Epidemiol. Infect. (1993), **111**, 407–412

Distinct yearly change of serotype distribution of human rotavirus in Thailand as determined by ELISA and PCR

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(*Accepted 17 May 1993*)

SUMMARY

A total of 241 group A rotavirus-positive stool samples collected from diarrhoeic patients in Thailand between July 1988 and June 1991 were characterized for their serotypes by enzyme-linked immunosorbent assay (ELISA) using serotype-specific monoclonal antibodies and by a polymerase chain reaction (PCR). In July 1988–June 1989, serotype 1 was the most prevalent (63·4%), followed by serotype 4 (11·0%) and serotype 2 (8·5%). In July 1989–June 1990, 59·8% were serotype 1, 24·3% were serotype 2, and 6·1% were serotype 3. In contrast, in July 1990–June 1991, serotype 3 was detected in the highest frequency (40·5%), 29·9% were serotype 1, and 27·3% were serotype 2. Thus, a distinct yearly change of serotype distribution of rotavirus in Thailand was observed in the three consecutive years. In particular, it was of note that the prevalence of serotype 3 greatly increased, in contrast to the previous studies in which almost no serotype 3 rotaviruses were detected in the years 1983–8 in Thailand.

Rotavirus is a major cause of diarrhoea in infants and young children worldwide [1, 2]. The mortality rate in rotavirus diarrhoea is high in areas where therapy for dehydration is not sufficiently available. Because of the importance of this disease as a major global health problem, the World Health Organization considers the development of an effective rotavirus vaccine to have a high public health priority. However, the antigenic complexity of rotavirus has hampered the development of an effective vaccine [1, 3]. Epidemiological studies on distribution of human rotavirus serotypes provide the basis for interpreting the results of field trials of candidate vaccines.

Serotypes 1–4, 8, 9, and 12 have been identified among human rotaviruses [1,

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3, 4]. Since the serotype specificity has been determined mainly with regard to the outer capsid protein VP7, an enzyme-linked immunosorbent assay (ELISA) using serotype-specific monoclonal antibodies directed to VP7 can be used for serotype determination [5, 6]. ELISA-serotyping has been widely and successfully employed to survey serotype distribution [7–15]. In addition, a polymerase chain reaction (PCR) to identify serotypes of rotavirus was also developed to assign rotavirus serotypes which could not been determined by ELISA-serotyping [16, 17].

We have previously examined the serotype distribution of human rotaviruses in Thailand between 1983 and 1984 and between 1987 and 1988 [12]. In this study, we extended the serotype surveillance in Thailand by characterizing rotaviruses in a total of 241 stool specimens obtained from diarrhoeal patients between 1988 and 1991 by ELISA-serotyping and PCR-typing.

ELISA-serotyping and ELISA-subgrouping were carried out with specific monoclonal antibodies as described previously [6, 18]. PCR-typing was performed in two steps (first and second amplifications) also as described previously [16, 17]. 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 the VP7 gene. The second amplification was performed using a mixture of six primers which were specific to each of six variable regions of the VP7 genes of serotypes 1–4, 8, and 9, paired with a primer to 3′ end of the VP7 gene.

The serotype of 161 (66·5%) of the 241 samples could be determined by ELISA-serotyping. Of the 80 rotavirus samples in which the serotype could not be determined by ELISA, 64 samples were examined by PCR, while the remaining 16 samples could not be subjected to PCR because the specimens were too small. The serotype of 56 (92·2%) of these 64 samples could be deduced by PCR-typing (Fig. 1) and, overall, 220 (91·3%) of 241 rotavirus samples could be assigned to serotypes by ELISA or PCR.

A distinct yearly change in the distribution of serotypes in Thailand was found in the three consecutive years (Table 1). In July 1988–July 1989, serotype 1 was the most prevalent serotype (52/82: 63·4%), 6 were serotype 2, 2 were serotype 3, and 7 were serotype 4. One rotavirus sample was assigned to serotype 9. Of 82 samples collected in July 1989–June 1990, 49 were serotype 1, 20 were serotype 2, 5 were serotype 3, and 1 was serotype 4. In July 1990–June 1991, serotype 3 was the predominant serotype (30/77: 39·0%), 23 were serotype 1, 21 were serotype 2, and 1 was serotype 4.

The subgroup specificity of 241 samples were also examined by ELISA using subgroup-specific monoclonal antibodies to assess whether antigenic specificities of the samples examined in this study conformed to the general relationship found between the subgroup and serotype antigens of human rotavirus: subgroup I human rotaviruses have serotype 2 or 8 specificity, and subgroup II human rotaviruses have serotype 1, 3, 4, or 9 specificity [1]. As shown in Table 1, of the 241 samples, 204 (84·6%) could be subgrouped: 47 were subgroup I, and 157 were subgroup II. Almost all the samples showed the above subgroup–serotype relationship, but one (subgroup I–serotype 1) sample obtained in July 1988–June 1989 and three (one subgroup I–serotype 1 and two subgroup II–serotype 2) samples collected in July 1989–June 1990 did not fit the usual antigenic pattern.

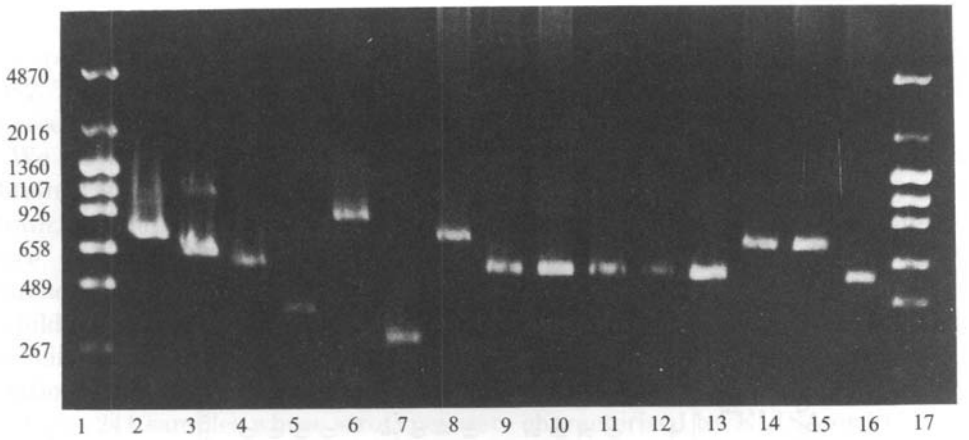

Fig. 1. PCR-typing: second amplification of six reference human rotavirus strains and nine stool samples unserotypable by ELISA. Lanes: 1, molecular weight markers; 2, serotype 1 (strain KU); 3, serotype 2 (strain S2); 4, serotype 3 (strain YO); 5, serotype 4 (strain ST-3); 6, serotype 8 (strain 69M); 7, serotype 9 (strain WI-61); 8, stool sample A (serotype 1); 9, stool sample B (serotype 3); 10, stool sample C (serotype 3); 11, stool sample D (serotype 3), 12, stool sample E (serotype 3); 13, stool sample F (serotype 3); 14, stool sample G (serotype 1); 15, stool sample H (serotype 1); 16, stool sample I (serotype 3); 17, molecular weight markers.

Rotavirus has two outer capsid proteins, VP7 and VP4, which are independent neutralization antigens [19]. Serotype specificity of rotaviruses is ascribed mainly to the antigenic identity of VP7. The two methods employed in this study are based on the presence of serotype-specific variable regions on VP7 [20]. ELISA using serotype-specific monoclonal antibodies directed to VP7 serologically detect the rotaviruses which carry the appropriate VP7 neutralization epitope, whereas the PCR detects the rotaviral RNA which possesses the VP7 nucleotide sequence specific to a given serotype [16, 20]. Although the two methods use different principles, they were found to detect a similar specificity. It has also been found that the sensitivity was higher in PCR-typing than in ELISA-serotyping [17, 18]. Indeed, the serotype of most samples whose serotype could not be defined by ELISA could be assigned by PCR-typing.

Surveys of serotype distribution of rotavirus have been performed widely by ELISA-serotyping. Overall, serotype 1 has been the most prevalent (61%), followed by serotypes 2, 3, and 4 in almost equal proportions [15]. It has also been found that the serotype distribution varies from time to time and from place to place. In the long-term surveillance of serotype distribution in Thailand which has been examined in this and previous studies, significant variations of serotype prevalence was observed [11–13]. In 1983–4, serotype 4 was the most prevalent and, in 1987–8, serotype 2 was detected with the highest frequency. Serotype 1 was the most prevalent serotype in 1988–9 and in 1989–90, while serotype 3 was the most common in 1990–1. In particular, the frequency of serotype 3 differed markedly in each year studied. Before 1988, we detected no serotype 3 rotaviruses. This was supported by two other serotyping studies. Sethabutr and colleagues [13] also failed to detect any serotype 3 rotaviruses by oligonucleotide hybridization method in 178 rotavirus specimens obtained in Thailand during 1987 and 1988.

Table 1. *Subgroup and serotype distribution of human rotavirus in Thailand between July 1988 and June 1991 as determined by ELISA and PCR*

Subgroup	Number of specimens	ELISA					PCR*							
		1	2	3	4	ND	1	2	3	4	8	9	ND†	NT‡
(A) July 1988–June 1989														
I	8	0	6	0	0	2	1	0	0	0	0	0	0	1
II	65	36	0	0	7	22	12	0	2	2	0	0	1	5
ND	9	0	0	0	0	9	3	1	0	0	0	1	0	4
Total	82	36	6	0	7	33	16	1	2	2	0	1	1	10
(B) July 1989–June 1990														
I	19	1	14	0	0	4	0	2	0	0	0	0	1	1
II	58	35	0	3	0	20	13	2	1	1	0	0	1	2
ND	5	0	2	0	0	3	0	0	1	0	0	0	0	2
Total	82	36	16	3	0	27	13	4	2	1	0	0	2	5
(C) July 1990–June 1991														
I	20	0	19	0	0	1	0	0	0	0	0	0	1	0
II	34	13	0	16	1	4	2	0	2	0	0	0	0	0
ND	23	5	0	4	0	14	3	2	8	0	0	0	0	1
Total	77	18	19	20	1	19	5	2	10	0	0	0	1	1

* PCR-typing was performed for only the specimens whose serotype could not be determined by ELISA.
† ND, Serotype or subgroup of the sample could not be determined.
‡ NT, PCR could not be applied to the sample because of the shortage.

Pipittajan and colleagues [11] found only one serotype 3 rotavirus by ELISA in 126 specimens collected between 1982 and 1983 and between July 1985 and July 1987. Thus, the prevalence of serotype 3 in Thailand appeared to increase rapidly in recent years although it had not been detected before June 1988.

We have also studied the properties of animal rotaviruses in Thailand and obtained some interesting findings. Serotype 3 porcine strains, a serotype 8 bovine strain, and numerous serotype 10 bovine strains rarely reported in other countries have been detected in diarrhoeic pigs or calves in Thailand [12, 21, 22]. Furthermore, a serotype 10 strain which is usually of calf origin was obtained from a child with diarrhoea in Chiang Mai, Thailand [33]. The continuation of rotavirus surveillance in Thailand will be useful in developing a deeper understanding of the relationship between human and animal rotaviruses.

Of the 241 samples whose serotypes were characterized by ELISA or PCR, the serotype of 20 samples could not be assigned. In addition, four strains (two subgroup I–serotype 1 strains and two subgroup II–serotype 2 strains) exhibited unusual combination of subgroup and serotype specificity, as reported elsewhere [24, 25]. Further precise examination of these strains by sequence analysis and hybridization assays would be required to elucidate the significance of these unusual strains in rotavirus epidemiology.

ACKNOWLEDGEMENTS

This study was supported in part by the Japan International Cooperation Agency and the Japanese Society for the Promotion of Sciences. We thank Dr Udom Leksomboon and the staff of the Children's Hospital; Dr Somchit Phomzoi, Dr Supaporn Pawutiyapong and the staff of Charoekrung Pracharug Hospital for providing stool specimens.

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