

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 60	Bacteriologic Survey of Stools from Patients with Acute Diarrhea
Project No. 3A 025601 A 81	Military Medical Research Program S. E. Asia
Task 01:	Military Medical Research Program S. E. Asia
Subtask 01:	Military Medical Research Program SEASIA (Thailand)
Reporting Installation:	US Army-SEATO Medical Research Laboratory APO 146, San Francisco, California Division of Medical Research Laboratories Department of Bacteriology and Immunology
Period Covered by Report:	April 1963 to 31 March 1964
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Reports Control Symbol:	MEDDH-288
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* This study was initiated by Lt. Col. Sidney Gaines and was under his direction until 8 September, 1963.

** Thai Department of Health

parts of the country except the northeastern portion. As in previous years approximately 12 percent of the specimens yielded salmonella, almost 47 percent yielded paracolons, and less than 2 percent yielded shigella. Fewer enteropathogenic Escherichia coli strains were isolated because of a curtailment of the study to exclude serotyping of isolates except those from children of six years or younger. All of the vibrios identified were either non-agglutinating (11.3 percent) or were El Tor. The Ogawa serotype predominated in all parts of the country except the southeastern area where Inaba was predominant. Vibrio cholerae El Tor Ogawa strain was isolated from the stool of an immunized American serviceman suffering from a moderately severe diarrhea. A concurrent parasitological examination revealed the presence of Endamoeba histolytica. The significance of this is that it is possible for immunized individuals to harbor and shed these organisms, and thus serve as potential sources of infection.

A survey of 470 patients with clinical cholera confirmed by various laboratories showed that this was not an epidemic of children but mostly of adults. Approximately 40 percent of these patients had been immunized against cholera. Most patients were in low economic groups as revealed by their annual income and sanitary facilities. Almost half of them had therapy before admission to hospitals and, when compared to classical cholera, their clinical course was mild. In spite of an intensive educational campaign in cholera areas only about one-fifth of the families boiled their drinking water and only about 10 percent thought that water was their source of infection. The efforts of the Royal Thai Department of Health undoubtedly reduced the severity of this outbreak.

BODY OF REPORT

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Objective: To determine the types, frequency of occurrence and distribution of salmonellae and shigellae in stools of patients with acute diarrhea and to determine the incidence of other Enterobacteriaceae and their relationships to this disease.

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Description: The 3,659 specimens included in this study were from 2,964 patients of both sexes from hospitals throughout Thailand. All were hospitalized for acute diarrhea, and most specimens were taken during the acute stage of the disease. In the Bangkok area 2 rectal swabs were taken from each patient. One was placed in a screw-capped tube of selenite-F enrichment broth and the second into a tube of alkaline peptone broth. Specimens from outside of Bangkok were submitted in a holding medium designed for transport of enteric bacteria. Upon arrival at the laboratory tubes of selenite-F and alkaline peptone broth were inoculated from the holding medium.

Each selenite-F broth was subcultured on desoxycholate-citrate and MacConkey agar plates. After overnight incubation, the selenite-F broth was again subcultured, this time to Salmonella-Shigella and eosin-methylene blue or MacConkey Agar plates. The alkaline peptone broth was subcultured on alkaline lauryl sulfate tellurite agar plates. All incubations were at 37 C.

All plates were examined after 24 and 48 hours incubation. Lactose negative colonies were transferred to Kligler's iron agar slants and subsequently into a variety of media to determine patterns of biochemical activity. Colonies typical of vibrios on alkaline lauryl sulfate tellurite agar were processed in the same way. Those isolates showing biochemical patterns typical of salmonella, shigella or vibrio were definitively identified serologically in accordance with methods described by Edwards and Ewing.

Progress: Results in Table I show that recognized diarrheal agents were isolated from approximately 25 percent of all specimens. This is somewhat higher than in previous years and the increase is explained by the continuing outbreak of cholera which began in July 1963.

The incidence of diarrhea associated with Salmonellae was 12.1 percent, (Table I) a percentage remarkably constant since 1961. Normal Thais were found to harbor the same percentage of this genus in their stools. The results in Table II show that 24 species of salmonella were identified from the 450 positive swabs. The percentage of children positive for Salmonellae was 17.7 as compared with 11.2 percent for adults. Most of this difference is accounted for by an outbreak of Salmonella paratyphi B in the nursery of Siriraj Hospital from April through September 1963. There were no further isolations of this organism from specimens submitted to SMRL after September 1963. Specimens of infant formulae tested at SMRL were negative for enteric pathogens and the cause - and cessation - of the outbreak remains unknown. Fifty-four of the 79 isolates of Salmonella montevideo were from Children's Hospital. These were distributed throughout the year indicating a focus of infection either in the geographic area served by the hospital, or in the hospital itself. The 45 isolates of Salmonella anatum were not localized in any one hospital and they were distributed throughout the country. In summary salmonellosis continues

TABLE I

Enterobacteriaceae isolated from Acute Diarrhea Cases in Thailand from
April 1963 through March 1964

Organism	Positive Specimens	Percent of Total Specimens Submitted
<u>Escherichia coli</u> (Enteropathogenic)	20	0.5
<u>Shigellae</u>	54	1.5
<u>Salmonellae</u>	444	12.1
<u>Vibrio cholerae</u> El Tor	451	12.3
Non-agglutinating vibrios	414	11.3
<u>Paracolo bacterium sp.</u>	1,715	46.9

TABLE II

Salmonella Species Isolated in Thailand from
April 1963 through March 1964

<u>Species</u>	<u>Group</u>	<u>Adult</u>	<u>Children</u>	<u>Total</u>
<u>Salmonella derby</u>	B	29	35	64
<u>Salmonella heidelberg</u>	B	1	3	4
<u>Salmonella paratyphi B</u>	B	7	85	92
<u>Salmonella san-diego</u>	B	1		1
<u>Salmonella saintpaul</u>	B			1
<u>Salmonella stanley</u>	B	5	4	9
<u>Salmonella typhimurium</u>	B	13	6	19
<u>Salmonella menston</u>	C ₁		1	1
<u>Salmonella montevideo</u>	C ₁	1	79	80
<u>Salmonella oslo</u>	C ₁	2	2	4
<u>Salmonella group C₁ species</u>		-	1	1
<u>Salmonella bovismorbificans</u>	C ₂		5	6
<u>Salmonella newport</u>	C ₂	6	8	14
<u>Salmonella group C₂ species</u>			1	1
<u>Salmonella bergedorf</u>	D	1		1
<u>Salmonella dublin</u>	D		2	2
<u>Salmonella enteritidis</u>	D		1	1
<u>Salmonella panama</u>	D		2	3
<u>Salmonella typhosa</u>	D	5	20	25
<u>Salmonella anatum</u>	E ₁	45	21	66
<u>Salmonella lexington</u>	E ₁	7	12	19
<u>Salmonella meleagridis</u>	E ₁	13	14	27
<u>Salmonella weltevreden</u>	E ₁	5	2	7
<u>Salmonella senftenberg var new castle</u>	E ₄	-	1	1
Total number isolated		144	306	450
Total patients examined		1,234	1,730	2,964
Number of positive patients		138	257	395

to be an important enteric disease and there is little indication that progress is being made in its eradication.

Only 54 shigella isolates (17 from adults and 37 from children) representing 7 species were isolated during this period. None of these were associated with outbreaks of any magnitude and the importance of these data is that the inoculum for outbreaks is present. As with the salmonella, the percentage of isolates of shigella from acute diarrheas was almost identical to the percentage of shigella isolated from normal Thais. (Table III)

Eight field trips into cholera areas outside of Bangkok were made by members of this department to provide diagnoses on the scene, transport specimens back to Bangkok and to arrange for submission of subsequent specimens to this laboratory. In addition support in terms of materials and personnel were provided to the U.S.A. Medical Research Team in Saigon, Viet Nam.

Using the Finkelstein and Mukerjee technique all isolates of agglutinable vibrios were El Tor. Early in the outbreak all strains were the Ogawa serotype and except for the southeastern portion of the country the ~~Ogawa~~ ^{Inaba} serotype predominated. The significance of the predominance of Inaba serotype in the southeastern portion of the country was thought to herald the end of the outbreak, but such was not the case. The outbreak has spread to all portions of Thailand except the extreme northeastern portion, and it shows no signs of terminating. There were 29 provinces reporting 609 cases of acute diarrhea with 35 deaths in March 1964. Many of these patients were in remote areas and in most instances stool specimens were not taken. Even so 73 cases were confirmed as cholera by various laboratories. Recent increases in the number of cases in two widely separated provinces indicate that cholera can become epidemic again in 1964. (Table IV)

Vibrio cholerae El Tor Ogawa strain was isolated from the stool of an immunized American serviceman suffering from a moderately severe diarrhea. The diagnosis was complicated by the concurrent finding of cysts of Endamoeba histolytica in his stool. Titrations of serum from this patient for agglutinating and vibriocidal antibodies were typical for sera from vaccinated individuals. While this can be considered as a possible case of mild cholera, a more important observation is that immunized individuals can harbor and shed these organisms, and thus serve as potential sources of infection.

A survey of 470 patients with clinical cholera confirmed by various laboratories showed that less than 20 percent of the patients were under 20 years. Historically cholera is a disease of the poor, and this outbreak was no exception. More than two-thirds of these patients were from families where the annual income was Baht 10,000 (approximately \$500) and the average

TABLE III

Distribution of Shigellae in Acute Diarrhea Cases in Adults and Children in Thailand

April 1963 through March 1964

Strain	Adults		Children		Total	
	No.	Percent of total isolated	No.	Percent of total isolated	No.	Percent of total isolated
<u>Shigella dysenteriae</u> 2	1	5.9	-	-	1	1.8
<u>Shigella flexneri</u> 2	2	11.8	7	19.0	9	16.7
<u>Shigella flexneri</u> 3	5	29.4	8	21.6	13	24.1
<u>Shigella flexneri</u> 4	3	17.6	1	2.7	4	7.4
<u>Shigella flexneri</u> 6	-	-	2	5.4	2	3.7
<u>Shigella sonnei</u> form 1	4	23.6	15	40.5	19	35.2
<u>Shigella sonnei</u> form 2	1	5.9	3	8.1	4	7.4
Alkalescens-Dispar	1	5.9	1	2.7	2	3.7
Total	17	100.0	37	100.0	54	100.0

TABLE IV

Vibrio Isolated from Acute Diarrhea Cases
from 22 July 1963 - 31 March 1964

Geographic Area	El Tor Inaba	El Tor Ogawa
Bangkok-Dhonburi	40	285
Southern Thailand	1	72
Southeast Thailand	22	5
Other areas of Thailand	-	26
Saigon, Viet Nam	-	50
Total	63	433

TABLE V

Occurrence of Paracolon Bacilli in Acute Diarrhea Cases in Thailand
April 1963 through March 1964 (all ages)

Number of patients	2,964
Number of specimens	
Number of patients positive for paracolon	1,599
Number of specimens positive for paracolon	1,715
Number of Paracolon bacilli isolated	2,109
<u>Paracolobactrum coliforme</u>	
<u>Paracolobactrum aerogenoides</u>	1,034
<u>Paracolobactrum intermedium</u>	396
Providencia	4

family size was seven. Sewage disposal was frequently unsanitary (Table VI) and water for drinking and domestic use (Table VII) frequently came from unsafe sources. In spite of intensive efforts on the part of Thai officials - posters, sound trucks, television appearances etc. - barely one-fifth of the people boiled their drinking water. The educational campaign did have some impact in that most of these patients realized cholera is acquired orally but only 10.4 percent thought water constituted the source of their infection. (Table VIII)

The educational programs were in conjunction with massive immunization campaigns. It was shown that almost half of these patients had been immunized against cholera on one or more occasions (Table IX), and the data in Table X suggest that immunization was of some value in that 54 of the 184 patients had been immunized four days or less before the onset of disease. It should be emphasized that these data do not reflect incidence of cholera, but rather are specific for a group with laboratory diagnosed cholera. There can be little doubt that the extensive efforts of the Thai health officials prevented a worse epidemic and that a final solution to the problem of cholera in Thailand will of necessity require many years.

A discussion of paracolons should be preceded with the clarification that this designation is artificial in that the only basis for its existence is the failure of gram negative bacilli to ferment lactose promptly. Their role in the production of diarrheal diseases is unproven, and they are included in this report for the sake of completeness. More than half of the patients were positive for one or more species of this organism with Paracolobactrum aerogenoides accounting for 49 percent of the isolates. There was no pattern of distribution of these organisms that suggested any role as an enteric pathogen except that no other recognized enteric pathogen was isolated from most of these specimens. (Table V)

Summary: Approximately 25 percent of 2,659 stool specimens were positive for known enteric pathogens and another 49 percent were positive for paracolons. All isolates of the 451 agglutinable vibrios were El Tor with the Ogawa serotype predominating. V. cholerae El Tor (Ogawa) was isolated from the stool of an American serviceman suffering from a mild case of diarrhea. His diagnosis was complicated by the concomitant finding of cysts of Endamoeba histolytica in his stool. A survey of 470 patients with clinical cholera confirmed by various laboratories showed that approximately 40 percent of them had been immunized against this disease. Other findings of this survey were presented and discussed.

Conclusion: Salmonellosis, shigellosis, and cholera remain serious medical problems of military significance in Thailand. There is no indication that these problems will diminish in the immediate future.

TABLE VI
 CHOLERA SURVEY STUDY
 Sewage Disposal

<u>Type</u>	<u>Percent</u>
Septic tank	3.2
Cesspool	20.0
Pit	31.2
Pail	3.2
On ground	32.3
In water	6.6
Not stated	3.3
Safe from flies	
Yes	27.8
No	62.5
Not stated	9.6

TABLE VII
 CHOLERA SURVEY STUDY 1963
 Patient's Source of Water

	<u>Drinking</u>	<u>Domestic</u>
Klong	3.6 %	10.4 %
River	0.2 %	0.6 %
Well	66.0 %	72.5 %
Rain	13.2 %	6.0 %
Tap	6.6 %	7.7 %
Not stated	10.4 %	10.6 %
Boiled	23.4 %	
Not boiled	62.3 %	
Not stated	14.2 %	

TABLE VIII

CHOLERA SURVEY STUDY 1963

Patients Opinion of Source of Infection

	<u>Percent</u>
Food	63.0
Drinking Water	5.3
Domestic Water	5.1
Other	2.4
Not stated	23.4

TABLE IX

CHOLERA SURVEY STUDY 1963

Prior Cholera Immunizations

<u>Number</u>	<u>Percent</u>
	39.8
2	4.5
3	1.1
4 or more	0.4
Immunized	33.6
Not stated	20.6

TABLE X

CHOLERA SURVEY STUDY 1963

Time before illness of last cholera immunization (days)

	<u>Percent</u>
	11.5
	5.5
10-14	3.2
15-19	4.0
20-24	1.1
25-29	1.5
30-39	0
40-59	7.0
60-89	0.9
90-180	4.5
Greater than 180	.3
Not stated	59.5

Ex.