trimeric form of protein [Gorziglia et al., 1985; Sabara et al., 1987]. Strain CMH222 was assigned initially SG non-I + II specificity based on the subgroup ELISA assay. However, sequencing of the *VP6* gene gave contradictory results and CMH222 was found to have SG I specificity. The *VP6* gene of CMH222 was found to have the highest amino acid and nucleotide sequence identities with the *VP6* gene of SG I simian strain TUCH. In addition, phylogenetic analysis revealed that the *VP6* genes of these strains may have emerged from the same ancestor.

Subgroup specificities have a broad correlation with G serotypes in humans, that is, SG I specificity is usually associated with serotype G2, G8, and G9 strains, while SG II specificity is more commonly associated with G1, G3, G4, and G9 strains. Molecular characterization of the *VP6* gene has shown that SG I and SG II epitopes are typically found in human rotavirus strains, and SG I, SG I + II, and SG non-I + II epitopes are typically in rotavirus strains of non-human origin [Gorziglia et al., 1988; Ito et al., 1997; Tang et al., 1997; Iturriza Gomara et al., 2002]. Additionally, among human rotaviruses, SG I specificity is associated with short electropherotype and SG II with long electropherotype. On the other hand, long electropherotype with SG I specificity is usually found in animal rotaviruses [Kapikian et al., 2001]. The detection of a human G3 strain (CMH222) with the VP7 sequence closely related to G3 simian strain RRV and carried SG I specificity may be indicative of an animal strain causing infection in a human subject. Unfortunately, the electropherotype of CMH222 could not be detected by PAGE even though its genome has been successfully amplified by RT-PCR.

It was unclear why CMH222 did not react with SG I specific MAb. It is more likely that the accumulation of point mutations may lead to amino acid changes on the epitopes and, therefore, could not be recognized by SG-specific MAbs. However, low concentration of the virus in the sample cannot be discounted. It has been suggested that an Ala residue at position 172 may play a significant contribution to the formation of SG I specific epitope [Lopez et al., 1994; Tang et al., 1997]. Indeed, the VP6 amino acid sequence of the CMH222 strain contained a unique change at amino acid 172 from Ala to Gln. An alternative possibility is that the addition of a nucleotide in the *VP6* gene may results in a frame-shift mutation that lead to the change of SG specificity. In this regard, the VP6 deduced amino acid sequence of CMH222 was compared with the reference strains, no frame-shift mutation was observed.

Although five rotavirus NSP4 genotypes have been identified to date [Cunliffe et al., 1997; Horie et al., 1997; Ciarlet et al., 2000; Mori et al., 2002a], the diversity of the NSP4 encoding genes among human rotaviruses has been mainly restricted to genotypes A or B, and a few genotype C. Conversely, all five NSP4 genotypes (A–E) have been identified in rotaviruses of animal origins. To our knowledge, only two strains of human rotaviruses, AU-1 and 09/92, have been reported to belong to NSP4 genotype C. In our analysis, the CMH222 strain also belonged to the NSP4 genotype C and formed phylogenetic cluster with other NSP4 genotype C rotavirus of animal origins. Again, this finding suggests that the NSP4 of the CMH222 strain may have originated from genotype C of animal origins by interspecies transmission events.

In conclusion, molecular genetic analyses provide strong evidence that CMH222 might be the reassortant, which resulted from multiple interspecies transmission events that lead to reassortment of the viruses of human, caprine, and simian origins.

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Outbreak of Sapovirus Infection Among Infants and Children With Acute Gastroenteritis in Osaka City, Japan During 2004–2005

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One hundred and twenty five fecal specimens were collected from sporadic cases of acute gastroenteritis in a pediatric clinic in Osaka City, Japan from July 2004 to June 2005 and tested for the presence of rotavirus, norovirus, sapovirus, astrovirus, and adenovirus by RT-multiplex PCR. Among diarrheal viruses detected, norovirus was the most prevalent (19.2%, 24 of 125), followed by group A rotavirus (18.4%, 23 of 125), astrovirus (1.6%, 2 of 125), and adenovirus (0.8%, 1 of 125), respectively. Interestingly, sapovirus infection was identified with high incidence of 17.6% (22 of 125). Sapovirus was subjected to molecular genetic analysis by sequencing. It was found that sapovirus detected in this study was classified into four genotypes (GI/1, GI/4, GI/6, and GI/8), and sapovirus GI/6 was predominant, followed by GI/8 and accounted for 81.8% (18 of 22) and 9.1% (2 of 22), respectively. It was noteworthy that sapovirus GI/6 infection was apparently confined within the period of 5 months (August 2004 through December 2004). This pattern of infection implied the outbreak of sapovirus GI/6 in these patients, which was the first outbreak of acute gastroenteritis attributed to sapovirus in Osaka City. Another interesting feature of the study was the appearance of two novel, naturally occurring recombinant sapoviruses circulating in the Japanese population studied. This report confirmed the presence as well as the importance of sapovirus causing acute gastroenteritis among Japanese infants and children. ***J. Med. Virol. 78:839–846, 2006.*** © 2006 Wiley-Liss, Inc.

KEY WORDS: sapovirus; Osaka; outbreak

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 developed countries. Acute gastroenteritis ranks consistently as one of the principal six causes of all deaths [Murray and Lopez, 1997; Parashar et al., 2003a]. Among different types of diarrheal viruses, rotavirus is the most important, being a major cause of severe gastroenteritis in infants and young children worldwide [Parashar et al., 2003b]. Sapovirus, however, is also considered to be a significant global enteropathogen in association with sporadic cases as well as outbreaks of gastroenteritis in such settings as kindergartens, schools, and nursing home for the elderly [Chiba et al., 1979; Matson et al., 1989; Chiba et al., 2000; Akihara et al., 2005; Zintz et al., 2005]. Etiologic studies of acute gastroenteritis in infants conducted in Japan with RT-PCR showed that sapovirus was one of the most common causes of outbreaks of viral gastroenteritis among infants in Sapporo [Chiba et al., 1979, 2000]. Immunological and seroepidemiological studies have indicated a worldwide distribution of sapovirus [Sakuma et al., 1981; Nakata et al., 1985; Lopman et al., 2002]. The age-related prevalence of antibody against this virus also has shown that infections commonly occur in children less than 5 years old. It was also found that serum antibody level to sapovirus was lowest in the first year of

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life, increasing after 2 years of age [Matsui and Greenberg, 2000].

Norovirus (formerly known as "Norwalk-like virus") and sapovirus (formerly known as "Sapporo-like virus") are distinct genera within the family *Caliciviridae*. These viruses contain a positive sense single-strand RNA genome surrounded by an icosahedral capsid. The norovirus genome contains three ORFs (ORF1, 2, and 3). The ORF1 encodes non-structural proteins, including the RNA-dependent RNA polymerase (RdRp), ORF 2 encodes the capsid proteis and ORF3 enodes a small capsid protein. In the two sapovirus ORFs (ORF 1 and 2), however, it is ORF 1 that encodes the non-structural as well as the capsid proteins while ORF2 encodes a small protein. The prototype strain of sapovirus is the Sapporo virus (Hu/SaV/Sapporo virus/1977/JP), which was discovered originally during an outbreak a home for infants in Sapporo, Japan in 1977 [Chiba et al., 1979]. Sapovirus has a typical "Star of David" configuration by electron microscopy (EM), and sapovirus strains are antigenically identical to each other by immune EM [Chiba et al., 2000]. Sapovirus is divided into five genogroups, among which only genogroups I, II, IV, and V are known to infect humans. Recently, the diversity of sapoviruses was described in which genogroup I and II sapoviruses could be classified into eight and five genotypes, respectively [Farkas et al., 2004; Akihara et al., 2005; Phan et al., 2005b].

The objectives of this study were: to determine the incidence of diarrheal virus infection in infants and young children with acute gastroenteritis in Osaka City, Japan; to characterize the detected sapovirus according to genogroup and genotype; and to analyze the genetic diversity among these viruses. Additionally, the age-related distribution and seasonal pattern of sapovirus infection were also studied.

MATERIALS AND METHODS

Fecal Specimens

One hundred twenty-five fecal specimens were collected from sporadic cases of acute gastroenteritis in a pediatric clinic in Osaka City, Japan during the period of July 2004 to June 2005. The fecal specimens were diluted with distilled water to 10% suspensions, and clarified by centrifugation at 10,000*g* for 10 min. The supernatants were collected and stored at −30°C until use for the detection of diarrheal viruses.

Extraction of Viral Genome

The viral genomes were extracted from 140 μl of 10% fecal suspensions using a QIAamp spin-column technique according to the manufacturer's instructions (QIAGEN®, Hilden, Germany).

Reverse Transcription (RT)

For reverse transcription (RT), except for adenovirus, 4 μl of extracted viral genome was added with a reagent mixture consisting of 5× first strand buffer (Invitrogen, Carlsbad, CA), 10 mM dNTPs (Roche, Mannheim, Germany), 10 mM DTT (Invitrogen), superscript reverse transcriptase III (200 U/μl) (Invitrogen), random primer (1μg/μl) (hexa-deoxyribonucleotide mixture) (Takara, Shiga, Japan), RNase inhibitor (33 U/μl) (Toyobo, Osaka, Japan), and MilliQ water. The total of reaction mixture is 8 μl. The RT step was carried out at 50°C for 1 hr, followed by 99°C for 5 min and then held at 4°C [Phan et al., 2005a].

Polymerase Chain Reaction (PCR)

The first group of viruses including astrovirus, norovirus (GI, GII), and sapovirus and the second group including group A, B, and C rotaviruses and adenovirus were detected by mutiplex PCR with specific primers as reported previously by Phan et al. [2005a]. These mutiplex PCR assays are the sensitive and specific methods that have been used with the success to accurately define the true burden of diarrheal disease due to viruses. The identification of the first group of viruses was performed with specific primers Beg9 and VP7-1', B5-2 and B3-3, G8NS1 and G8NA2, Ad1 and Ad2 for group A, B, and C rotaviruses and adenovirus with four different amplicon sizes of 395, 814, 352, and 482 bp, respectively in one PCR tube. For the detection of the second group of viruses, specific primers PreCAP1 and 82b; G1SKF and G1SKR; COG2F and G2SKR, SLV5317 and SLV5749 specifically generated four different sizes of amplicons of 719, 330, 387, and 434 bp for astrovirus, norovirus (GI, GII), and sapovirus, respectively (Table I). Sapovirus polymerase region was also amplified to identify recombinant sapovirus using primers P290 and P289 [Jiang et al., 1999]. To eliminate the possibility of co-infection of two different genotypes in case of sapovirus recombination, flanking polymerase and capsid regions with their junction was amplified with primers P290 and SLV5749 to generate 1,163-bp product [Jiang et al., 1999; Phan et al., 2005a]. PCR was carried out with 1 μl of cDNA in 10 μl of the reagent mixture containing 10× Taq DNA polymerase buffer (Promega, Madison, WI), dNTPs (2.5 mM/μl), primers (33 μM/μl), Taq DNA polymerase (5 U/μl) (Promega) and MilliQ water. PCR was performed at 94°C for 3 min followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 60 sec, and a final extension at 72°C for 7 min, and then held at 4°C. In order to monitor the quality of extraction and RT-PCR, Hu/SVGII/Karachi/928/1994, and MilliQ water were used as positive and negative controls, respectively. In experiment, to avoid false positive results instructions (autoclaved solutions, reagents into aliquots, disposable gloves, avoidance of splashes, and positive displacement pipettes) to prevent cross contamination were followed strictly [Kwok and Higuchi, 1989].

Electrophoresis

The PCR products were electrophoresed in a 1.5% agarose gel, followed by staining with ethidium bromide

TABLE I. A List of Specific Primers Used in the Study to Detect Multiple Target Viruses Among Infants and Children With Diarrhea in Japan by RT-PCR

Virus	Target region	Primer name	Polarity[a]	Sequence (5′–3′)[b]	Amplicon size (bp)	Position (nt number)	Representative strain's accession number
Group A rotavirus	VP7	Beg9	+	GGCTTTAAAAGAGAGAATTTCCGTCTGG	395	1–395	D16343
		VP7-1′	−	ACTGATCCTGTTGGCCATCCTTT			
Group B rotavirus	VP7	B5-2	+	GGCAATAAAATGGCTTCATTGC	814	1–814	AY539856
		B3-3	−	GGGTTTTTACAGCTTCGGCT			
Group C rotavirus	VP7	NG8S1	+	ATTATGCTCAGACTATCGCCAC	352	353–704	AB086966
		NG8S2	−	GTTTCTGTACTAGCTGGTGAAC			
Adenovirus	Hexon	Ad1	+	TTCCCCATGGCICAYAACAC	482	1834–2296	M12411
		Ad2	−	CCCTGGTAKCCRATRTTGTA			
Astrovirus	Capsid	PreCAP1	+	GGACTGCAAAGCAGCTTCGTG	719	4235–4953	L23513
		82b	−	GTGAGCCACCAGCCATCCCT			
Norovirus GI	Capsid	G1-SKF	+	CTGCCCGAATTYGTAAATGA	330	5342–5671	M87661
		G1-SKR	−	CCAACCCARCCATTRTACA			
Norovirus GII	Capsid	COG2F	+	CARGARBCNATGTTYAGRTGGATGAG	387	5003–5389	X86557
		G2-SKR	−	CCRCCNGCATRHCCRTTRTACAT			
Sapovirus	Capsid	SLV5317	+	CTCGCCACCTACRAWGCBTGGTT	434	5083–5516	X86560
		SLV5749	−	CGGRCYTCAAAVSTACCBCCCCA			
Sapovirus	Polymerase	P290	+	GATTACTCCAAGTGGGACTCCAC	331	4354–4684	X86560
		P289	−	TGACAATGTAATCATCACCATA			

[a]+, Forward primer; −, Reverse primer.
[b]Within nucleotide sequence of primers, B = C, G or T; H = A, C or T; N: Any base; R = A or G; S = G or C; V = A, C or G; W = A or T; Y = C or T.

for 20 min, then visualized under ultraviolet (UV) light, and the results were recorded by photography.

Nucleotide Sequencing and Phylogenetic Analysis

The nucleotide sequences of PCR products (DNA) positive for sapovirus were determined directly with the Big-Dye terminator cycle sequencing kit and an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA). Sequence analysis was performed using CLUSTAL X software (Version 1.6). Phylogenetic tree with 100 bootstrap resamples of the nucleotide alignment data sets was generated using the neighbor-joining method with CLUSTAL X. The genetic distance was calculated using Kimura's two-parameter method (PHYLIP). SimPlot software (Version 1.3) was used to compare recombinant sapovirus sequences. The capsid and polymerase sequences of sapovirus strains 5786/Osaka/JP, 5800/Osaka/JP, 5836/Osaka/JP, 5862/Osaka/JP, 5862/Osaka/JP, and 5821/Osaka/JP, had been submitted to the DDBJ DNA/GenBank database and had been assigned accession numbers AB242322 and DQ307499, AB242323 and DQ 307500, AB242324 and DQ307501, DQ401095 and DQ 401096, AB242325, and AB242326, respectively. Reference sapovirus strains and accession numbers used in this study were as follows: PEC (AF182760), Lyon/598/97/F (AJ271056), London/92 (U95645), Mex340/90 (AF435812), Cruiseship/00 (AY289804), Hou7-1181/90 (AF435814), Arg39 (AY289803), Parkville/94 (U73124), Sapporo/82 (U65427), Manchester/93 (X86560), Karachi/730/1992 (AB126249), Karachi/874/1992 (AB181129), Karachi/928/1994 (AB181128), Karachi/1017/1990 (AB181227), Karachi/876/1993 (AB181132), Houston/90 (U95644), Stockholm/97 (AF194182), 12/DCC/Tokyo/Japan/44 (AB236380), Karachi/872/1991 (AB181231), 4408/Maizuru/Japan (AB180209), and Mex14917/2000 (AF435813).

RESULTS

Detection of Diarrheal Viruses

A total of 125 fecal specimens were collected from infants and children with acute gastroenteritis in Osaka City, Japan, during the period of July 2004 to June 2005. For the pediatric population, the lowest age was 5 months, the highest was 8 years, and the average age was 1.2 years (14 months). Among all children with acute gastroenteritis, 91.2% were aged less than 36 months. Males accounted for 57%. All fecal specimens were tested for the presence of rotavirus, norovirus, sapovirus, astrovirus, and adenovirus by RT-multiplex PCR. Diarrheal viruses were detected in 72 out of 125 (57.6%) specimens tested. Among diarrheal viruses detected, norovirus was the most prevalent (19.2%), followed by 18.4% of group A rotavirus, 1.6% of astrovirus, and 0.8% of adenovirus, respectively. It was interesting that sapovirus infection was identified with the high incidence and accounted for 17.6%. No group B and C rotaviruses were found in these patients.

Nucleotide Sequencing and Phylogenetic Analysis of Sapovirus Genogroup and Genotype

The PCR products of sapovirus were sequenced in order to characterize further the genetic relationship among the sapovirus isolates detected in infants and children with acute gastroenteritis in Osaka City, Japan. Their partial nucleotide sequences were compared to each other as well as to those of reference sapovirus strains available in the DDBJ DNA/GenBank database by BLAST. The nucleotide sequence of the 5′ ends of the sapovirus capsid gene was determined by direct sequencing with the amplified fragments. This region has been shown to be suitable for genotyping [Farkas et al., 2004; Phan et al., 2005b]. A total of 22 sapovirus sequences were analyzed by phylogenetic analysis and grouped using the recent sapovirus capsid region classification scheme [Farkas et al., 2004; Akihara et al., 2005; Phan et al., 2005b]. In the present study, all of the sapovirus sequences were classified into only one distinct genogroup I (Fig. 1). The results indicated that sapovirus group I was a dominant genogroup. The sapovirus GI sequences clustered into four genotypes (GI/1, GI/4, GI/6, and GI/8) and these presented 4.5% (1 of 22), 4.5% (1 of 22), 81.8% (18 of 22), and 9.1% (2 of 22), respectively. Using CLUSTAL X, it was also noted that these sapoviruses had a high identity on the amino acid level and the nucleotide level with other reference sapovirus strains previously registered in the DDBJ DNA/GenBank database in the same genotype ranged from 90% to 100%.

Nucleotide Sequencing and Genetic Analysis of Sapovirus Polymerase

The polymerase regions of all sapovirus strains were amplified and sequenced to examine genetically the virus found during this study. In 20 sapoviruses belonging to GI/1, GI/4, and GI/6, the genotypes were maintained in both polymerase and capsid regions. They also shared a high identity at the nucleotide level and the amino acid level of the polymerase with other reference sapovirus strains registered previously in the DDBJ DNA/GenBank database in the same genotype ranged from 95% to 100%. It was found that two sapoviruses with GI/8 capsid, the 5862/Osaka/JP and the 5821/Osaka/JP, were classified into the Sapporo/82 cluster (known as GI/1), not into the 12/DCC/Tokyo/Japan/44 cluster (known as GI/8) when polymerase-based grouping was performed (Fig. 2).

Novel Recombinant Sapoviruses

It was found that the polymerase and capsid regions of the 5862/Osaka/JP and the 5821/Osaka/JP shared 96% and 85% of the amino acid similarity with the Sapporo/82, respectively. However, they had a high homology (99%) at the amino acid level of the capsid region with the 12/DCC/Tokyo/Japan/44. Therefore, these two strains were all recombinant viruses with a GI/8 capsid and GI/1 polymerase. When the sequences of the 5862/Osaka/JP and the 5821/Osaka/JP were compared with that of the Sapporo/82 using the SimPlot, an apparent region of genetic recombination was found at the polymerase- and capsid-junction. Before this junction, the 5862/Osaka/JP, the 5821/Osaka/JP, and the Sapporo/82 were homologous. After the junction, however, the homology was notably different. The SimPlot analysis showed a sudden drop in the identity for the 5862/Osaka/JP and the 5821/Osaka/JP. The 100% homology at the nucleotide as well as the amino acid levels of polymerase and capsid among the 5862/Osaka/JP and the 5821/Osaka/JP was also noted.

Outbreak of Sapovirus

The results shown in Figure 3 indicated that although the fecal specimens were collected over the period of 12 months (July 2004 to June 2005), the sapovirus GI/6 infection was apparently confined within a period of 5 months (August 2004 through December 2004). This pattern of infection indicated an outbreak of sapovirus GI/6 in these subjects and this would be the first outbreak of acute gastroenteritis attributed to sapovirus in Osaka City. It was found that the nucleotide as well as the amino acid sequences of the capsid and polymerase regions among 18 sapovirus isolates were identical (100%). A phylogenetic tree of the amino acid sequences of these isolates and the reference strains was constructed and all of 18 sapovirus GI/6 isolates formed a distinct cluster. These results indicated that sapovirus GI/6 isolates detected in infants and children in Osaka City were closely homologous to each other, suggesting that they were probably derived from the same source of infection. In addition, majority (94.4%, 17 of 18) of sapovirus GI/6 infected cases were confined to infants and young children with an age of less than 3 years. This observation demonstrated that sapovirus infection in this outbreak occurred mainly in infants and young children.

DISCUSSION

Viral gastroenteritis is still a health burden and one of the most frequently encountered problems in developed and developing countries [Parashar et al., 2003a; Mulholland, 2004]. In this study, diarrheal viruses were detected in 57.6% of fecal specimens tested. These findings suggested that acute gastroenteritis in infants and children in Osaka City about 57.6% might be due to the diarrheal viruses and 42.4% caused by other etiologic agents. Among the diarrheal viruses detected, norovirus was found to be the most prevalent and became a leading cause of viral gastroenteritis in infants and children in Osaka City, followed by group A rotavirus, sapovirus, astrovirus, and adenovirus. These viruses were also considered to be significant global enteropathogens and were associated with sporadic cases as well as outbreaks of gastroenteritis [Noel and Cubitt, 1994; Carter and Willcocks, 1996; Bon et al., 1999; Deneen et al., 2000; Marks et al., 2000].

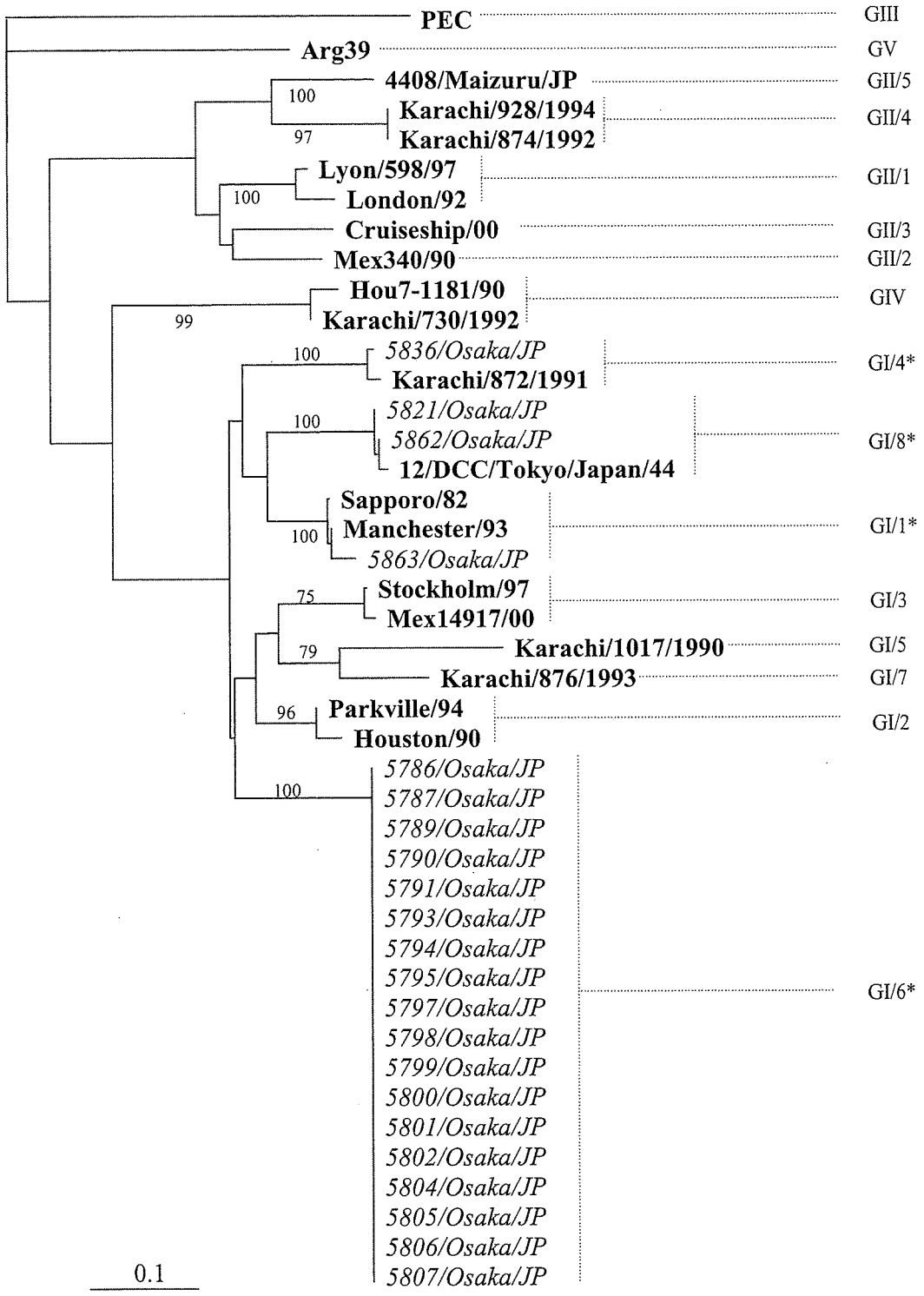

Fig. 1. Phylogenetic tree of nucleotide sequences of Japanese sapovirus. All of the sapovirus sequences were classified into only one distinct genogroup I and the sapovirus genogroup I sequences clustered into four genotypes (GI/1, GI/4, GI/6, and GI/8). The tree was constructed from partial amino acid sequences of capsid region of sapovirus isolates detected in Osaka City, Japan. Reference strains of sapovirus were selected from DDBJ/GenBank under the accession number indicated in the text. Japanese sapovirus is highlighted in italic. PEC strain was used as an out-group strain for phylogenetic analysis. The scale indicates nucleotide substitutions per position. The numbers in the branches indicate the bootstrap values. *, Genotype contains Japanese sapovirus detected in the study.

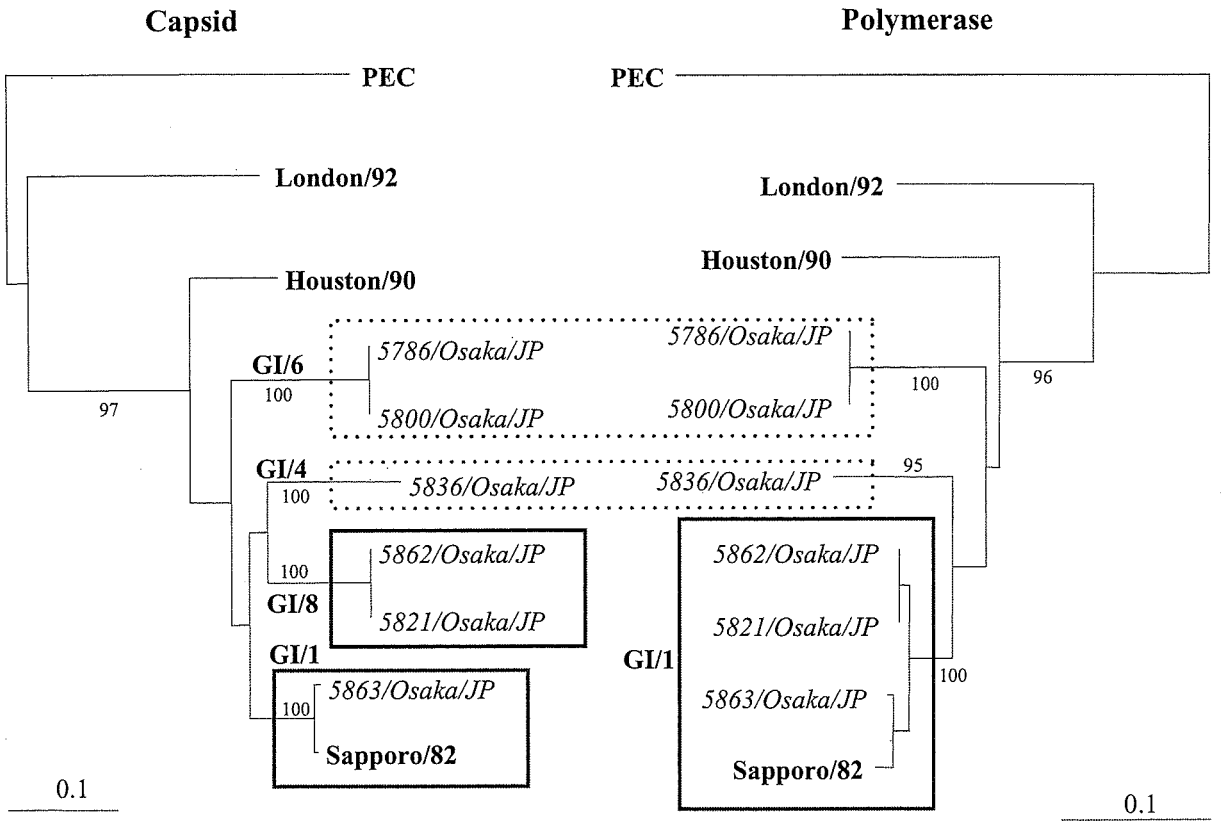

Fig. 2. Observation of changes of sapovirus genotypes on the basis of phylogentic trees of amino acid sequences. The novel recombinant sapoviruses, the 5862/Osaka/JP and the 5821/Osaka/JP, were classified into two distinct genotypes GI/1 and GI/8 when the polymerase- and capsid-based groupings were performed. In other sapoviruses detected, the genotypes were maintained in both polymerase and capsid regions. The trees were constructed from partial amino acid sequences of capsid and polymerase regions of Japanese sapovirus isolates. Reference strains of sapovirus were selected from DDBJ/GenBank under the accession number indicated in the text. Japanese sapovirus was highlighted in italic. PEC strain was used as an outgroup strain for phylogenetic analysis. The scale indicates nucleotide substitutions per position. The numbers in the branches indicate the bootstrap values.

Sapovirus is recognized as one of the causes of the diarrheal illness in humans worldwide and its prevalence was shown to range from 0.3% to 9.3% and usually much lower than norovirus [Oh et al., 2003; Yan et al., 2003; Phan et al., 2004]. It was interesting that sapovirus infection was identified with high prevalence, accounting for 17.6%. The results in this study showed that all Japanese sapovirus sequences belonged to only one sapovirus genogroup I with four distinct genotypes (GI/1, GI/4, GI/6, and GI/8). Of note, the sapovirus GI/6 isolates detected were predominant (81.8%) and closely homologous to each other, suggesting that they came from the same source of infection. It was noted that the sapovirus GI/6 infection was confined only within a short period of 5 months (August 2004 through December 2004). Moreover, only two sapovirus GI/6 strains were found in 2002–2003 during a 7-year (1996–2004) survey of sapovirus infection in diarrheal fecal specimens of infants and children collected from Osaka City, Japan and no sapovirus GI/6 was detected in 2003–2004 [Phan et al., 2005b]. Taken together, this is the first report of an outbreak attributed to the sapovirus GI/6 among infants and children in Japan and this would be the first outbreak of acute gastroenteritis attributed to sapovirus in Osaka City.

In this outbreak, the majority of infants and children with sapovirus infection (94.4%) were aged less than 36 months. This result was in line with published reports on sapovirus epidemiology worldwide in which sapovirus infection causes acute gastroenteritis in all age group, through it occurs predominantly in infants and young children [Chiba et al., 2000; Okada et al., 2002; Akihara et al., 2005]. Our findings also confirmed sapovirus as one of the enteropathogens responsible for viral gastroenteritis among infants and children in Japan. According to most studies, sapovirus infection has been found mainly during the cold season [Chiba et al., 1979; Phan et al., 2004, Yan et al., 2005]. In contrast, the present study has demonstrated that the outbreak associated with sapovirus lasted continuously from August to December as the middle of summer to the end of autumn in Japan. This observation clearly indicated that sapovirus infection could occur not only in the cold season but also in the hot season.

Recombination is among the major driving forces of viral evolution. Recombination of viral RNA is known to

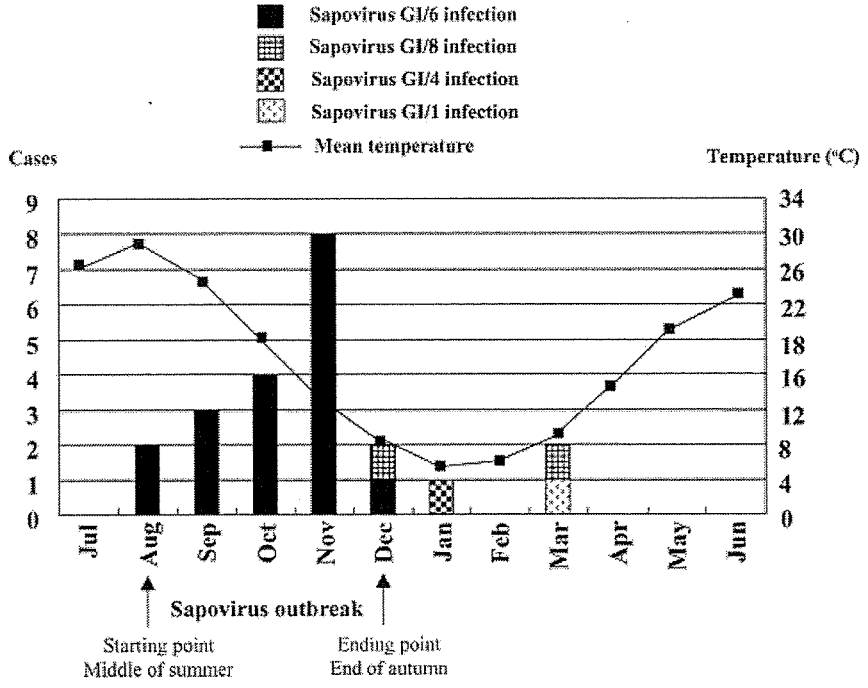

Fig. 3. Monthly distribution of sapovirus infection in infants and children with acute gastroenteritis in Osaka City, Japan, during the period July 2004 to June 2005. The sapovirus GI/6 infection was apparently confined within a period of 5 months (August 2004 through December 2004). The duration of outbreak of sapovirus GI/6 infection is shown.

depend upon various immunological and intracellular constraints that may allow the emergence of viable recombinant [Worobey and Holmes, 1999]. Recombination in viruses can greatly affect phylogenetic groupings, confuse molecular epidemiologic studies, limit the virus control programs, and have major implications in viral vaccine design [Matson, 2003; Bull et al., 2005]. Although several recombinant noroviruses causing sporadic cases and outbreaks of acute gastroenteritis have been reported, the data of recombinant sapovirus are limited. Another interesting feature of this study was the presence of two novel, naturally occurring recombinant sapoviruses circulating in the Japanese population studied. These isolates were obtained from a male patient and a female patient both aged 1 year and with clinical manifestations of acute gastroenteritis in Osaka City, Japan. These isolates had total homology (100%) at the nucleotide as well as amino acid levels of the capsid and polymerase. The findings demonstrated that they likely represented the same strain. Based on genetic analysis, these isolates shared a high level of sequence identity (96%) in the polymerase region and a low level of amino acid identity in the capsid region (85%) with the Sapporo/82 cluster (known as GI/1). However, they had a high level of amino acid identity (99%) in capsid region with 12/DCC/Tokyo/Japan/44 cluster (known as GI/8). Altogether, these sapovirus strains demonstrated a novel recombination with GI/1 polymerase and GI/8 capsid.

In conclusion, this is the first report of outbreak associated with the sapovirus GI/6 infection among infants and children with acute gastroenteritis in Osaka City, Japan and warns of the threat they pose. This is also the first study, to our best knowledge, demonstrating the existence of the novel recombinant sapovirus causing diarrheal illness in Osaka City, Japan.

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Existence of Multiple Genotypes Associated With Acute Gastroenteritis During 6-Year Survey of Norovirus Infection in Japan

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Norovirus (NoV) is recognized as one of the most common causative agent of diarrheal disease in young children worldwide. The current study was undertaken to determine the distribution of NoV genotypes in Japan. A total of 3,864 fecal specimens from children with acute gastroenteritis in five regions (Tokyo, Maizuru, Saga, Sapporo, and Osaka) of Japan from July 1995 to June 2001 were collected and then tested for the presence of NoV by RT-PCR. Three hundred sixty four were found to be positive for NoV, accounting for 11%. The highest prevalence of NoV infection was in November, December, and January as the early winter months in Japan. NoV was subjected to be further characterized to sequencing analysis. All NoVs belonged to two different genogroups I and II and these represented 3% and 97%, respectively. This finding indicated that NoV genogroup II was the dominant group causing acute gastroenteritis in Japan. Interestingly, NoV strains were classified into 16 distinct genotypes including genogroup II genotype 9 that was firstly identified in Japan. Of these, NoV genogroup II genotypes 3 and 4 dominated over other genotypes and became the leading strains in Japanese pediatric population. In conclusion, diarrhea due to NoV infection is still a health burden in Japan. This report also stresses the great genetic diversity as well as the importance of NoV causing the diarrhea in Japan. ***J. Med. Virol. 78:1318–1324, 2006.*** © 2006 Wiley-Liss, Inc.

KEY WORDS: norovirus; genotype; diversity; Japan

INTRODUCTION

Norovirus (NoV) is in the family *Caliciviridae* and contains a single-stranded positive-sense RNA genome, approximately 7.7 kb in size. The NoV genome composes of three open reading frames (ORFs). ORF1 encodes non-structural proteins, including the RNA-dependent RNA polymerase, ORF 2 encodes the capsid protein, and ORF3 a small capsid protein. To date, NoV can be genetically divided into three genogroups (GI, GII, and GIII) based on genome sequence. Of these, NoV GI and GII are known to infect humans and NoV GIII infects animals including bovine and murine. NoV cannot be cultivated in cell culture or experimental animal models. Detection of NoV has relied mainly on RT-PCR using specific primers with the binding sites at the polymerase region or the capsid region [Katayama et al., 2002]. For the genetic classification of NoV, the polymerase region or the capsid region has been used independently. Recently, genetic classification of NoV has described at least 14 and 17 different genotypes for NoV GI and GII, respectively [Kageyama et al., 2004] in which strain Alphatron belongs to NoV genogroup II genotype 17. This capsid region-based classification appeared to distinguish successfully the antigenicity determined by both antigen and antibody ELISA with recombinant virus-like particle [Kobayashi et al., 2000a,b]. Hardy et al. [1997] reported a naturally, occurring recombinant in NoV, then several NoV strains have been described as recombinants and the recombination site were found at the junction of ORF1 and ORF2 [Jiang et al., 1999; Hansman et al., 2004].

Norovirus has been reported as one of the major causative agents of non-bacterial gastroenteritis in all

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age groups [Inouye et al., 2000; Lopman et al., 2002]. NoV is highly infectious and associated with food-borne and water-borne outbreaks of acute gastroenteritis worldwide in different epidemiologic settings such as hospitals, hotels, schools, cruise ship, and restaurants [Inouye et al., 2000; Billgren et al., 2002; Kageyama et al., 2004]. However, the diarrheal illness due to NoV is usually mild and self-limiting. Global outbreaks of gastroenteritis have been caused previously by different strains of NoV GI and II. Since a study reported by Noel et al. [1999] found the "95/96-US" strain which is grouped into genogoup II genotype 4 (GII/4, known as a Lordsdale cluster) having a global distribution, an unusual increase in the number of NoV outbreaks was reported in Europe and the United States [Lopman et al., 2004; Vipond et al., 2004]. Even NoV infection has a great impact on people in both developing and developed countries; and effective anti-NoV drugs have not been developed. Molecular epidemiology of NoV infection is needed in order to successfully control and prevent illnesses caused by NoV.

The objectives of this study were: to determine the incidence of NoV infections in children with acute gastroenteritis in five different regions of Japan from 1995 to 2001; to characterize NoV detected according to genogroup and genotype; and to describe the genetic diversity among them. Additionally, the age-related distribution and seasonal pattern of NoV infection were determined.

MATERIALS AND METHODS

Fecal Specimens

A total of 3,864 fecal specimens were collected from children with acute gastroenteritis in Sapporo, Tokyo, Maizuru, Osaka, and Saga of Japan from July 1995 to June 2001. The fecal specimens were diluted with distilled water to 10% suspensions, and clarified by centrifugation at 10,000*g* for 10 min. The supernatants were collected and stored at −30°C until use for the detection of NoV.

Extraction of Viral Genome

The viral genomes were extracted from 140 μl of 10% fecal suspensions applying the QIAamp spin-column technique according to the manufacturer's instructions (QIAGEN®, Hilden, Germany).

Reverse Transcription (RT)

For RT, 7.5 μl of extracted viral genome was added with a reagent mixture consisting of 2.05 μl of 5× First strand buffer (Invitrogen, Carlsbad, CA), 0.75 μl of 10 mM dNTPs (Roche, Mannheim, Germany), 0.75 μl of 10 mM DTT (Invitrogen), 0.75 μl (200 U/μl) of superscript reverse transcriptase III (Invitrogen), 0.375 μl (1 μg/μl) of random primer (hexa-deoxyribonucleotide mixture) (Takara, Shiga, Japan), 0.5 μl (33 U/μl) of RNase Inhibitor (Toyobo, Osaka, Japan), and 2.325 μl MilliQ water. The total of the reaction mixture was 15 μl [Yan et al., 2003]. RT step was carried out at 50°C for 1 hr, followed by 99°C for 5 min and then held at 4°C.

Polymerase Chain Reaction (PCR)

Using PCR with specific primers as previously reported resulted in the identification of two genogroups of NoV [Yan et al., 2003]. Two pairs of specific primers G1SKF (CTGCCCGAATTYGTAAATGA) and G1SKR (CCAACCCARCCATTRTACA), and COG2F (CARGAR BCNATGTTYAGRTGGATGAG) and G2SKR (CCRCC NGCATRHCCRTTRTACAT) [where B is C, G, or T; H is A, C, or T; N is any base; R is A or G; and Y is C or T] that amplify capsid gene of NoV were used to detect NoV GI and GII, respectively. These primers were generated specifically for two different sizes of amplicons of 330 bp and 387 bp for NoV GI and NoV GII, respectively. PCR was carried out with 2.5 μl of cDNA in 22.5 μl of the reagent mixture containing 10× Taq DNA polymerase buffer (Promega, Madison, WI), dNTPs (2.5 mM/μl), primers (33 μM), Taq DNA polymerase (5 U/μl) (Promega), and MilliQ water. PCR was performed at 94°C for 3 min followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 60 sec, and a final extension at 72°C for 7 min, and then held at 4°C.

Electrophoresis

The PCR products were electrophoresed in a 1.5% agarose gel, followed by staining with ethidium bromide for 20 min then visualized under ultraviolet (UV) light, and the results were recorded by photography.

Nucleotide Sequencing and Phylogenetic Analysis

The nucleotide sequences of PCR products (DNA) positive for NoV were determined with the Big-Dye terminator cycle sequencing kit and an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA). Sequence analysis was performed using CLUSTAL X software (Version 1.6). Phylogenetic tree with 100 bootstrap resamples of the nucleotide sequence alignment data sets was generated using the neighbor-joining method with CLUSTAL X. The genetic distance was calculated using Kimura's two-parameter method (PHYLIP). Reference NoV strains and accession numbers used in this study were as follows: Manchester (X86560), Melksham (X81879), Chitta (AB032758), Wortley (AJ277618), Hillington (AJ277607), Toronto (U02030), Lordsdale (X86557), Fayetteville/1998/US (AY113106), Erfurt/546/00/DE (AF42118), M7/99/US (AY130761), Saitama U1 (AB039775), Camberwell (AF145896), Snow (U70059), Arg320 (AF190817), Mexico (U22498), MD145 (AY032605), Mora/97/SE (AY081134), Saitama-KU80aGII/99/JP (AB058582), Bristol (X76716), SaitamaU16 (AB039778), SaitamaU17 (AB039779), WUG1 (AB081723), Chiba (AB022679), Birmingham (AJ277612), and Saitama KU8/99/JP (AB058547).

TABLE I. Distribution of NoV Infection Among Children by Age Group From 1995 to 2001

	0 m	6 m	1 y	2 y	3 y	4 y	5 y	6 y	7 y	8 y	9 y	10 y	ND	Total
1995/1996	1	4	11	7	2	3	1	0	0	0	0	0	6	35
1996/1997	2	6	16	5	2	0	0	0	0	0	0	0	0	31
1997/1998	7	14	27	8	3	1	0	0	0	0	1	1	9	71
1998/1999	3	9	24	9	2	2	3	0	1	0	0	3	4	60
1999/2000	2	14	30	15	9	2	2	3	1	1	2	2	13	96
2000/2001	0	18	21	13	3	2	2	4	3	2	0	2	1	71
Total	15	65	129	57	21	10	8	7	5	3	3	8	33	364

Note: m, month; y, year; ND, not determined.

RESULTS

Epidemiology of NoV Infection

A total of 3,314 fecal specimens collected from children with acute gastroenteritis in Sapporo, Tokyo, Maizuru, Osaka, and Saga of Japan during July 1995 and June 2001 were examined for NoV. In the pediatric population, the lowest age was 0 month and the highest was 10 years. Of 3,314 fecal specimens tested, 364 were detected to be positive for NoV and this represented 11%. Table I showed that the highest NoV infection was in the 1-year old group (35.4%; 129 of 364). The NoV infection was identified among children aged less than 6 months (4.1%; 15 of 364). It was also found that children younger than 3 years had a high rate of NoV infection (73.1%, 266 of 364).

Seasonal Variation of NoV Infection

The NoV detection rate was analyzed between July 1995 and June 2001. Figure 1 shows that NoV was detected continuously for 10 months (September to June). No NoV was found in both July and August. The highest prevalence of NoV infection was in December (41.5%; 151 of 364), followed by January and November with 15.7% (57 of 364) and 13% (47 of 364), respectively. The lowest NoV detection rate was in October (0.3%; 1 of 364).

Distribution of NoV G-Types

The nucleotide sequence of the 5′ ends of the NoV capsid gene was determined by direct sequencing with the amplified fragments. This region has been shown to be suitable for genotyping. All NoV sequences were analyzed by phylogenetics and grouped using the NoV capsid region classification scheme of Kageyama et al. [2004]. In the present study, all of the NoV sequences were classified into two distinct genogroupes I and II and these represented 3% (11 cases) and 97% (353 cases), respectively. The NoV GI sequences clustered into four genotypes (GI/3, GI/4, GI/8, and GI/11), accounting for 27.3% (3 of 11), 54.5% (6 of 11), 9.1% (1 of 11), and 9.1% (1 of 11), respectively. In NoV GII, genotype 4 was dominant every year, from 41.9% (1996–1997) to 80% (1995–1996) followed by genotype 3 as second predominant strain, ranging from 19.1% (1999–2000) to 38% (1997–1998) (Table II). Moreover, many other NoV genotypes including GII/1, GII/2, GII/5, GII/6, GII/9, GII/10, GII/12, GII/13, GII/14, and GII/15 were found

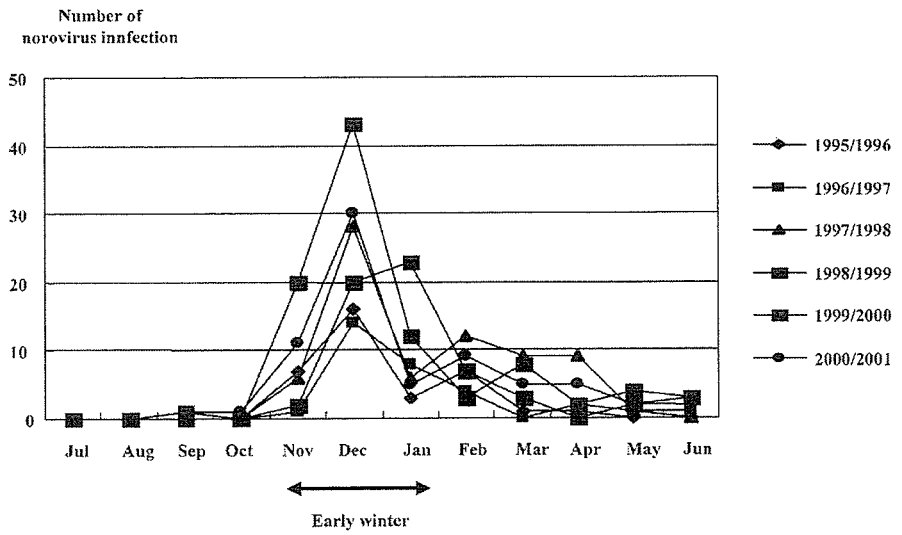

Fig. 1. Seasonal pattern of NoV detected among children with acute gastroenteritis during 6-year survey of NoV infection in Sapporo, Tokyo, Maizuru, Osaka, and Saga of Japan during July 1995 and June 2001. The cold season was also indicated.

TABLE II. Distribution of NoV Genotypes in Five Regions of Japan From 1995 to 2001

				No. (%) of genotypes of GII (1995–1996)						
Regions	Total	GI	GII	GII/2	GII/3	GII/4	GII/5	GII/6	GII/12	Rare
Sapporo	73	0	0	0	0	0	0	0	0	0
Tokyo	98	0	11	0	0	8 (72.7)	0	3 (27.3)	0	0
Maizuru	265	0	24	0	0	20 (83.3)	0	3 (12.5)	1 (4.2)	0
Osaka	—	—	—	—	—	—	—	—	—	—
Saga	—	—	—	—	—	—	—	—	—	—
Total	436	0	35	0	0	28 (80)	0	6 (17.1)	1 (2.9)	0
				No. (%) of genotype of GII (1996–1997)						
Regions	Total	GI	GII	GII/2	GII/3	GII/4	GII/5	GII/6	GII/12	Rare
Sapporo	50	0	2	0	0	0	0	1 (50)	1 (50)	0
Tokyo	71	0	13	0	10 (76.9)	3 (23.1)	0	0	0	0
Maizuru	239	0	16	1 (6.3)	1 (6.3)	10 (62.5)	1 (6.3)	0	1 (6.3)	G9, G10
Osaka	—	—	—	—	—	—	—	—	—	—
Saga	—	—	—	—	—	—	—	—	—	—
Total	360	0	31	1 (3.2)	11 (35.5)	13 (41.9)	1 (3.2)	1 (3.2)	2 (6.5)	2 (6.5)
				No. (%) of genotypes of GII (1997–1998)						
Regions	Total	GI	GII	GII/2	GII/3	GII/4	GII/5	GII/6	GII/12	Rare
Sapporo	62	0	16	0	6 (37.5)	4 (25)	0	5 (31.3)	1 (6.3)	0
Tokyo	93	0	16	0	0	16 (100)	0	0	0	0
Maizuru	249	0	16	0	11 (68.8)	5 (31.3)	0	0	0	0
Osaka	96	0	23	0	10 (43.5)	11 (47.8)	0	1 (4.4)	1 (4.4)	0
Saga	—	—	—	—	—	—	—	—	—	—
Total	500	0	71	0	27 (38.0)	36 (50.7)	0	6 (8.5)	2 (2.8)	0
				No. (%) of genotypes of GII (1998–1999)						
Regions	Total	GI	GII	GII/2	GII/3	GII/4	GII/5	GII/6	GII/12	Rare
Sapporo	43	0	2	0	0	2 (100)	0	0	0	0
Tokyo	80	0	7	0	0	5 (71.4)	0	1 (14.3)	1 (14.3)	0
Maizuru	248	0	21	0	3 (14.3)	12 (57.1)	0	6 (28.6)	0	0
Osaka	134	0	23	0	7 (30.4)	12 (52.2)	0	4 (17.4)	0	0
Saga	87	0	7	0	7 (100)	0	0	0	0	0
Total	592	0	60	0	17 (28.3)	31 (51.7)	0	11 (18.3)	1 (1.7)	0
				No. (%) of genotypes of GII (1999–2000)						
Regions	Total	GI	GII	GII/2	GII/3	GII/4	GII/5	GII/6	GII/12	Rare
Sapporo	56	0	3	0	3 (100)	0	0	0	0	0
Tokyo	49	GI/4, GI/11	7	0	2 (28.6)	4 (57.1)	1 (14.3)	0	0	0
Maizuru	387	GI/4, GI/3	57	5 (8.8)	5 (8.8)	44 (77.2)	1 (1.8)	1 (1.8)	0	G14
Osaka	121	GI/4, GI/8	14	0	6 (42.9)	7 (50)	0	1 (7.1)	0	0
Saga	153	GI/4	8	0	1 (12.5)	3 (37.5)	0	3 (37.5)	1 (12.5)	0
Total	766	7	89	5 (5.6)	17 (19.1)	58 (65.2)	2 (2.3)	5 (5.6)	1 (1.1)	1 (1.1)
				No. (%) of genotypes of GII (2000–2001)						
Regions	Total	GI	GII	GII/2	GII/3	GII/4	GII/5	GII/6	GII/12	Rare
Sapporo	44	0	5	0	1 (20)	4 (80)	0	0	0	0
Tokyo	37	0	2	0	0	2 (100)	0	0	0	0
Maizuru	365	0	22	0	3 (13.6)	16 (72.7)	0	0	0	G10, G13
Osaka	108	GI/3, GI/4	23	0	7 (30.4)	7 (30.4)	2 (8.7)	4 (17.4)	1 (4.3)	G14, G15
Saga	106	0	15	0	3 (20)	11 (73.3)	0	0	0	G1
Total	660	4	67	0	14 (20.9)	40 (59.7)	2 (3)	4 (6)	1(1.5)	6(9)

co-circulating in Japanese children with acute gastroenteritis (Fig. 2). It was found that NoV strains in the study with the same genotype had high homology with each other, ranging from 95% to 100% even when they were detected in different years and different areas in Japan.

DISCUSSION

Norovirus is one of the important causes of sporadic cases and outbreaks of gastroenteritis worldwide [Koo et al., 1996; Holtby et al., 2001; Lopman et al., 2004]. Out of 3,864 fecal specimens tested in the study, 11% were positive for NoV by RT-PCR. This finding is in agreement with previous reports on molecular epidemiology of NoV infection worldwide in which its prevalence was shown ranging from 10% to 60% [Love et al., 2002; Marks et al., 2003]. The finding also suggested that from acute gastroenteritis cases in children in five regions of Japan about 11% might be due to NoV and 89% might be caused by other etiologic agents. The result also confirmed NoV as one of the important enteropathogens responsible for viral gastroenteritis among children in Japan. In some reports, NoV was prevalent during the cold season, and several studies did not find a seasonal correlation [Noel et al., 1997; Mounts et al., 2000; Otsu

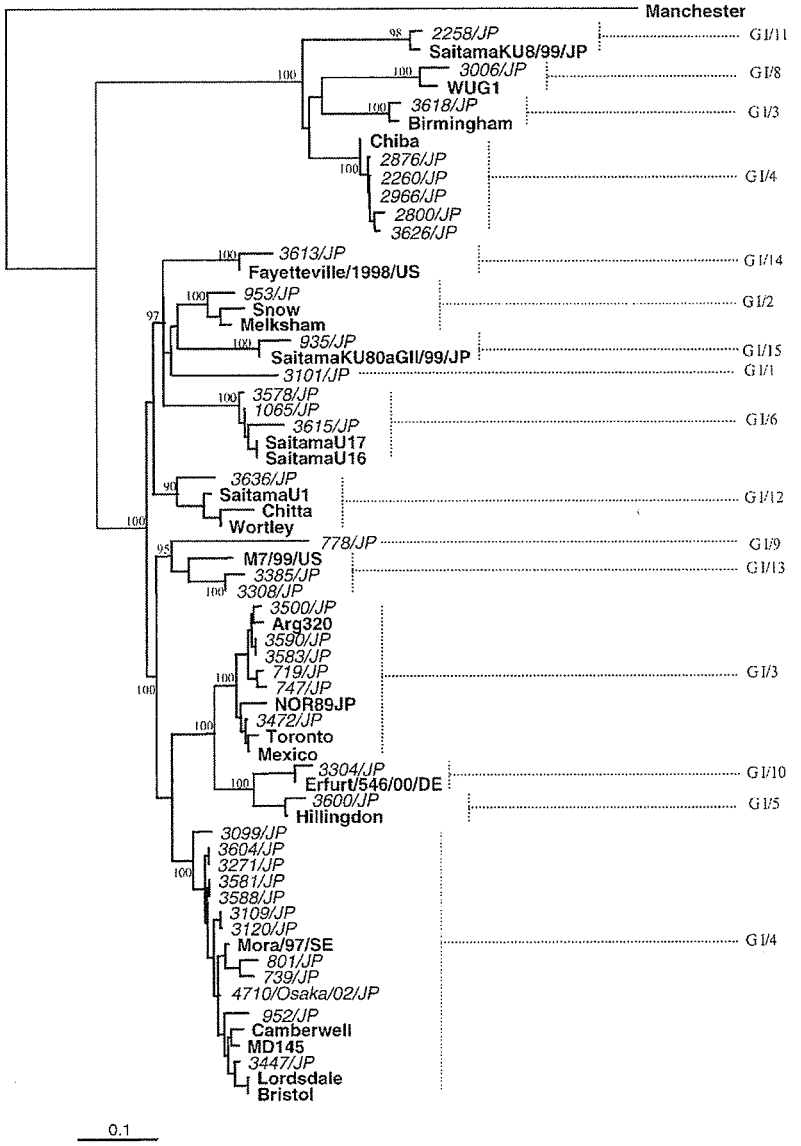

Fig. 2. Phylogenetic tree of nucleotide sequences of NoV. The tree was constructed from partial nucleotide sequences of capsid region of NoV isolates detected in Japan. Reference strains of NoV were selected from the DDBJ/GenBank database under the accession numbers indicated in the text. Japanese NoV was highlighted in italic. Manchester strain was used as an out-group strain for phylogenetic analysis. The scale indicates nucleotide substitutions per position. The numbers in the branches indicate the bootstrap values.

et al., 2003]. The main peak of NoV infection in Japan was between November, December, and January. Obviously, there are two peaks of viral infection causing acute gastroenteritis in Japan; one peak associated with NoV infection lasts from November to January known as early winter in this study and another peak associated with rotavirus infection lasts from February to April known as late winter [Yoshinaga et al., 2006]. Furthermore, it was interesting that the highest incidence of NoV infection fell into the 1-year old group, and the rate of incidence decreased with increasing age over 2 years. Quite possibly, children aged 1 year might lack antibody protection to NoV; whereas by the time children have reached 2 years old they begun to acquire viral immunity.

The results of this study showed that all Japanese NoV isolates belonged to two distinct genogroups I and II (GI, GII) and these represented 3% and 97%, respectively. This indicated that NoV GII was the dominant group causing acute gastroenteritis among Japanese pediatric population. The distribution of NoV genotypes was also investigated within a time-line period from 1995 to 2001. Interestingly, phylogenetic analysis of the partial capsid gene of NoVs identified a wide range of genotypes (up to 16) had been co-circulating and caused diarrheal illness among children in Japan during that time. In this study, a rare NoV genotype (GII/9), which has not been detected in Japan, was identified. Moreover, there was only one strain, which matched closely this GII/9 strain in the DDBJ/GenBank database. The other NoV strains, such as GII/13, which have a few homologous strains in the DDBJ/GenBank database, were also detected. Taken together, it is noteworthy that NoV strains detected among Japanese pediatric population with acute gastroenteritis demonstrated a great genetic diversity.

According to other reports published by different groups of investigators, NoV belonging to the Lordsdale cluster (GII/4) represented the highest detection in sporadic gastroenteritis among infants and children not only in Japan but also many other countries who conduct NoV surveillance [Lopman et al., 2002; Nicollier-Jamot et al., 2003; Ueki et al., 2004]. Generally, GII/4 detected in the present study was found to be the dominant genotype in causing acute gastroenteritis in Japan. However, it was interesting that GII/3 sometimes dominated GII/4 in some regions of Japan during different periods of time. In 1996–1997, the detection rate of GII/3 in Tokyo was very high as 76.9% whereas that of GII/4 was only 23.1%. A similar pattern of NoV infection was also identified in Sapporo and Maizuru in 1997–1998. Further epidemiologic studies should be conducted to determine whether strains from GII/3 continue to be dominant in Japan in future.

Another interesting feature of the present study was the temporary increase of NoV GII/6 strains, which became the second predominant NoV genotype causing the illness among children in Japan in Maizuru during 1995–1996 and 1998–1999. These results suggested that the NoV GII/6 were also an important cause of sporadic cases of acute gastroenteritis. This sudden predominance of NoV GII/6 strains indicated that the pediatric population might lack antibody protection to these strains, whereas by the time they have begun to acquire viral immunity or NoV GII/6 strains could be more virulent at that time.

In conclusion, diarrhea due to NoV infection is still a health burden in Japan. This report also stresses the great genetic diversity and the importance of NoV causing the diarrhea in Japan. Moreover, such study of the molecular epidemiology of NoV provides knowledge on the diversity of genotypes found in humans. Continuous monitoring of the NoV genotypes should be continued for the control of diarrheal disease due to NoV infection to be successful.

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Clin. Lab. 2006;52:515-518

SHORT COMMUNICATION

Human Adenovirus Type 1 Related To Feline Adenovirus: Evidence of Interspecies Transmission

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SUMMARY

Adenovirus is recognized to be a significant global enteropathogen in association with sporadic cases as well as outbreaks of acute gastroenteritis in humans. Based on the genetic analysis, one adenovirus strain bearing feline adenovirus gene was detected in a fecal specimen collected from a 1-year old female child with acute gastroenteritis in Japan. The human adenovirus detected and feline adenovirus shared high identities (100% and 97%) at the amino acid levels of hexon and fiber genes, respectively, and they belonged to the same human Ad1 cluster (known as the prototype Adenoid 71). These findings suggest that the interspecies transmission of adenovirus between humans and felines might occur in nature. This report is noteworthy because it is the first, to the best of our knowledge, providing evidence of adenovirus type 1 transmission between humans and animals, and highlights possible zoonoses in humans. Further epidemiological studies should be conducted to determine whether this adenovirus strain will be emergent in future. (Clin. Lab. 2006;52:515-518)

KEY WORDS

Human, Feline, Adenovirus

THE STUDY

The adenoviruses constitute the *Adenoviridae* family, which is divided into two genera, *Mastadenovirus* and *Aviadenovirus*. Whereas the *Aviadenovirus* genus is limited to viruses of birds, the *Mastadenovirus* genus is found to infect humans, and other animals such as murine, equine, fowl, porcine, ovine, canine, and caprine [1]. Human adenovirus causes a variety of diseases such as acute respiratory, gastrointestinal, and urinary tract infections. To date, 51 human adenovirus serotypes have been recognized and classified into six subgenera from A to F. This classification scheme is generally consistent with subgroupings of human adenoviruses on the basis of their physicochemical, biological, and genetic properties [1]. Among six subgenera, subgenus F, represented by two human adenoviruses, type 40 (Ad40) and Ad41, was the most important in association with acute gastroenteritis both in outpatients and hospitalized children in developed and developing countries [2, 3].

During the epidemiological surveillance of diarrheal viruses in Maizuru City, Japan, in March 2005 one adenovirus strain bearing feline adenovirus gene was detected in a fecal specimen collected from a 1-year old female child with acute gastroenteritis. Clinical symptoms of acute gastroenteritis included diarrhea, vomiting, and fever. The child vomited from 1 to 4 times per day. The fever rose to 39.4 °C. No mucus or blood was found in the feces. The duration of diarrhea was 4 days. None of the surrounding family members had acute gastroenteritis. The viral genome was extracted from the fecal specimen by using a QIAamp spin column (QIAGEN®, Hilden, Germany). Using RT-multiplex PCR previously described resulted in the identification of diarrheal viruses, including human astrovirus, norovirus, sapovirus, rotavirus, and adenovirus [4]. For iden-

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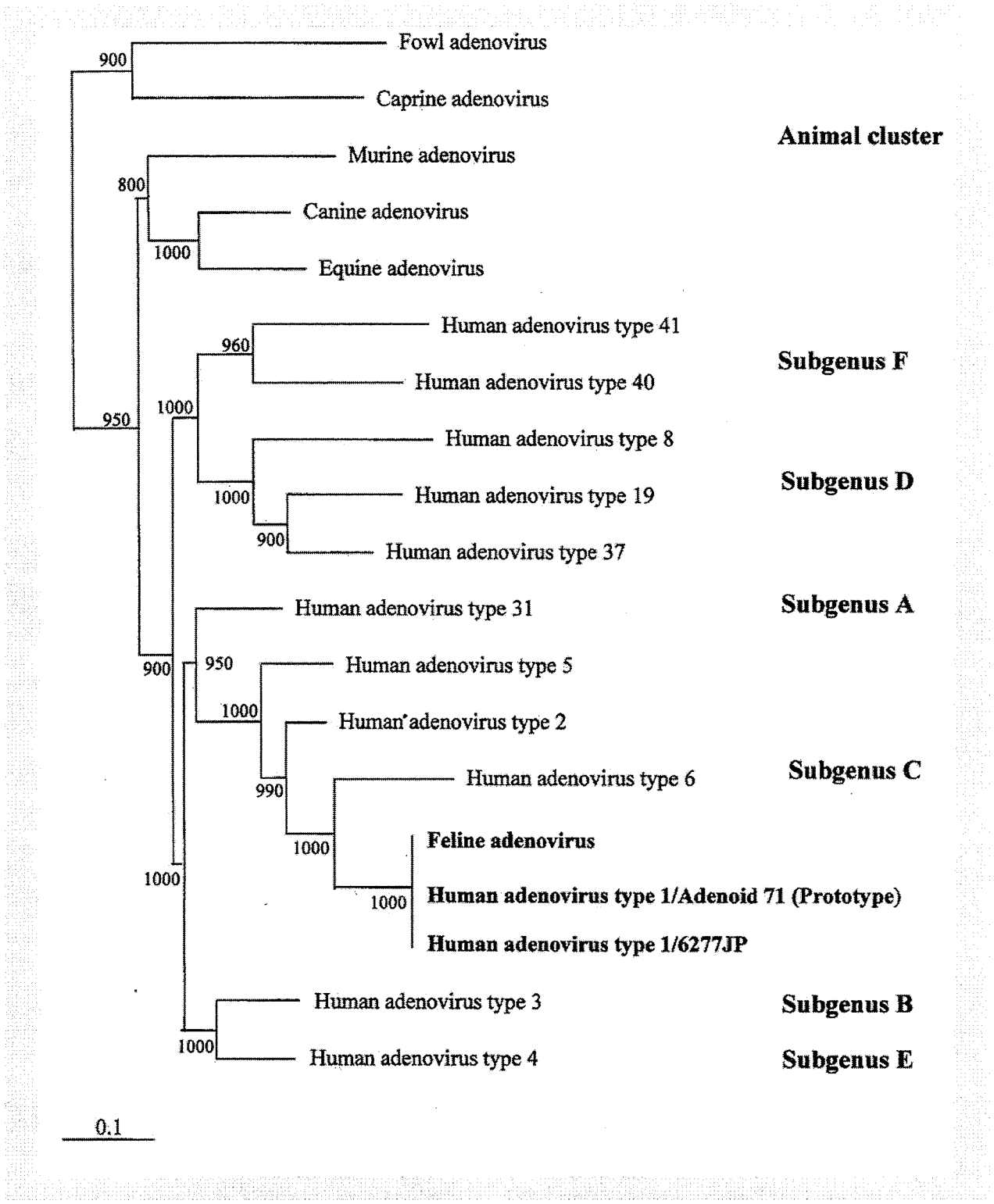

Figure. Phylogenetic tree of amino acid sequences of adenovirus strain 6277JP. The tree was constructed from amino acid sequences of seven hypervariable regions of the hexon gene of adenovirus strain 6277JP detected in Maizuru City, Japan. The 6277JP, the prototype Adenoid 71 and feline adenovirus are highlighted in bold type. The scale indicates amino acid substitutions per position. The numbers in the branches indicate the bootstrap values.