SALMONELLA ENTERITIDIS OUTBREAK IN THAILAND : STUDY BY RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS

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Abstract. An outbreak of *Salmonella enteritidis* in Thailand was reported in 1990. The majority of isolates were found in chicken and human throughout the country. The continuation of a high rate of spreading which is presently continuing prompted us to investigate possible clonal involvement in the outbreak. One hundred and twenty five isolates of *S. enteritidis* which were isolated between 1990-1993 were clonally identified by the technique of Random Amplified Polymorphic DNA (RAPD) analysis. Eight profiles were found indicating the presence of 8 clones, designated no. 1-8. The predominant clone was profile no. 4 which was encountered in 93.6% of tested isolates while the rest of the profile comprised only 0.8-1.6%. The predominant clone was distributed mainly in isolates from chickens and humans which is suggestive that the profile no. 4 is the major clone involved in this outbreak and that chickens were the source of S. *enteritidis* infection. The information from the Microbiology Laboratory at Ramathibodi Hospital revealed that nearly 40% of *S. enteritidis* were isolated from blood specimens. This may reffect the invasiveness of *S. enteritidis* in Thailand. We concluded that the outbreak involved the single clone, RAPD profile no. 4 which may disperse dominantly during the epidemic.

INTRODUCTION

An increase in non-typhoidal salmonellosis has been observed in Thailand (Trikalsaransukh *et al*, 1986; Sirinavin *et al*, 1988; Sricharmorn *et al*, 1979). Epidemics of certain *Salmonella* serotypes have been reported, such as the outbreak of *S. krefeld* during the years 1976-1978 (Boozaya-angkool *et al*, 1979; Jayanetra *et al*, 1990). Currently *S. enteritidis* is the most common serotype encountered in extraintestinal infection in humans and infection in poultry (Bangtrakulnonth *et al*, 1993). Prior to 1990, the isolation of *S. enteritidis* in human was negligible, the average rate of isolation was less than 1%. Since then, the isolation rate of this serotype increased from 1.33% in 1990 to 2.98%, 9.54% and 16.98% between 1991 and 1993, respectively. The increase incidence of *S. enteritidis* in Thailand followed the worldwide spreading of this serotype, the prevalence of which has been reported since 1980s in Europe and the Americas (Anonymous, 1988). In Thailand, the sudden outbreak of this particular serotype in humans and in chickens is highly suggestive that chicken is the source of *S. enteritidis* infections in humans. To identify if there are particular strains associated with the outbreak in Thailand, we subtyped *S. enteritidis* collected during the epidemic years by Random Amplified Polymorphic DNA (RAPD) analysis. The method was described by Welsh and McClelland (1990) and Williams *et al* (1990). DNA polymorphism was presented by DNA fragments amplified with short arbitrary primers via Polymerase Chain Reaction (PCR).

MATERIAL AND METHODS

Bacteria

125 isolates of *S. enteritidis* were obtained from the WHO National Salmonella and Shigella Center, Thailand. They were isolated from various types of specimens and locations during 1990–1993.

DNA isolation

The method employed in this study followed those in Sambrook *et al* (1989).

Random Amplified Polymorphic DNA (RAPD) analysis

Bacterial DNA was amplified by the method of polymerase chain reaction (PCR). The PCR reac-

tion mixture was composed of: 100 mM Tris–HCl, 50 mM KCl 0.4 mM MgCl, 400 µM dNTP, 0.4 µM primer, 15 ng DNA, 1 U Taq polymerase in a final volume of 50 µl.

Six primers were initially tested singly and pairwise for best discrimination of bacterial DNA. The effective primer was then selected for the entire study, namely ATCTGGCAGC. The reaction was carried out for 35 cycles with the following program, 30 seconds 95°C, 1 minute 35°C, 2 minutes 72°C. PCR product was electrophoresed on 1.5% agarose gel in Tris–borate/EDTA buffer.

Information on *Salmonella* serotype in Thailand

The national data of *Salmonella* serotypes during 1991-1994 were obtained from the WHO National Salmonella and Shigella Center, Thailand. The data of *Salmonella* serotype from clinical specimen during 1990-1994 was available from the division of Clinical Microbiology, Department of Pathology, Ramathibodi Hospital, Bangkok.

RESULTS

Evidence for emergence of *S. enteritidis*

The numbers of *S. enteritidis* in Thailand during 1972-1993 from 2 major sources, humans and chickens are shown in Table 1. In 1972-1989, the isolation rate of this serotype from humans was 0.59%. In 1990, an initial increase was observed, continuing up till the present, with a rate of 16.98% in 1993. A similar increasing rate of isolates from chicken meat was observed.

The national data of first ten ranking of *Salmonella* serotypes isolated from humans, non-humans are shown in Table 2 and 3 . Table 4 gives information from a University Hospital, where one can clearly see that *S. enteritidis* became one of the top ten *Salmonella* serotypes in 1990 and progressively moved to 2nd-3rd rank in 1992 to the first rank in 1993 onwards. Similar observations were made in Ramathibodi Hospital and in the national data as

Table 1

S. enteritidis (SE) isolates from human and chicken meat in Thailand.

Year	Human SE*	Chicken meat SE*
1972-1989	266/44,718 (0.59)	
1990	51/ 3,839 (1.33)	15/ 1,073 (1.40)
1991	107/ 3,648 (2.93)	43/ 815 (5.28)
1992	308/ 3,227 (9.54)	108/ 958 (11.27)
1993	236/ 1,390 (16.98)	159/ 949 (16.75)

(*) Number of *S. enteritidis*/total *Salmonella* isolates. (%)

Table 2

The most frequently *Salmonella* serovars isolated from human specimens, Thailand.*

	1991	1992	1993	1994
1	*S. derby*	*S. weltevreden*	*S. enteritidis*	*S. enteritidis*
2	*S. weltevreden*	*S. derby*	*S. weltevereden*	*S. derby*
3	*S. agona*	*S. enteritidis*	*S. derby*	*S. weltevreden*
4	*S. krefeld*	*S. typhimurium*	S. 1, 1, 4,5 12:i:-	*S. anatum*
5	*S. typhimurium*	S. I,4,512:i:-	*S. typhimurium*	S. I,4,5,12:i:-
6	*S. virchow*	*S. agona*	*S. krefeld*	*S. typhimurium*
7	*S. anatum*	*S. anatum*	*S. anatum*	*S. agona*
8	*S. enteritidis*	*S. krefeld*	*S. agona*	*S. kedougonu*
9	*S. stanley*	*S. emek*	*S. choleraesuis*	*S. rissen*
10	*S. paratyphi* A	*S. blockley*	*S. blockley*	*S. stanley*

*Data from WHO National Salmonella and Shigella Center, Thailand.

Table 3

The most frequently *Salmonella* serovar isolates from non-human specimens, Thailand.*

	1991	1992	1993	1994
1	*S. paratyphi B* biovar Java	*S. virchow*	*S. enteritidis*	*S. enteritidis*
2	*S. hadar*	*S. enteritidis*	*S. blockley*	*S. hadar*
3	*S. virchow*	*S. hadar*	*S. hadar*	*S. blockley*
4	*S. blockley*	*S. blockley*	S. *paratyphi B* biovar Java	S. *paratyphi* B biovar Java
5	*S. weltevreden*	*S. anatum*	*S. derby*	*S. typhimurium*
6	*S. enteritidis*	S. *paratyphi* B biovar Java	*S. anatum*	*S. chester*
7	*S. anatum*	*S. derby*	*S. amsterdam*	*S. amsterdam*
8	*S. typhimurium*	*S. amsterdam*	*S. emek*	*S. virchow*
9	*S. derby*	*S. agona*	*S. virchow*	*S. emek*
10	*S. senftenberg*	*S. senftenberg*	*S. weltevreden*	*S. derby*

*Data from WHO National Salmonella and Shigella Center, Thailand.

Table 4

The most frequently isolate *Salmonella* serovar from clinical specimen.*

	1990	1992	1993	1994
1	*S. typhimurium*	S. I,4,5,12:i:–	*S. enteritidis*	*S. enteritidis*
2	*S. derby*	*S. enteritidis*	*S.* I,4,5,12:i:–	*S. derby*
3	S. I,4,512:i:–	*S. derby*	*S. derby*	S. I,4,5,12:i:–
4	*S. weltevreden*	*S. typhimurium*	*S. anatum*	*S. anatum*
5	*S. paratyphi* A	*S. paratyphi* A	*S. weltevreden*	*S. paratyphi* A
6	*S. agona*	*S. anatum*	*S. blockley*	*S. weltevreden*
7	*S. choleraesuis*	*S. weltevreden*	*S. agona*	*S. typhimurium*
8	*S. stanley*	*S. krefeld*	*S. typhimurium*	*S. agona*
9	*S. enteritidis*	*S. agona*	*S. virchow*	*S. anatum,* *S. rissen,* *S. choleraesuis*
10	*S. blockley*	*S. stanley*	*S. choleraesuis*	*S. stanley*

* Data from Ramathibodi Hospital.
No data available in 1991.

well. Furthermore, the important finding was that the majority of stains were isolated from blood, body fluids and sites other than the gastrointestinal tract, which was indicative of the invasiveness of the epidemic strain (Table 5).

Random Amplified Polymorphic DNA (RAPD) analysis

The molecular size of DNA amplified by primer ATCTGGCAGC was found to be in the range of 200-4,000 bp. However the demonstration of DNA fragments higher than 2,000 bp was inconsistent, hence they were excluded from the analysis for clonal identification.

From 125 isolates of *S. enteritidis*, 8 profiles were generated (Fig 1). The frequency of each profile is shown in Table 6. It is obvious that the majority of isolates showed RAPD profile no. 4 (93.6%). Moreover these isolates were the main ones from humans and chickens (Fig 2). Profiles no. 5 and 6 presented rarely in chicken specimens and profile no. 1, 3, 7 and 8 were found in specimens other than chickens. The result indicated that a clone having DNA profile no. 4 was most likely

Table 5

The isolates of *S. enteritidis* from clinical specimen, Ramathibodi Hospital, Bangkok, 1992-1994.

Specimens	Percentage of isolates		
	1992 N=51*	1993 N=70*	1994 N=85*
Blood	38	43	32
Rectal	22	14	32
Pus	28	23	17
Urine	10	6	8
Throat	4	1	2
Joint fluid	-	3	4
Ascitic fluid	-	-	2
CSF	-	7	-
Cervi	-	3	-

* Total no. of *S. enteritidis*

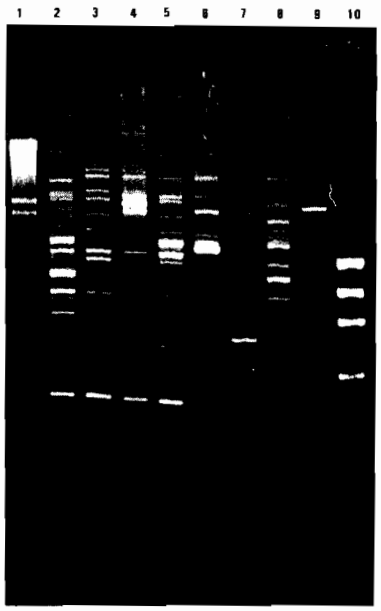

Fig 1–RAPD profile of *Salmonella enteritidis* amplified with 10 nucleotide length primer, ATCTGGCAGC. Lane 2–9; represent profile no. 1–8, respectively. Note the dissimilarity of the pattern of DNA fragment. Lane 1; λ/Hind III and Lane 10; θx174/Hae III markers.

the epidemic clone contributing to human transmission.

DISCUSSION

The initial increase of *Salmonella enteritidis* in Thailand occurred in 1990. It was encountered in humans and poultry infections and has been the most frequent serotype since 1993.

From our study, 125 randomly sampled isolates during the outbreak could be discriminated by RAPD into 8 profiles. The frequency of each profile was very different *ie* 0.8% in profile no. 1, 2, 5, 6, 7 and 8; 1.6% in profile no. 3 and 93% in profile no. 4. This evidence showed the diversity of the strains in the *S. enteritidis* population in Thailand. However the highest frequency of profile no. 4 indicated that the strain with this profile was involved in the outbreak. To demonstrate that there is no subtype among the large population of *S. enteritidis* with profile no. 4, some of isolates were subjected to RAPD with another 2 primers; the result showed the same pattern of every isolate, suggestive of strain homogeneity. Therefore it is highly likely that there was no other subtype among the remaining strains with profile no. 4.

Isolates with profile no. 4 came from 2 major sources; humans and chickens. This finding suggested that *S. enteritidis* could be transmitted from chickens to humans via food consumption. Chicken meat was reported to be the contaminated vehicle in UK while intact shell eggs or food containing eggs were the source of the organism in many countries (Dorn *et al*, 1993; Coyle *et al*, 1988; St Louis *et al*, 1988). In 1993, Saithanu *et al* (1994 a,b) studied the contamination of egg shells and contents with *S. enteritidis* in Thailand. Surprisingly they showed that only 0.24% of hen eggs and 0.71% of duck eggs were contaminated with *S. enteritidis* and it was concluded that the spreading of *S. enteritidis* in Thailand may related to the chicken meat only (Bangtrakulnonth *et al*, 1993).

The persistence of *S. enteritidis* in the poultry industry was believed to be maintained by consumption of contaminated feed (Hinton *et al*, 1989) and by vertical transmission in broiler and laying hen breeding stock (Lister, 1988). As the organism generally caused inapparent infection in poultry, the propagation of *S. enteritidis* continued in the

Table 6

RAPD profile of *Salmonella enteritidis* from various sources and location in Thailand, 1990-1993.

RAPD profile no.	Frequency (%)	Source of specimen	Location
1	0.8	non-human - eel	Bangkok
2	0.8	human - blood	Bangkok
3	1.6	human - blood	Bangkok
4	93.6	non-human - chicken meat, fluff, yolk	Chantaburi
		squid, animal feed	Khon Kaen
			Bangkok
		human - blood, fluid, pus,stool,	Songkhla
		urine	
5	0.8	non-human - chicken meat	Bangkok
6	0.8	non-human - chicken meat	Bangkok
7	0.8	human - pus	Bangkok
8	0.8	non-human - chicken meat	Bangkok

Non-human : chicken meat (40%), fluff (2.4%), yolk (2.2%), squid (0.8%), animal feed (0.8%), litter (0.8%)
Human : blood (20.8%), stool (17.6%), rectal swab (3.2%), pus (2.4%), urine (2.4%), fluid (0.8%)

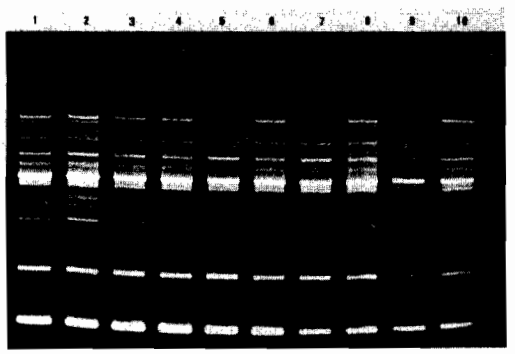

Fig 2–The RAPD profile no. 4 of islates from various sources of human specimens and chicken. Note the clearly identical pattern of DNA fragment. Lane 1-5; Human specimens from blood, urine, rectal, CSF and pus. Lane 6-10; chicken specimens from 3 meats, 1 fluff and 1 yolk.

flocks. Hopper *et al* (1988) isolated *S. enteritidis* from eggs with either ovarian or oviduct infection. Lister (1998) suggested that isolation of *S. enteritidis* from ovarian tissue was indicative of yolk contamination. Although the initial number of organism may be low, but the long storage of eggs may result in high population of organism in eggs (Humphrey *et al*, 1991). *S. enteritidis* has been isolated from tissues such as heart, spleen and cecum of broilers with macroscopic pericarditis, and from swabs of the carcass cavity. However, the frequency of such infection was only 1 in 1,000 broilers (O'Brien, 1988). This could mean that *S. enteritidis* may be invasive in broilers and hens. Dissemination of *S. enteritidis* in poultry flocks appears to be the contamination vehicle to humans.

Considering the clinical significance of *S. enteritidis*, the information from Ramathibodi Hospital showed that nearly 70% of *S. enteritidis* were isolated from extraintestinal sources. Nearly 40% of specimens were blood and the remainder were body fluids, urine and pus. This suggested that *S. enteritidis* is invasive among the Thai population. Wong *et al* (1994) also reported a high rate of extraintestinal infection of *S. enteritidis* in Hong Kong. They also were very concerned that *S. enteritidis* is likely to become a major serotype in extraintestinal salmonellosis, should the worldwide problem of contamination still continue.

WHO *Salmonella* surveillance data from 1979-1987 revealed that *S. enteritidis* appeared to be increasing in America and in Europe (Rodrigue *et al*, 1990). In 1970 only 2 (10%) of 21 countries reported *S. enteritidis* as their most common sero-

type. The figure increased to 9 of 21 (43%) countries in 1987; 8 of 9 of these were European countries. In Scottland *S. enteritidis* has been among the ten most prevalent serotypes during the past two decades and dominant since 1987 (Rankin *et al*, 1995). In Hong Kong and Japan *S. enteritidis* become the problem since 1989 (Wong *et al*, 1994; Taguchi *et al*, 1992). Epidemiological studies by phagetyping revealed that the predominant type in the United States and Canada is phage type 8 while phage type 4 predominates in Europe and UK (Hickman-Brenner *et al*, 1991; Amonymous, 1988; Coyle *et al*, 1988; Cowden *et al*, 1989; Dorn *et al*, 1993). In Germany prior epidemic isolates have been studied; this revealed that the predominant line in the epidemic was a new strain (Stanley *et al*, 1992). In Switzerland the predominant phagetype prior to the epidemic was phage type 8. During the epidemic the common type was phage type 4 (Stanley *et al*, 1992). In Scottland the outbreak has also been attributed to phage type 4 (Rankin *et al*, 1995). In Thailand *S. enteritidis* was a rare serotype before 1990, dramatically increasing since 1991. There was no information regarding the phagetype of strains either before or during the outbreak. Our typing results based on RAPD done on the isolates from the collection of the WHO National Salmonella and Shigella Center, Thailand is currently the only source that provides the epidemiological data. It is concluded that *S. enteritidis* RAPD profile no. 4 type dispersed and expanded during the time of the epidemic. Previously being a rare serotype, the emergence of this serotype reflects the worldwide situation and alert that *S. enteritidis* may have a long lasting impact on Public Health in Thailand.

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