

Laboratory Testing of *Salmonella paratyphi A* in Taiwan, 1987-1996

Abstract

In Taiwan between 1987 and 1996, 45 sporadic cases of *Salmonella paratyphi A* were detected. The majority of cases, a total of 24, occurred in the year 1996, thus accounting for 53% of all cases. 33 cases (73%) were imported; 8 (18%) were local sporadic cases; and 4 (9%) were of unknown origin. Most imported cases were infected in Southeast Asia: 31% in Thailand, 27% in Indonesia, 7% on mainland China, and 2% each in Singapore, Malaysia, Cambodia, and Myanmar. The pathogenic agents of 37 cases (82.2%) were detected by blood culture, 6 (5 carriers) (13.3%) from feces, one each (2.2%) from bile and bone marrow. There were slightly more affected males than females; and more cases were in the 21-40 and 41-50 age groups. Of the five carriers, one was male, and the other four were female. Cases were detected in each month except February and November, with more cases in March through October. The 42 strains of *Salmonella paratyphi A* detected in the past ten years were tested with Bio-test No. 1 kit (Eiken, Japan). When electronic codes of the 21 biochemical characteristics obtained from the strains were converted into computer codes, the strains were found to belong to four biological types. The typical biological type of *Salmonella paratyphi A* had 35 strains; the other 7 strains belonged to the other three biological types. They differed from the typical biological type by only one or two characteristics.

Key word: *Salmonella paratyphi A*

Introduction

Enteropathogenic bacteria include *Salmonella typhi* and *Salmonella paratyphi*. Enteric fever has been an infectious disease affecting humans since the beginning of history. It has not been completely eradicated, and cases are reported each year.

Cases of paratyphoid, however, have not been reported in Taiwan for the last ten years. With an increase in tourism, there have been more imported cases of infectious diseases including paratyphoid. The first case of paratyphoid was detected in Taiwan in 1987⁽¹⁾. Through effective control measures, the disease did not spread. Every year or every other year, however, some sporadic cases, more so in recent years, have been reported. It is likely that the disease will recur in outbreaks and pose some threat to the health of the population.

This report presents an analysis of *Salmonella paratyphi* A strains isolated from sporadic cases and imported cases from Southeast Asian countries in the last ten years, which will include their biochemical characteristics, countries in which the disease was likely transmitted, specimens, age, and monthly distribution. When visiting these countries, some precautions should be taken.

Materials and Method

- 1) Strains for testing: In the period between 1987 and 1996, 42 strains of *Salmonella paratyphi* A were isolated by medical care institutions and the former Division of Bacterial Diseases of the National Institute of Preventive Medicine from sporadic cases occurring in tourists, local patients and carriers. They were tested for their biochemical characteristics using basic and other agars including: triple sugar iron agar (TSIA), lysine decarboxylase medium, and sulfide indole motility medium (SIM), using biochemical test kit available on market (Bio-test No. 1, Eiken, Japan) for further biochemical testing. The findings were converted into computer codes to determine names of the bacteria. Serological method was also applied to analyze and assess antigen structures.
- 2) Detection of *Salmonella paratyphi* A: Cases of *Salmonella paratyphi* A detected in the ten years between 1987 and 1996 in Taiwan were analyzed.
- 3) Confirmation by serological method⁽²⁾:
 - (1) Analysis of O (somatic) Antigen: *Salmonella paratyphi* A strains to be tested were inoculated on tryptic soy agar (TSA), placed in the culture box at 37 °C for 18-20 hours, and tested by slide agglutination method for O antigen. A drop of O2 antiserum (Seiken, Japan) was placed on the slide. Colonies were collected with a platinum loop for mixing with antisera and shaken to accelerate agglutination. It was read positive if significant particles could be noted by the naked eye in 30 seconds.

- (2) Analysis of H (flagellum) Antigen: *Salmonella paratyphi* A strains to be tested were inoculated on H broth (Difco), placed in the culture box at 37°C for 6-8 hours, and 0.6% formalin normal saline of the same quantity was added to form H-antigen fluid. 0.5 mL of the H-antigen fluid was placed in a test tube (13x100 mm), one drop of *Salmonella* H-antigen (Seiken, Japan) was added and the test tube was then shaken. The specimen was placed in a water tank at 50°C for observation of any cloud-like particles one hour later. If there were particles, it was read positive.

Results

The first sporadic case of paratyphoid A was detected in Taiwan in 1987. In the last ten years, except for the years 1988 and 1990, sporadic cases were reported each year, totaling 45 cases. The number has been increasing, for example in 1996 to 24 cases (53% of all cases). Of all cases, 33 (73%) were imported; 8 (18%) were local sporadic cases; and 4 (9%) of unknown origin. Most of the patients reported having visited Indonesia, Thailand, Malaysia, Singapore, Vietnam or Mainland China as tourists, for commercial or occupational reasons or to meet relatives. They developed a typhoid like fever either while in route or soon after their return. Most (82%) cases were of *Salmonella paratyphi* A were detected by blood culture; 6 (5 carriers) (13.3%) from feces; and one each (2.2%) from bile and bone marrow. 31% of them were infected in Thailand, 27% in Indonesia, 7% in mainland China, and 2% each in Singapore, Malaysia, Cambodia, and Myanmar. There were 26 male and 19 female cases with a sex ratio of one male to 0.73 females. Most of them were in the 21-40 and 41-50 age groups. Of the five carriers, one was male and the other four were females (Table 1).

Cases were reported in each month with the exception of February and November, most cases occurring in the warmer months between March and October (Figure 1).

The 42 *Salmonella paratyphi* A strains of the last ten years were tested for basic biochemical reactions by differentiation media such as TSIA, lysine decarboxylase medium, and SIM, and by Bio-test No. 1 kit available on the market, for more biochemical characteristics. Computer codes were used. 20 test items included H₂S, ONPG, IPA, indole, citrate, lysine, ornithine, arginine, urea, malonate, glucose, mannitol, adonitol, arabinose, inositol, rhamnose, sorbitol, maltose, sucrose, and nitrate. For the 21 biochemical characteristics obtained, every three reaction tanks were assigned a computer code, and given respectively scores of 4, 2, and 1. The seven-digit code constituted the code number for the bacterium. The code number was cross-referenced to the computer codebook for the name of the bacterium.

The 42 stains of *Salmonella paratyphi* A thus tested were grouped into four different biotypes. Of them, the 35 strains coded 0021535 were the most

typical.

The other two strains coded 4021535 and 4031535 were different from the typical ones by one or two characteristics. Five other strains coded 0031535 showed positive reaction to arginine. Further testing by serological methods such as slide and test tube agglutination tests demonstrated that in the O and H antigens, the O₂ antigen and H-a antigen of *Salmonella paratyphi A* were positive. The reaction of arginine and hydrogen sulfide was slightly different from that of the typical strain (Table 2).

Discussion

In the ten years between 1987 and 1996, 45 *Salmonella paratyphi A* cases were detected; of them, 73.3% (33 cases) were imported, 17.7% were local cases, and 9% of unknown origin. In a Japanese analysis of 88 *Salmonella paratyphi A* cases detected between 1988 and 1994, it was estimated that 79.6% of them were imported, 17% were local cases, and 3.4% of unknown origin. The features common to the infection in Japan and Taiwan were that there were a large number of imported cases; the cases had visited Southeast Asian countries and developed, upon return, acute symptoms of enteric fever, which were later confirmed by culture to be caused by typhoid-like pathogens. In Japan, 54.6% of the cases had pathogens detected by blood culture, 25% by blood and feces, and 15.9% by feces alone. This was close to the 82.2% detection rate by blood culture in Taiwan⁽³⁾. Eight of the cases or carriers had never been abroad, suggesting that there are still some unknown sources of potential infection in Taiwan. Though there have been sporadic cases in the past ten years, due to effective disease control measures, the infection did not spread. They were only individual cases. In Japan, however, many cases were imported from Southeast Asia each year resulting in a winter outbreak from December 1993 through January 1994; and another outbreak in August-September 1995. The number of paratyphoid cases in those two years exceeded the number of typhoid cases^(3,4). In May and August 1966, some tourist groups from Taiwan visited Thailand. Upon return, some of the members became ill. They were confirmed to be infected by *Salmonella paratyphi A* after specimen testing, and were treated under isolation. This was the most serious imported infection in recent years. A similar incident occurred in Japan in March of the same year. A group of university students on a study tour visited Singapore, Malaysia, and Indonesia and became infected by typhoid and paratyphoid bacteria. There are still cases of *Salmonella paratyphi A* in Japan in the winter. Statistics of Japan shows that imported cases occur throughout the year, especially in March and April^(3,4). In Taiwan, except for the months of February and November, imported cases were reported every month. It is, therefore, reasonable to assume that the disease is present in Southeast Asian countries throughout the year, especially in the summer time. Visitors to these areas should take necessary precautions.

Statistics collected in Japan from January 1994 through September 1996 revealed that more *Salmonella paratyphi* A cases or carriers were in the 20-29 age groups, followed by the 30-39 and 40-49 age groups; according to the statistics for 1988 through 1994, more cases were in the 30-39 age groups, followed by the 20-29 and 40-49 age groups^(3,4). In Taiwan in the last ten years, more cases were found in the 21-50 age groups. The age distributions of cases in the two countries were similar. It is likely that because of the similarity in cultures, more people of the same age groups visit Southeast Asia for tourism, business, and meeting friends. Japanese have different eating habits, therefore it is possible that their chances of being infected are higher. With the exception of a larger number of cases in 1996, Taiwan has thus far only sporadic cases. Cases each year in Japan far exceed those of Taiwan⁽⁴⁾. The other reasons are that Japan has a better disease reporting system and that more of them visit Southeast Asia each year.

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Table 1. Detection of Salmonella paratyphi A in Taiwan, 1987-1996

Year	Case	Specimen ^a	Age							Sex		^b Country Visited	
			1-10	11-20	21-30	31-40	41-50	51-60	71-80	unknown	M		F
1987	1(2.2)	blood/1				1					1		unknown
1989	1(2.2)	blood/1								1			unknown
1991	2(4.4)	blood/2				1	1				1	1	Indonesia/1 ; Taiwan/1
1992	1(2.2)	blood/1				1					1		unknown
1993	6(13.3)	blood/5 feces/1	1	1	1	2				1	4	2	Indonesia/3 ; Vietnam 、 Malaysia/1 ; Taiwan/2
1994	6(13.3)	blood/4 feces/1 bone marrow/1			1	1	3				1	6	Thailand/1 ; Myanmar/1 ; Singapore/1 ; mainland China /1 ; Cambodia/1 ; Taiwan/1
1995	4(8.8)	blood/3 bile/1				2	1	1			2	2	Indonesia/3 ; unknown/1
1996	24(53.3)	blood/20 feces/4		2	11	6	2	2	1		10	14	Indonesia/5 ; Thailand/13 ; mainland China /2 ; Taiwan/4
Total	45(100)		1	3	13	14	7	3	3	1	26	19	

^a : Specimen/Cases () : %

blood/37(82.2)

feces/6(13.3)

bile/1(2.2)

bone marrow/1(2.2)

^b : Country/No. () : %

Thailand/14(31) ; Indonesia/12(27) ; mainland China/ 3(7) ;

Singapore/1(2) ; Cambodia/1(2) ; Vietnam 、Malaysia/1(2) ;

Myanmar/1(2)

Total : Imported/33(73) ; local/8(18) ; unknown/4(9)

Table 2. Biochemical Characteristics of Salmonella paratyphi A tested with Bio-test

Code No.	No. of Strains	Name of Bacterium	Serological Finding	Year Isolated
0021535 ^a	35	<i>S. paratyphi</i> A	O2:+++H:a+++	1987.1991-1996
4021535 ^b	1	<i>S. paratyphi</i> A	O2:+++H:a+++	1993
4031535 ^c	1	<i>S. paratyphi</i> A	O2:+++H:a+++	1993
0031535 ^d	1	<i>S. paratyphi</i> A	O2:+++H:a+++	1991
0031535 ^d	1	<i>S. paratyphi</i> A	O2:+++H:a+++	1993
0031535 ^d	1	<i>S. paratyphi</i> A	O2:+++H:a+++	1996
0031535 ^d	1	<i>S. paratyphi</i> A	O2:+++H:a+++	1996
0031535 ^d	1	<i>S. paratyphi</i> A	O2:+++H:a+++	1996
Total	42			

^a bio-types with most typical characteristics.

^b bio-types with one non-typical characteristics.

^c bio-types with two non-typical characteristics.

^d bio-types with one non-typical characteristics.

Figure 1. The Month Distribution of paratyphoid A (1987-1996)