongsuwanna Y, Guntapong R, Chiwakul M *et al.* Detection of a human rotavirus with G12 and P[9] specificity in Thailand. *J. Clin. Microbiol.* 2002; **40**: 1390–4.
33 Desselberger U, Iturriza-Gomara M, Gray JJ. Rotavirus epidemiology and surveillance. In: Chadwick D, Goode JA (eds). *Gastroenteritis Viruses (Novaritis Foundation Symposium 238).* Wiley, Chishester, 2001; 125–52.
34 Das BK, Gensch JR, Hoshino Y *et al.* Characterization of the G serotype and P genogroup of New Dehli newborn rotavirus strain 116E. *Virology* 1993; **197**: 99–107.
35 Inouye S, Yamashita K, Yamadera S, Yoshikawa M, Kato N, Okabe N. Surveillance of viral gastroenteritis in Japan: Pediatric cases and outbreaks incidents. *J. Infect. Dis.* 2000; **181**: S270–4.
36 Glass PJ, White LJ, Ball JM, Leparc-Goffart I, Hardy ME, Estes MK. Norwalk virus open reading frame 3 encodes a minor structural protein. *J. Virol.* 2000; **74**: 6581–91.
37 Bertolotti-Ciarlet A, Crawford SE, Hutson AM, Estes MK. The 3' end Norwalk virus mRNA contains determinants that regulate the expression and stability of the viral capsid protein VP1: a novel function for the VP2 protein. *J. Virol.* 2003; **77**: 11 603–15.
38 Ando T, Noel JS, Fankhauser RL. Genetic classification of 'Norwalk-like viruses'. *J. Infect. Dis.* 2000; **181**: S336–48.
39 Vinje J, Green N, Lewis DC, Gallimore CI, Brown DWG, Koopmans MPG. Genetic polymorphism across regions of the three open reading frames of 'Norwalk-like viruses'. *Arch. Virol.* 2000; **145**: 223–41.
40 Koopmans M, Vinje J, Duizer E, de Wit M, van Duijnhoven Y. Molecular epidemiology of human enteric caliciviruses in the Netherland. In: Chadwick D, Goode JA (eds). *Gastroenteritis Viruses (Novaritis Foundation Symposium 238).* Wiley, Chishester 2001; 197–218.
41 Katayama K, Shirato-Horikoshi H, Kojima S *et al.* Phylogenetic analysis of the complete genome of 18 Norwalk-like viruses. *Virology* 2000; **299**: 225–39.
42 Cubitt WD, Jiang XJ, Wang J, Estes MK. Sequence similarity of human calciviruses and small round structured viruses. *J. Med. Virol.* 1994; **48**: 252–8.
43 Jiang X, Cubitt D, Hu J *et al.* Development of an ELISA to detect MX virus, a human calcivirus in the snow Mountain agent genogroup. *J. Gen. Virol.* 1995; **76**: 2739–47.
44 Kojima S, Kageyama T, Fukushi S *et al.* Genogroup-specific PCR primers for detection of Norwalk-like viruses. *J. Virol. Meth.* 2002; **100**: 107–14.
45 Kageyama T, Kojima S, Shinohara M *et al.* Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *J. Clin. Microbiol.* 2003; **41**: 1548–57.
46 Pang X-L, Honma S, Nakata S, Vesikari T. Human caliciviruses in acute gastroenteritis of young children in the community. *J. Infect. Dis.* 2000; **181**: S288–94.
47 Glass RI, Bresee J, Jiang B *et al.* Gastroenteritis viruses: an overview. In: Chadwick D, Goode JA (eds). *Gastroenteritis Viruses (Novaritis Foundation Symposium 238).* Wiley, Chishester, 2001; 5–25.
48 Honma S, Nakata S, Numata K *et al.* Epidemiological study of prevalence of genogroup II human calicivirus (Mexico virus) infections in Japan and southeast Asia as determined by enzyme-linked immunosorbent assays. *J. Clin. Microbiol.* 1998; **36**: 2481–4.

49 Jing Y, Quan Y, Huo Y, Wang L-P, Jiang X. Seroprevalence against Norwalk-like human caliciviruses in Beijing, China. *J. Med. Virol.* 2000; **60**: 97–101.
50 Iritani N, Seto Y, Kudo H *et al.* Prevalence of Norwalk-like virus infections in cases of viral gastroenteritis among children in Osaka city, Japan. *J. Clin. Microbiol.* 2003; **41**: 1756–9.
51 Ando T, Monroe SS, Gensch JR, Jin Q, Lewis DC, Glass RI. Detection and differentiation of antigenically distinct small round-structured viruses (Norwalk-like viruses) by reverse transcription-PCR and southern hybridization. *J. Clin. Microbiol.* 1995; **33**: 64–71.
52 Noel JS, Frankhauser RL, Ando T, Monroe SS, Glass RI. Identification of a distinct common strains of 'Norwalk-like viruses' having a global distribution. *J. Infect. Dis.* 1999; **179**: 1334–4.
53 Sakamoto T, Negishi H, Wang Q-H *et al.* Molecular epidemiology of astroviruses in Japan from 1995–1998 by reverse transcription-polymerase chain reaction with serotype-specific primers (1–8). *J. Med. Virol.* 2000; **61**: 326–31.
54 Sakon N, Yamazaki K, Utagawa E, Okuno Y, Oishi I. Genomic characterization of human astrovirus type 6 Katno virus and the establishment of a rapid and effective reverse transcription-polymerase chain reaction to detect all serotypes of human astrovirus. *J. Med. Virol.* 2000; **61**: 125–31.
55 O'Neil HJ, McCaughey C, Coyle PV, Wyatt DE, Mitchell F. Clinical utility of nested multiples RT-PCR for group F adenovirus, rotavirus and Norwalk-like viruses in acute viral gastroenteritis in children and adults. *J. Clin. Virol.* 2002; **25**: 335–43.
56 Yah H, Yaghu H, Okitsu S, Nishio O, Ushijima H. Detection of norovirus (GI, GII), sapovirus and astrovirus in fecal samples using reverse transcription single-round multiplex PCR. *J. Virol. Meth.* 2003; **114**: 37–44.
57 Simpson R, Aliyu S, Iturriza-Gomara M, Desselberger U, Gray J. Infantile viral gastroenteritis: on the way to closing the diagnostic gap. *J. Med.* 2003; **70**: 258–62.
58 Oh D-Y, Gaedicke G, Schreier E. Viral agents of acute gastroenteritis in German children: Prevalence and molecular diversity. *J. Med. Virol.* 2003; **71**: 82–93.

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Feature Article

Foreward: Molecular epidemiology of viral infection in Asia

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Key words Asia, arthropod-borne virus, emerging virus, molecular epidemiology, zoonotic virus.

Since the late 20th century, new viral infections, that is emerging virus infections, have been spreading across the world. Due to the effect of globalization and the ease of international travel, local viruses have spread to countries where they were previously unheard-of. West Nile fever/encephalitis, HIV, Nipah virus infection and severe acute respiratory syndrome (SARS) are typical examples. In Asia, Japanese encephalitis, dengue fever/dengue hemorrhagic fever, hantavirus infection, rabies, hepatitis B and C, enterovirus encephalitis including polio, and virus diarrhea are also important infections.[1]

Molecular epidemiological studies on viruses using genome amplification by polymerase chain reaction (PCR) and gene analyses began in the 1990s.[2,3] The source and route of infections are better understood, and the effect of therapy, existence of resistant viruses, and seasonal or geographic distributions have also become more clearly defined and understood. The results of these investigations have contributed to vaccine development. In this series of feature articles, specific viral infections in children such as rubella virus, measles virus, hepatitis B and C viruses, HIV, picorna viruses and diarrheal viruses are individually described. In this forward to the feature article series on viruses in Asia, other viruses such as dengue virus, Japanese encephalitis virus, yellow fever virus, West Nile virus, rabies virus, hantavirus, Nipah virus, metapneumovirus and SARS corona virus are also discussed with special reference to Asia.

Flavivirus

Flaviviridae is divided into four genuses based on antigenic and genetic analyses: dengue virus, Japanese encephalitis virus, tick-borne encephalitis virus and yellow fever virus.

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Dengue virus, Japanese encephalitis virus and tick-borne encephalitis virus are further divided into 4, 5 and 5 species, respectively (Table 1), through mainly envelope gene analyses.[4,5] Envelope protein and gene analyses are important for understanding pathogenesis and classification. These viruses are arthropod-borne and difficult to control because the viruses are usually transmitted through vectors such as mosquitoes and ticks. Moreover, birds and vertebrates also act as natural hosts and along with mosquitoes and ticks they travel easily.[4,5]

Dengue virus

Dengue virus belongs to *Flaviviridae* and is transmitted in a cycle involving humans and *Aedes* mosquitoes. In forest areas, a cycle including primates and mosquitoes also exist.[6] To date, two manifestations, dengue fever and dengue hemorrhagic fever are known.[4,7] The symptoms are same among the four serotypes. Once immunity is developed in a human, it remains life-long and re-infection with the same serotype does not occur. However other serotypes may then cause disease because of the incomplete protection of the first infection. More than tens of millions of people have dengue fever and more than hundreds of thousands of people are reported to have dengue hemorrhagic fever every year. Dengue virus is approximately 11 kb positive single stranded RNA virus. It consists of structural proteins of core, membrane and envelope, and seven non-structural proteins. Envelope protein is the target of neutralizing antibody and hemagglutinin inhibition antibody. Serotypes of dengue virus 1, 2, 3, and 4 are originally classified using antiserum, but recently they have also been classified with envelope gene analyzes. The similarity of envelope gene is more than 90% at the same serotype and 65% at a different serotype. Moreover, dengue virus serotype 1, 2, 3 and 4 are divided into three, six, four and one genotypes, respectively.[8] There are common and different genotypes in serotypes 1–3 in Asia, Africa, Pacific islands and Caribbean islands. Some genotypes appear in specific areas during a limited period each year. Variation of envelope gene affects the virulence of these