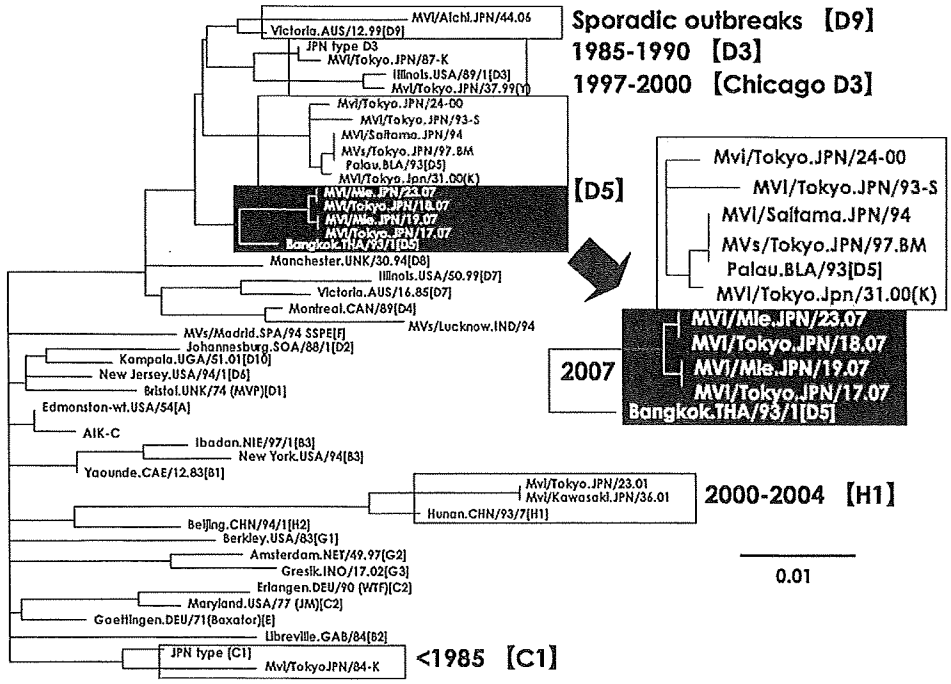


Fig. 2. Phylogenetic analysis of measles virus in the H gene. Strains in gray square are Palau type D5 and those in black square Bangkok type D5.

adjusted to 100 TCID50 and the results are shown in Figure 3. There was no significant difference in NT antibody titers within fourfold dilutions against the different genotypes of A, Palau type D5, Bangkok type D5, and D9.

### Characteristics of Wild-Type Measles Virus

Seven wild-type measles virus strains were isolated and the current wild type did not induce cell fusion in Vero cells. D5 and D9 strains infected and replicated in Vero cells without demonstrating typical cell fusion. Infectious titers in Vero cell culture fluid detected 7 days after infection were similar or 1/2 or1/3 lower than those in the B95a cell culture (data not shown).

Measles virus grew well at 35–37°C and virus growth at 39°C differs from strain to strain [Nakayama et al., 2003]. Virus growth of MVi/Aichi.JPN/44.06 at different temperatures of 33, 35, 37, and 39°C was investigated in B95a cells, and the results are shown in Figure 4. It grew well at 33, 35, and 37°C, but the infective titers at 39°C decreased to approximately 1/100 in comparison with those observed at 33 or 35°C. Three D5 strains isolated in 2007 and MVi/Tokyo.JPN/93-S [Palau type D5] were cultured in B95a cells at 35°C and 39°C and the infective titers on day 7 of culture are also shown in Figure 4. As previously reported, MVi/Tokyo.JPN/93-S grew well at 39°C as it did at 35°C, but the infectivity of the Bangkok type D5 was lower at 39°C than at 35°C.

## DISCUSSION

The WHO global measles and rubella laboratory network (LabNet) has been established and the Western Pacific Region adopted the goal of measles elimination by increasing the use of laboratory testing as an integral component of its surveillance. They recommended that effective surveillance comprised laboratory confirmation by the detection of IgM antibody in single serum, together with virus isolation for genotyping. However, the sensitivity of virus isolation is low and depends on the timing of sample collection and transporting conditions. The detection of measles virus-specific IgM antibodies in serum is a standard serological diagnostic

TABLE II. Nucleotide and Amino Acid Differences in D5 and D9 Strains

| | AIK-C | Palau | Bangkok | Tokyo, 2000 | Tokyo, 2007 | Aichi, 2006 |
|---|---|---|---|---|---|---|
| AIK-C | | 52 (2.8%) | 53 (2.9%) | 59 (3.2%) | 57 (3.1%) | 66 (3.6%) |
| Palau | 16 (2.6%) | | 25 (1.3%) | 10 (0.5%) | 35 (1.9%) | 45 (2.4%) |
| Bangkok | 18 (2.9%) | 8 (1.3%) | | 33 (1.8%) | 16 (0.9%) | 39 (2.1%) |
| Tokyo.JPN/2000 | 17 (2.8%) | 5 (0.8%) | 11 (1.8%) | | 43 (2.3%) | 54 (2.9%) |
| Tokyo.JPN/17.07 | 16 (2.6%) | 8 (1.3%) | 4 (0.7%) | 11 (1.8%) | | 50 (2.7%) |
| Aichi.JPN/44.06 | 19 (3.1%) | 11 (1.8%) | 10 (1.6%) | 14 (2.3%) | 10 (1.6%) | |

Values present diagonally below in the table body indicate amino acid differences and the values present diagonally above in the table body indicate nucleotide differences.

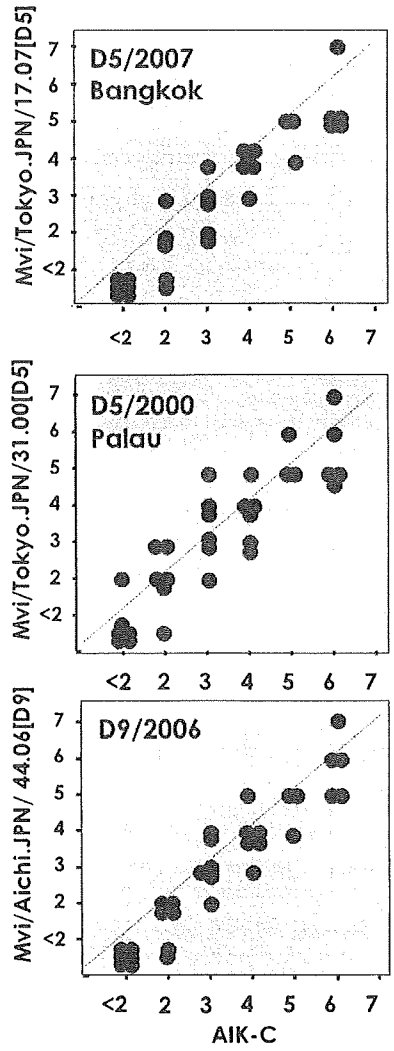


Fig. 3. Neutralization test antibodies against the AIK-C vaccine strain (genotype A), MVi/Tokyo.JPN/17.07 (genotype D5 Bangkok type), MVi/Tokyo.JPN/31.00 (genotype D5 Palau type), and MVi/Aichi.JPN/44.06 (genotype D9).

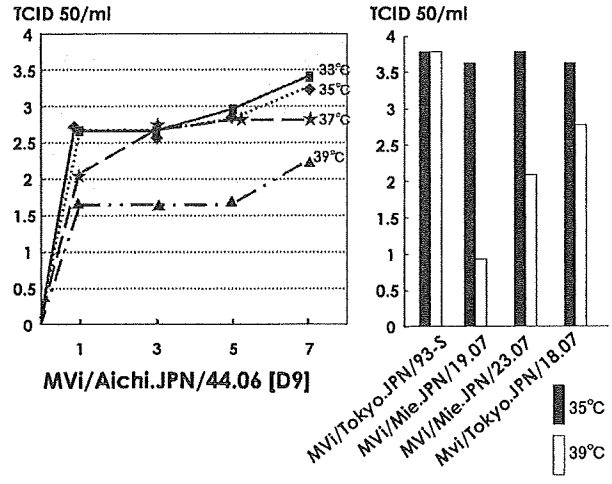


Fig. 4. Virus growth at different temperatures of 33, 35, 37, and 39°C.

method when serum samples are obtained on day 4 or 5 of rash onset and later [WHO, 2005b]. These methods were effective in the case of the primary infection causing typical measles illness. However, in the case of modified or subclinical measles, the detection rate of serum IgM antibodies was reported to be extremely low and the genomic detection by RT-PCR should be examined [Mosquera et al., 2005]. In this report, serum IgM antibody against measles virus was initially examined in several patients, but it was negative because all patients visited the clinics within day 1 or 2 after rash onset. In addition, the patients over 15 years of age having a single dose of measles immunization demonstrated very mild febrile illness with eruptions. Among 18 patients, 12 were diagnosed by RT-LAMP. The remaining six patients were not confirmed, because paired sera were obtained rarely from adults with mild symptoms. Detection of the measles genome is reliable and sensitive method and these six patients were supposed to have some other virus infections.

van den Hof et al. [2003] examined 791 serum samples from Dutch persons aged 2–49 years. The sero-prevalence and mean titers of measles EIA and NT were lower in the vaccinated populations than in older individuals infected naturally, and vaccine-acquired immunity weakened year by year. Glass and Grenfell [2004] developed a mathematical model to predict clinical and subclinical measles cases. Vaccination levels dropped from >90% in the 1990s to 84% in 2001/2002 in England, and they considered that clinical measles cases would remain fairly stable over time if vaccine coverage is maintained at 90%, but that there would be a pronounced increase in the numbers of clinical cases if vaccine coverage is around 84% or below. In any case, they predicted an increase in the number of subclinical measles. In the measles outbreak in Japan 2007–2008, a large proportion of cases of measles comprised adults or teenagers with very mild illness, and many cases went undiagnosed. These cases were confirmed by detection of the measles genome in this study and the diagnostic approach is now combining genome detection with virus isolation and the detection of IgM antibodies [CDC, 2008c].

Circulating wild-type measles virus genotypes have been investigated since 1984 and dynamic changes in the major circulating genotype have been reported [Nakayama et al., 1995; Yamaguchi, 1997; Zhou et al., 2003; Nakayama et al., 2004; Okafuji et al., 2006]. The dominant circulating genotypes changed drastically in large outbreaks in 1984, 1987–1988, 1991–1993, and 2001–2002 and each outbreak was caused by a different genotype in Japan, as summarized in Figure 5. Genotype C1 was an indigenous strain for a long period before 1985, D3 was involved in the 1987–1988 outbreak, and D5 in 1990–1993. The genotype of measles virus was studied in India from 1994 to 1997. The indigenous strain in India was D4, and a large outbreak was observed in 1997, caused by the Chicago type D3 strain [Nakayama et al., 2004]. In 1997, the Chicago type D3 was isolated in Japan, which was a different cluster from

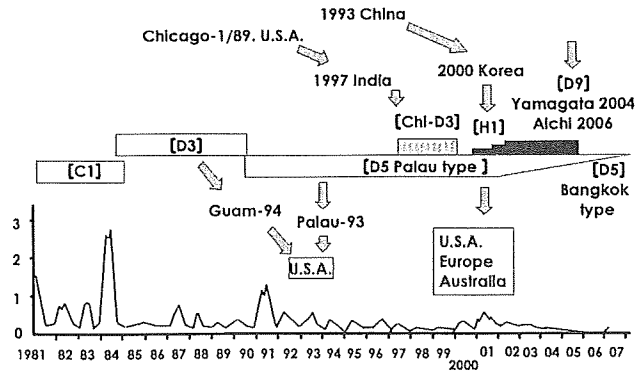


Fig. 5. Major circulating measles genotypes since 1984.

the D3 strains in 1987–1988. The Chicago-type D3 in 1997–1999 was supposed to have been imported from outside, and it was replaced by D5 in 2000, similar to the previous Palau type D5 before 1997. The H1 genotype was isolated from adult measles patients, and this genotype became a dominant strain after 2000 [Zhou et al., 2003]. In 2004, a sporadic outbreak was reported at a junior high school in Yamagata Prefecture and several measles strains were isolated. They were identified as genotype D9, which had not previously been reported in Japan [Mizuta et al., 2005]. In 2005, sporadic outbreaks were reported in Chiba and Ibaraki Prefectures, caused by the Palau type D5. In Asian countries, several different indigenous genotypes have been reported: H1 in China [Xu et al., 1998], G2 in Indonesia and Malaysia [Rota et al., 2000], D4 in India [Wairagkar et al., 2002], D3 in Papua New Guinea [Miki et al., 2002], and D7 in Australia [Chibo et al., 2003]. D8 in Nepal [Truong et al., 2001] and D9 in Australia [Chibo et al., 2003] were also reported. The sporadic outbreak caused by D9 in Yamagata in 2004 was suggested to have been imported from outside. In this study, D9 was also isolated in a sporadic outbreak in Aichi Prefecture in 2006, and seven nucleotide differences in the partial N gene (7/456: 1.5%) were observed between the two D9 strains of the MVi/Yamagata.JPN/7.04 and MVi/Aichi.JPN/44.06. There was no information on the isolation of D9 during 2004–2006 and no epidemiological linkage. Thus, they seemed to have been imported independently from other Asian counties, considering the narrow transmission chain in Japan.

The Palau type D5 was an indigenous strain since 1990 in Japan but was interrupted by outbreaks of the Chicago type D3 from 1997 to 2000 and of H1 from 2001 to 2004. Genotype D5 was detected in a sporadic outbreak in 2006 around Tokyo and transmitted to Okinawa, which was identified as the Bangkok type D5, which was different from the Palau type circulating during1990–2005 in Japan [National Institute of Infectious Diseases, 2007; Morita et al., 2007]. The Bangkok type D5 isolated in Japan in 2007 would have been imported from other Asian countries rather than being a result of the accumulation of mutations of the indigenous Palau type D5. Similar Bangkok type D5 strains (MVi/Queensland.AU/37.03, MVi/Maldives. MAL/32.05, MVs/Phnom Penh.KHM/19.02, MVs/Taichung. TWN/45.03) were reported in Asian countries and also in the UK (MVs/Chelmsford.GBR/30.07).

In 2007 and 2008, similar strains were reported in Europe and the USA and epidemiological linkage to the outbreak in Switzerland was identified [Delaportel et al., 2007; Richard et al., 2008; CDC, 2008b]. In this report they were thought to have been transmitted from Japan, demonstrating high-level sequence homology to Japanese isolates. Asia is the major reservoir of the wild measles virus and several Asian genotypes have been detected in the USA. and Europe. Global knowledge on the distribution of genotypes is imperative in identifying the geographical regions where more aggressive vaccination campaigns should be implemented in order to eliminate measles.

Minor antigenic changes in the Chicago type D3 and H1 strains were reported in comparison with the NT titers against the AIK-C vaccine strain [Zhou et al., 2003; Nakayama et al., 2004]. In this study, 1.3–1.8% amino acid differences were observed between the D5 Bangkok and Palau types. Therefore, the difference in the antigenicity was investigated and there were no significant differences in NT antibody titers against the Bangkok type D5, Palau type D5, and D9 strains in comparison with those against the AIK-C vaccine strain. No significant difference was demonstrated in terms of antigenicity. Some virus strains of the Chicago type D3 and Palau type D5 grew as well at 39°C as they did at 33, 35, and 37°C. However, the Bangkok type D5 and D9 showed poor growth at 39°C. The responsible genomic region(s) for virus growth at different temperatures is now under investigation.

Recommendations to prevent further transmission from the index case of importation noted that the outbreak investigation was conducted when the index case of a Japanese boy had a measles-like illness and only six additional cases were identified [CDC, 2008a]. Transmission was interrupted because of the high vaccination coverage rates of two-dose MMR among children and adolescents. The prompt response was sustained by strong and effective surveillance systems. The WHO has recommended that all children should be provided with a second opportunity for measles vaccination [WHO, 2006]. This second opportunity is scheduled just before entry to primary school in most countries, and a two-dose schedule of combined measles–rubella vaccine was launched in 2006 in Japan. The scheduled timing of the two doses is at the age of 12–24 months and 5–6 years. Supplemental immunization for the other populations was not considered, and most school children (over 8 years of age) did not have the benefit of the two-dose schedule. Okafuji et al. [2006] reported that NT antibodies decreased to undetectable levels in approximately 10% of vaccine recipients 6–7 years after vaccination when the measles outbreak was controlled. The outbreak in Japan 2007 highlighted the inadequacy of the immunization strategy, leading to a gap in immunization among teenagers without

supplemental immunization. Additionally, several had no immunization history because of a distrust of the vaccine due to the MMR scandal from 1989 to 1993 in Japan [Ueda et al., 1995]. All school children and young teenagers should have a second dose of measles vaccine to attain the goal of measles elimination. Thus, the Japanese government has decided to launch a catch-up campaign targeting young teenager at 13 and 18 years of age for the next 5 years, anticipating elimination of measles by 2012. The infrastructure for the surveillance system based on laboratory-based diagnosis is now in preparation.

## ACKNOWLEDGMENTS

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# A Long-Term Survey on the Distribution of the Human Rotavirus G Type in Thailand

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The distribution of the G type of human rotavirus was surveyed in Thailand between July 1993 and June 2007. A significant yearly change in the distribution of the G type distribution was found. From 1993–1994 to 1998–1999, the G1 type was the most dominant. In 1999–2000, G9 began to appear at a high frequency. In 2000–2001, 2001–2002, and 2002–2003, G9 was very common. In 2003–2004, G1 became the most prevalent type again, and since then it has been detected at the highest frequency. G12 strains, which were first detected in 1998–1999, were also found in 2004–2005 and 2006–2007. The G4 and G3 types were moderately prevalent in 2001–2002 and 2004–2005, respectively. Nucleotide sequence analysis of the VP7 genes of the G9 and G12 strains which reemerged in Thailand showed that they were each similar to the contemporary strains in other countries. ***J. Med. Virol. 82:157–163, 2010.*** © 2009 Wiley-Liss, Inc.

**KEY WORDS:** rotavirus; G type; Thailand; sequence analysis; VP7

## INTRODUCTION

Group A human rotavirus is the most common etiologic agent of severe gastroenteritis in infants and young children worldwide. It has been estimated that all children will be infected at least once by the age of 5 years, and that rotavirus is responsible globally for ~600,000 deaths each year, mostly in developing countries [Parashar et al., 2006]. In both developed and developing countries, rotavirus infection leads to a high rate of hospital admission related to dehydration.

Rotaviruses possess a genome comprising 11 double-stranded RNA (dsRNA) segments, enclosed in a triple-layered protein capsid [Estes and Kapikian, 2007]. The outermost layer is composed of two proteins, VP7 and VP4, which are associated with the G type and P type, respectively. At least 15 different G types (G1–G15) and 26 P types (P[1]–P[26]) have been found in humans and animals. Among them, 11 G types (G1–G6, G8–G11, and G12) and 10 P types (P[3]–P[6], P[8]–P[11], P[14], and P[19]) have been isolated from humans. The common G types worldwide are G1–G4, and G9 [Gentsch et al., 1996; Santos and Hoshino, 2005]. However, the distribution of the G type varies each year, and a distinct G type distribution has been found in different countries. Knowledge of the prevalence of the G type in each country has become more relevant, since two types of human rotavirus vaccine, RotaTeq (Merck & Co., Inc., Whitehouse Station, NJ) and Rotarix (GlaxoSmithKline Biologicals, Rixensart, Belgium), were developed in 2006 [Ruiz-Palacios et al., 2006; Vesikari et al., 2006]. The efficacy of both vaccines as to the heterotypic human rotaviruses in circulation is a serious concern. With this background, fundamental data on the distribution of the G type worldwide is important for the prevention of infection with human rotavirus. In many studies, however, surveys on the distribution of the G type have been carried out for a short-term or on small numbers of samples.

In this study, the yearly change of the distribution of the G type of human rotavirus was surveyed long-term and on a large-scale in Thailand between July 1993 and June 2007. Nucleotide sequence analyses of rotavirus strains with the G9 or G12 specificity, which were detected in this study are also described.

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## MATERIALS AND METHODS

### Stool Specimens and Study Sites

A total of 7,452 stool samples were collected from the same or different study sites in each period between July 1993 and June 2007 (1993–2007) in Thailand (Table I). In 1993–1994, 1,150 samples were collected from four hospitals: the Nakorn Ping Hospital in Chiang Mai, the Nakhon Phanom Hospital in Nakhon Panom, the Children's Hospital in Bangkok, and the Hadyai Hospital in the Songkhla Province (near Malaysia), which are located in the northern, northeastern, central, and southern parts of Thailand, respectively. In 1994–1997 and 1999–2000, 359 samples were collected only from the Queen Sirikit National Institute of Child Health in Bangkok. In 1997–1999 and 2000–2001, 593 samples were collected from Nakhon Panom. In 2001–2003, 2,153 samples were collected from six hospitals located throughout Thailand: the Maesod Hospital, Tak Province on the Myanmar border, the Nong Khai Hospital, Nong Khai Province on the Laos border, Chantaburi Hospital, Chanthaburi Province near the Cambodia border, Sa Kaeo Hospital, Sa Kaeo Province on the Cambodia border, and Hadyai Hospital. Between July 2003 and June 2007, 3,197 stool specimens were examined at three or four study sites: the Maesod Hospital, the Nong Khai Hospital, the Chanthaburi Hospital, and the Hadyai Hospital.

### Detection of Rotavirus

All stool specimens were screened for rotavirus by polyacrylamide gel electrophoresis (PAGE) of the segmented rotaviral genome as described previously [Pongsuwanna et al., 1996], since non-group A rotaviruses and picobirnaviruses can also be detected by this method, although PAGE of RNA exhibits relatively low sensitivity. In brief, rotaviral RNA was extracted from stool specimens with a disruption solution comprising sodium dodecyl sulfate (SDS), 2-mercaptoethanol, and EDTA, and then with phenol–chloroform. The RNA was electrophoresed on 10% acrylamide gels (2-mm thick) for 16 hr at 20 mA at room temperature. RNA segments were visualized by silver staining.

### RT-PCR

PCR-typing was undertaken in two steps (first and second amplifications) as described previously [Gouvea et al., 1990; Taniguchi et al., 1992]. In the first amplification, complementary DNA corresponding to the full-length VP7 gene was amplified with a pair of primers for the 3′ and 5′ ends of VP7 genes. The second amplification was carried out using a mixture of primers that are specific to each of six variable regions of the VP7 genes of G1–4, G8, and G9 paired with a primer for the 3′ end of the VP7 gene.

### Nucleotide Sequence Determination

Full-length cDNAs of the VP7 genes of 16 G9 strains and 7 G12 strains were prepared by RT-PCR. Direct sequencing was carried out using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kits (AB Applied Biosystems, Foster City, CA) with an automated sequencer, an ABI Prism 3100 Genetic Analyzer (AB Applied Biosystems). Nucleotide sequences were analyzed for the construction of phylogenic trees using Clustal W 1.8.

### Nucleotide Sequence Accession Numbers

The nucleotide sequence data described in this article for the VP7 genes of Thai rotavirus strains were submitted to the GenBank database and have been assigned the accession numbers: AB436813 (G12 strain MS064-05), AB436814 (G12 strain MS310-06), AB436815 (G12 strain MS329-06), AB436816 (G12 strain MS038-07), AB436817 (G12 strain MS040-07), AB436818 (G12 strain MS041-07), AB436819 (G12 strain MS051-07), AB436820 (G9 strain HRV-205-99), AB436821 (G9 strain HRV00-01), AB436822 (G9 strain HY006-01), AB436823 (G9 strain CHB010-01), AB436824 (G9 strain NK002-01), AB436825 (G9 strain MS057-02), AB436826 (G9 strain HY070-02), AB436827 (G9 strain SK066-02), AB436828 (G9 strain NK018-02), AB436829 (G9 strain CHB058-02), AB436830 (G9 strain HY095-03), AB436831 (G9 strain MS037-03), AB436832 (G9 strain SK041-03), AB436833 (G9 strain NK022-03), AB436834 (G9 strain CHB002-04), and AB436835 (G9 strain NK008-04).

## RESULTS

### Detection of Rotavirus

Of the 7,452 stool specimens screened for rotavirus RNA by PAGE, 2,560 (34.4%) were positive for rotavirus (Table I). The survey of the G type distribution in Thailand since 1988 showed that the incidence of rotavirus was high between September and February, particularly in November, December, and January, the coldest months of the year in Thailand, and that there were few incidences of rotavirus infection in June and July (data not shown), and therefore the year between July of a given year and the next June was considered as one rotavirus season. For example, 1993–1994 means the year between July 1993 and June 1994.

### Distribution of the G Type

In the survey between July 1993 and June 2007, G1 (47.0%) was overall the most dominant G type in Thailand, followed by G9 (22.6%), G2 (9.1%), and G4 (3.5%) (Table I). G1 was the most prevalent from 1993–1994 to 1998–1999 and from 2003–2004 to 2006–2007. G9 appeared in 1999–2000 at a high frequency, and was the most prevalent in 2000–2001, 2001–2002, and 2002–2003, the prevalence rates being 79.2%, 82.6%, and 49.1%, respectively. In 2002–2003, G2 was highly prevalent type (35.8%). G3 was rare in Thailand throughout the years studied, but G3 was a highly prevalent (22.0%) in 2004–2005. G4 showed a high prevalence only in 2001–2002. A very unusual G8 strain was

TABLE I. G Type Distribution of Human Rotavirus in Thailand Between July 1993 and July 2007

| Collection year | Collection sites | No. of specimens collected | No. of specimens positive for rotavirus (positive rate %) | G type (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | G1 | G2 | G3 | G4 | G8 | G9 | G12 | Not determined |
| **1993–1994** | **Total** | **1,150** | **368 (32.0)** | **316 (85.9)** | **7 (1.9)** | **0** | **11 (3.0)** | **0** | **0** | **0** | **34 (9.2)** |
| | Bangkok | 414 | 125 (30.2) | 116 (92.8) | 0 | 0 | 2 (1.8) | 0 | 0 | 0 | 7 (5.6) |
| | Chiang Mai | 200 | 54 (27.0) | 44 (81.5) | 5 (9.3) | 0 | 0 | 0 | 0 | 0 | 5 (9.3) |
| | Nakorn Panom | 119 | 37 (31.1) | 35 (94.6) | 0 | 0 | 2 (5.4) | 0 | 0 | 0 | 0 |
| | Songkhla | 417 | 152 (36.5) | 121 (79.6) | 2 (1.3) | 0 | 7 (4.6) | 0 | 0 | 0 | 22 (14.5) |
| **1994–1995** | **Bangkok** | **163** | **99 (60.7)** | **79 (79.8)** | **4 (4.0)** | **0** | **4 (4.0)** | **0** | **1 (1.0)** | **0** | **11 (11.1)** |
| **1995–1996** | **Bangkok** | **47** | **31 (66.0)** | **26 (83.9)** | **4 (12.9)** | **0** | **1 (3.2)** | **0** | **0** | **0** | **0** |
| **1996–1997** | **Bangkok** | **113** | **51 (45.1)** | **32 (62.8)** | **9 (17.6)** | **0** | **3 (5.9)** | **0** | **0** | **0** | **7 (13.7)** |
| **1997–1998** | **Nakorn Panom** | **147** | **29 (19.7)** | **26 (89.7)** | **1 (3.4)** | **0** | **0** | **0** | **0** | **0** | **2 (6.9)** |
| **1998–1999** | **Nakorn Panom** | **262** | **101 (38.5)** | **91 (90.1)** | **2 (2.0)** | **0** | **0** | **0** | **0** | **1 (1.0)** | **7 (6.9)** |
| **1999–2000** | **Bangkok** | **36** | **14 (38.9)** | **1 (7.1)** | **6 (42.9)** | **0** | **0** | **0** | **5 (35.7)** | **0** | **2 (14.3)** |
| **2000–2001** | **Nakorn Panom** | **184** | **53 (28.8)** | **3 (5.7)** | **0** | **1 (1.9)** | **3 (5.7)** | **0** | **42 (79.2)** | **0** | **4 (7.6)** |
| **2001–2002** | **Total** | **1,061** | **356 (33.6)** | **4 (1.1)** | **0** | **1 (0.3)** | **57 (16.0)** | **0** | **294 (82.6)** | **0** | **0** |
| | Nong Khai | 156 | 62 (39.7) | 0 | 0 | 0 | 1 (0.6) | 0 | 61 (98.4) | 0 | 0 |
| | Chanthaburi | 242 | 73 (30.2) | 2 (2.7) | 0 | 0 | 9 (12.3) | 0 | 62 (84.9) | 0 | 0 |
| | Tak | 271 | 84 (31.0) | 2 (2.4) | 0 | 1 (1.2) | 18 (21.4) | 0 | 63 (75.0) | 0 | 0 |
| | Songkhla | 235 | 80 (34.0) | 0 | 0 | 0 | 29 (36.3) | 0 | 51 (63.8) | 0 | 0 |
| | Sa Kaeo | 157 | 57 (36.3) | 0 | 0 | 0 | 0 | 0 | 57 (100.0) | 0 | 0 |
| **2002–2003** | **Total** | **1,092** | **428 (39.2)** | **2 (0.5)** | **153 (35.8)** | **0** | **2 (0.5)** | **0** | **210 (49.1)** | **0** | **61 (14.3)** |
| | Nong Khai | 250 | 107 (42.8) | 0 | 9 (8.4) | 0 | 2 (1.9) | 0 | 78 (72.9) | 0 | 18 (16.8) |
| | Chanthaburi | 164 | 59 (36.0) | 1 (1.7) | 22 (37.3) | 0 | 0 | 0 | 21 (35.6) | 0 | 15 (25.4) |
| | Tak | 143 | 50 (35.0) | 1 (2.0) | 36 (72.0) | 0 | 0 | 0 | 5 (10.0) | 0 | 8 (16.0) |
| | Songkhla | 343 | 131 (38.2) | 0 | 16 (12.2) | 0 | 0 | 0 | 105 (80.2) | 0 | 10 (7.6) |
| | Sa Kaeo | 192 | 81 (42.2) | 0 | 70 (86.4) | 0 | 0 | 0 | 1 (1.2) | 0 | 10 (12.3) |
| **2003–2004** | **Total** | **543** | **174 (32.0)** | **107 (61.5)** | **14 (8.1)** | **0** | **6 (3.5)** | **1 (0.6)** | **18 (10.3)** | **0** | **28 (16.1)** |
| | Nong Khai | 156 | 47 (30.1) | 30 (63.8) | 4 (8.5) | 0 | 1 (2.1) | 0 | 6 (12.8) | 0 | 6 (12.8) |
| | Chanthaburi | 207 | 72 (34.8) | 43 (59.7) | 8 (18.6) | 0 | 5 (11.6) | 0 | 8 (18.6) | 0 | 8 (18.6) |
| | Tak | 155 | 47 (30.3) | 34 (72.3) | 0 | 0 | 0 | 0 | 0 | 0 | 13 (27.7) |
| | Songkhla | 25 | 8 (32.0) | 0 | 2 (25.0) | 0 | 0 | 1 (12.5) | 4 (50.0) | 0 | 1 (12.5) |
| **2004–2005** | **Total** | **422** | **109 (25.8)** | **36 (33.0)** | **5 (4.6)** | **24 (22.0)** | **2 (1.8)** | **0** | **2 (1.8)** | **2 (1.8)** | **38 (34.9)** |
| | Nong Khai | 119 | 49 (41.2) | 13 (26.5) | 0 | 8 (16.3) | 0 | 0 | 1 (2.0) | 0 | 27 (55.1) |
| | Chanthaburi | 164 | 21 (12.8) | 18 (85.7) | 3 (14.3) | 0 | 0 | 0 | 0 | 0 | 0 |
| | Tak | 139 | 39 (28.1) | 5 (12.8) | 2 (5.1) | 16 (41.0) | 2 (5.1) | 0 | 1 (2.6) | 2 (5.1) | 11 (28.2) |
| **2005–2006** | **Total** | **1,105** | **428 (38.7)** | **361 (84.4)** | **6 (1.4)** | **6 (1.4)** | **0** | **0** | **5 (1.2)** | **0** | **50 (11.7)** |
| | Nong Khai | 141 | 55 (39.0) | 52 (94.5) | 0 | 0 | 0 | 0 | 0 | 0 | 3 (5.5) |
| | Chanthaburi | 569 | 273 (48.0) | 233 (85.3) | 5 (1.8) | 1 (0.4) | 0 | 0 | 3 (1.1) | 0 | 31 (11.4) |
| | Tak | 202 | 63 (31.2) | 45 (71.4) | 1 (1.6) | 5 (7.9) | 0 | 0 | 0 | 0 | 12 (19.0) |
| | Songkhla | 193 | 37 (19.2) | 31 (83.8) | 0 | 0 | 0 | 0 | 2 (5.4) | 0 | 4 (10.8) |
| **2006–2007** | **Total** | **1,127** | **289 (25.6)** | **120 (41.5)** | **21 (7.2)** | **6 (2.1)** | **0** | **0** | **2 (0.1)** | **6 (2.1)** | **134 (46.4)** |
| | Nong Khai | 136 | 16 (11.8) | 14 (87.5) | 0 | 0 | 0 | 0 | 0 | 0 | 2 (12.5) |
| | Chantaburi | 520 | 127 (24.4) | 61 (48.0) | 16 (12.6) | 0 | 0 | 0 | 0 | 0 | 50 (39.4) |
| | Tak | 343 | 128 (37.3) | 34 (26.6) | 5 (3.9) | 6 (4.7) | 0 | 0 | 2 (1.6) | 6 (4.7) | 75 (58.6) |
| | Songkhla | 128 | 18 (14.1) | 11 (61.1) | 0 | 0 | 0 | 0 | 0 | 0 | 7 (38.9) |
| **Total** | | **7,452** | **2,560 (34.4)** | **1,204 (47.0)** | **232 (9.1)** | **38 (1.5)** | **89 (3.5)** | **1 (0.04)** | **579 (22.6)** | **9 (0.4)** | **378 (14.8)** |

Rotavirus was detected by PAGE analysis of RNA, and the G type was determined by RT-PCR. Data as a total in each collection year are shown in bold.

detected in 2003–2004. G12 (strain T152) was first detected in Thailand in 1998–1999 [Pongsuwanna et al., 2002]. G12 strains were also found in 2004–2005 and 2006–2007, although the incidence was low. Although strain T152 was detected in the Children's Hospital in Bangkok, the G12 strains detected after 2004 were all in the Maesod Hospital, Tak Province (Myanmar border). Thus, a drastic yearly change in the G type distribution was observed in Thailand in 1993–2007. It was of note that G9 and G12 rotaviruses had reemerged in Thailand.

The trend of the changes in the distribution of the G type in different regions of Thailand was almost the same. However, there were also some differences in the distribution of the G type depending on the regions where collections were undertaken, for example, G2 was highly prevalent in the Tak and Sa Kaeo regions in 2002–2003 compared to the other regions, and the prevalence of G3 was much higher in the Tak region than in the other regions in 2004–2005 (Table I).

### Sequence Analysis of G9 Human Rotaviruses

In order to characterize the G9 strains that reemerged in Thailand, the complete nucleotide sequences of the VP7 genes of 16 representative G9 strains collected in four different districts and different years in this study were determined, the sequences being compared with each other and with those of the representative G9 strains detected in Thailand and other countries. The identity was very high among the 16 Thai G9 strains detected between 1999 and 2004 in this study: 99.1–99.9% at the nucleotide level and 98.2–100.0% at the amino acid level. On comparison with Thai G9 strains in other studies, the 16 present Thai G9 strains showed identities of 99.4–99.6%, 98.0–98.2%, and 95.0–95.2% at the nucleotide level with strains CMH319Thai and CMH045Thai detected in 2000–2001, 97CM86 in 1997, and Mc345 in 1988, respectively. It has been shown that there are three lineages (I–III) of G9 strains on phylogenetic analysis [Hoshino et al., 2004]: I comprises prototypes WI61, F45, and AU32; II comprises strain 116E; and III comprises the G9 strains that reemerged. The Thai 16 G9 strains that reemerged in Thailand are also included in lineage III (Fig. 1).

### Sequence Analysis of G12 Human Rotavirus

The RNA profiles of the seven Thai G12 strains were examined by PAGE. They all exhibited similar RNA patterns (Fig. 2). The complete VP7 nucleotide sequences of the seven G12 strains detected in 2004–2005 and 2006–2007 were also determined. The VP7 nucleotide sequences of the seven Thai G12 strains showed very high identities (99.3–100%). In contrast, they exhibited 90.2–90.4% identity to prototype strain L26, 90–90.2% to porcine RU172 strain, 97.2–97.6% to a Thai strain, T152, detected in 1998–1999, and 97.7–98.5% to the G12 strains that reemerged in other countries. On phylogenetic analysis, the seven Thai G12 strains were found to be closely related to the strains detected after 2000 and thus were included in lineage III (Fig. 3).

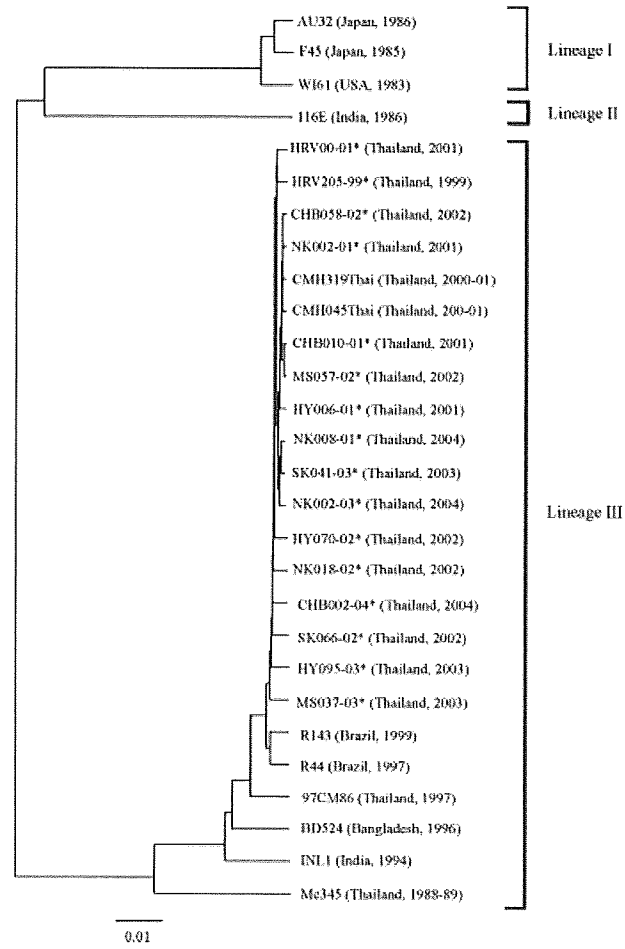


Fig. 1. Phylogenetic analysis of nucleotide sequences of the full VP7 genes of human G9 rotavirus strains detected in Thailand. The strain names with asterisks are those detected in this study. Reference sequences were obtained from the GenBank and EMBL databases. The bar indicates the variation scale.

## DISCUSSION

In Thailand, diarrhea remains an important cause of morbidity and mortality among infants and young children, and rotavirus infection is a common cause of hospital admission [Pipittajan et al., 1991; Maneekarn and Ushijima, 2000; Bresee et al., 2004; Veeravigrom et al., 2004; Jiraphongsa et al., 2005; Sungkapalee et al., 2006]. It has been estimated that in Thai patients with diarrhea, the risks of rotavirus diarrhea, of a health care visit, and of hospital admission are 1 in 8, 1 in 36, and 1 in 85, respectively [Jiraphongsa et al., 2005]. The occurrence of rotavirus diarrhea in Thailand has a unimodal distribution pattern with a peak in October through to February [Jiraphongsa et al., 2005; data not shown], and rotavirus infection was found most frequently in children aged 6–11 months up to 2 years (data not shown).

In Thailand, a number of interesting epidemiological features were revealed by continuous surveys of

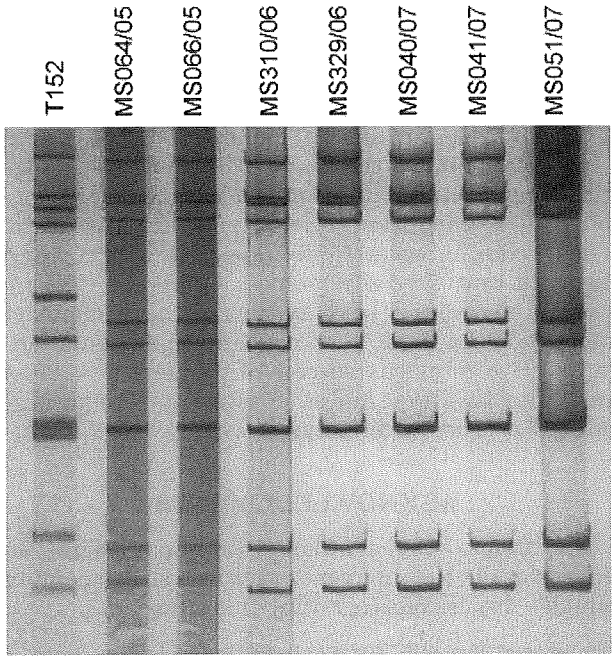


Fig. 2. RNA profiles of human G12 rotavirus strains detected in Thailand.

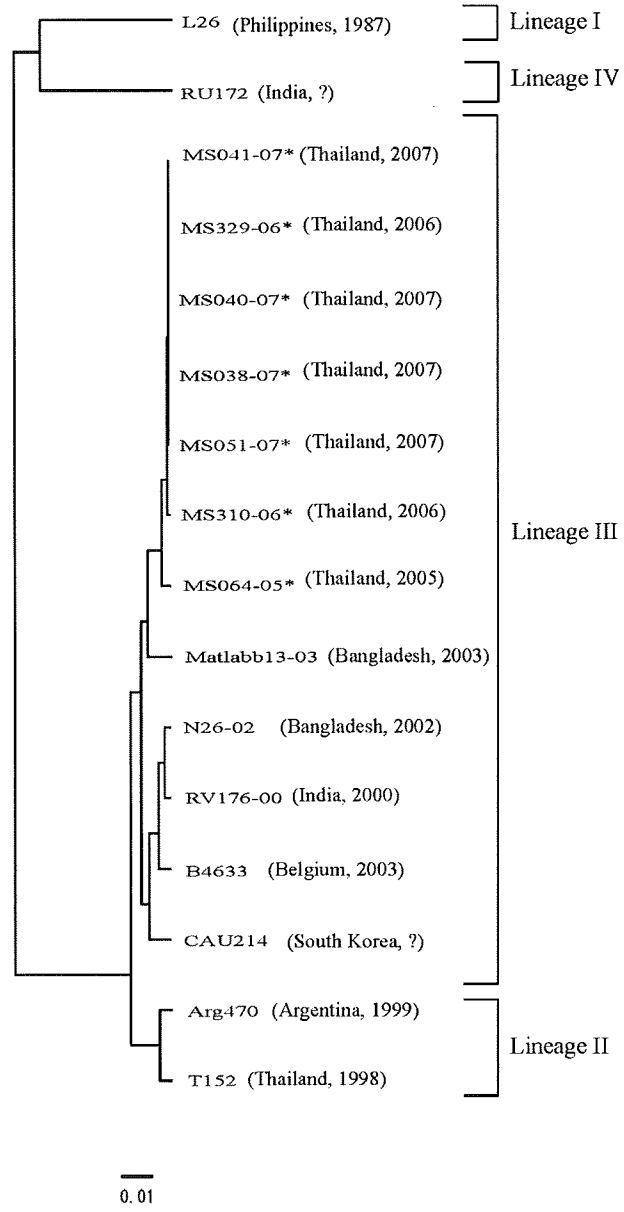


Fig. 3. Phylogenentic analysis of nucleotide sequences of the full VP7 genes of human G12 rotavirus strains detected in Thailand. The strain names with asterisks are those detected in this study. Reference sequences were obtained from the GenBank and EMBL databases. The bar indicates the variation scale.

gastroenteritis virus among humans and animals: (1) unusual G10 human and porcine rotaviruses were detected [Pongsuwanna et al., 1996], (2) various combinations of G type and P type of bovine rotaviruses were found [Taniguchi et al., 1991], (3) a G12 human rotavirus with P[9] specificity was detected [Pongsuwanna et al., 2002], (4) group B and C porcine rotaviruses were detected [Pongsuwanna et al., 1996; unpublished data], and (5) picobirnaviruses were detected in pigs and humans [Pongsuwanna et al., 1996; Wakuda et al., 2005].

Regarding the G type distribution in Thailand, a yearly change in the distribution of the G type was also found in previous studies [Pongsuwanna et al., 1989, 1993]. In 1988–1989, 1989–1990, and 1991–1992, G1 was most prevalent. In 1990–1991 and 1992–1993, G3 and G2 were predominant, respectively. Thus, following the previous studies, a 19-year survey of the distribution of the G type in Thailand was carried out. Such a long-term survey in the same country will be useful for understanding the epidemiology of human rotavirus, and will provide fundamental data useful for future introduction of rotavirus vaccines.

Other studies on the distribution of G type studies have been performed in Thailand. In Chiang Mai between 1995 and 1997, G1, G2, G4, and G9 were detected at frequencies of 47%, 40%, 3%, and 6%, respectively, and G9 was found to have reemerged in 1996–1997 in Thailand [Zhou et al., 2001]. In the present study, interestingly, G9 rotavirus was detected earlier and in 1994–1995. However, the genome could not be characterized because the stool sample is not available now. In Bangkok, Thailand, between November 2002 and March 2004, the G types of 36 rotavirus-positive specimens were determined. Types G1, G2, and G9 were detected in 5.6%, 69.4%, and 25.0%, respectively [Theamboonlers et al., 2005]. In 2000–2001, 107 samples were subjected to G type determination: G9 was the most prevalent (91.6%), followed by G3 (5.6%) and G2 (2.8%) in Chiang Mai [Khamrin et al., 2006]. In 2003 and 2004, G2 and G1 were identified as the most dominant types, respectively [Khamrin et al., 2007]. The present long-term and large-scale study extended these small-scale studies and strengthened the data.

A changing distribution of rotavirus G types has been reported in other countries, although some differences

were observed. In Australia, G1 was the most prevalent in 1999–2001, G9 was highly prevalent in 2001–2003, and G1 was again the most prevalent type after 2004 [O'Ryan, 2009]. In Italy, G1 was highly prevalent in 2001, 2002, and 2004, and G9 was the most common type in 2005 [De Grazia et al., 2007]. In Ireland, G9 was the most prevalent type in 2001–2002, and G1 was identified as the most common G type in 2002–2004 [Reidy et al., 2005].

G9 rotavirus was first isolated in the United States in 1983 and then in Japan in 1985. After that, G9 rotaviruses were not detected for about a decade but reemerged in the mid-1990s. The G9 strains that reemerged were distinct genetically from those in the 1980s. At present, G9 rotaviruses have emerged as the fifth most common G type worldwide [Ramachandran et al., 2000; Santos and Hoshino, 2005]. Therefore, it has been suggested that G9 should be incorporated into candidate rotavirus vaccines [Montenegro et al., 2007]. At least three phylogenetic sequence lineages have been reported among the VP7 nucleotides of G9 rotaviruses [Hoshino et al., 2004]. Lineage 1 includes those isolated in the 1980s, lineage 2 G9 strains have been detected only in asymptomatic neonates in India, and the majority of G9 strains that are prevalent around the world today belong to lineage 3. The VP7 genes of the Thai G9 strains detected in this study were found to have very similar nucleotide and amino acid sequences, and were closely related to lineage 3 G9 strains.

G12 was first identified and characterized in 1990 among rotaviruses causing diarrhea in children in the Philippines [Taniguchi et al., 1990; Urasawa et al., 1990]. After a long period, the G12 strains have reemerged in Thailand [Pongsuwanna et al., 2002; Wakuda et al., 2003], the United States [Griffin et al., 2002], Japan [Shinozaki et al., 2004], Argentina [Castello et al., 2006], Nepal [Uchida et al., 2006], India [Ray et al., 2007], Bangladesh [Rahman et al., 2007], Belgium [Rahman et al., 2007], Slovenia [Steyer et al., 2007], South Korea [Le et al., 2008], Saudi Arabia [Kheyami et al., 2008], South Africa [Page et al., 2009], Hungary [Banyai et al., 2009], and Malawi [Cunliffe et al., 2009]. One porcine G12 virus was also detected in India [Ghosh et al., 2006]. A high incidence has been reported in several countries such as Nepal. There is a possibility that extensive spread of the G12 type at high frequency has occurred, as found for G9. Using phylogenetic analysis, G12 strains are grouped into four lineages: lineage I includes prototype strain L26; lineage II consists of strains from Thailand (strain T152), Japan and Argentina; lineage III includes the US strain, and most Indian and Bangladesh strains; and lineage IV comprises only a porcine strain, RU172 [Rahman et al., 2007]. Following the detection of G12 strain T152 in Thailand in 1998–1999, the G12 rotavirus was detected in this Thai study in 2004–2005 and 2006–2007. Differing from strain T152 detected in 1998–1999, the seven Thai G12 strains detected in 2004–2005 and 2006–2007 are included in lineage III. These results indicate that G12 rotavirus is now endemic in Thailand. However, the G12 strains were detected in the same district in different years, and they showed almost the same RNA profiles. This indicates that the G12 strains have not spread throughout Thailand. It is of interest to follow the spread of G12 rotavirus strains in Thailand.

The distribution of the G type in Thailand has changed with time. Continuous monitoring of epidemiology of rotavirus is important, especially for the introduction of a vaccine, in order to document its impact and to ensure its continued effectiveness. Comprehensive analysis of the diversity of rotavirus may have significant implications for the development and implementation of an effective rotavirus vaccine of the next generation. Analysis of the P type and other segments is required for more precise characterization.

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■外来患者の感染制御 ―― ❸

# 感染管理に必要な予防接種について

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**Key Point**

- 予防接種により能動免疫が付与される．これは疾患特異的で非常に効果的な感染予防策である．
- ワクチンで予防が可能な疾患に対しての対策を怠れば，管理責任や法律的問題に発展する可能性がある．
- 医療関係者は各疾患に対する防御免疫能が保持できるように，日常から心がけたい．
- 防御免疫を有しない可能性がある者に対しては，積極的に予防接種を行う．
- 麻疹と水痘については，患者発生後の曝露後免疫も感染拡大制御策の１つである．

## はじめに

基礎疾患や治療薬の影響により感染症が重症化しやすい患者に交差感染が及べば，健康被害は甚大なものとなる．患者同士の感染伝播以外に，病院職員が疾患を媒介することもあり，管理責任や医療訴訟に発展する可能性がある．また，職員の間で疾患が流行すれば多くの欠勤者が発生し，業務に多大な支障をきたす．

予期せぬ感染伝播を完全になくすことは現実的に困難であるが，リスクを最小限に抑えるように努めることが院内感染予防対策の基本である．ワクチンで予防することができる疾患については，それを最大限に活用することが大切である．本稿では，麻疹・風疹・水痘・ムンプスについて概説する．

## 職員管理

患者が発生してから慌てるのではなく，医療関係者はワクチン予防可能疾患に対する自身の防御免疫能を日頃から知っておくことが肝要である．そしてその情報は，施設責任者とも共有する必要がある．

具体的には，①既往歴，②予防接種歴，③血清抗体価による防御免疫の評価である．

既往歴については，医師による明確な記録や病因診断があれば信頼性は高いが，漠然とした記憶のみでは不正確なこともしばしばで

ある．予防接種歴も，母子手帳への記載のような正確な記録が残ってない場合は信頼できない．抗体価については後述する．

現場での対応として，2009年2月に日本環境感染学会が公開した「院内感染対策としてのワクチンガイドライン」にあるチャートがわかりやすい[1]．本ガイドラインでは，防御免疫が不十分な可能性のある医療関係者をより広く抽出して，前もってワクチンを接種しておくことが推奨されている．これはワクチンを用いた疾患予防の原則であり，既往歴や接種歴が曖昧な場合は，接種により予防を心がける．免疫を有する個体にワクチンを接種しても，そのために副反応が増強することはない．

## 抗体価測定法と判定

### 1 測定方法

どのような方法で抗体価を測定するかは大切な事項である．例えば補体結合(compliment fixation；CF)法は，感染初期の抗体を同定することはできるが，その後早期に陰性化するので，個体の防御免疫を評価する方法としては適していない．

中和(neutralization；NT)法は当該ウイルスに対する中和抗体を測定し，感染防御能を評価する本質的な測定法であり特異性も高い．しかし，手技が煩雑で結果を得るまでに時間を要する．

酵素抗体(enzyme immunoassay；EIA)法によるIgG抗体は感度・特異性とも良好で，多数検体の処理が可能である．ただし感度が非常に鋭敏で，ごく低値の抗体も検出され，どの程度の抗体価を有すれば個体が感染防御能をもつかの評価が困難な場合がある．また，測定費用が高価な点，キットごとにカットオフ値が異なり測定値の絶対値が標準化されていない点も短所である．

赤血球凝集能をもつウイルスについては，赤血球凝集抑制(hemagglutination inhibition；HI)法により抗体価を測定できる．本法はNT法に比して手技が簡単，EIA法よりも安価などの利点はあるが，感度に劣り，例えば麻疹抗体の検査にHI法を用いると，相当数の偽陰性者がでる．医療関係者は麻疹罹患を是非とも回避したい対象であり，接種対象を広く選定するという意味では有用かもしれないが，高価なワクチンを多数消費することになる．また，HI法の測定に用いる血球の入手は，近年は困難となってきている．

これらの点を総合すると，防御免疫の有無を評価するためにはEIA法でIgG抗体を測定するのがもっとも有用であろう．ただし費用が高価となる点を考慮して，麻疹に対してはゼラチン粒子凝集(particle agglutination；PA)法，水痘に対しては免疫粘着血球凝集反応(immune adherence hemagglutination；IAHA)法などの方法を用いることができる．また，風疹HI法の感度は麻疹と比較すると遥かに優れているので，風疹抗体についてはHI法による判定でもよいであろう．ムンプス抗体に関しては，EIA法を代用できる測定法が見当たらない(**表1**)[2]．

### 2 抗体価の判定

どれだけの抗体価を有すれば発病を100%

**表1** ワクチン予防可能疾患に対する抗体検査法

| 疾患名 | 個体防御免疫有無の判定に用いる抗体検査法 |
|---|---|
| 麻　疹 | EIA(IgG抗体)，NT，HI，PA |
| 風　疹 | HI，EIA(IgG抗体) |
| 水　痘 | IAHA，EIA(IgG抗体) |
| ムンプス | EIA(IgG抗体) |

(文献2)より引用)

防ぐことができるかは確定できないが，医療関係者に対しては積極的な予防策の実践が推奨される．著者ら国立病院機構三重病院は三重大学医学部と共同で，実習開始前の医学部学生に対しては**表2**に示す基準で過去数年にわたって院内感染予防策を実施してきた[2]．なお本基準での接種に際して，抗体価は株式会社エスアールエルによる測定値を用いた．

日本環境感染学会によるガイドライン[1]では**表3**に示す基準を提案している．そして，①医療関係者を対象としたものであるから“接種を考慮する抗体価の基準値”が高く設定してある，②検査時点での免疫状態を判断する基準であり長期の免疫状態を示すものではない，という注釈が述べられている．

## 患者発生時の対応

患者発生時にもワクチンを用いて対処できる手段がある[2]．一般的に“曝露後免疫”と呼ばれる方法であるが，各疾患による相違点を**表4**にまとめた．

### 1 麻疹

米国小児科学会は“免疫学的に正常な1歳以上の小児・成人に対しては，感染源曝露後72時間以内に麻疹生ワクチンを接種すれば，発症予防が期待できる”という見解である[3]．わが国の麻疹ワクチン・MRワクチンの添付文書には曝露後予防に関する記載がないが，国立感染症研究所感染症情報センターによる

**表2** 実習開始前の医学部学生へのワクチン接種基準

| | |
|---|---|
| 麻　疹 | HI抗体価8倍未満の場合，EIA-IgGを再検査し，EIA-IgG8.0未満の者に対して麻疹ワクチン(MRワクチン)を接種する．<br>EIA-IgGによる再検査を行わない場合は，HI抗体価8倍未満の者に対して麻疹ワクチン(MRワクチン)接種を行う． |
| 風　疹 | HI抗体価32倍未満の者に対して風疹ワクチン(MRワクチン)を接種する． |
| 水　痘 | IAHA抗体価4倍未満の者に対して水痘ワクチンを接種する． |
| ムンプス | EIA-IgG抗体価4.0未満の者に対してムンプスワクチンを接種する． |

(文献2)より引用)

**表3** 医療関係者用ワクチン接種判断基準の目安

| | |
|---|---|
| 麻　疹 | 中和法で8倍未満<br>あるいは，PA法で256倍未満<br>あるいは，EIA-IgG 16.0未満 |
| 風　疹 | HI抗体価32倍未満<br>あるいは，EIA-IgG 8.0未満 |
| 水　痘 | IAHA抗体価8倍未満<br>あるいは，EIA-IgG 4.0未満(陰性者とEIA-IgG抗体価±の者)<br>あるいは，水痘抗原皮内テスト陰性者 |
| ムンプス | EIA-IgG 4.0未満(陰性者とEIA-IgG抗体価±の者) |

(文献1)より引用)

**表4** 各疾患に対する曝露後免疫

| | 麻　疹 | 水　痘 | 風　疹 | ムンプス |
|---|---|---|---|---|
| ワクチン(能動免疫) | 感染源との接触後72時間以内であれば，効果が期待できる可能性あり | 感染源との接触後72時間以内であれば，効果が期待できる可能性あり | 曝露後免疫の有効性は明らかでない | 曝露後免疫の有効性は明らかでない |
| 免疫グロブリン製剤(受動免疫) | 感染源との接触後6日以内であれば，発症予防や軽症化が期待できる可能性あり | 感染源との接触後96時間以内であれば，発症予防や軽症化が期待できる可能性あり* | 曝露後免疫の有効性は明らかでない | 曝露後免疫の有効性は明らかでない |

*わが国では水痘高力価免疫グロブリン製剤は認可されておらず，通常の免疫グロブリン製剤を用いることになる．また，保険適用未収載である．
(文献2)より引用)

「医療機関での麻疹対応ガイドライン」[4]では，曝露後3日以内の接種であれば効果が期待できる可能性が述べられている．

能動免疫であるワクチン以外に，受動免疫を与える免疫グロブリン製剤による予防も選択手段である．感染源との接触から6日以内に投与すれば，発症予防あるいは軽症化効果が期待できる．ワクチンが接種できない免疫不全宿主においても対処可能な手段であることも知っておきたい．ただし，わが国で保険適用が認められているのは，筋注用免疫グロブリン製剤のみである．

## 2 水痘

米国小児科学会によれば“免疫学的に正常な1歳以上の小児・成人に対しては，感染源曝露後72時間以内に水痘生ワクチンを接種すれば発症予防が期待できる”とされており[3]，わが国の水痘ワクチン添付文書にも，同様の記載がある．

米国では水痘高力価免疫グロブリン製剤が認可されており，曝露後96時間以内の投与は発症予防あるいは軽症化効果の期待ができるとされる[3]．しかしわが国では，水痘高力価免疫グロブリン製剤は認可されていないので，免疫不全宿主などに対応する際には通常の免疫グロブリン製剤を用いることになる（保険適用未収載）．

## 3 風疹・ムンプス

風疹とムンプスは麻疹や水痘とは異なり，“曝露後の生ワクチン接種により，発症予防効果があるとは限らない”というのが，現状での考え方である．ただし，今後の免疫付与を考えるならば，曝露後でも接種するメリットはある．すでに感染を受けた個体に接種をしたとしても，それによる症状の増悪や合併症は報告されていない．

## おわりに

ワクチンにより予防が可能な疾患については，日常の備えと患者発生時の対処を心がけることにより，感染リスクマネジメントを向上させることが可能である．


文献

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▒ このワクチン必要か

# ヘモフィルスインフルエンザ菌b型ワクチン

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〔論文要旨〕

ヘモフィルスインフルエンザ菌b型（Hib）ワクチンがわが国にもようやく導入された．現状では希望者のみに対する任意接種であるが，是非とも総ての子どもたちに接種したいワクチンである．Hibが原因となる疾患は，髄膜炎をはじめ重篤なものばかりであり，生命や後遺症に関わる．また，病初期からの診断は必ずしも容易ではなく，確定診断のための検査に医師は熟練した技術を要し，患者には苦痛が伴う．罹患した場合の治療期間や費用は多大なものとなり，Hibは子どもたちにも家族にも大きな疾病負担である．そして費用対効果分析の結果では，Hibワクチン導入による費用削減効果が示されている．さらに，インフルエンザ菌では耐性菌が増加しており，治療に難渋する可能性も考えられ，制御策としては予防が大切である．海外ではすでに20年以上前から広く使われており，高い評価の確立したワクチンである．

## 1. はじめに

ヘモフィルスインフルエンザ菌b型（以下Hib）ワクチンは，子どもたちの健康を守るために不可欠である．それは，**表1**に示すような理由があるからである．以下に概説する．

## 2. Hibが引き起こす疾患

Hibは組織侵襲性が強く，重篤な感染症の原因となる．ワクチンが導入される以前にHibによって起っていたと推計される疾患の頻度を図1に示した[1]．髄膜炎，喉頭蓋炎，菌血症，肺炎，骨髄炎，関節炎などは，英語では“Invasive Infection～身体を侵襲する感染症”と総称され，本来は無菌である部位にHibの感染が起る．Hib Invasive Infectionの中で最も頻度が高いのは髄膜炎で全体の半数以上を占めるが，他の疾患も重篤なものばかりである．

当院の現状を紹介する．1996年から2005年までの10年間に，国立病院機構三重病院小児科病

**表1**　Hibワクチンが必要である理由

1．髄膜炎はもちろん，他のHib疾患も重症なものが多く，生命や後遺症に関わる．
2．Hib疾患は，病初期からの診断が必ずしも容易ではない．
3．診断のためには各種検査が必要で，患者は苦痛を伴う．
4．治療期間や費用が多大である．
5．耐性菌の増加により，治療に難渋する場合がある．
6．Hibワクチンを導入した海外諸国では，顕著な予防効果が確認されている．

Haemophilus influenza type b(Hib) conjugate vaccine
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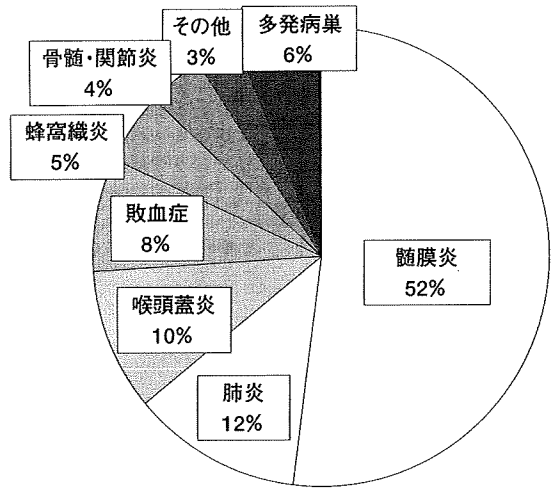


図1　Hibによって起こる疾患の頻度分類（ワクチン導入前）　（文献1より引用）

棟に入院した症例を集計した（図2）[2]．当該期間中に診療した細菌性髄膜炎27例のうち，17例（63.0%）の起因菌はHibであった．急性喉頭蓋炎は3例あり，全例Hibが原因であった．細菌性骨髄炎・関節炎の症例は10例あり，うち2例の起因菌はHibであった．病院を受診する背景人口（津市）から5歳未満人口10万人当りのHib疾患年間罹患率を計算すると，髄膜炎8.9，喉頭蓋炎2.4，化膿性関節炎・骨髄炎1.6という結果であった．

1990年代後半に実施された全国サーベイランス調査では，わが国におけるHib髄膜炎の発生頻度は5歳未満人口10万人当り年間7.5という結果であった[3]．この罹患率と国内の5歳未満総人口から推計すると，わが国では1年間に500人程度のHib髄膜炎の患者が発生していることになる．

石和田らは千葉県全域の調査を毎年継続して実施し[4]，全身性Hib感染症とHib髄膜炎それぞれの5歳未満人口10万人当り罹患率が，2003年8.3，6.1，2004年13.4，8.7，2005年16.5，11.7という結果で，津市や全国サーベイよりも高い数値であり，かつ近年は増加傾向であった．

これらの結果より，Hibによって引き起こされるInvasive Infectionは髄膜炎をはじめ重症疾患ばかりであり，わが国でも決して稀な頻度ではないということがわかる．

### 3. Hib髄膜炎について

#### (1) 発症年齢と臨床症状

当院で10年間に経験したHib髄膜炎17例の患者年齢は，生後3ヶ月から5歳1ヶ月（平均22.8ヶ月）に分布し，1歳未満児が6例(35.3%)，1歳以上2歳未満児が4例(23.5%)で，2歳未満の小児が全体の約6割を占めた（図3）[2]．

これら症例が化膿性髄膜炎と診断されたのは，発症から1.47±1.43日目であった（発症日を病日0とした）が，診断されるまでに担当医

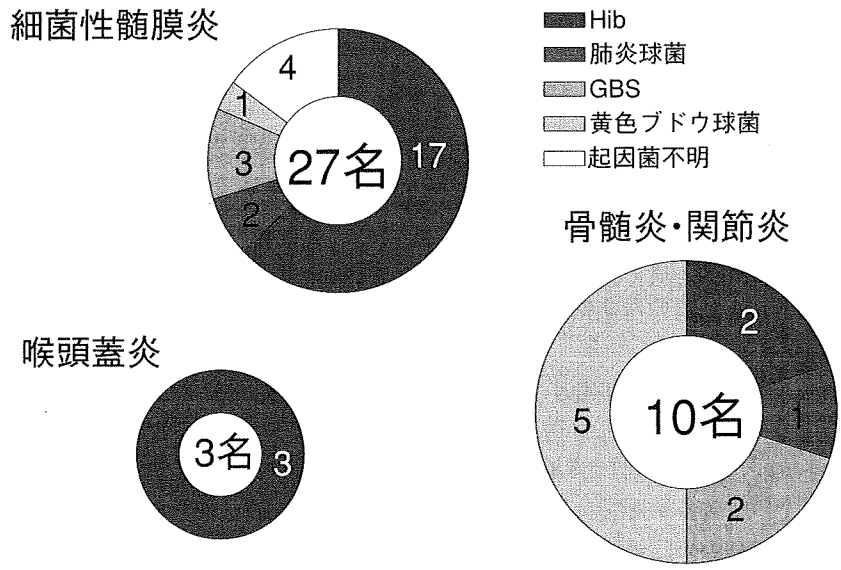


図2　疾患別の起因菌（1996～2005年；三重病院）　（文献2より引用）

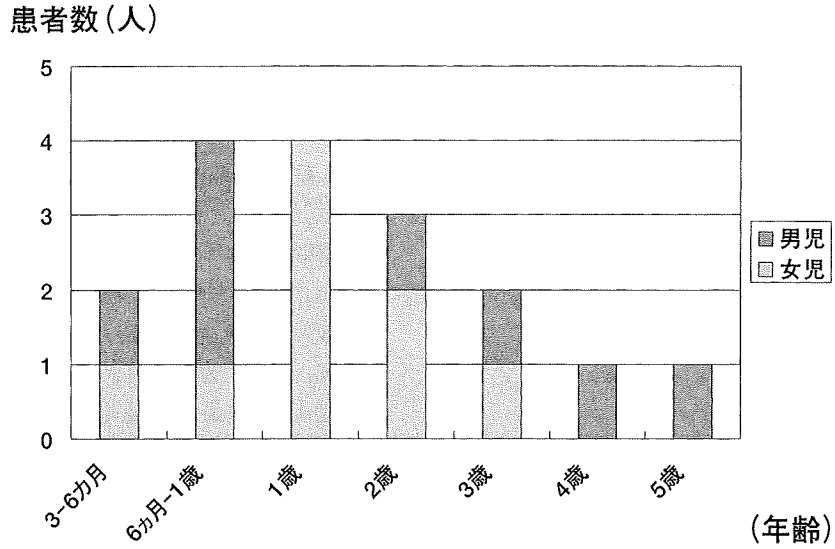


図3　Hib 髄膜炎患者の年齢と性別（三重病院；n＝17）　（文献2より引用）

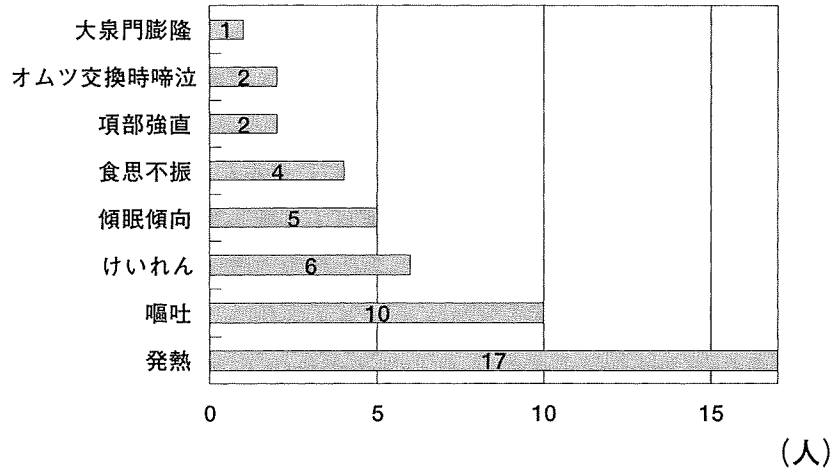


図4　Hib 髄膜炎患者に認められた症状（三重病院；n＝17）　（文献2より引用）

がカルテに記載した症状を図4に示した．発熱は全例で認めたが，次いで多かった症状は嘔吐（10例，58.8%）であった．けいれん，意識障害，髄膜刺激徴候，大泉門膨隆など中枢神経系異常を疑わせる特異的な所見を認めた症例は，いずれも半数にも満たなかった．

Hib 髄膜炎は主に2歳未満の低年齢小児に発症するために，所見の判定は決して容易ではなく，臨床症状のみからでは病初期に的確な診断できない場合もしばしばあると考えられた．診断の遅れは予後への悪影響や，場合によっては法律的問題にも発展する可能性があり，本疾患は予防することが何よりも大切である．

(2)　予後

当院における10年間の集計[2]では，幸い死亡例はなかったが，3例（17.6%）に後遺症を認めた．後遺症の内容は，てんかん，高度難聴，発達障害・てんかん・水頭症・四肢麻痺であった．後遺症を認めた3例は，認めなかった14例に比して，髄膜炎発症が低年齢で，発症してから抗菌薬の全身投与を開始されるまでの日数が長く（後遺症例；2.4±2.4日，中央値2日――非後遺症例；1.33±1.23日，中央値1日），治療開始時の髄液糖濃度が低い（後遺症例；12.0

±20.5mg/dl, 中央値3.0mg/dl——非後遺症例；41.7±34.2mg/dl，中央値38.0mg/dl）という結果であったが，統計学的有意差は認めなかった．

Hib 髄膜炎患者の入院期間は17日～51日（平均27.7日，中央値23日）であり，後遺症群ではより長期の入院を要した．子どもたちが Hib 髄膜炎に罹患すれば，本人にも家族にもいろんな意味で大きな負担となることは明らかである．

### 4. Hib 感染症の的確な早期診断

Hib 髄膜炎を病初期に診断することは，臨床症状のみからは決して容易でないことをすでに述べた．化膿性髄膜炎を疑ったら迅速に髄液検査を実施し，確定診断とあわせて病原体を同定し，適切な治療を計画実施しなければならない．小児の髄液検査は，患児にとって大きな苦痛や不安を伴う検査であるとともに，医師にとっても熟練と高度の技術を要する．そして，時には検査による合併症や医療事故に繋がることもある．それを恐れて診断と治療が先延ばしになるようなことがあってはならないが，本疾患がワクチンで制御され，髄液検査をしなければならない機会が少しでも減ることは，患者・医師の双方にとってもありがたいことであろう．

喉頭蓋炎は，最も大切な救急疾患のひとつである．発症頻度は決して高くないが，数時間の経過で急激に病状が進行する．早期に診断し適切な治療を開始しないと，窒息により死亡したり後遺症を残す．急を要する疾患と診断されずに帰宅した後に大事に至ったり，救急診察室や処置室での急変もしばしばであり，医療訴訟に発展することもある．幸い当院で経験した3例は，後遺症を残すことなく全例治癒したが，初診の時点で適切に正確な診断が出来ていた例はなかった[2]．ひとつ対処を間違えば，大切な子どもの将来を台無しにしてしまう疾患である．喉頭蓋炎の大多数は Hib が原因であり，ワクチンを導入した国々では喉頭蓋炎の患者も Hib 髄膜炎と同様に大いに減少した[1,4]．喉頭蓋炎制御の観点からも，やはり Hib ワクチンの導入は不可欠である．

骨関節感染症も低年齢児の罹患が多く，初発症状は非特異的であり早期からの的確な診断は困難である．そして当院の集計[2]では，Hib による骨髄炎・関節炎の患者年齢は，黄色ブドウ球菌など他の細菌による同疾患の患者と比較して低年齢であった．すなわち，Hib 疾患を予防すれば，症状の訴えが乏しく診断困難な低年齢児の骨関節感染症を予防できることが期待された．

### 5. 耐性菌の増加

Hib においては，薬剤耐性菌の増加が大きな問題となっている．当院の化膿性髄膜炎8例，骨髄炎1例の計9例から分離された Hib に対して，抗菌薬感受性を検討した結果[2]を表2に示した．βラクタマーゼ産生菌は1株のみであったが，PCR 法による *pbp* 遺伝子変異を検討した結果，*pbp* 3-1，*pbp* 3-2の双方に変異を認めた株（g-BLNAR）が2株，*pbp* 3-1のみに変異を認めた株（g-Low-BLNAR）が4株であった．すなわち，βラクタマーゼ非産生アンピシリン耐性菌（β-lactamase negative ampicillin resistant strain, BLNAR）が全体の6割以上を占めていた．CTX や MEPM といった元来インフルエンザ菌に強い抗菌力を示す薬剤の MIC が高い菌もあり，治療に難渋することも考えられた．BLNAR の増加が近年著しいことは，全国調査の結果[5]でも報告されている．Hib 耐性菌増加の現状を踏まえた対策としても，ワクチンによる予防が大切である．

### 6. 海外における Hib ワクチンの評価

欧米では1980年代後半からすでに Hib ワクチンが使用され，しかも大多数の国では定期接種として全小児を対象に接種が行われている．ワクチンを導入した海外諸国では，髄膜炎をはじめとする小児期 Hib Invasive Infection が明らかに減少したことが報告されている（表3）[1]．

世界保健機関（World Health Organization, WHO）も，Hib ワクチンをすべての子どもたちに接種することを推奨しており[6]，すでに世界で高い評価の確立したワクチンである．わが国での導入は欧米より約20年遅れた上に，希望する者のみが接種する「任意接種」の扱いである．一刻も早く日本の子どもたち総てが，定期接種として Hib ワクチンを接種できる日が来ることを願いたい．

表2　Hib invasive infection の患者から分離された菌の抗菌薬感受性

| | 年齢 | 疾患 | ABPC (MIC μg/ml) | CTX (MIC μg/ml) | MEPM (MIC μg/ml) | β-L 産生能 (Nitrocefin 法) | TEM 型 β-L 産生遺伝子の有無 | 変異を認めた *pbp* 遺伝子 (遺伝子による耐性識別判定) |
|---|---|---|---|---|---|---|---|---|
| 1 | 3ヶ月 | 髄膜炎 | 2 | 0.06 | 0.125 | — | 無 | *pbp3-1* (g-Low-BLNAR) |
| 2 | 5ヶ月 | 髄膜炎 | 0.125 | ≦0.015 | 0.06 | — | 無 | 無 (感受性菌) |
| 3 | 9ヶ月 | 髄膜炎 | 0.125 | ≦0.015 | 0.03 | — | 無 | 無 (感受性菌) |
| 4 | 1歳1ヶ月 | 髄膜炎 | 1 | 0.06 | 0.125 | — | 無 | *pbp3-1* (g-Low-BLNAR) |
| 5 | 1歳5ヶ月 | 髄膜炎 | 2 | 検索せず | 検索せず | — | 無 | *pbp3-1, pbp3-2* (g-BLNAR) |
| 6 | 2歳3ヶ月 | 髄膜炎 | 0.25 | ≦0.015 | 0.03 | — | 無 | 無 (感受性菌) |
| 7 | 2歳10ヶ月 | 髄膜炎 | ＞32 | 0.06 | 2 | 産生 | 有り | *pbp3-1* (g-Low-BLNAR) |
| 8 | 3歳1ヶ月 | 髄膜炎 | 16 | 2 | 0.5 | — | 無 | *pbp3-1, pbp3-2* (g-BLNAR) |
| 9 | 1歳7ヶ月 | 骨髄炎 | 1 | 検索せず | 検索せず | — | 無 | *pbp3-1* (g-Low-BLNAR) |

MIC；Minimum Inhibitory Concentration（最小発育阻止濃度）　（文献2より引用）
ABPC；アンピシリン，CTX；セフォタキシム，MEPM；メロペネム
β-L；βラクタマーゼ，*pbp*；penicillin binding protein
BLNAR；β-lactamase negative ABPC resistant（βラクタマーゼ非産生アンピシリン耐性）

表3　Hib ワクチン導入による効果

| 国・地域 | 5歳未満人口 (千人) | Hib 髄膜炎罹患率 (導入前→導入後) | Hib Invasive Infection 罹患率 (導入前→導入後) |
|---|---|---|---|
| 米国 | 20,524 | 54 → ＜1 | 88 → 1.6 |
| 英国 | 3,831 | 24 → 0.6 | 36 → 1 |
| ドイツ | 4,115 | 23 → 0.9 | 46 → 1.3 |
| スカンジナビア | 1,581 | 31 → ＜1 | 51 → 1 |
| オーストラリア | 1,360 | 25 → 6 | 59 → 16 |
| イスラエル | 566 | 18 → ＜1 | 34 → ＜1 |
| チリ | 1,500 | 40 → ＜2 | ～ |

（文献1より引用）