BODY OF REPORT

SEATO Medic Study No. 67	Cholera and Enterobacteriaciae Study in Chiengmai
Project No. 3A 025601 A 811	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 San Francisco 96346
	Division of Medical Research Laboratories
	Department of Bacteriology and Immunology
Period Covered by Report:	1 December 1963 to 11 March 1965
Principal Investigator:	Leon J. LeBeau, Ph.D.
Associate Investigator:	Howard E. Noyes, Ph.D.
Assistant Investigators:	Anan Phuangsab, M.Sc. Parimol Khanjanasthiti, M.Sc. Wicha Poonvit, M.Sc. Kampol Panas-Ampol, M.D. Prayut Thitasut, M.D., M.Sc. Pien Chiowanich, M.D. Majourieya Suksuvan, B.S. Chongjin Swasilpa, B.S. Chumnum Promikotgow, M.D. Mr. Amnuay Sethakorn Mr. Kasem Watanachi Mr. John Wilhite
Reports Control Symbol:	MEDDH-288
Security Classification:	UNCLASSIFIED

Objective: Determination of factors which influence the epidemiology and endemicity of enteric infection in Chiengmai has been the objective of this work.

Since enteric infection is a serious deterrent to troop effectiveness in tropical regions, it would be of value to know the seasonal periodicity of these agents as well as the factors which contribute to the persistence and dissemination of the etiologic agents in order to estimate potential risk to troops at all times. Very little such work has been done in Northern Thailand. This study has investigated the situation in Chiengmai, using this city as an example of a northern urban environment.

Description: This work consists of seven basic studies which relate to the epidemiology of enteric infection.

1. Investigation of the various water sources in Chiengmai to determine seasonal frequency of enteropathogenic flora as well as the survival rate of *Vibrio cholerae*, *Salmonella typhosa* and *Shigella flexneri* has been undertaken. Five hundred milliliters to one liter water samples were collected at least once per week from November 1963 to the present from the following sources: (a) Mae Ping (the major river draining the entire Ping valley and surrounding mountains) (b) Mae Kha (a small river which flows through the city and serves as a major sewage run off.) This river is considered particularly dangerous because its bed dries up to a mere trickle in the hot season, and frequently overflows its banks in the wet season (c) the klong surrounding the old, walled city (a shallow canal which is stagnent except in the rainy season) (d) water jars and shallow wells in six major markets which sell general merchandise as well as food (e) water from a deep, driven well at the medical school (untreated but piped to all buildings on the grounds) (f) municipal water supply (this is obtained from the Mae Ping river, and is precipitated and chlorinated--treatment is well controlled at the plant but the piping is subject to leakage and contamination in the vicinity of the consumer). All water samples examined in this study were passed through millipore filters and the membranes were cultured in enrichment media for 12-18 hours followed by subculture on McConkeys and Salmonella-Shigella or Desoxycholate Citrate agar plates from the Selinite F broth and on alkaline tellurite agar from the alkaline peptone broth. All suspected pathogens were confirmed serologically using Difco* grouping serum.

The water from various sources was also examined for iron and calcium content, pH determination and bacterial quantitation by aerobic plate count. One hundred liter volumes of water from each source were impounded in each of three in Shanghai jars (unglazed earthen vessels) and contaminated to a final concentration of approximately 10^6 of each organism per milliliter using infusion broth cultures of *S. typhosa*, *Sh. flexneri* and *V. cholerae El Tor-Ogawa*. One liter samples were collected periodically from O hours to two weeks and checked by the millipore filter technic and enrichment and culture method previously described.

2. Studies on the role of flies and cockroaches in the markets as vectors of

* Difco Laboratories, Inc. Detroit 1, Michigan.

intestinal infection have been in progress since July of 1964. Each week day fly and cockroach traps were placed in six major markets (fly traps by day and roach traps by night) for a collection time of eight hour for flies and 15 hours for cockroaches. Traps containing the flies were placed in the freezing compartment of the refrigerator (-10°C) for 20 minutes and then the contents were poured in respective, preweighed, one liter bottles containing 300 ml of mannitol buffer* and glass beads. Each collection was weighed and recorded. The bottles were shaken for 20 minutes on a reciprocal Kahn shaker and the contents poured through the rough filter pad of a millipore filter unit. The pad was placed in a sterile petri dish and given to the parasitologist for examination by wet mount. The filtrate was passed through the millipore filter membrane and the membrane divided into two parts, one was placed in Selinite F broth and the other was dropped into alkaline peptone broth. After 12 hours enrichment subcultures were made and the organisms characterized as described previously. A similar procedure with omission of the freezing step was used in the study of cockroaches.

3. The study of the role of fresh fruit and vegetables in the dissemination of enteric pathogens was initiated in September 1964 and is still in progress. Various fruits and vegetables which are frequently eaten uncooked were examined to determine surface flora. One kilogram of each type was placed in a three liter, screw-cap jar along with one liter of mannitol buffer*, pH 7.0, and subjected to the agitation of a Kahn shaker for 20 minutes. The broth was filtered through the rough millipore filter pad and then through the millipore membrane. The pads were examined for parasites and the membrane for bacteria by enrichment followed by subculture on selective media. Isolates were screened biochemically and suspicious cultures were checked serologically.

In addition, the survival of V. cholerae, El Tor-Ogawa, S. typhosa, and Sh. flexneir III on the surface of artifically contaminated fruit and vegetables was determined. Produce was coated by immersion in a mannitol buffer containing the three organism, each in concentration of approximately 10^6/ml. The basket of fruit or vegetalbes was allowed to drain-dry and remain in an ant-proof, screened cabinet at room temperature. At intervals from O hours to two weeks, portions of the product were placed in one liter bottles along with 240 ml. of sterile mannitol buffer and processed as in flora studies.

4. Two carrier rate studies have been initiated. Rectal swabs were taken on the day of admission from all children in the pediatrics department; and weekly rectal swabs were obtained from a group of 100 boys, aged 6-7, enrolled in a very low tuition school. Children absent from school on day of sampling were examined in the home on the same or following day. All specimens were taken in duplicate, one swab placed immediately into Selinite F broth and the other into alkaline peptone broth. After 12 hours incubation subcultures were made and the suspicious

* Mannitol Buffer: Mannitol 5.0 gm, Peptone 5.0 gm, KH_2PO_4 1.1 gm, $Na_2HPO_4 .12H_2O$ 4.4 gm, (or $Na_2 HPO_4 .7 H_2O$ 3.3 gm) H_2O q.s. ad 1000 ml pH 7.0, molarity 0.0 4M, Sterilized at 121°C for 20 minutes.

Table I

SALMONELLA, SHIGELLA AND VIBRIO CHOLERAE ISOLATIONS FROM THE WATERS OF CHIENGMAI

Total Samples Examined - 1437	1963 Dec	1964 Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov Dec	1965 Jan Mar
Klongs in Chiengmai 24 specimens per month	0	Sal	2 Sal	0	6 Sal Inaba	6 Sal 2 Shig	10 Sal	4 Sal	2 Sal	0	2 Sal	0	0
Mae Ping river 8 specimens per month	Inaba	Sal	0	0	0	2 Sal	2 Sal	4 Sal	0	0	0	0	0
Mae Kha river 8 specimens per month	0	0	Sal	2 Sal	3 Sal	Sal Shig	2 Sal	4 Sal	Sal	0	0	0	0
Standing water 2 specimens per month	0	0	0	Sal	0	0	0	0	0	0	0	0	0
Drainage Ditches 4 specimens per month	0	0	0	0	3 Sal	0	Sal	3 Sal	0	2 Sal	0	0	0
Chiengmai Municipal Water 4 specimens per month	0	0	0	0	0	0	0	0	0	0	0	0	0
Ice Cream (frozen milk bars) 4 specimens per month	0	0	0	0	0	Sal	Sal	0	0	0	0	0	0
Ice from Ice Plants 3 specimens per month	0	0	0	0	Sal	0	0	Sal	Sal	0	0	0	0
Wells at selected Wats 2 specimens per month	Sal	0	Sal	0	0	0	2 Sal Shig	0	Sal	Sal	0	0	0
Market Wells 18 specimens per month	2 Sal	0	Sal	Sal	0	Sal	Sal	4 Sal	Sal Inaba	Sal	0	0	0

Sal=Salmonella sp.; Shig=Shigella sp.; Inaba=V. cholerae El Tor-Inaba

Table II

SURVIVAL OF SALMONELLAE, SHIGELLAE AND CHOLERA VIBRIOS IN CHIENGMAI WATERS

(Examined in Triplicate)

Source of Water	mg./100 cc. Ca	Fe	Initial Plate Count	pH	Survival in days 0	1	2	4	7	10	20
Mae Ping river	0.48	0.034	$5x10^5$	8.2							
Vibrio cholera Ogawa					3/3	0/3	2/3	0/3	0/3	0/3	0/3
Salmonella typhosa					3/3	2/3	1/3	0/3	0/3	0/3	0/3
Shigella flexneri III					2/3	1/3	0/3	0/3	0/3	0/3	0/3
Mae Kha river	2.16	0.152	$4x10^9$	7.7							
Vibrio cholera Ogawa					2/3	0/3	1/3	3/3	1/3	0/3	0/3
Salmonella typhosa					2/3	1/3	0/3	0/3	0/3	0/3	0/3
Shigella flexneri III					1/3	0/3	0/3	0/3	0/3	0/3	0/3
Deep (driven) Well	Trace	0.906**	$4x10^3$	6.0							
Vibrio cholera Ogawa					3/3	3/3	3/3	3/3	2/3	2/3	0/3
Salmonella typhosa					3/3	3/3	3/3	3/3	3/3	1/3	0/3
Shigella flexneri III					1/3	1/3	0/3	0/3	0/3	0/3	0/3
Klong Water	0.88	0.012	$1x10^4$	8.5							
Vibrio cholera Ogawa					3/3	3/3	3/3	3/3	3/3	0/3	*
Salmonella typhosa					2/3	2/3	1/3	0/3	0/3	0/3	*
Shigella flexneri III					1/3	1/3	0/3	0/3	0/3	0/3	*
Shallow Well in Market	2.04	0.005	$7x10^4$	6.9							
Vibrio cholera Ogawa					3/3	3/3	3/3	3/3	3/3	1/3	*
Salmonella typhosa					2/3	2/3	0/3	0/3	0/3	0/3	*
Shigella flexneri III					1/3	0/3	0/3	0/3	0/3	0/3	*
Municipal Tap Water	*	*	$4x10^2$	7.4							
Vibrio cholera Ogawa					3/3	3/3	3/3	1/3	*	*	*
Salmonella typhosa					3/3	3/3	1/3	0/3	*	*	*
Shigella flexneri III					1/3	0/3	0/3	0/3	*	*	*

* Work is still in progress and results are pending.
** 0.018 after precipitations on standing.

The question of Chiengmai water as a major source of infection may be clouded by our inability to isolate agents repeatedly from a given source. Except for one instance all sites containing pathogens on one day did not do so 1-3 days later at the next collection time. This emphasizes the importance of survival studies for the offending agents. In Table II preliminary figures are given to show the survival of V. cholerae El Tor-Ogawa S. typhosa and Sh. flexneri in water from different sources in the city. The calcium content was rather low but the iron content was relatively high in deep well and river water.

These results parallel those shown for the waters of India in that heavily contaminated water did not support the survival of the pathogens as well as relatively uncontaminated water, presumably because of biologic competition. Table II shows that Sh. flexneri could not be found other than initially, while S. typhosa and V. cholerae could persist in unpolluted water as long as 10 days and in heavily polluted water, at the most, a few days.

When one relates this survival data to a recognition of dilution and dispersal of the organisms of pollution caused by the current and flow of the rivers, streams and ditches, it becomes more understandable why repeated positives were not found. Further, this would indicate that sporadic polution and chance sampling of the moving water in various stages of dilution has been observed rather than constant contamination at the sampling sites. On the other hand, in shallow wells dispersal would not be a factor so that lack of survival and dilution could explain our inability to isolate salmonella subsequent to an initial isolation even though the wells were untreated during the course of this study.

The frequently ignored role of ice cream and ice as enteric disease hazards is highlighted by the isolation of Salmonellae from these products during the last few days of the dry season and the beginning of the rainy season. It would be reasonable to assume that ice and ice cream are more likely means of dissemination of enteric pathogens than some of the waters because the dirty river and klong waters are not used much for drinking purposes. This ice and ice cream could rank with the well waters as a means of spread. A given well, however has limited patronage and therefore, is, at best, responsible for a focal problem while the ice and ice cream are encountered throughout most of the city. This work on water flora is being terminated at this time while the survival studies are being extended to the other sources of water in Chiengmai. The tentative completion date for existing water studies is 15 May 1965.

The results of the cultures taken from flies are provided in Tables III and IV and for cockroaches in Table V, while the data for parasite ova and cysts on flies are given in Table VI. No ova or cysts suspected of being human parasites were recovered from cockroaches. It was noted that flies harbored ova and cysts at a fairly consistent rate all year long, but enteropathogenic bacteria were found especially in the months of July, August and early September. (The flies have not been examined yet during April, May and June--this work will be forthcoming.) This information along with the consistent isolation of Escherichia coli from the

Table III

ENTERIC PATHOGENIC BACILLI ISOLATED FROM MARKET PLACE FLIES

Market Places from which Samples were Taken	Number of Fly Pools	Escherichia coli	Paracolons	Pseudomonas aeruginosa	Alcaligenes fecalis	Alcaligenes (H_2S+)	Proteus species	Salmonella species	Shigella species	Vibrio cholerae Inaba	Salmonella-like but nonagglutinating
Chang Purg	128	128	69	4	38	36	90	6	0	0	22
Varoros	110	110	52	5	54	16	71	5	2	0	24
Chiengmai Gate	113	113	60	3	53	21	66	6	0	1	16
Lumyi	111	111	59	3	49	19	62	7	1	1	29
Navarot	116	116	51	7	59	21	85	8	1	0	17
Sanpakoi	42	42	18	1	13	9	18	0	0	0	2
TOTAL	620	620	309	23	257	122	392	32	4	2	110

Table IV

SEASONAL ISOLATION OF ENTEROPATHOGENIC BACTERIA FROM FLIES

Organism	Total + isolates to July 1964-March 1965	July through September	October through December	January through March
	Number	Number	Number	Number
Vibrio cholera Inaba	2	2	0	0
Salmonella species	32	29	1	2
Shigella species	4	3	0	1

Table V

ENTERIC PACTERIA ISOLATED FROM COCKROACHES IN CHIENGMAI

Sites from which the Samples were Taken	Number of Cockroach Pools	Escherichia coli	Paracolons	Pseudomonas aeruginosa	Alcaligenes fecalis	Alcaligenes (H_2S+)	Proteus species	Salmonella species	Shigella species	Vibrio cholera Inaba	Salmonella-like but nonagglutinating
Home on Vulai Road	13	8	7	0	4	0	6	0	1	0	2
Home at Changlao	18	15	9	0	12	1	2	0	0	0	0
Home at Puag pia	10	9	5	0	6	1	1	0	0	0	0
Home on Tapa Road	3	2	2	0	1	0	1	0	0	0	1
Railway Station	4	3	3	0	0.	3	3	0	0	0	0
Wat Supan	7	4	2	1	6	1	2	0	1	0	0
Chiengmai Gate Market	41	29	14	9	23	2	16	0	0	0	3
Lumyi Market	10	6	2	2	4	3	0	0	0	0	1
Navarot Market	10	7	3	2	10	2	4	1	0	0	1
Varoros Market	23	18	6	3	22	0	2	1	0	0	3
Chang Purg Market	9	7	6	2	3	0	1	0	0	0	2
TOTAL	148	108	59	19	91	13	38	2	2	0	13

Table VI

HUMAN PARASITES RECOVERED FROM FLIES IN CHIENGMAI

Sampling Sites	Fly Pools Examined	Number Pools Positive	Percent of Pools Positive
Chang Purg Market	136	80	58.8
Navarot Market	130	27	20.8
Varoros Market	115	20	17.4
Lumyi Market	120	26	21.7
Chiengmai Gate Market	128	28	21.9
Sanpakoi Market	52	9	17.3
TOTAL	681	190	27.9
HELMINTHIC OVA AND LARVAE			
Trichuris ova		20	2.94
Ascaris ova		19	2.79
Opistorchis ova		69	10.13
Hookworm ova		37	5.43
Hymenolepis diminuta ova		13	1.91
Taenia ova		10	1.45
Enterobius ova		1	0.14
Echinostomata ova		1	0.14
Rhabditoid larvae of Strongyloides		17	2.50
Rhabditoid larva of Hookworm		1	0.14
PROTOZOAN CYSTS			
Endamoeba histolytica		17	2.50
Protozoan cysts suspected of being E. histolytica		1	0.14
Endamoeba coli		41	6.02
Endolimax nana		5	0.73
Giardia lamblia		6	0.88
MIXED INFESTATION		58	8.52

NOTE: No ova or cysts suspected of being human parasites were recovered from cockroaches.

Table VII

CARRIER RATES FOR ENTEROPATHOGENIC BACILLI AMONGST CHILDREN IN CHIENGMAI

	Cultures Recovered During Routine Rectal Swab Examination on all Pediatric Admissions at Chiengmai Hospital			Cultures Recovered in Survey of 100 School Boys followed weekly by Rectal Swab Culture		
	Salmonella species	Shigella species	Vibrio cholerae Ogawa	Salmonella species	Shigella species	Bibrio Ogawa
May 1964	3	1	0			
June	4	0	2			
July	2	1	0			
August	2	1	0	Study begun in September		
September	1	0	0	1	0	0
October	0	0	0	0	0	0
November	1	0	0	0	0	0
December	0	0	0	0	0	0
January 1965	0	0	0	0	0	0
February	0	0	0	0	0	0
March	1	0	0	School Vacation		

Table VIII

PRELIMINARY REPORT OF PARASITE INFESTATION OF SCHOOL CHILDREN IN CHIENGMAI

	Number Found	Percent Positive
HELMINTHIC OVA		
Ascaris lumbricoides	87	15.2
Trichuris trichiura	98	17.1
Opisthorchis	77	13.4
Hookworm*	153	26.7
Enterobius vermicularis	19	3.2
Taenia	12	2.1
HELMINTHIC LARVAE		
Strongyloides	2	0.4
PROTOZOAN CYSTS		
Endamoeba histolytica	63	11.0
large race	33	5.8
small race	30	5.2
Giardia lamblia	114	19.9
Endamoeba coli	117	20.4
Endolimax nana	48	8.4
Iodamoeba butschlii	17	3.0
MIXED INFESTATIONS	228	39.7
Protozoa with Protozoa	25	4.4
Protozoa with Helminths	147	25.7
Helminths with Helminths	56	9.8
TOTAL STOOLS EXAMINED---573	395	68.9

* 233 specimens of the 573 total examined to date came from Chung Kian School. Of these, 110 contained hookworm ova for a percentage of 47.2. The remaining 340 specimens from four other schools yielded only 43 with ova of hookworm, or 12.7%.

flies would lead one to conclude that flies come in contact with feces throughout the year, but that only during the rainy season is the carrier rate in humans for intestinal pathogens high enough to be reflected in the fly isolates. This is further demonstrated in Table VII where the carrier rate in routine pediatric admissions and school children indicates a seasonal distribution for the enteropathogenic bacteria. In contrast, Table VIII shows that amongst the first 573 school children examined for intestinal parasites even at the height of the hot, dry season a carrier rate of 59.4% was found. It is interesting to observe that a comparison of Tables VI and VIII reveals a close correlation in percent recovery in all instances save that for Trichuris, Ascaris and Giardia in which the carrier rate in humans far exceeds the relative incidence of recovery from flies. The high carrier rate for Hookworm shown in Table VII is unreal inasmuch as 72% of the ova observed came from students at a single school. The children from other schools had a hookworm carrier rate of 12.7%. These data emphasize the danger contracting certain parasitic diseases from the fly-vegetable route throughout the entire year. It should be recognized, of course, that many of the parasites listed in the tables are no risk to the populace through the fly-vegetable route since the ova would not represent the infectious stage for humans.

Cockroach cultures have yielded very few pathogens to date. One strain of Salmonella was isolated from roaches in a market in November and another strain from a different market in December. Samples collected at a home and at a Wat (temple), also included in Table V, have yielded different Shigella species in each instance. At the same time no parasites of human significance have ever been recovered from the cockroaches. If one considers that not only were parasites not found, but coliform bacteria could not be isolated from the roaches with any consistancy, as compared with flies, one might assume that the cockroaches at the markets do not have as much opportunity to come in contact with feces as do flies. This is partly understood by their nocturnal habits and their limited area coverage. The instances where coliforms were recovered may reflect not infrequent contact with dog or pig feces in the market place. The two salmonella isolates may represent contact with meat, a broken egg or even the feces of infants since infants and small children are often brought by mothers to the market, and, as is the custom, they rarely are clothed below the waist. On this basis the role of the cockroach in the dissemination of enteric infection in Chiengmai may be extremely limited; however, they are present in large numbers in the markets and could be a potential source of spread if given access to fecal pollution. The enterobacterial flora found on fruit and vegetables routinely purchased from the market as well as survival data for V. cholerae, Sh. flexneri and S. typhosa are given in Table IX. It is significant that flora studies show coliform contamination of 17% of the fruit and 79% of the vegetables while 83% of all the produce examined harbored Alkaligenes fecalis. In addition lettuce, chinese cabage and coriander had ova of Ascaris or cysts of Endomoeba histolytica. From lettuce, the larvae of hookworm were also obtained. This is particularly important since Japanese workers have indicated a high rate of hookworm infectivity through ingestion of larvae. It should be noted that such larva was recovered once from flies as well. Thus the likelihood of fecal contamination is great, but enteric

Table IX

FLORA AND SURVIVAL OF ENTEROPATHOGENIC BACILLI ON FRUITS AND VEGETABLES

Name	Initial Enterobacteria Flora	Survival Time (In Duplicate) V.cholerae	Sal.typhosa	Shig.flexneri
Lumyai (Euphoria longana)	E. coli, Alc. fecalis Ps. aeruginosa	12 hours	5 days	5 days
Langsad (Lansium domesticum)	Serratia marcescens	2 days	5 days	5 days 4 days
Rambutans (Nephelium lappacium)	Prot. morgani Ps. aeruginosa	2 days	3 days	3 days 4 days
Lamuds (Manilkara kaki)	Prot. mirabilis Ps. aeruginosa	24 hours	5 days	3 days
Jujubes (Zizyphus jujube)	Alc. fecalis, Paracolon Prot. mirabilis	4 days	4 days	5 days
Yam beans (Pachyrizus erosus)	Alc. fecalis Prot. mirabilis	4 days	6 days	2 days
Emblica (Phyllantus emblica)	E. coli, Alc. fecalis Prot.rettgeri, Paracolon	2 days	6 days	4 days
Water chestnuts (Eleocharis tuberosa)	Alc.fecalis, Paracolon	6 days	3 days	24 hours
Tomatoes (Lycopersicon esculentum)	Alc.fecalis, Cetrobacter E. coli, Paracolon	24 hours	3 days	3 days
Lettuce (Lactuca sativa)	E. coli, A. aerogenes Alc. fecalis	22 hours	3 days	4 days
Chinese cabbage (Brassica chinensis)	E. coli, Alc. fecalis Prot. mirabilis, Paracolon	2 days	5 days	4 days
(Ipomaea repitans)	E. coli, Alc. fecalis Paracolon	2 days	4 days	2 days
Green Onions Allium cepa	Alc.fecalis, Citrobacter E. coli, Paracolon	3 days	5 days	5 days
Cucumbers (Cucumis salivus)	Alc. fecalis, E. coli Prot. rettgeri	3 days	2 days	3 days
Cow peas (Vigna sinensis)	Alc.fecalis, Citrobacter A. aerogenes	24 hours	2 days	3 days
Potatoes (Solanum tuberosum)	Alc. fecalis A. aerogenes	2 days	3 days	6 days

Table IX (Continued)

Name	Initial Enterobacteria Flora	Survival Time (In Duplicate) V.cholerae	Sal.typhosa	Shig.flexneri
Canton cabbage	Alc.fecalis, Citrobacter Prot. rettgeri, Paracolon	3 days	3 days	2 days
Corianders (Coriandrum sativum)	E. coli, Prot. rettgeri Alc. fecalis, Paracolon	24 hours	4 days	2 days
Bell peppers (Capscum annum)	Paracolon	4 days	2 days	3 days
Garlic (Allium sativum)	Paracolon, Prot.mirabilis	2 days	5 days	4 days
Egg Plant (Solanum xanthocaroum)	Citrobacter, P.aeruginosa	24 hours	3 days	24 hours
Carrots (Daucus carota)	Seratia marcescens	2 days	24 hours	3 days
Sugarpeas (Pisum sativum)	Paracolon, Alc.fecalis	5 days	6 days	2 days
Rhizomea (Kaempferia pandurata)	E. coli, Paracolon Alc. fecalis	4 days	3 days	4 days
Radishes (Raphanus sativus)	Alc. fecalis Prot. morganii	24 hours	2 days	3 days
Wing beans (Psophocarpus tetragonobolus)	Prot. morganii. Alc.fecalis	2 days	2 days	24 hours
Bryonies (Coccinia indica)	Alc.fecalis, Paracolon E. coli	2 days	6 days	2 days
Bryonia Magosa	Paracolon	2 days	4 days	2 days
Celery Apium graveolens	Paracolon, Alc.fecalis	2 days	6 days	4 days
Mint (Mentha arvensis)	Alc. fecalis Intermediate coliforms	2 days	4 days	2 days

Table X

STUDY OF THE SANITARY FACILITIES OF MAJOR MARKETS IN CHIENGMAI

Markets observed	8
Cement Slab privies	7
Wet and dirty floors	8
Toilet paper provided	
Rice paper squares	3
Old newspapers in room	2
Nothing provided	3
Running water flush	1
Water jar and dip pan	7
Hand washing facilities	2*
Fly-proofing	0

*Two markets were equiped with sinks and taps; however both were caked with dirt and showed signs of disuse. One of the taps did not produce water when opened.

Table XI

PRELIMINARY RESULTS OF SERUM ANTIBODIES FOR SALMONELLA IN CHILDREN

(These results are taken from the first 427 of 2000 sera to be studied.)

Salmonella Group Antigen	Negative Sera Number	Positive Sera for Each Dilution				
		1-20 No.	1-40 No.	1-80 No.	1-160 No.	1-320 No.
A	302	47	50	22	4	2
B	147	117	102	30	13	23
C	13	23	94	142	81	74
D	188	120	73	33	10	3
E	0	11	51	132	96	127

pathogenic bacilli have not yet been isolated. This can be understood since this work has been in progress since October 1964 and the carrier rate as well as water and fly pollution during this interval appears to be lowest. These studies of flora will be repeated during May and July in an effort to determine if fresh foods harbor intestinal pathogens and are likely means of dissemination during the height of the enteric infection season.

The survival of salmonella, shigella and V. cholerae on fruit and vegetables ranged from 12 hours to 6 days. This is, as a rule, more than ample time for sale and consumption of the product, since most of the produce is sold on the day it arrives at the market and is held but a short while in the home before it is eaten.

The contamination of produce with fecal organisms may take place through contact with flies. In addition, freshening with water is a common practice, and since it has been shown that most water in Chiengmai, save municipal water, consistently shows evidence of fecal pollution, this practice may be a source of contamination. A third factor in contamination may be suggested by the data in Table X. Inadequate sanitary facilities at the market place would be most evident just at the time when a merchant suffering from diarrhea might require frequent use. Not only would the merchant carry organisms back to the stalls on her hands, but it would be difficult for her to keep from contaminating the jars of bulk water used in flushing the toilets since handleless pans are used to transport the water to the toilet. Thus the hands of many other merchants using the facilities that day, and even several succeeding days, may become soiled resulting in eventual contamination of the produce. In addition, open home privies nearby as well as sewage polluted ditches of rivers are within cruising distance of flies for at least two of the markets.

The opportunity for the dissemination of intestinal pathogens seems so evident that one might assume a high annual rate of acute infection during the season. Table XI corroborates this point in that an exceptionally high proportion of the school children have antibodies against salmonella. These data cover only 427 of some 2000 sera, but the preliminary information indicates that better than 90% show titers of 1-40 or higher for antigens of Group C and 97% for Group E; while less than 20% have such a titer for Group A, 39% for Group B and 28% for Group D. This must be considered in the light of very rare use of Typhoid-Para A-B vaccine in this area.

Such high titers not only reflect high infection rate (probably the result of numerous inapparent infections) and may provide initial immunity against severe infections during intervals of high carrier rates. Thus, a heavy dose of the infectious agent is actually required in order to produce apparent infection in most of the populace. It may also mean that frequent inapparent infections keep the organism in the epidemiological cycle during intervals outside of the case load peak.

The serological work using both Salmonella and V. cholerae antigens will be

completed by May and the statistical analysis should be carried out during June 1965.

Summary: This work has indicated that carrier state and infection in children as well as water pollution and contamination of flies were most evident during the months of May through August. In contrast a stable parasite carrier rate of 69% existed in school children. This may be reflected in the data which show that ova and cysts of parasites were recovered from flies all year long. Fecal contamination of fruits and vegetables has been shown to be common and enteropathogens were able to survive on their surface for 2-6 days. Survival of such organisms in water was a function of degree of pollution in that high indigenous flora decreased the survival time. Ice cream and ice were likewise potential sources of dissemination of enteric pathogens. Usually high antibody titers against Salmonella Groups B, C and E were found in the sera of school children.

Conclusion: The waters of Chiengmai are essentially fecally polluted so that anyone not native to the area and not having high levels of immunity would very likely succumb to enteric infection if he drank the unboiled or untreated water. This hazard is particularly evident from April until September. During this same time interval ice and ice cream can be dangerous and the flies may transport the enteropathogens. Flies harbor parasites all year long and are, therefore, always a threat. Fruit and vegetables have not yet shown a flora of enteropathogenic bacilli, but they do show evidence of fecal contamination and a flora of human intestinal parasites throughout the year. Thus they cannot be eaten uncooked or untreated without the threat of infection or infestation. The evidence of this hazard is further manifest in the high parasite carrier rate in the populace of 59% as well as the high antibody levels in school children against Salmonella Groups B, C and E.