

A PROSPECTIVE STUDY OF TRAVELERS' DIARRHEA AMONG AMERICAN
PEACE CORPS VOLUNTEERS IN RURAL THAILAND: INFECTIONS
WITH MULTIRESISTANT ENTERIC PATHOGENS

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OBJECTIVE : To determine the etiology of travelers' diarrhea in Thailand and the extent of resistance among enteric pathogens.

BACKGROUND : Travelers' diarrhea (TD) is a well studied syndrome of acute watery diarrhea, often accompanied by tenesmus, occurring in new arrivals in developing countries (1-8). In Mexico and Africa episodes of diarrhea are associated with a variety of enteropathogens (4-8), the most common of which are enterotoxigenic *Escherichia coli* (tox+ *E. coli*). Sixty-three percent of Peace Corps volunteers (PCV) arriving in Kenya (6) were infected with this enteric pathogen. Antibiotic resistance was less common among tox+ *E. coli* (88% multiply sensitive) in comparison to tox- *E. coli* (56% multiply sensitive).

In studies of tox+ *E. coli* infections in the Far East widespread R factor mediated antibiotic resistance of this enteric pathogen has been reported in Taiwan, the Philippines, Korea, Indonesia (9), and Thailand (10). In this report Peace Corps volunteers arriving in Thailand were studied prospectively to determine the etiology of their diarrhea, and the extent of antibiotic resistance among infecting enteropathogens.

METHODS :

Study Population : Thirty-seven PCVs, 21 to 57 years of age, who arrived in Thailand July 13, 1979 gave informed written consent to participate in the study; thirty-five remained in the country for ten weeks. Eighteen had previously travelled to developing countries, and 14 had experienced travelers' diarrhea. The volunteers travelled from Seattle, Washington to Narita Airport, Japan where they were delayed for several hours. While in Japan, they were served "box lunches". Sausage and cheese included in the food was described as "spoiled". After travelling to Hong Kong where they remained overnight and few ate, they arrived in Bangkok the following morning. The principal investigator (PE) and two Thai nurses met the group four hours after their arrival and remained with the volunteers during their first five weeks in Thailand. During this period PCVs were attending an orientation program in U'thong, a

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small town 110 kilometers northwest of Bangkok. After five weeks, volunteers visited other PCVs' work sites in different areas of the country so it was impossible to follow the group closely after they left U'thong.

Collection of Specimens : Stool and sera were collected from the participants within 24 hours after their arrival. During their initial five weeks PCVs were questioned each morning to determine if they had any abnormal gastrointestinal symptoms. Individuals with any watery, or unformed stools were asked about the time of onset, the character of their stool, the frequency of their bowel movements, and their associated symptoms. Oral temperatures were recorded. Three or more watery stools, or two watery stools accompanied with abdominal cramps in a 24 hour period was considered to be an episode of TD. Two or more unformed, but not necessarily watery stools, with or without symptoms, was defined as mild diarrhea (MD). Attacks of diarrhea separated by a period of at least 48 hours, during which time the PCVs passed formed stool and did not experience any gastrointestinal symptoms, were considered repeat episodes. Affected participants were asked to submit multiple stool specimens (up to three) on each day they were ill. Stool and sera were also collected from PCVs after five and ten weeks in Thailand.

Processing of Specimens : Stools were cultured on selective media, and incubated under appropriate conditions immediately after collection. Specimens for rotavirus detection were frozen immediately in dry ice, and later stored at -70°C until tested. Stools were also preserved in ten percent formalin and polyvinyl alcohol (PVS), and examined for ova and parasites.

Bacteriology : Stools were cultured onto MacConkey, Heckteon (HE), Thio-sulfate Citrate Bile Salt (TCBS) agar media, and inoculated into Hajna Broth, alkaline peptone water (pH = 8.0), and Christenson's media (11). Hajna broth was incubated at 37°C and subsequently subcultured after 24 hours onto HE and Desoxycholate media, while APW was subcultured on TCBS after six hours. The Christenson's media was incubated at 4°C for 21 days, and then subcultured onto MacConkey and *Salmonella shigella* agar at 25°C for 48 hours. Stools were also cultured directly on 10% sheep blood agar containing vancomycin (10 µg/ml), trimethoprim (5 µg/ml), and polymyxin B (2.5 I.U./ml), and incubated microaerophilically (GASPAK without the catalyst) at 42°C for 48 hours. Small gram negative rods which were oxidase and catalase positive and would grow microaerophilically, but not aerobically, at 42°C and 37°C, but not at 25°C were identified as *Campylobacter jejuni/coli*. *Salmonella*, *Shigella*, vibrios, *Aeromonas hydrophila*, *Plesiomonas shigelloides* and *Yersinia enterocolitica* were identified according to standard procedures (12), with the API-20E system, and with the use of commercial antisera. *P. shigelloides* were tested for agglutination in *Shigella* antisera (group A-D) (DIFCO, Detroit, MI).

Ten lactose positive colonies were selected from the MacConkey plate, and stored on nutrient agar slants. All lactose positive colonies were tested for enterotoxin production. Colonies that did not ferment citrate and were indole and methyl red positive were initially identified as *E. coli*. Tox+ and five tox- *E. coli*, *Shigella*, *Salmonella*, and *Y. enterocolitica* isolated from each patient were tested on Mueller-Hinton agar by the Kirby Bauer method (13) for antimicrobial sensitivity to the following antibiotics; ampicillin Ap (10 µg), cephalothin Cf (30 µg), chloramphenicol Cm (30 µg), doxycycline Do (30 µg), gentamicin Gm (10 µg), kanamycin Km (30 µg), neomycin Nm (30 µg), Streptomycin

Sm (10 µg), sulfisoxazole Su (250 µg), sulfisoxazole-trimethoprim SXT (23.75 and 1.25 µg), and tetracycline Tc (30 µg). *E. coli*, ATCC 25922, sensitive to all antibiotics tested, *Pseudomonas aeruginosa* ATCC 27858 resistant to Ap, Cm, Km, Nm, and SXT, *E. coli* 203, a clinical isolate from the Philippines, resistant to Ap, Cm, Do, Gm, Km, Nm, Sm, Su, and Tc, and *E. coli* Gm, a clinical isolate from Thailand, resistant to Ap, Cm, Do, Gm, Km, Nm, Sm, Su, SXT, and Tc were included as controls. Isolates that were resistant to SXT on Mueller-Hinton agar were retested on Isosensitivity agar (Oxoid Ltd, England). The minimum inhibitory concentration of Do was determined for tox+ *E. coli*, and *Shigella* by the tube dilution method (12).

Tests for Enteropathogenicity : Lactose positive isolates were inoculated into 5 mls of Trypticase soy broth (BBL, Cockeysville, MD) with 0.6% yeast extract (DIFCO, Detroit, MI), and incubated on a rotating tissue culture tube apparatus at eight revolutions per minute at 37°C for 24 hours. Supernatants were tested for heat-labile toxin, LT, in the Y-1 adrenal cell tissue culture assay (14), and heat-stable toxin, ST, in the suckling mouse assay (15) simultaneously. Tox+ isolates were confirmed as *E. coli* in the API-20E system.

A. hydrophilia and *P. shigelloides* isolates were cultured in a similar manner, and tested for LT and ST production, and cytotoxicity in Y-1 adrenal cell tissue cultures. Isolates were also tested for hemolysis of rabbit erythrocytes by incubating two-fold dilutions of culture supernatants with a one percent suspension of rabbit erythrocytes at 37°C for one hour (16). *P. shigelloides* that agglutinated in *Shigella* antisera were tested in the Sereny test (17).

Anti-LT antibody titers were determined in duplicate by the adrenal cell neutralizing assay (18). Units of antitoxin were determined for each sample by comparing the specimen with a standard serum assigned the value of 1000 U/ml against LT (Swiss Serum Institute Anticholera Serum) (7).

Virology : Stool specimens were examined for rotavirus employing a modification of an ELISA technique (19) with goat (coating) (20) and guinea pig (detecting) antibodies to SA-11 virus (21). Antibodies to rotavirus were determined by complement fixation employing SA-11 as a substitute antigen (22). Antibody titers to Norwalk agent were determined by a previously described radioimmunoassay blocking test (23).

Examination for Ova and Parasites : Stools were examined microscopically for intestinal parasites after fixation in PVA and trichrome staining of smears, and by formalin-ether concentration (24).

RESULTS :

Clinical : Eighty-six percent (30/35) of volunteers developed unformed or watery stools, and 57 percent (20/35) developed TD during their first five weeks in Thailand. Only five (14%) remained well. Episodes of diarrhea began within four days after they arrived, and continued throughout their stay in U'thong, Figure 1. Of the 20 PCVs who developed TD, seven had one, eight had two, four had three, and one had four attacks of TD. The mean duration of TD was 3.35 days, range 1-12 days. Four volunteers were confined to their room for greater than two days, and one volunteer was hospitalized after 12 days of continuous

diarrhea. Four individuals, who had experienced multiple attacks of diarrhea, lost in excess of 15 lbs over the five week period. Symptoms associated with 39 episodes of TD in 20 PCVs are listed in Table 1. The attack rate of TD was 12/18 (67%) among PCVs who had previously travelled to developing tropical countries vs 8/17 (47%) of those who had not. Of 14 PCVs who had a history of previous TD, 11 developed TD again in Thailand. Nineteen PCVs (54%) reported experiencing diarrhea after leaving U'thong, and before the final serum was collected after ten weeks.

Bacteriologic Results : Thirty-seven volunteers submitted stool specimens immediately after arriving in Bangkok. Tox+ *E. coli* were isolated from five, and *Y. enterocolitica* from one of these asymptomatic individuals, Table 2. All of these "arrival" tox+ *E. coli* were multiply sensitive to antibiotics. Of the five volunteers from whom tox+ *E. coli* were isolated (1-3 tox+ *E. coli*/volunteer) three remembered having experienced mild abdominal cramps after leaving Japan, but none had had diarrhea.

Two hundred and eighteen stools were collected during 164 days of diarrhea (1.3 cultures/volunteer/day of diarrhea). Stools were also collected from each of the 35 volunteers after five and ten weeks in Thailand (at which time none had gastrointestinal symptoms). Bacteria belonging to the genus *Aeromonas* were the most common organisms associated with episodes of TD, Table 2. *A. hydrophilia* and *P. shigelloides* were isolated more frequently during attacks of TD than when PCVs were well (15/39 vs 5/70; 13/39 vs 6/70) ($p < 0.001$). In four attacks of TD *P. shigelloides* were isolated, in the absence of other enteric pathogens, within 24 hours of the onset of symptoms. *A. hydrophilia* was the only potential enteric pathogen isolated from two PCVs within 24 hours after the onset of TD.

Eighty-three percent (15/18) of the *A. hydrophilia* isolates associated with diarrheal disease (13TD and 2 MD) (Table 2), and 80 percent (4/5) of those recovered from the PCVs when they were well were cytotoxic for Y-1 adrenal tissue cultures, and hemolyzed rabbit erythrocytes. Forty-seven percent (7/18) of isolates from participants with diarrhea, and 40 percent (2/5) of isolates from those without diarrhea were positive in the suckling mouse assay in addition to being cytotoxic and hemolytic. Culture supernatants which were heated at 100°C for 15 minutes were negative in the suckling mouse assay.

None of 27 *P. shigelloides* (Table 2) were cytotoxic, hemolytic, or positive in the mouse assay. Twenty-six percent (7/27) agglutinated in either group A and D (1), C(1), C and D (4) antisera, however none of the cross-agglutinating isolates were positive in the Sereny test (17).

2354 lactose positive colonies isolated from 218 diarrheal, 35 "five week", and 35 "ten week" stools were tested for LT and ST production. 2189 (93%) of these lactose positive colonies were identified as *E. coli* (eight/specimen). Tox+ *E. coli* were isolated from 26 percent (10/39) of episodes of TD (Table 2). LT+ST+ and LT+ *E. coli* were more commonly isolated from PCVs during attacks of TD than when they were well (10/39 vs 4/70) ($p < 0.005$).

Shigella were isolated from 13 percent (5/39) of episodes of TD (Table 2). *C. jejuni/coli*, *Y. enterocolitica*, and *Salmonella* were each associated with single episodes of TD. Multiple potential bacterial pathogens were isolated

during 12 of 39 episodes of TD (Table 3). Seventeen percent (6/35) of PCVs developed a four-fold rise in antibody titer to LT during their first five weeks in Thailand. Two of seven (29%) individuals from whom LT+ *E. coli* were isolated during episodes of TD developed a significant rise in antibody titer. Four individuals, three of whom had TD, also had \geq four-fold rises in serum antibody titer to LT, but tox+ *E. coli* were never isolated from their stools. Thus, fifty percent (10/20) of PCVs with TD had serologic and/or bacteriologic evidence of infections with LT+ organisms over the initial five week period. There was no correlation between initial serum antibody titer to LT, and subsequent protection from infection with tox+ *E. coli*.

The antibiotic sensitivity of 185 "arrival", 175 "five week", and 175 "ten week" tox- *E. coli* (five tox- *E. coli*/ volunteer) are shown in figure 2. The antibiotic sensitivity of tox+ and tox- *E. coli*, *Shigella*, *Salmonella*, *Y. enterocolitica*, *C. jejuni/coli*, and aeromonads isolated during episodes of diarrhea are shown in Table 4. Forty-eight percent (42/88) of tox+ *E. coli* acquired in Thailand were multiply resistant (ie. resistant to more than one antibiotic). In comparison, sixty-three percent (687/1090) of tox- *E. coli* were multiply resistant. Sixty-one percent of tox+ *E. coli*, 59 percent of tox- *E. coli*, and 86 percent of *Shigella* acquired in Thailand were resistant to doxycycline.

Virology Results : Rotavirus was not detected from any stools collected from volunteers. No PCV developed a greater than or equal to four-fold rise in complement fixing antibody titer to SA-11 virus, the substitute antigen used for human rotavirus, over the first five weeks. One volunteer developed a significant antibody titer rise during the second five week period (1:2 to 1:256). One of 35 volunteers had serologic evidence of infection with Norwalk agent over the ten week period (<1:50 to 1:100).

Parasitic Results : Two PCVs, one of whom had been a recent volunteer in Afghanistan, were infected with *Giardia lamblia* on arrival. Another, who had never been outside of the United States, was infected with *Endolimax nana* when he arrived. All three continued to excrete these protozoa during episodes of diarrhea. Six of 35 (17%) acquired intestinal parasites during their first ten weeks in Thailand, Table 5. *Entamoeba histolytica* was found in two volunteers, six and 29 days after their arrival, during attacks of diarrhea.

This study is complete.

Table 1. Symptoms Associated with 39 Episodes of Travelers' Diarrhea Among 20 Peace Corps Volunteers

	<u>Yes</u>
URI	2 (5%)
Cough	2 (5%)
Headache	15 (38%)
Photophobia	4 (10%)
Sore throat	4 (10%)
Anorexia	24 (61%)
Malaise	16 (41%)
Chills	6 (15%)
Feverish feeling	14 (36%)
Temperature >38.3°C	4 (10%)
Abdominal cramps	32 (82%)
Nausea	23 (59%)
Vomiting	4 (10%)
Prostration	26 (67%)
Rash	0 (0%)
Flatulence	16 (41%)

<u>Stools</u>	<u>No. in 24 hours</u>
< 3	3
3-5	20
5-10	10
> 10	6

Table 2. Bacterial Enteric Pathogens Isolated from PCVs During Their First Ten Weeks in Thailand

Enteric Pathogen	Arrival (37) ⁺	Episodes of diarrhea			
		TD (39) ^o	MD (37) ^o	5 wks ⁺ (35)	10 wks ⁺ (35)
Tox+ <i>E. coli</i>	5(13%)	10(26%)	3(8%)	2(6%)	2(6%)
LT-ST	0	6	2	2	0
LT	3	4	1	0	2
ST	2	0	0	0	0
<i>Salmonella</i>	0	1(3%)	0	0	0
<i>Shigella</i>	0	5(13%)	5(11%)	2(6%)	0
<i>S. dysenteriae</i>	0	1	0	0	0
<i>S. flexneri</i>	0	4	2	1	0
<i>S. sonnei</i>	0	0	1	0	0
<i>S. boydii</i>	0	0	2	1	0
<i>C. jejuni/coli</i>	0	1(3%)	0	0	0
<i>Y. enterocolitica</i>	1(3%)	1(3%)	0	0	0
<i>A. hydrophilia</i>	0	12(31%)	6(16%)	3(9%)	2(6%)
<i>P. shigelloides</i>	0	13(33%)	8(22%)	5(14%)	1(3%)

+ Number of PCVs cultured

o Number of episodes cultured

Table 3. Twelve Multiple Bacterial Infections Among 39 Episodes of Travelers' Diarrhea

<u>PCV No.</u>	<u>Episode</u>	<u>Pathogens</u>
2	1st	LT+ <i>E. coli</i> , <i>Y. enterocolitica</i> , <i>A. hydrophilia</i> , <i>P. shigelloides</i>
6	2nd	<i>A. hydrophilia</i> , <i>P. shigelloides</i>
7	2nd	<i>A. hydrophilia</i> , <i>P. shigelloides</i>
8	1st	LT+ST+ <i>E. coli</i> , <i>P. shigelloides</i>
15	1st	LT+ST+ <i>E. coli</i> , <i>S. flexneri</i> , <i>A. hydrophilia</i>
16	1st	<i>Salmonella</i> Group B, <i>P. shigelloides</i>
	3rd	<i>S. dysenteriae</i> , <i>A. hydrophilia</i>
21	1st	LT+ <i>E. coli</i> , <i>A. hydrophilia</i> , <i>P. shigelloides</i>
28	1st	LT+ <i>E. coli</i> , <i>P. shigelloides</i>
32	1st	LT+ <i>E. coli</i> , <i>P. shigelloides</i>
	2nd	<i>S. flexneri</i> , <i>A. hydrophilia</i>
37	1st	LT+ST+ <i>E. coli</i> , <i>P. shigelloides</i>

Table 4. Antibiotic Resistance of Enteric Pathogens and Tox- *E. coli* Isolated from PCVs During Their First Ten Weeks in Rural Thailand

Enteric Pathogens	Percent Resistance										
	Ap	Cf	Cm	Do	Gm	Km	Nm	Sm	Su	SXT	Tc
Tox- <i>E. coli</i> (N = 1090)	26	1	36	59	0	21	21	64	64	0	59
Tox+ <i>E. coli</i> (N = 88)	31	1	42	61 ^Δ	0	11	11	42	42	0	61 ⁺
<i>Shigella</i> (N = 10)	57	0	79	86 ^o	0	14	14	64	64	0	86
<i>Salmonella</i> (N = 1)	0	0	0	0	0	0	0	0	0	0	0
<i>Y. enterocolitica</i> (N = 2)	100	100	0	0	0	0	0	0	0	0	0
<i>C. jejuni/coli</i> (N = 1)	0*	0	0	0	0	0	0	0	0	0	0
Possible Enteric Pathogens											
<i>A. hydrophilia</i> (N = 23)	92	38	3	5	0	3	3	46	92	54	8
<i>A. plesiomonas</i> (N = 27)	54	0	0	12	6	28	28	86	35	13	26

+ The mean MICs was 191 µg/ml (range 62.5 - 250 µg/ml)

Δ The mean MIC was 23.5 µg/ml (range 3.9 - 31.2 µg/ml)

* Resistant to Penicillin (10 µg disc)

o The mean MIC was 16.9 µg/ml (range 15.6 - 31.2 µg/ml)

Antibiotic sensitivity of *C. jejuni/coli*, was determined by spreading 10⁸ organisms on blood agar, adding sensitivities discs, and incubating the culture microaerophilically at 37°C for 48 hours.

Table 5. Intestinal Parasites Found in Seven of 35 PCVs During Their First 10 Weeks in Thailand

<u>Number of PCVs infected</u>		<u>Intestinal Parasites</u>
	ARRIVAL SPECIMEN	
2*		<i>Giardia lamblia</i>
1		<i>Endolimax nana</i>
	5 WEEKS SPECIMEN	
1		<i>Ascaris lumbricoides</i>
1		<i>E. nana</i>
		<i>Entamoeba coli</i>
1		<i>Trichuris Trichiura</i>
		<i>G. lamblia</i>
1		Unidentified Amoeba
	10 WEEKS SPECIMEN	
1		<i>E. coli</i>
		<i>Iosamoeba butschlii</i>
1		<i>Entamoeba histolytica</i>
		<i>E. nana</i>
		<i>I. butschlii</i>
2*		<i>G. lamblia</i>
	DIARRHEAL EPISODE SPECIMEN	
1		<i>E. histolytica</i>
		<i>E. nana</i>
		<i>E. coli</i>
		<i>I. butschlii</i>
2*		<i>G. lamblia</i>
1		<i>E. coli</i>
		<i>I. butschlii</i>
		<i>E. histolytica</i>
1		<i>E. coli</i>
		<i>I. butschlii</i>

* *G. lamblia* was found in stools of two PCVs on arrival, during episodes of diarrhea and after ten weeks.

ANTIBIOTIC RESISTANCE OF E. COLI FLORA IN PCV

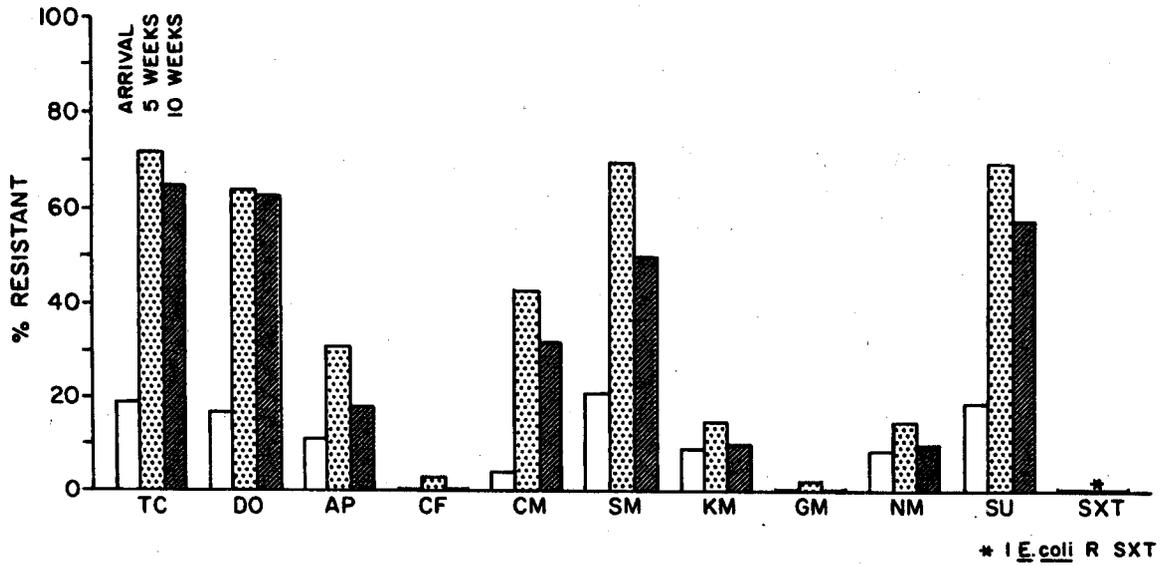


Figure 2. Antibiotic sensitivity of 185 "arrival", "five week", and "ten week" tox- *E. coli* isolated from PCVs during their first five weeks in Thailand.

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