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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

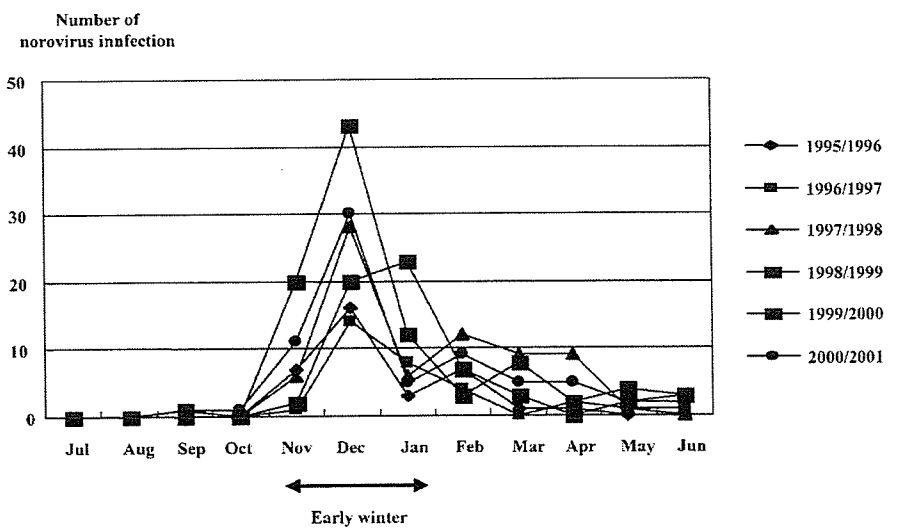

Fig. 1. Seasonal pattern of NoV detected among children with acute gastroenteritis during 6-year survey of NoV infection in Sapporo, Tokyo, Maizuru, Osaka, and Saga of Japan during July 1995 and June 2001. The cold season was also indicated.

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

Regions	Total	GI	GII	No. (%) of genotypes of GII (1995–1996) 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

Regions	Total	GI	GII	No. (%) of genotype of GII (1996–1997) 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)

Regions	Total	GI	GII	No. (%) of genotypes of GII (1997–1998) 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

Regions	Total	GI	GII	No. (%) of genotypes of GII (1998–1999) 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

Regions	Total	GI	GII	No. (%) of genotypes of GII (1999–2000) 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)

Regions	Total	GI	GII	No. (%) of genotypes of GII (2000–2001) 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

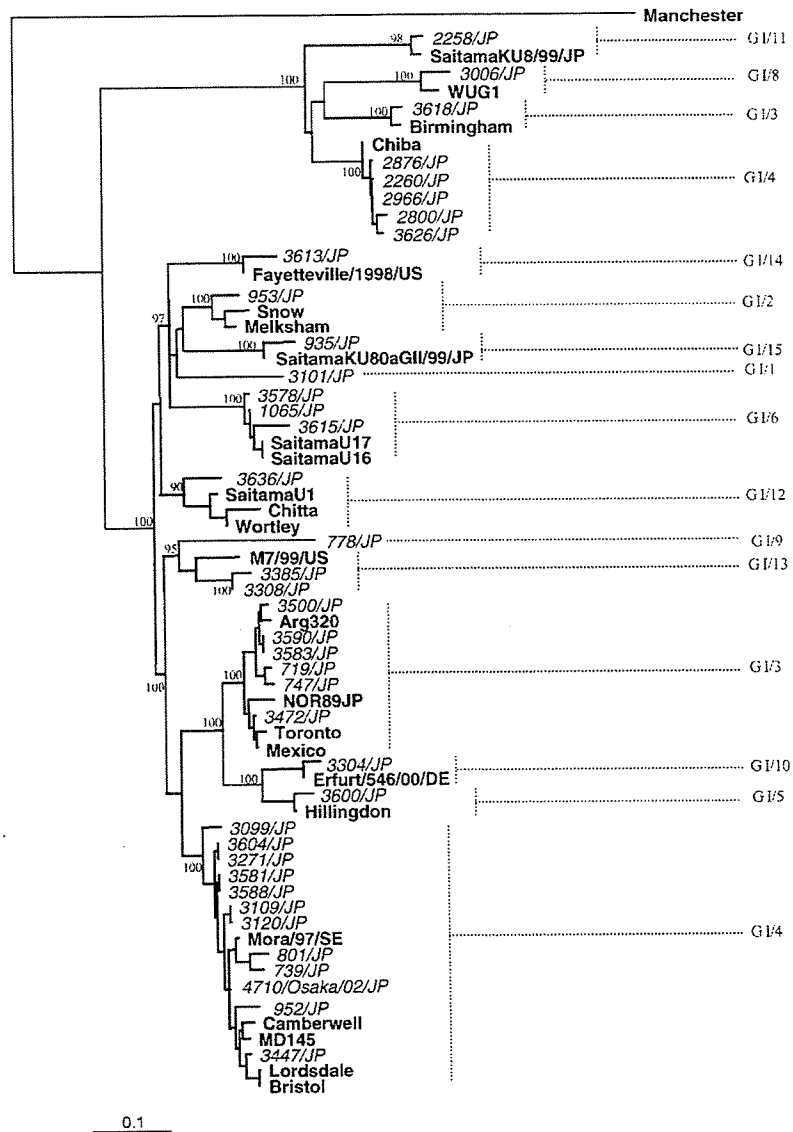

Fig. 2. Phylogenetic tree of nucleotide sequences of NoV. The tree was constructed from partial nucleotide sequences of capsid region of NoV isolates detected in Japan. Reference strains of NoV were selected from the DDBJ/GenBank database under the accession numbers indicated in the text. Japanese NoV 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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Novel Recombinant Norovirus in China

To the Editor: Norovirus (NoV), the distinct genus within the family *Caliciviridae*, is a major cause of sporadic cases and outbreaks of acute gastroenteritis in humans (*1*). NoV possesses a positive-sense, single-stranded RNA genome surrounded by an icosahedral capsid. The NoV genome contains 3 open reading frames (ORFs). ORF1 encodes nonstructural proteins, ORF 2 encodes capsid protein (VP1), and ORF3 encodes a small capsid protein (VP2). NoV is still uncultivable by standard culture with different cell lines. However, expression of either VP1 or both VP1 and VP2 with recombinant baculoviruses formed viruslike particles that are morphologically and antigenically similar to the native virion (*2*).

A fecal specimen was collected from an infant hospitalized with acute gastroenteritis in Kunming, China, in November 2004 and was tested for diarrheal viruses in a cooperative laboratory in Japan. The viral genome was extracted by using a Qiagen kit (Qiagen, Hilden, Germany). Polymerase chain reaction with specific primers resulted in the identification of astrovirus, rotavirus, sapovirus, adenovirus, and NoV genogroup I (GI) and GII (*3*). NoV polymerase was also amplified to identify recombinant NoV with primers Yuri22F and Yuri22R (*4*). Products were sequenced directly, and sequence analysis was performed by using ClustalX and SimPlot.

The fecal specimen was positive for NoV GII. The Figure shows that the 146/Kunming/04/China sequence clustered into the distinct GII genotype 7 (Leeds/90/UK cluster). 146/Kunming/04/China was classified into the Saitama U4 cluster (GI/6) when polymerase-based grouping was performed. Altogether, 146/Kunming/04/China was expected to be the recombinant NoV with GII/7 capsid and GII/6 polymerase.

To eliminate the possibility of coinfection with 2 different NoV genotypes, to localize the potential recombination site, and to clarify a possible recombination mechanism, the ORF1/ORF2 overlap and flanking polymerase and capsid regions of 146/Kunming/04/China was amplified with primers Yuri22F and GIISKR to produce a 1,158-bp amplicon (*3,4*). When the sequence of 146/Kunming/04/China was compared with that of Saitama U4 by using SimPlot, a recombination site was found at the ORF1/ORF2 overlap. Before this junction, 146/Kunming/04/China and Saitama U4 were homologous. After the ORF1/ORF2 overlap, however, the homology was notably different. SimPlot showed a sudden drop in the nucleotide identity for 146/Kunming/04/China. ClustalX showed that 146/Kunming/04/China shared a high identity (93%) in the polymerase region and a low identity (78%) in the capsid region with Saitama U4. In contrast, high identity (95%) in the capsid region was found between 146/Kunming/04/China and Leeds/90/UK. Since Leeds/90/UK polymerase was not available in GenBank, the polymerase homology between 146/Kunming/04/China and Leeds/90/UK was unknown. Polymerase of 146/Kunming/04/China was almost

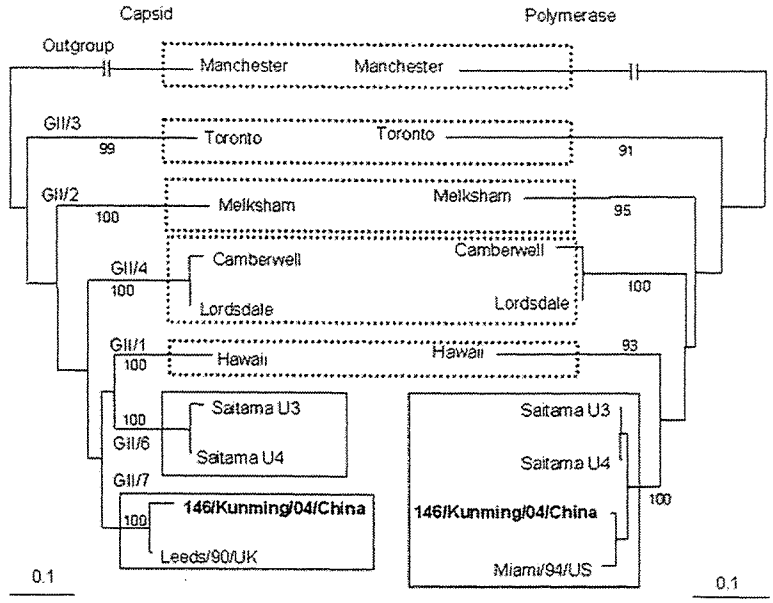

Figure. Changes in norovirus (NoV) genotypes on the basis of phylogentic trees of nucleotide sequences of 146/Kunming/04/China. Trees were constructed from partial nucleotide sequences of capsid and polymerase regions of 146/Kunming/04/China. 146/Kunming/04/China is **boldface**. Dashed boxes indicate the maintenance of genotypes of reference NoV strains, and solid boxes indicate the involvement of NoV genotypes with recombinant NoV 146/Kunming/04/China. A phylogenetic tree with 100 bootstrap resamples of the nucleotide alignment datasets was generated by using the neighbor-joining method with ClustalX. The genetic distance was calculated by using the Kimura 2-parameter method (PHYLIP). The scale indicates nucleotide substitutions per position. The numbers in the branches indicate the bootstrap values. Manchester strain was used as an outgroup strain for phylogenetic analysis. The nucleotide sequence of NoV strain 146/Kunming/04/China had been submitted to GenBank and has been assigned accession no. DQ304651. Reference NoV strains and accession nos. used in this study are as follows: Manchester (X86560), Toronto (U02030), Melksham (X81879), Camberwell (AF145896), Leeds/90/UK (AJ277608), Lordsdale (X86557), Hawaii (U07611), Saitama U3 (AB039776), Saitama U4 (AB039777), and Miami/94/US (AF414410).

identical with that of Saitama U4, but the capsids of 146/Kunming/04/China and Leeds/90/UK were distinctly different from that of Saitama U4. This genetic pattern of 146/Kunming/04/China implied a novel, naturally occurring recombinant NoV with GII/7 capsid and GII/6 polymerase.

RNA recombination is a mechanism for virus evolution (*5*). Literature documenting recombination in NoV is fairly rich, but none is from China (*6*). Therefore, 146/ Kunming/04/China was not only the first but also the first recombinant NoV from China. This isolate shared the closest sequences of polymerase and capsid with Saitama U4 and Leeds/90/UK, respectively. Strain Saitama U4 was detected in 1997 in Japan (*7*), whereas strain Leeds/90/ UK was detected in 1990 in the United Kingdom (*8*). Quite possibly, Saitama U4 and Leeds/90/UK were parental strains of 146/Kunming/04/China. However, the distant geographic relationship of these strains obscured evidence of where and when the recombination event occurred. This phenomenon also suggested that these parent strains or this progeny strain might be more prevalent than is often assumed.

Recombination depends on various immunologic and intracellular constraints. Recombinant viruses are all alike in that they successfully pass through 5 stages: 1) successful co-infection of a single host, 2) successful co-infection of a single cell, 3) efficient replication of both parental strains, 4) template switching, and 5) purifying selection (*9*). In this study, 146/Kunming/04/China was recovered from a patient with diarrhea, fever, and vomiting. This observation indicated that this strain theoretically fulfilled all prerequisites for recombination.

The NoV capsid is predicted to be well suited for genotype classification (*10*). In this study, 146/Kunming/04/China belonged to 2 distinct genotypes, 7 and 6, by capsid- and polymerase-based groupings, respectively. Moreover, the recent demonstration of recombination in an increasing number of NoVs suggests that it is a more widespread event than was previously realized. Consequently, the phylogenetic classification of NoV on the basis of on capsid sequence is questionable. We suggest that classification of NoV strains should rely on not only capsid sequence but also polymerase sequence.

In conclusion, our results described the genetic characterization of novel, naturally occurring recombinant NoV and increased evidence for the worldwide distribution of recombinant NoV. This report is the first to describe acute gastroenteritis caused by recombinant NoV in China and warns of the threat it poses.

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closely related to *E. coli*, as was previously observed in Tanzania (*9*). This finding is also the first report of CTX-M-3 in sub-Saharan Africa.

Multidrug resistance profiles involving non–β-lactam antimicrobial drugs coselected these ESBL-producing isolates. We suggest that the misuse of antimicrobial drugs in the Central African Republic and the migratory flux of regional populations could result in emergence and selection of these ESBL phenotypes in the community. We could not establish a relationship between the different strains isolated in hospitalized and ambulatory patients. Because of the implications for treating such infections, particularly in developing countries, the spread of ESBL-producing *Enterobacteriaceae* merits close surveillance in the Central African Republic.

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Novel Recombinant Sapovirus, Japan

To the Editor: *Sapovirus* is the distinct genus within the family *Caliciviridae*; these viruses cause sporadic cases and outbreaks of gastroenteritis in humans worldwide (*1*). The sapovirus genome contains 2 open reading frames (ORFs). ORF1 encodes nonstructural and capsid proteins while ORF2 encodes a small protein (*2*). Sapovirus has a typical "Star of David" configuration by electron microscopic examination. The prototype sapovirus is the Sapporo virus (Hu/SaV/Sapporo virus/1977/ JP), which was originally discovered from an outbreak in a home for infants in Sapporo, Japan, in 1977 (*3*). Sapovirus is divided into 5 genogroups, among which only genogroups I, II, IV, and V are known to infect humans (*4*).

A fecal specimen was collected from a 1-year-old boy with acute gastroenteritis in Osaka, Japan, in March 2005. The viral genome was extracted by using a QIAamp kit (Quigen, Hilden, Germany). By using multiplex reverse transcription–polymerase chain reaction (RT-PCR), 2 groups of diarrheal viruses were identified. The first group included astrovirus, norovirus, and sapovirus; the second group included rotavirus and adenovirus (*5*). Sapovirus polymerase region was also amplified to identify recombinant sapovirus by using primers P290 and P289 (*6*). To eliminate the possibility of co-infection of 2 different sapovirus genotypes, to localize the potential recombination site, and to understand a possible recombination mechanism of recombinant sapovirus, flanking polymerase and capsid regions, with their junction of HU/5862/Osaka/JP, were amplified with primers P290 and SLV5749 to produce a 1,162-bp product (*5,6*). Products were directly sequenced, and capsid- and polymerase-based phylogenetic trees showed recombinant sapovirus.

The fecal specimen was positive for sapovirus. HU/5862/Osaka/JP clustered into the genogroup I genotype 8 (GI/8 the 8/DCC/Tokyo/JP/44 cluster) (Figure) by using the recent sapovirus capsid region classification (*7*). HU/5862/Osaka/JP with GI/8 capsid was classified into GI/1 (the Sapporo/82 cluster) when polymerase-based grouping was performed. When the sequence of HU/5862/Osaka/JP was compared with that of Sapporo/82 by using SimPlot Version 1.3 (available from http://sray.med.som.jhmi.edu/SCRoftware/simplot), the recombination site was identified at the polymerase-capsid junction. Before this junction, sequences of HU/5862/Osaka/JP and Sapporo/82 were highly homologous. However, homology between them was notably different after the junction, with a sudden drop in the identity for HU/5862/Osaka/JP. By using ClustalX, HU/5862/Osaka/JP shared a 96% identity in polymerase sequence and an 85% identity in capsid sequence with Sapporo/82. In contrast, homology was 99% in the capsid region between HU/5862/Osaka/JP and 8/DCC/Tokyo/JP/44. Since a polymerase sequence of 8/DCC/Tokyo/JP/44 was not available in GenBank because of the unsuccessful amplification, homology in the polymerase region between HU/5862/Osaka/JP and 8/DCC/Tokyo/JP/44 was unknown.

Altogether, the findings underscored that HU/5862/Osaka/JP represented a novel, naturally occurring, recombinant sapovirus with GI/8 capsid and GI/1 polymerase. To determine whether the child was infected with this novel recombinant sapovirus or whether the novel recombinant sapovirus resulted from co-infection with 2 different viruses, Svppo (Sapporo/82-specific primer), Svdcc (8/DCC/Tokyo/JP/44-specific primer), and SLV5749 were used to amplify the capsid region (*5*). However, no amplicon was found. These negative results indicate no co-infection in this child.

Even though many molecular epidemiologic studies on sapovirus infection have been performed worldwide, reports documenting recombination in sapovirus are still limited. The first recombinant sapovirus identified was the Thai isolate Mc10 or the Japanese isolate C12 (*8*); the Japanese isolate Ehime1107 and the SW278 isolate from Sweden were identified later (*9*). Recombination occurred only in sapovirus genogroup II, which is more capable of recombination than other genogroups (*8,9*). In this study, we identified HU/5862/Osaka/JP with a novel recombination between 2 distinct genotypes within genogroup I. This is the first report of acute gastroenteritis caused by recombinant sapovirus genogroup I. The findings underscore that natural recombination occurs not only in sapovirus genogroup II but also in genogroup I.

In recent studies of sapovirus recombination, evidence for the location of the recombination event is lacking because of the distant geographic relationship of parent and progeny strains. HU/5862/Osaka/JP shared the closest sequences of polymerase and capsid with Sapporo/82 and 8/DCC/Tokyo/JP/44, respectively. Sapporo/82 was first isolated in 1982, and 8/DCC/Tokyo/JP/44 was isolated in 2000, both in Japan. Possibly, Sapporo/82 and 8/DCC/Tokyo/JP/44 were parental strains of HU/5862/Osaka/JP, and the event leading to the novel recombination might have occurred in Japan.

The capsid region was used for genotype classification of sapovirus (*7*). When capsid-based grouping was performed, HU/5862/Osaka/JP distinctly belonged to genotype 8. When polymerase-based grouping was

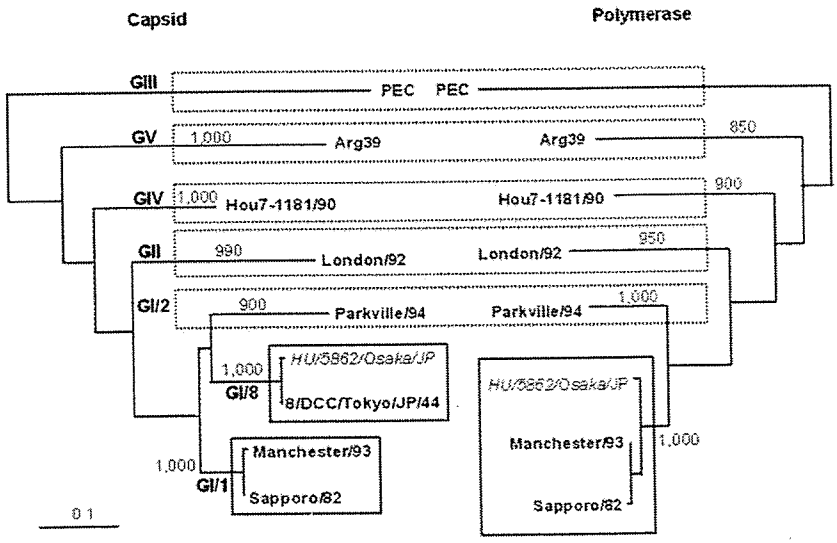

Figure. Changing genotypes of sapovirus on the basis of phylogenetic trees. Trees were constructed from partial amino acid sequences of capsid and polymerase of HU/5862/Osaka/JP highlighted in *italics*. Phylogenetic tree with 1,000 bootstrap resamples of the nucleotide alignment datasets was generated by using the neighbor-joining method with ClustalX. The genetic distance was calculated by using Kimura 2-parameter method (PHYLIP). The scale indicates amino acid substitutions per position. The numbers in branches indicate bootstrap values. Porcine enteric calicivirus was used as an outgroup strain for phylogenetic analysis. The nucleotide sequence data of sapovirus strain HU/5862/Osaka/JP has been submitted to GenBank and has been assigned accession no. DQ318530. Reference sapovirus strains and accession nos. used in this study were as follows: PEC (AF182760), London/92 (U95645), Arg39 (AY289803), Parkville/94 (U73124), Manchester/93 (X86560), Sapporo/82 (U65427), Hou7-1181/90 (AF435814), and 8/DCC/Tokyo/Japan/44 (AB236377).

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Originals

Clinical Evaluation of Breech Deliveries Over a Fifteen-Year Period at a Hospital in Ota, Japan

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SUMMARY

Objective : To examine the characteristics and perinatal outcome of pregnancies with breech presentation.

Methods : Breech deliveries were divided into four groups : primipara vaginal delivery group (PV-G), multipara vaginal delivery group (MV-G), planned cesarean section group (PC-G), and emergency cesarean section group (EC-G). The maternal age, gestational week, neonatal birth weight, and Apgar score were compared.

Results : There were no significant differences in maternal age, gestational week as well as neonatal birth weight among the four groups. An Apgar score at 1 minute of less than 6 points was seen in 0 %, 11.1 %, 15.3 %, and 20 % of the PC, MV, PV and EC-Gs, respectively. (PV-G and PC-G were compared to obtain $p < 0.05$) Although, no neonate in this study had an Apgar score at 5 minutes of less than 6 points.

Conclusion : There was no significant difference of perinatal outcome between vaginal delivery and cesarean section for breech presentation at term.

Key Words : breech delivery, planned caesarean section, neonatal outcome

INTRODUCTION

Breech deliveries occur in approximately 3.5 % of all deliveries [1]. Compared to vertex deliveries, there are high frequencies of fetal hypoxemia due to umbilical cord compression at delivery, forelying and prolapse of the umbilical cord among breech deliveries [2]. In addition, a subsequent fetal head delivery at a breech delivery may be difficult [3]. Therefore, there are high risks associated with breech deliveries. Due to the recent increase in malpractice lawsuits, cesarean section (C-section) is frequently chosen for breech deliveries in many countries. Although C-section is a relatively safe surgery, some potential problems may occur. The frequency of maternal complications after a C-section delivery (for example hemorrhagic shock, septic shock, postoperative pulmonary embolism, bowel obstruction) is significantly higher than that after a vaginal delivery [4], and the length of hospitalization is longer among women who undergo C-section delivery. Therefore, simply electing to perform C-sections for all breech deliveries should be avoided. It is essential to carefully decide how to deliver a breech presentation on a case-by-case basis. We performed a retrospective study of cases of breech delivery at Motojima General Hospital over a fifteen-year period to study the indications for vaginal breech delivery.

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Table 1 The following criteria for trying a vaginal delivery in our department

1) More than 37 weeks of gestation
2) An estimated body weight of fetus 2,500–3,500 grams
3) A frank breech or complete breech position
4) No overextension of the fetal neck
5) No placental site abnormality
6) No forelying of the umbilical cord
7) No maternal complications that may affect the delivery.
8) The pelvis is in no way contracted by X-ray pelvimetry

Table 2 Total number of deliveries and breech delivery situations during the past fifteen years in our department.

years	Total deliveries	Breech pregnancies	Vaginal deliveries	Planned C-sections	Emergency C-sections
1990	95	3	2	1	0
1991	85	4	2	2	0
1992	134	3	3	0	0
1993	152	4	3	1	0
1994	81	2	0	2	0
1995	201	5	1	4	0
1996	161	2	0	2	0
1997	154	6	0	3	3
1998	180	6	0	6	0
1999	193	3	1	2	0
2000	209	4	2	2	0
2001	178	5	3	2	0
2002	190	4	2	1	1
2003	187	2	2	0	0
2004	205	3	1	1	1
Total	2405	56 (2.32)[a]	22 (39.2)[b]	29 (51.7)[b]	5 (8.9)[b]

[a] values are numbers (%) to total deliveries.
[b] values are numbers (%) to breech pregnancies.

PATIENTS AND METHODS

This study included cases of singleton breech presentation that were delivered after 37 weeks of gestation during the period between January 1989 and December 2003 at Motojima General Hospital. 56 cases of singleton breech presentation (38 primiparas, 18 multiparas) were included in this study. The cases were divided into four groups according to the method of delivery : primipara vaginal delivery group (PV-G), multipara vaginal delivery group (MV-G), planned C-section group (PC-G), and emergency C-section group (EC-G). Among the four groups, the following factors were compared and evaluated. The annual rate of breech deliveries, the percentages of primiparas and multiparas, maternal age, gestational week, neonatal birth weight, and the neonatal Apgar scores.

In addition, we studied the difference in the method of breech delivery by the presence or absence of a previous delivery and the reason for emergency C-section. Table 1 shows the criteria for trying a vaginal delivery in breech presentation cases used in our department.

After the risks of the different delivery methods were explained, the method of delivery was decided by the physicians and the patient. Informed consent for the planned method of delivery was obtained.

Statistical analysis ; Data were analyzed using the

Table 3 Comparison of breech delivery methods by presence or absence of previous deliveries.

	Primipara breech Delivery (N = 38)	Multipara breech Delivery (N = 18)	*p*
Vagina Delivery	13 (34.1)[a]	9 (50.0)	0.243
Planned Cesarean Section	21 (55.3)	8 (44.4)	
Emergency Cesarean Section	4 (10.6)	1 (5.6)	

[a] Values are numbers (%)

χ^2 test. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS II software (version 11.0.1 for Windows ; SPSS, Inc., Chicago, IL) .

RESULTS

The total number of deliveries at our hospital during the 15-year period between 1989 and 2003 is shown in Table 2. The annual number of breech presentations was 2-6 cases per year, and the annual rate of breech delivery among the total number of deliveries averaged 2.32%. Among the 56 cases of breech presentation, 22 cases (39.3%) underwent vaginal delivery, 29 cases (51.8%) underwent planned C-section, and 5 cases (8.9%) underwent emergency C-section. Therefore, the number of cases in which vaginal delivery was planned was 27 cases, and the success rate of vaginal deliveries was 81.5% (22/27), which is similar to that observed at other hospitals[5,6].

Compared with the multiparas, the primiparas had a lower rate of vaginal delivery and higher rates of planned C-section and emergency C-section, although the differences were not significant (Table 3).

The reasons for emergency C-section in the 5 cases are shown in Table 4. Among the 5 cases who underwent emergency C-section, 3 cases had an unsuccessful vaginal delivery because of prolonged labor. 1 case had a change in presentation from breech presentation to footling presentation during the delivery, and 1 case showed variable deceleration of the fetal heart rate on electronic fetal heart rate monitoring. Among the 5 cases, 1 case had a neonatal Apgar score at 1 minute of less than 6 points. Although, at 5 minutes, all five cases had an Apgar score above 8 points. There were no complications during the C-section. The length of time between the time point at which it was decided to perform C-section and the time of delivery was within 30 minutes in all five cases.

Table 4 Causes for emergency cesarean section

(1) Unsuccessful of vaginal delivery : 3cases
Prolonged labor of the first stage ; 2 cases
Prolonged labor of the second stage ; 1case
(2) Variable deceleration : 1 case
(3) Change of presentation during delivery : 1 case
breech presentation → footling presentation

Table 5 shows the maternal profiles and neonatal prognosis in the different breech delivery methods. There were no significant differences in the maternal age, gestational week, nor neonatal birth weight among the four groups.

The length of the delivery time was significantly shorter in the MV-G than in the PV-G.

A neonatal Apgar score at 1 minute of less than 6 points was not seen in the PC-G, but was seen in 15.3% of the PV-G, 11.1% of the MV-G, and 20% of the EC-G.

As for the Apgar score at 5 minutes, no neonate in this study had a score less than 6 points.

Table 5 Comparison of maternal profiles and neonatal prognosis in each method of breech delivery.

	Primipara Vaginal Delivery	Multipara vaginal delivery	Planned cesarean section	Emergency cesarean section	*p*
Maternal age (mean age)	28.0	30.7	30.3	27.1	
Gestation length (mean weeks, day)	39 w 4 d	38 w 1d	37 w 6 d	38 w 3 d	
Length of delivery (mean hours minutes)	10 h 44 m	6 h 2 m	.....	.....	0.01
Neonatal birth weight (mean grams)	3068	3076	2968	3016	
Apgar score less than 6 points (at one minute)	2	1	0	1	*0.04
Apgar score less than 6 points (at five minute)	0	0	0	0	

* compared to data of primipara vaginal delivery and planned cesarean section

DISCUSSION

In our study, the number of neonates with an Apgar score at 1 minute of less than 6 points was 2 cases (15.3 %) in the PV-G, 1 case (11.1 %) in the MV-G, and no case in the PC-G. However, no case in the vaginal delivery groups had an Apgar score at 5 minutes of less than 6 points. The neonatal Apgar scores of the PV-G and MV-G did not significantly differ. It is suggested that the neonatal prognosis after vaginal delivery of breech presentation in primiparas is not worse than that in multiparas. If the criteria for vaginal delivery trial are fulfilled, vaginal delivery of breech presentation in primiparas is fully possible. There were no cases with an apger score at 5 minutes of less than 6 points in all groups. It is therefore suggested that the neonatal prognosis after a planned C-section is not necessarily better than that after a vaginal delivery for breech presentation.

Further studies are needed to determine whether vaginal delivery or planned C-section for breech presentation results in better neonatal prognosis. In a recent study[7], 2,088 cases with frank or complete breech presentation after 37 weeks of gestation who fulfilled vaginal breech delivery criteria were randomly divided into two groups, the planned C-section group and vaginal delivery group, and the maternal and neonatal prognoses were compared ; the study found that neonatal morbidity and mortality in the perinatal period were significantly lower in the planned C-section group than in the vaginal delivery group. There were no significant differences in the rates of maternal morbidity and mortality between the two groups. Therefore, the authors concluded that it was desirable to choose planned C-section for singleton breech presentation after 37 weeks of gestation. However, estimation of fetal body weight by ultrasonography was performed in only 60 % of the cases in their study. As for assessment of the pelvis, clinical evaluation alone was performed in 90 % of the cases and x-ray, MRI, or CT was performed in only 10 % of the cases. The management of breech presentation is not sufficient compared to the numerous other facilities. It cannot be denied that there was a possibility that vaginal delivery was tried with having overlooked the cases which were unsuitable for vaginal breech delivery.

Some studies reported that there was no significant difference in neonatal prognosis between the vaginal delivery and planned C-section groups for breech presentation, and that the rate of maternal complications was significantly higher in the planned C-section group[5,6]. In studies in which vaginal delivery was performed for breech presentation under the following criteria, i.e., delivery at term or near term, estimated fetal body weight of 2,000 – 4,000 grams, pelvic diameter was certain size, simple breech presentation and no fetal head overextension, the perinatal mortality was 0 % and the rate of serious morbidity was 0 – 1.2 %[8,9].

Umbilical cord prolapse is seen in 4-7 % of all breech presentations [2]. However, umbilical cord relapse was seen in about 0.4 % of frank breech presentation cases, which was similar to the rate among vertex presentation cases [10]. There is no consensus at present as to whether planned C-section should be performed for breech presentation or whether vaginal delivery can be performed if the criteria are fulfilled.

In our study, there was no significant difference in the short-term prognosis of infants who had been born by vaginal delivery and infants who had been born by planned C-section. In a study that followed infants for over two years, infants who had been born without deformity by vaginal simple breech delivery did not show increased risk of neural damage compared with infants with vertex presentation [11]. A study reported that there were no significant differences in the rates of serious physical damage, mental development damage, nor neurological damage in long-term prognosis between infants who had been born by planned C-section and infants who had been born by vaginal delivery for breech presentation [12].

There are some limitations in our study. The number of cases in our study was small compared with that in other reports, and long-term follow-up of the infants was not performed. It is necessary to study a larger number of cases of breech presentation and to perform long-term follow-up of such children.

Our data are of assistance in counseling women with breech presentation at term and in advising a trial of labor and vaginal delivery if that is the preference of the mother. Our data may help balance the relative safety of selected breech delivery for the infant against the potential maternal risks of cesarean delivery.

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妊婦HIVスクリーニングの実態と問題点

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はじめに

我が国におけるHIV感染は近年増加傾向にあり，この傾向は先進国の中では唯一の例外である．隣国の中国ではエイズ患者の急増にその対策が追いつかず，「棄民政策」さえ台頭しつつある（中日育児シンポジウム，2004年9月，西安）．我が国でも女性感染者の増加が顕著であり，感染妊婦と母子感染の急増が危惧される．我々は厚生労働省のエイズ対策研究事業の研究班としてHIV母子感染ゼロを目指して，①周産期におけるHIV感染対策の現状把握，②日本の国情に合致した最も有効な母子感染防止対策の確立と標準化，③HIV母子感染及びその対策に関する医療関係者のみならず一般国民に対する啓発教育・広報活動の推進を一貫して行ってきた．

①周産期におけるHIV感染対策の現状把握については，a）妊婦HIVスクリーニングの実施状況の一次，二次アンケート調査研究（産科施設1,570，小児科施設3,142）（和田分担），b）HIV感染妊婦並びにその出生児の後方視的調査研究（喜多，外川分担），c）HIV母子感染予防対策未施行例の社会疫学的解析と予防対策に関する研究（戸谷分担），d）妊婦HIVスクリーニング検査における偽陽性率の検討と陽性例への対応（塚原分担）などを後方視的または前方視的に調査検討を実施した．

本稿では当班の平成15年，16年度研究報告書の成績をもとに妊婦HIVスクリーニングの実態とその問題点について以下述べたい．

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1．妊婦HIVスクリーニングの実態

当班は，平成11年度より厚労省編「全国病院便覧」に記載されている産科または産婦人科を標榜する施設のうち個人の開設するものを除く

表1　都道府県別HIVスクリーニング検査実施率

都道府県	分娩件数	HIV検査件数	HIV検査率
山梨	2,690	2,690	100.0％
滋賀	2,762	2,762	100.0％
埼玉	19,606	19,602	100.0％
三重	5,793	5,789	99.9％
静岡	12,327	12,309	99.9％
奈良	3,440	3,432	99.8％
新潟	10,349	10,285	99.4％
長野	10,197	10,119	99.2％
京都	10,116	10,033	99.2％
石川	5,233	5,168	98.8％
茨城	11,496	11,330	98.6％
群馬	6,085	5,955	97.9％
福島	8,358	8,158	97.6％
愛媛	4,121	3,986	96.7％
宮城	7,993	7,716	96.5％
岡山	7,112	6,819	95.9％
大阪	38,159	36,497	95.6％
千葉	10,040	9,595	95.6％
栃木	4,916	4,668	95.0％
兵庫	18,631	17,532	94.1％
富山	3,725	3,479	93.4％
東京	43,696	40,593	92.9％
神奈川	31,789	29,378	92.4％
岐阜	5,871	5,411	92.2％
佐賀	1,598	1,465	91.7％
鹿児島	6,417	5,779	90.1％
高知	2,584	2,308	89.3％
北海道	21,106	18,669	88.5％
香川	4,329	3,828	88.4％
愛知	28,213	24,943	88.4％
広島	10,900	9,617	88.2％
秋田	5,755	5,068	88.1％
福井	3,532	2,940	83.2％
和歌山	3,813	3,117	81.7％
徳島	2,423	1,948	80.4％
山形	6,355	5,073	79.8％
大分	2,514	1,956	77.8％
山口	5,199	3,928	75.6％
沖縄	7,423	5,552	74.8％
岩手	5,674	4,238	74.7％
青森	4,405	3,231	73.3％
熊本	6,342	4,651	73.3％
島根	3,429	2,438	71.1％
福岡	9,933	6,813	68.6％
長崎	4,512	3,064	67.9％
鳥取	2,191	1,318	60.2％
宮崎	2,124	1,144	53.9％
全国	435,276	396,394	91.1％

1,570施設に対して，妊婦HIV検査率について調査を行ってきた．今年度は1,557件の有効送付数に対して最終回答数は1,168件で，最終回答率は75.0％であった．質問項目は次の5点である．①昨年度全国調査（平成15年10月）以後に診療し，本調査に未報告のHIV感染妊婦数，②昨年度全国調査（平成15年10月）以前に診療し，本調査に未報告または報告したかどうか不明のHIV感染妊婦数，③妊婦に対するHIV抗体検査の実施率，④平成14年1月から12月までの分娩件数，⑤回答者氏名と医療機関名．上記質問に対しての有効回答の統計学的解析を行った．

送付数は1,570件であり，回収数は843件，回収率54.0％，産婦人科廃止等による返送は10施設であり，有効送付数1,560件であった．「回収率」とは，送付数に対しての本研究班に戻ってきた葉書の数から算出したものであり，「回答率」とは，送付数から産婦人科廃止等で返信された葉書の枚数を差し引いたもの（有効件数）に対して回答のあった葉書の数から算出したものである．有効回答率は56.2％で，都道府県別有効回答率は83.3％（大分県）～10.4％（兵庫県）に分布した．回答率にばらつきがあり，さらに回答率が低かったために，平成15年11月10日に未回答施設に対して再調査を行った．最終的に有効回答率は本年は75.0％（昨年比0.2％減）に達し，都道府県別有効回答率は91.7％（徳島県）～55.6％（栃木県）であった．本調査では日本全国での年間分娩件数1,153,660件（平成15年：母子保健の主なる統計－平成15年度刊行－編集：財団法人母子衛生研究会，発行：母子保健事業団，東京）のうち病院調査で435,276人（37.7％，昨年比1.1％減）の妊婦を捕捉したことになる．

妊婦HIVスクリーニング実施率は，「各病院での分娩件数」×「各病院でのHIVスクリーニング検査実施率」＝「各病院での検査件数」，「総検査件数」÷「総分娩件数」×100＝「検査率（％）」とした．検査率は全国平均で91.1％（昨年比1.4％増）であった（表1）．もっとも検査率の高かった県は山梨県，滋賀県で100.0％，もっとも検査率の低かった県は宮崎県で53.9％であった．依然として地域差があるが，その差が縮小される

表2 都道府県別HIVスクリーニング検査実施率の年次推移

都道府県	抗体検査率						昨年度比	11年度比
	16年度	15年度	14年度	13年度	12年度	11年度		
佐賀	91.7%	91.8%	33.9%	0.1%	0.1%	2.3%	−0.1%	89.4%
沖縄	74.8%	72.0%	36.8%	30.3%	6.3%	5.1%	2.8%	69.7%
和歌山	81.7%	85.1%	67.7%	48.9%	34.5%	13.9%	−3.4%	67.8%
島根	71.1%	57.5%	42.8%	21.3%	20.5%	17.6%	13.6%	53.5%
愛媛	96.7%	95.2%	61.4%	73.1%	40.8%	45.6%	1.5%	51.1%
高知	89.3%	78.7%	47.2%	53.9%	33.4%	40.0%	10.6%	49.3%
大分	77.8%	68.3%	50.2%	74.6%	31.0%	31.2%	9.5%	46.6%
山口	75.6%	70.4%	64.6%	38.0%	32.2%	29.9%	5.2%	45.7%
山形	79.8%	74.6%	66.3%	64.2%	49.7%	34.5%	5.2%	45.3%
香川	88.4%	93.2%	84.1%	76.9%	45.8%	44.2%	−4.8%	44.2%
徳島	80.4%	85.3%	79.3%	50.3%	50.1%	37.9%	−4.9%	42.5%
福岡	68.6%	56.9%	40.5%	34.8%	36.0%	32.7%	11.7%	35.9%
兵庫	94.1%	84.1%	80.0%	68.9%	73.0%	58.5%	10.0%	35.6%
鹿児島	90.1%	88.2%	88.9%	85.6%	71.6%	55.2%	1.9%	34.9%
奈良	99.8%	94.0%	87.1%	96.4%	85.2%	68.7%	5.8%	31.1%
岡山	95.9%	85.9%	85.2%	75.8%	69.2%	66.6%	10.0%	29.3%
岩手	74.7%	59.5%	58.3%	58.9%	56.6%	46.9%	15.2%	27.8%
滋賀	100.0%	98.0%	76.7%	71.5%	75.6%	73.0%	2.0%	27.0%
北海道	88.5%	81.9%	79.9%	71.5%	69.8%	64.0%	6.6%	24.5%
熊本	73.3%	83.7%	68.5%	68.0%	60.8%	49.7%	−10.4%	23.6%
広島	88.2%	83.3%	78.6%	81.1%	76.8%	65.0%	4.9%	23.2%
秋田	88.1%	95.5%	96.0%	68.9%	72.1%	65.0%	−7.4%	23.1%
大阪	95.6%	93.4%	87.0%	81.1%	83.3%	74.0%	2.2%	21.6%
栃木	95.0%	99.6%	99.3%	87.4%	90.2%	75.0%	−4.6%	20.0%
宮崎	53.9%	48.6%	32.5%	47.0%	22.0%	34.0%	5.3%	19.9%
福井	83.2%	100.0%	75.6%	54.1%	71.7%	65.3%	−16.8%	17.9%
京都	99.2%	89.1%	94.5%	95.1%	91.5%	81.4%	10.1%	17.8%
長野	99.2%	98.3%	97.4%	95.1%	98.4%	82.8%	0.9%	16.4%
三重	99.9%	91.3%	93.9%	90.8%	96.5%	83.6%	8.6%	16.3%
愛知	88.4%	95.0%	89.9%	90.9%	83.6%	73.8%	−6.6%	14.6%
長崎	67.9%	58.1%	58.8%	59.7%	56.5%	55.2%	9.8%	12.7%
富山	93.4%	90.5%	89.3%	81.3%	79.4%	80.7%	2.9%	12.7%
岐阜	92.2%	93.3%	97.0%	94.9%	97.0%	80.6%	−1.1%	11.6%
静岡	99.9%	100.0%	100.0%	98.4%	98.4%	88.4%	−0.1%	11.5%
新潟	99.4%	99.1%	99.5%	99.9%	95.1%	88.4%	0.3%	11.0%
群馬	97.9%	97.7%	98.9%	94.2%	95.7%	87.1%	0.2%	10.8%
石川	98.8%	98.7%	94.9%	97.3%	92.1%	89.3%	0.1%	9.5%
鳥取	60.2%	44.2%	52.2%	49.6%	59.6%	52.2%	16.0%	8.0%
福島	97.6%	98.6%	99.5%	92.8%	96.0%	89.9%	−1.0%	7.7%
茨城	98.6%	98.3%	98.7%	98.4%	94.7%	91.2%	0.3%	7.4%
山梨	100.0%	100.0%	99.9%	100.0%	95.7%	94.8%	0.0%	5.2%
宮城	96.5%	95.4%	88.8%	95.7%	95.1%	91.5%	1.1%	5.0%
東京	92.9%	95.2%	93.8%	96.5%	91.5%	88.8%	−2.3%	4.1%
埼玉	100.0%	99.5%	99.0%	99.1%	99.6%	96.1%	0.5%	3.9%
千葉	95.6%	98.7%	95.0%	98.6%	97.5%	95.1%	−3.1%	0.5%
神奈川	92.4%	96.8%	96.0%	95.8%	97.0%	93.1%	−4.4%	−0.7%
青森	73.3%	57.7%	41.1%	42.6%	69.0%	87.8%	15.6%	−14.5%
全国	91.1%	89.7%	85.0%	82.6%	79.7%	73.2%	1.4%	17.9%

傾向にあることが明らかになった．昨年比で10%以上検査率が上昇した府県は，鳥取県（16.0%増），青森県（15.6%増），岩手県（15.2%増），島根県（13.6%増），福岡県（11.7%増），高知県（10.6%増），京都府（10.1%増），兵庫県（10.0%増），岡山県（10.0%増）（昨年比）の9府県であった（**表2**）．また，調査を開始した平成11年度との比較で30%以上検査率が上昇し

表3　病院区分別HIVスクリーニング検査実施率（エイズ拠点病院・大学病院）

区分	有効送付数	回答数	回答率	分娩件数	検査件数	検査率	未実施施設数	未実施施設率
拠点病院	309	257	83.2％	121,612	116,049	95.4％	9	3.5％
拠点病院以外	1,248	911	73.0％	313,664	280,345	89.4％	93	10.2％
合計	1,557	1,168	75.0％	435,276	396,394	91.1％	102	8.7％
大学病院	112	101	90.2％	38,612	36,768	95.2％	4	4.0％
大学病院以外	1,445	1,067	73.8％	396,664	359,626	90.7％	98	9.2％
合計	1,557	1,168	75.0％	435,276	396,394	91.1％	102	8.7％

た県は，佐賀県（89.4％増），沖縄県（69.7％増），和歌山県（67.8％増），島根県（53.5％増），愛媛県（51.1％増），高知県（49.3％増），大分県（46.6％増），山口県（45.7％増），山形県（45.3％増），香川県（44.2％増），徳島県（42.5％増），福岡県（35.9％増），兵庫県（35.6％増），鹿児島県（34.9％増），奈良県（31.1％増）（11年度比）の15県であった．調査を開始した平成11年度との比較では，47都道府県で青森県と神奈川県を除く45都道府県で検査率が上昇していた．昨年比で検査率が減少していたのは1都14県あった．このうち1都12県は10％未満の変動であり，さらにこのうちの1都10県は5％未満の変動であった．福井県では，昨年度比で16.8％の検査率の減少が見られた．昨年度と今年度ともに回答した施設は福井県内で9施設あったが，このうち1施設で昨年は全例にHIVスクリーニング検査を行っていたが，今年は1例も検査を行わなかった施設があったため，県内の検査率が大きく減少したと考えられた．他の8施設では昨年と今年で検査率に変化はなかった．青森県では平成11年度調査開始以降，検査率が減少し続けていたが（41.1％：平成14年度調査），平成15年度調査以降検査率が上昇に転じた（73.3％：平成16年度調査）．青森県は，平成11年4月より県によるHIV抗体検査の公的補助を中止したため検査率が急激に減少したが，検査率の減少に歯止めがかかったと推測する．千葉県でも平成15年に県の全額公費負担を中止しているが，検査率は平成11年度調査開始以来95％以上で推移している（**表2**）．

全国平均検査率（91.1％）を上回る県はほとんど関東・甲信越，東海・北陸，近畿ブロックに所属する県であった．ブロック別の検査率は，北海道・東北ブロックで87.4％，関東・甲信越ブロックで95.6％，東海・北陸ブロックで92.8％，近畿ブロックで95.4％，中国・四国ブロックで85.6％，九州ブロックで74.5％であった．昨年比では，北海道・東北，近畿，中国・四国，九州の各ブロックで約5％程度検査率が上昇したが，関東・甲信越，東海・北陸ブロックでは，それぞれ1.7％，2.7％検査率が減少していた．平成11年度では，関東・甲信越ブロックと九州ブロックで52.9％の差があったのに対し，今年度では21.1％にまで差が縮小していた（平成11年度「厚生省HIV感染症の疫学研究班・母子感染に関する研究グループ」報告書および平成12～14年度「厚生労働省　妊産婦のSTD及びHIV陽性率と妊婦のSTD及びHIVの出生児に与える影響に関する研究班・HIV母子感染予防の臨床的研究班」報告書の数値を含む）．

拠点病院・拠点病院以外の病院との区別によるHIVスクリーニング検査率を**表3**に示す．回答率は拠点病院で約10％上回っていた．検査率は拠点病院で95.4％，拠点病院以外の病院で89.4％であり，その差は6.0％であった．拠点病院では回答のあった257施設中9施設（3.5％）でまったく検査を行っていなかった．大学病院・大学