

Molecular Epidemiology of Wild Type 1 Poliovirus in Thailand during 1992-1995

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Abstract

Twenty years ago, poliomyelitis was one of the main health problem in Thailand and most cases are associated with type 1. Since the reception of the Expanded Program on Immunization in 1977, the incidence has been dropping dramatically. In the last 4 years, number of confirmed indigenous polio cases were under 10 per year. Most of them lived in bordering provinces and some of them in refugee camp. To trace the source of reservoirs and transmission in Thai children and the distribution of wild poliovirus type 1, the molecular epidemiological studies were used. A total of 25 strains of wild type 1 poliovirus isolated from patients were studied. Among these, 12, 6, 2, 1 and 4 strains were isolated from patients living in Thailand, hill tribe, Cambodia, Myanmar and Nepal respectively and were analyzed by partial genomic sequencing of viral genome. One hundred fifty bases of genomic sequence which encoding parts of the capsid protein VP1 and noncapsid protein 2A at position 3,508-3,527 were analyzed. The result of genomic sequencing dendrogram showed that these strains could be grouped into 2 genotypes. The first genotype included 9 strains most of which were isolated from patients in the eastern provinces of Thailand and their genotype were closely related to strains recently isolated from Cambodian and hill tribe patients living in provinces near eastern border of Thailand. Another independent genotype, 16 strains, were related to strains found in Myanmar and Nepal patients. These genotypes were found in Thai patients living in other regions in the years later which started from 1992-1995. The study indicated that the primary source of cases-associated viruses imported into Thailand must be from these neighboring countries. The combination of surveillance and molecular epidemiological studies are useful to attain more effective eradication of poliomyelitis in Thailand. (*J Infect Dis Antimicrob Agents* 1997;14:79-86.)

INTRODUCTION

The first epidemic of poliomyelitis in Thailand was traced back to 1952 (Netsiri et al, 1957). At that time, over 1,000 cases per year were reported and the incidence rate

was calculated to be over 2 per 100,000 population (Annual Epidemiology Report, 1957-1996). Since the nationwide application of oral polio vaccine immunization in 1979, the incidence of poliomyelitis has fallen dramatically. At present, at least 80 percent of infants in Thailand have

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received 3 doses of poliovaccine (OPV) by the age of one year. The impact of OPV immunization was evidenced all over the world since the global incidence of poliomyelitis has fallen sharply. The incidence has reached a stage in 1988 when the World Health Organization has committed to eradicate poliomyelitis worldwide by the year 2000 (World Health Assembly, 1988). Since then, the poliomyelitis eradication program has been established in all WHO regions. The strategies for achieving poliomyelitis eradication as recommended by WHO demonstrated to be effective in many countries (Hull et al, 1994). These are: 1) maintaining high coverage with oral polio vaccines; 2) conduct of national immunization days; 3) implementation of effective acute flaccid paralysis surveillance; 4) aggressive outbreak control by mopping up.

In Thailand during the last four years, the incidence of poliomyelitis was changed from endemic to focal transmission. The indigenous polio cases were confirmed to be less than 10 cases per year and type 1 was still the most common isolate (Pongsuwanna et al, 1995). One of the factors that may have had a role of poliomyelitis occurrence in Thailand is an endemicity of poliovirus in neighboring countries which still have political problems and achieved low coverage rate of immunization.

Since the National Institute of Health (NIH), Department of Medical Sciences, Ministry of Public Health, Thailand is one of WHO Regional Reference Laboratory for poliovirus diagnosis, isolation and serotyping. The centre received stool samples obtained from patients with acute flaccid paralysis (AFP), contact cases living not only in Thailand but also in Cambodia, Laos, Nepal and Myanmar. We were able to characterise the isolated stains from AFP by hybridization to determine whether the viruses are wild or vaccine-derived (De et al, 1995). Wild stains identified by these procedures were further characterized by partial nucleotide sequencing of viral genome (Rico et al, 1987) which reveals the genetic relationship among virus isolates. We took this opportunity to study the molecular epidemiology of wild poliovirus isolated from cases in Thailand and neighboring countries and the result was reported herewith.

MATERIALS AND METHODS

Virus and cell culture

All of the wild-type 1 (25 strains) used in the study were isolated in Virus Research Institute, NIH, Thailand from stool specimens of AFP patients and some contacts.

They consisted of 12, 6, 2, 1 and 4 strains isolated from patients in Thailand, hill tribe, Cambodia, Myanmar and Nepal respectively. Both HEp-2C and RD cells in Eagle's minimum essential medium supplemented with 2 percent FBS were used for virus isolation as described by WHO (WHO manual, 1992). Type-specific hyperimmune sera for virus identification were provided by WHO Polio Special Reference Laboratory (RIVM, Netherlands). The original isolates were propagated in RD monolayer cells to produce high titered inoculation stocks and submitted for molecular studies.

Molecular characterization

Intratype differentiation. The isolated poliovirus strains were characterized by hybridization with Sabin-specific nucleic acid probes which specifically hybridize with the genomes of OPV-related isolates, but not with those of wild polioviruses as described previously (De et al, 1995, Pongsuwanna et al, 1996). Briefly, cultures of isolated strains in RD cells were treated by 37 percent formaldehyde solution, and mixtures were heated at 65°C for 15 minutes. Samples were immobilized onto positively charged nylon membranes (Boehringer Mannheim) by using a 96 manifold (Schleicher & Schuell), RNAs were bound to the filters by UV cross linking ($\lambda = 254 \text{ nm}$). Hybridization was performed by incubation of the filters in hybridization buffer containing DIG-labeled Sabin-specific probes at 65°C for 18-24 hours. Detection the hybrids by the Boehringer Mannheim non-radioactive detection kit. The filters were exposed to X-ray film (Kodak XAR2). This characterization determines whether the isolate is wild or vaccine derived strain.

Nucleotide sequence of PCR products. All wild type 1 poliovirus strains detected by this method were further characterized by partial genomic sequencing as described (Rico et al, 1987, Kew et al, 1990). Briefly, RNA from culture of isolates served as templates for RT-PCR of a 150-nucleotide segment in the encoding parts of the capsid protein VP1 (90 nucleotides) and non-capsid protein 2A (60 nucleotides) at position 3,508-3,527 with a set of primer Q8 (Yang et al, 1991). The PCR products (c DNA) were purified and sequence by the dideoxy method using the Tag DyeDeoxy™ Terminator Cycle sequencing kit (Applied Biosystems, Inc., U.S.A.). The primers were the same as used in the PCR. Samples were electrophoresed through 4.75 percent polyacrylamide gel with 8.3M urea and analyzed on a 373A automate sequencer (Applied

Biosystems, Inc., U.S.A.). The CLUSTAL program (Higgins et al, 1988) was used for determination of sequence relationships among poliovirus genomes which summarized in dendrograms.

RESULTS

Twenty-five strains of wild type 1 which were isolated from AFP patients in different countries and provinces (Table 1) were used for sequencing. These sequences could be grouped into 2 distinct genotypes by dendrogram analysis (Fig. 1,2). In Fig. 1 showed that 8 strains belonged to the first genotype; 4 strains were isolated from patients

who lived in Udon Thani (THA92, UDON THANI), Yasothon (THA92, YASOTHON), Sakon Nakhon (THA92 SAKON NAKHON) and Sing Buri (THA92, SING BURI). The remaining 4 strains were isolated from hill tribe refugee and contact in Chon Buri (RC92, CHON BURI and RC92c, CHON BURI), Cambodian refugee camp in Trat and Prachin Buri (RC92, KAM, TRAT; RC92, KAM, PRACHIN BURI). The provinces where the 1st genotype were found were mostly located in the eastern part of Thailand near Cambodia. There were fourteen strains of the second genotype which included wild type 1 isolated in 1992. The provinces where most patients

Table 1. Wild type 1 polioviruses isolated in Thailand during 1992-1995.

Genotype	Code	Source	Nationality/Province	Onset	Note
I	RC92, KAM, PRACHIN BURI	AFP	Cambodian refugee camp	13 Aug'92	**
I	THA92, SING BURI	AFP	Thai/Sing Buri	17 Aug'92	-
I	THA92, YASOTHON	AFP	Thai/Yasothon	26 Aug'92	-
I	RC92, KAM, TRAT	AFP	Cambodian refugee camp	29 Aug'92	**
I	THA92, SAKON NAKHON	AFP	Thai/Sakon Nakhon	1 Sep'92	-
I	THA92, UDON THANI	AFP	Thai/Udon Thani	5 Oct'92	-
I	RC92, HT, CHON BURI	AFP	Hill tribe refugee camp	6 Oct'92	***
I	RC92C, HT, CHON BURI	contact	Hill tribe refugee camp	-	***
I	THA95, KHON KAEN	AFP	Thai/Khon Kaen	30 Nov'95	-
II	NEP92-001	AFP	Nepalese	24 Dec'91	-
II	THA92, SUPHAN BURI	AFP	Thai/Suphan Buri	29 May'92	-
II	NEP92-005	AFP	Nepalese	- Jun'92	-
II	MMR92, RANONG	AFP	Myanmar	3 Aug'92	*
II	NEP92-010	AFP	Nepalese	2 Sep'92	-
II	THA92, KANCHANABURI	AFP	Thai/Kanchanaburi	4 Sep'92	-
II	RC92, HT, TAK 1	AFP	Hill tribe refugee camp	9 Sep'92	***
II	THA93, CHON BURI	AFP	Thai/Chon Buri	27 Jan'93	-
II	RC93, HT, TAK	AFP	Hill tribe refugee camp	27 Mar'93	***
II	RC93, HT, SARABURI	AFP	Hill tribe refugee camp	17 Apr'93	***
II	THA93, LOP BURI	AFP	Thai/Lop Buri	31 Aug'93	-
II	THA93, PATTANI 1	AFP	Thai/Pattani	5 Sep'93	-
II	THA93c, PATTANI 1	contact	Thai/Pattani	-	-
II	THA93, PATTANI 2	AFP	Thai/Pattani	29 Nov'93	-
II	RC94, HT, CHIANG MAI	AFP	Hill tribe/Chiang Mai	27 Jun'94	***
III	NEP92-002	AFP	Nepalese	14 Jan'92	-
Sabin	THA93c, LOP BURI	contact	Thai/Lop Buri	-	-

* Myanmar patient hospitalized in Thai hospital

** camp in Thailand

*** camp in Thailand

lived were in western part of Thailand such as Tak, Kanchanaburi, Suphan Buri. Poliovirus strains of the second genotype were spread into other parts of the country in 1993 and caused illness in Lop Buri, Pattani, Saraburi and Chon Buri. This genotype recurred in 1994 since it was isolated from hill tribe patient in Chiang Mai and its

genetic sequence was related to the 2nd genotype as shown in the first dendrogram.

Wild type 1 isolated strain in 1995 from Thai patient who lived in Khon Kaen Province (THA95, KHON KAEN) was added for dendrogram analysis as shown in Fig. 2. The genetic sequence of this strain was related to the

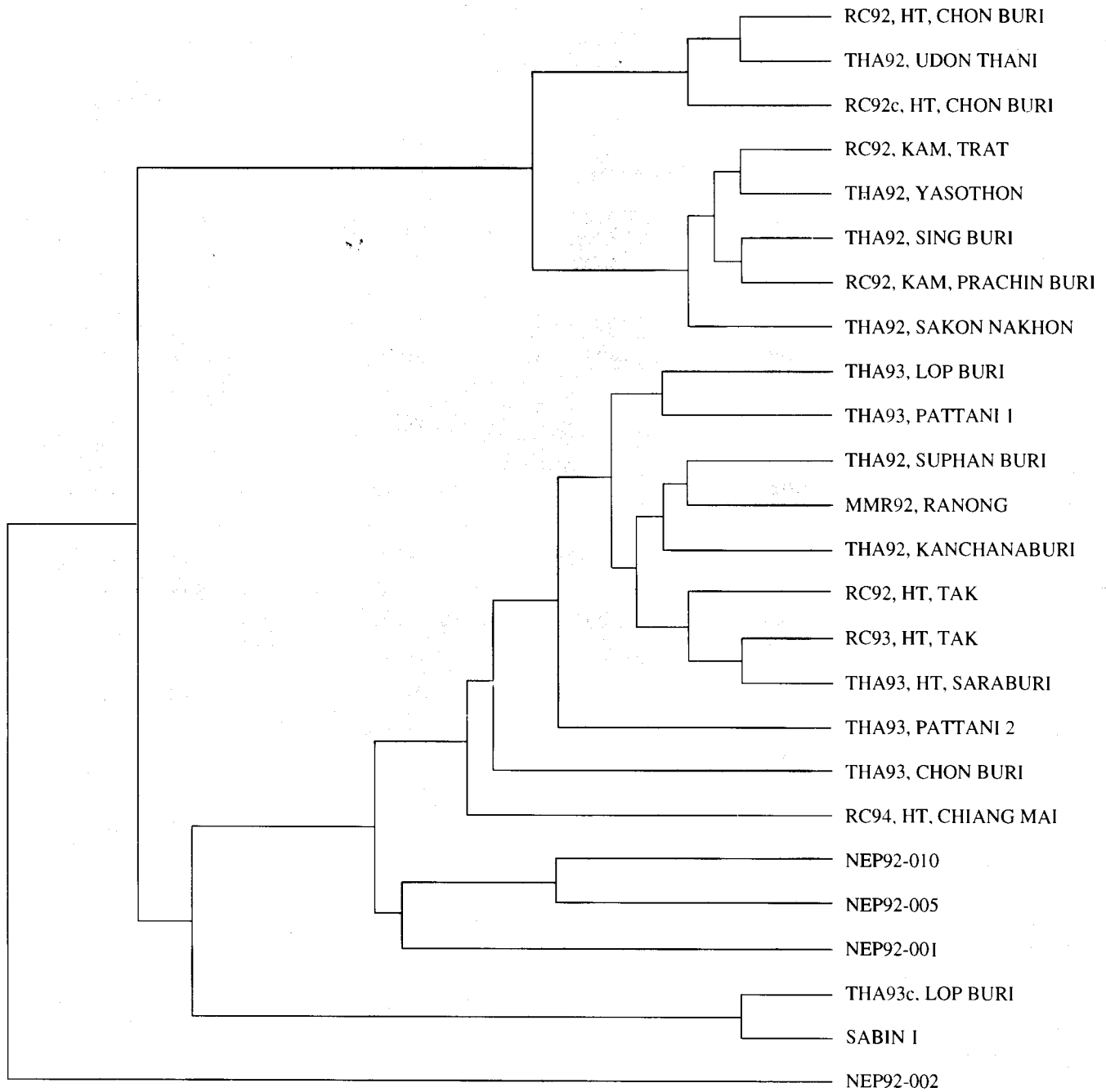


Fig. 1 Sequence relationships of VP1-2A region; nucleotide 3295 to 3444; numbered by alignment with the reference Sabin 1 among 23 wild type 1 polioviruses isolated in Thailand.

1st genotype of this study. The spot map in Fig. 3, presented polio confirmed cases in Thailand during 1992 to 1995 which showed the incidence of poliomyelitis has been decreased dramatically and revealed the focal epidemic of poliomyelitis in border provinces and the provinces that hosted refugee camps.

DISCUSSION

AFP surveillance showed a nearly constant incidence of acute flaccid paralysis in children less than 15 years of age in Thailand. Eleven, 14 and 1 of wild type were isolated in 1992 to 1994 respectively and in 1995, only 2

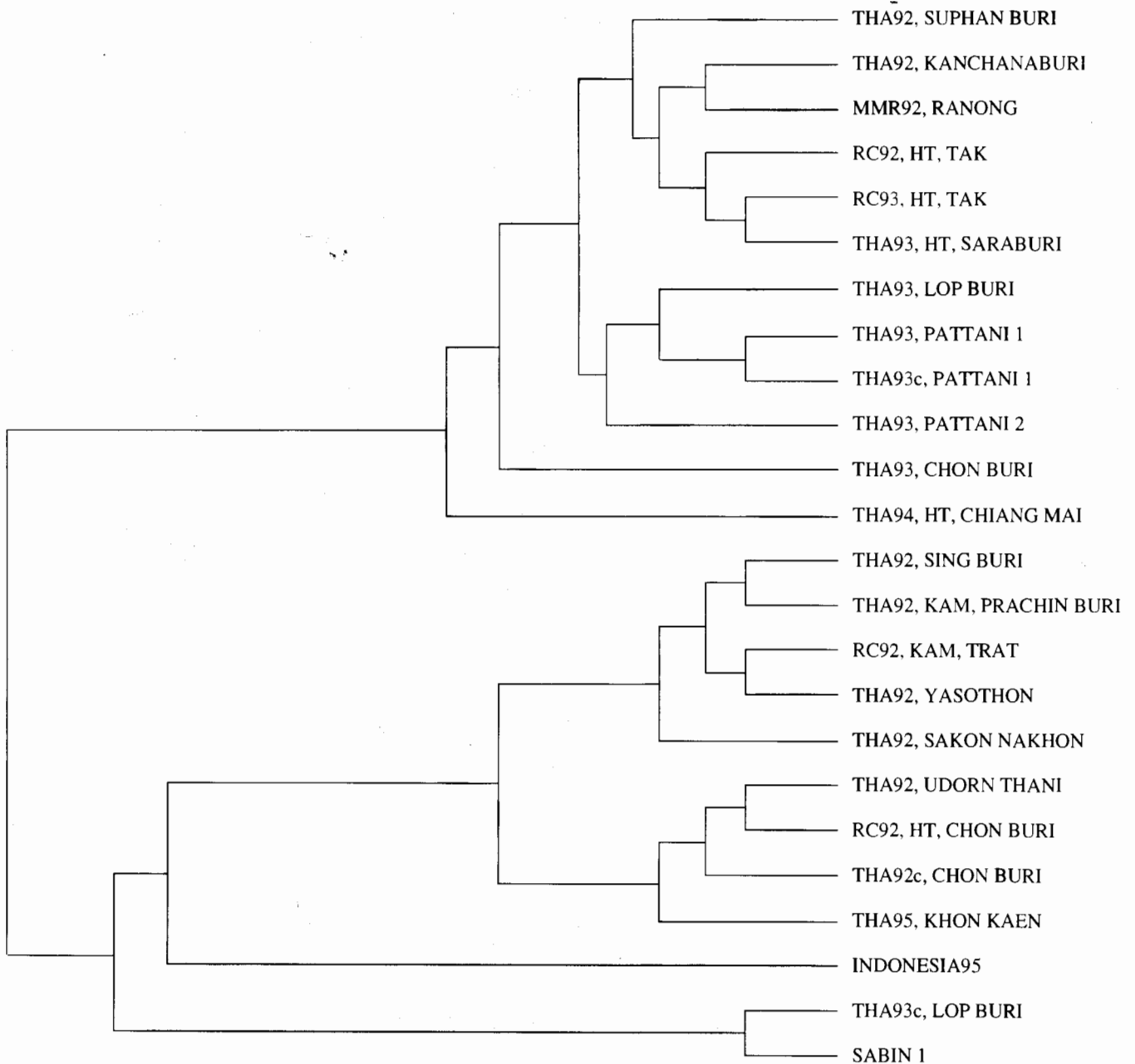


Fig. 2 Sequence relationship of VP1-2A region; nucleotide 3295 to 3444; numbered by alignment with the reference Sabin 1 among 22 wild type 1 polioviruses isolated in Thailand.

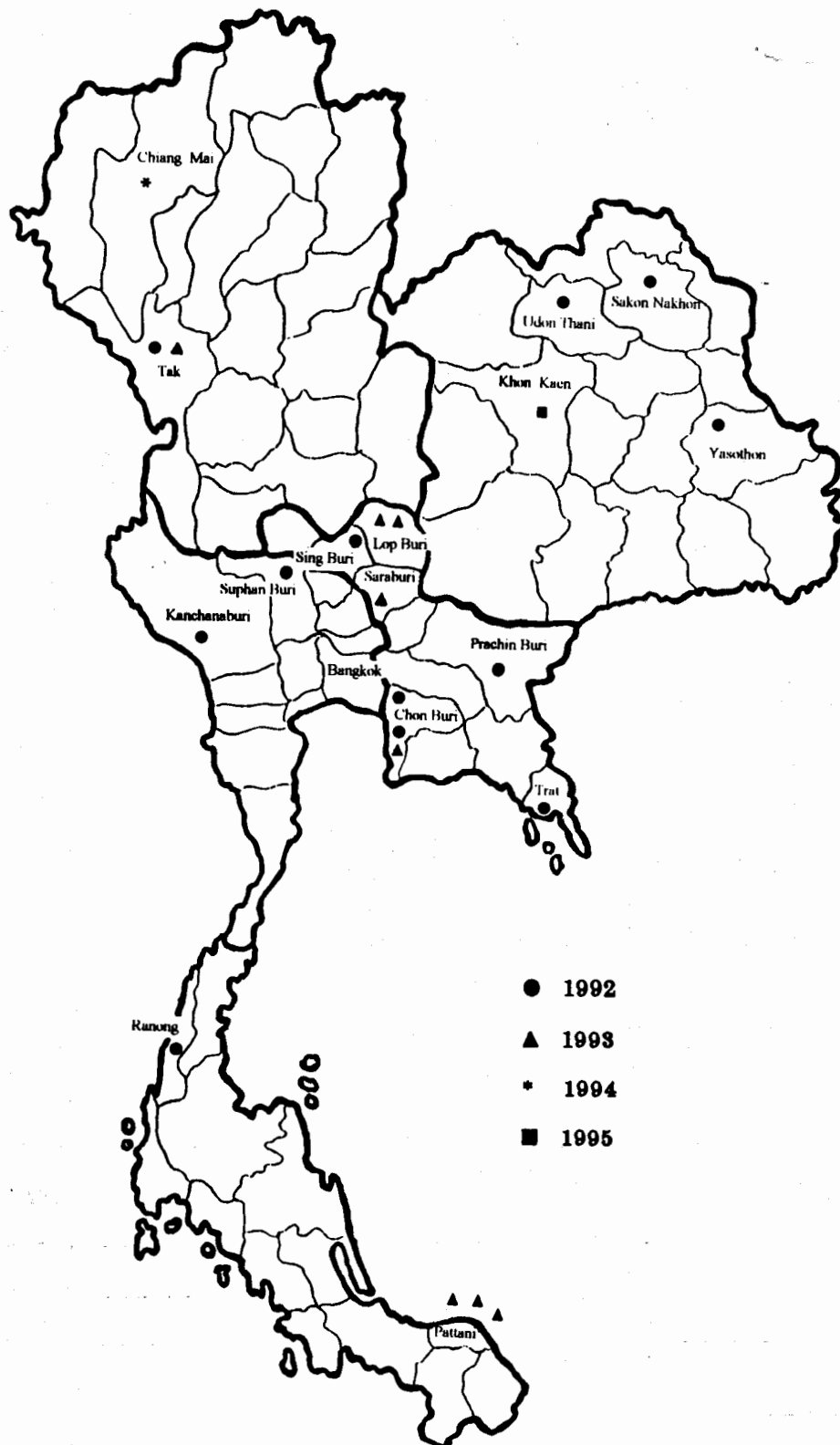


Fig. 3 Wild type 1 polioviruses isolated in Thailand during 1992-1995.

wild poliovirus associated cases were detected (Pongsuwanna et al, 1996). Although, over 80 percent of children aged one year in Thailand have received 3 or more doses of OPV but the disease is still present in provinces at the

border of Thailand. It is conceivable that the cases were detected mostly in the migration camp. In addition foreign children who entered Thailand from endemic countries were also the remaining pockets of endemicity in the urban

and rural poor area as ever happened in Netherlands and Namibia in 1992 and 1994 (Van Spaendonck et al, 1996, Van Niekerk et al, 1994). Our molecular epidemiological studies revealed that the existing risk of importation and circulation of wild type 1 from endemic areas of neighboring countries to well-immunized countries such as Thailand. Fortunately, it could not establish endemicity and caused only focal epidemic and transmission. The 1st strain of genotype 1 was detected from AFP patients in Cambodian refugee camp of Prachin Buri (RC92, KAM, PRACHINBURI) on 13 August 1992 which shared the same source with strains isolated in Sing Buri (THA92, SING BURI), Yasothon (THA92, YASOTHON), and Trat (RC92, KAM, TRAT). All were detected in the same month on 17, 26 and 29 August 1992 respectively. Other isolated strains (3 strains) of the same genotype were detected in September and October 1992. The remaining had some genetic difference from the previous strains because the poliovirus genome evolves rapidly during replication in humans (Nottay et al, 1981; Minor et al, 1986b). This genotype could not be detected from AFP patient in Thailand since 1992 until 30 November 1995, the wild type 1 strain from AFP patient in Khon Kaen was isolated. This isolate is closely related to viruses found previously in Thailand and also found in Cambodia and southern Vietnam (Kew, personal communication).

The 1st strain of another independent genotype was found in Nepal on December 24, 1991 (NEP92-001) and the other strains of the same genotype were found in Thai and refugee camp patients from 1992 to 1994. The last case of this genotype was a hill tribe patient in Chiang Mai in 1994. The dendrogram in Fig. 1 showed the cases in Tak (RC93, HT, TAK) and Saraburi (RC93, HT, SARABURI) acquired the virus from the same source because there were some hill tribe communities in Saraburi and also in Tak. Hill tribe people in Saraburi came from the community in Tak and that means there were very close contact between the two communities. Another point in the dendrogram (Fig. 2) showed the epidemiological linkage between wild type which was isolated from case and contact of the same community. These two strains were THA93, PATTANI 1 and THA93c, PATTANI 1. The information from wild strain surveillance and partial genomic sequence for molecular epidemiological study in Thailand, is useful to understand the importation of wild strain from endemic area to Thailand and revealed two directions of the importation; eastern must be imported from Cambodia and southern Vietnam, and western must be leaked from Nepal. This similar situation used to be occur in United States and

Mexico (Rico-Hesse et al, 1987) and also in the northern Andean region and the Caribbean coast of Colombia.

Since polio eradication program and the AFP surveillance have been implemented, the outbreak response and mop-up immunization were operated to respond to the AFP case reports. Learning from experience of polio eradication in the Americas that the success of eradication relied on improvement of immunization coverage through organization of national immunization days (NIDs) in which OPV is administered to all children under 5 years regardless of their previous immunization status. Our data in this study revealed the risk of importation of wild type from endemic area to high coverage OPV immunized area. To close the pocket of endemicity area and to prevent the leakage of wild strain transmission into Thailand, the NIDs program must be performed in each country during the same period. In 1996, cooperation between Thailand and neighboring countries to jointly work toward regional eradication of poliomyelitis was initiated. The information from this studies can be used to make anyone involved in the program understood more about the wild strain transmission in each region and to achieve more effective eradication of poliomyelitis from Thailand.

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