

PROPHYLACTIC DOXYCYCLINE FOR TRAVELERS' DIARRHEA  
IN THAILAND

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OBJECTIVE : To prevent travelers' diarrhea among Americans during their first five weeks in Thailand.

BACKGROUND : In a previous study, we found that 57 percent of 35 American Peace Corps volunteers developed travelers' diarrhea during their first five weeks in Thailand (1). The predominant recognized pathogen in Thailand (1) as in Mexico and Africa (2-6) was ETEC. In contrast to other regions of the world where antibiotic resistance was distinctly unusual among ETEC (3-6), in Thailand 48 percent were resistant to multiple antibiotics and sixty-one percent were resistant to doxycycline (1).

Doxycycline has effectively prevented 80-90% of travelers' diarrhea in Kenya and Morocco (5, 6) countries where ETEC are nearly all sensitive to tetracycline. Since doxycycline is primarily excreted by diffusion across the small bowel mucosa (7) which results in relatively high concentrations at least transiently in the area where ETEC colonization occurs, it is possible that ETEC classified as "resistant" by the usual criteria (based on serum levels) might indeed be "sensitive" to these higher concentrations of antibiotics in the small bowel. In addition, *in vitro* experiments (8) suggest that doxycycline may also inhibit adherence of *E. coli* to mucosal surfaces. For these reasons we elected to study the efficacy of doxycycline in preventing travelers' diarrhea in an area where doxycycline resistant enteric pathogens are prevalent (1, 9).

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## METHODS :

Subjects : Sixty-three Peace Corps volunteers, 22 to 70 years of age, who travelled to Thailand either in March (N=40) or July (N=23), 1980 participated in the study. Nineteen had previously travelled to developing countries and eleven of these had previously developed travelers' diarrhea.

Vials containing capsules of doxycycline (100 mg) or a placebo (prepared and provided by Pfizer Laboratories) were randomly assigned to volunteers with instructions to take one capsule daily with meals during their first three weeks in Thailand. Volunteers who had submitted pre-travel stools in San Francisco began to take their capsules on the airplane the day they left the United States. The group from whom pre-travel stools were collected in Thailand ingested their first tablet within 36 hours after their arrival. Unannounced urine collections obtained weekly during the first three weeks were tested for antibiotic activity (5, 10). The volunteers spent their first three days in Bangkok, after which they travelled to one of several villages (Pranburi, Inburi, and Sri Prachan) where they spent the remaining part of the study. Volunteers were interviewed each morning to determine if they developed gastrointestinal symptoms. Travelers' diarrhea was defined as the occurrence of three or more watery stools, or at least two watery stools with abdominal cramps, vomiting, fever, or prostration. Volunteers having one or two watery stools without other symptoms were said to have loose motions. A recurrence of diarrhea was separated from a previous episode by at least two asymptomatic days.

Laboratory studies : Stools were collected from the group of volunteers who travelled to Thailand in March, 1980 immediately before they left San Francisco with the use of modified Stewart's transport medium (Culture Caddie, Inolex Corporation). The second group of volunteers who travelled from Seattle and arrived in July, 1980 submitted initial stool specimens within 36 hours after their arrival in Thailand. Stools were collected routinely again after two weeks (during the treatment period) and at the end of five weeks (two weeks after discontinuation of treatment). Stool specimens were also obtained from volunteers at the onset and on each day of diarrhea. Stool specimens were cultured immediately after collection as previously described (1).

Ten lactose positive colonies were selected from the MacConkey plate, streaked on nutrient agar slants, and tested for heat-labile enterotoxin (LT) in the Y-1 adrenal cell assay (11) and heat-stable enterotoxin (ST) in the suckling mouse assay (12). Serotypes of toxigenic *E. coli* were determined with standard techniques (13).

Culture supernatants of *Y. enterocolitica* which were grown at 22°C were tested for heat-labile and heat-stable enterotoxin. Sterile culture supernatants of *A. hydrophila* were tested for cytotoxicity in Y-1 adrenal tissue cultures, and ability to distend suckling mouse intestine (1, 12, 14). Serotypes of *Y. enterocolitica* were determined as previously described (15).

Antibiotic resistance : ETEC and five non-ETEC, *Shigella*, *Salmonella*, *Y. enterocolitica*, and *A. hydrophila* isolated from each patient were tested on Mueller-Hinton agar by the Bauer-Kirby method (16). Isolates which were resistant to trimethoprim and sulfamethoxazole on Mueller-Hinton agar were retested on Isosensitivity agar (CM 471, Oxoid Ltd, Basingstoke, England). Antibiotic sensitivities of *C. fetus* subsp. *jejuni* were determined by inoculating  $10^8$  organisms on a blood agar plate, adding sensitivity discs, and incubating the culture microaerophilically at  $37^{\circ}\text{C}$  for 48 hours.

Serological tests : Serum specimens were collected either immediately before volunteers left the United States or within 24 hours after arrival in Thailand, and after they had been in country for three and five weeks. Titers of antibody to LT were determined in duplicate with the microtiter adrenal cell neutralization assay (17). Antibody titers to human rotavirus were determined by complement fixation assay with SA-11 virus as a substitute antigen (18). Titers of antibody to Norwalk virus were determined by a radioimmunoassay blocking test (19).

## RESULTS :

Clinical : Seventy-two volunteers were initially enrolled in the study. One volunteer was removed from the study because she developed malaise and vomiting after taking doxycycline (three other volunteers experienced similar milder symptoms, but continued to take doxycycline). Eight volunteers (four in each treatment group) who had spent two weeks in Guatemala within a week prior to traveling to Thailand were excluded from the analysis since we felt that these individuals were not similar to other volunteers who had just arrived in Thailand from a developed country. Although seven of these eight suffered from episodes of travelers' diarrhea in Guatemala, none became ill in Thailand.

Of the 63 volunteers included in the study 30 were randomized to the doxycycline group and 33 to the placebo group. Antibacterial activity was found in all weekly urines collected from volunteers in the doxycycline group, but was not present in specimens collected from those in the placebo group. The episodes of diarrhea experienced by each group are shown in Figure 1. During the three week treatment period fewer volunteers taking doxycycline experienced episodes of travelers' diarrhea than those receiving placebo (3/30 vs 8/33) ( $p=0.12$ ) (Fisher's Exact test). Episodes of loose motions occurred in five (17 percent) of 30 in the doxycycline group and seven (21 percent) of 33 in the placebo group. One volunteer in the placebo group had a repeat episode of travelers' diarrhea within his first three weeks in Thailand.

Bacteriology : No enteric pathogens were isolated from the 40 volunteers prior to leaving the United States. However eleven of 22 volunteers from whom stools were collected within 36 hours after their arrival in Thailand were infected asymptotically with bacterial enteric pathogens. Six were infected with non-enterotoxigenic *Y. enterocolitica*, four with ETEC, and one with *S. sonnei*. None of these infected volunteers had previously travelled outside of the United States in the six months prior to arriving in Thailand.

As shown in Table 1, LT-ST+ *E. coli* were isolated from one volunteer with travelers' diarrhea and another with loose motions, both in the placebo group. *S. sonnei* was isolated from one participant with travelers' diarrhea in the placebo group, but after the treatment period. No enteric pathogens were found in stools collected during the other 20 episodes of travelers' diarrhea which occurred among both groups during their first five weeks in Thailand. *C. fetus* subsp. *jejuni* which was resistant to doxycycline group was isolated from one volunteer with loose motions in the doxycycline group. This individual was asymptomatic within 48 hours after the onset of diarrhea. Each of the three participants who had travelers' diarrhea while taking doxycycline had watery stools for less than 36 hours. Recognizable enteric pathogens were not found in their stools during acute episodes. Two volunteers infected with doxycycline resistant LT+ST- *E. coli*, and one infected with resistant *S. sonnei* prior to doxycycline administration did not develop diarrhea after doxycycline prophylaxis was started.

LT+ST- *E. coli* were isolated from one asymptomatic volunteer in the doxycycline group and two in the placebo group after they had been in Thailand two weeks. The one volunteer in the doxycycline group and one of the two in the placebo group were infected with doxycycline resistant ETEC. None of these individuals had or developed diarrhea within the next five days.

Seventy-seven percent (24/31) of heat-labile and 54 percent (12/22) of heat-stable enterotoxigenic *Escherichia coli* were resistant to two or more different antibiotics. As shown in Figure 2, the percent of antibiotic resistant *E. coli* of volunteers (five non-ETEC/volunteer) in both the treatment and the placebo group increased after two weeks in Thailand.

During the treatment period (first three weeks in Thailand) *A. hydrophila* were isolated from four (50%) of eight volunteers with travelers' diarrhea in the placebo group, but none of three with travelers' diarrhea in the treatment group ( $p=0.21$ ). *A. hydrophila* was isolated from four (44 percent) of nine episodes of loose motions that occurred in the placebo group, and from only one (11 percent) of nine episodes of loose motions that occurred among participants in the doxycycline group ( $p=0.13$ ). After the treatment period *A. hydrophila* was isolated from as many volunteers with travelers' diarrhea in the treatment group as in the placebo group. *A. hydrophila* was isolated more frequently during episodes of diarrhea than when the participants were passing normal stools (15/26 vs 15/59) ( $p<0.001$ ) (Chi-square). Nine (60 percent) of 15 *A. hydrophila* isolated from volunteers with diarrhea and eight (53 percent) of 15 isolated from participants when they were well were cytotoxic to Y-1 adrenal cells and hemolyzed rabbit erythrocytes. Six of nine cytotoxic *A. hydrophila* isolated from volunteers with diarrhea and four of eight from those who were well distended suckling mouse intestine (gut to remaining body weight ratio  $>0.83$ ).

Multiple pathogens : One participant with travelers' diarrhea was infected with more than one potential bacterial pathogen (*A. hydrophila*, and *S. sonnei*). One volunteer with loose motions was infected with *Salmonella* group B and *S. sonnei*.

Serological results : Serial serum specimens obtained from 62 volunteers were measured for antibodies to LT, rotavirus and Norwalk agent. No volunteer had a rise in antibody titer to LT or rotavirus. Two of 62 volunteers did, however, develop greater than or equal to fourfold rises in antibody titer to Norwalk agent. Only one of these volunteers suffered loose motions and that was after being in Thailand four weeks.

Serotypes of bacterial pathogens : The serotypes and antibiotic resistance of ETEC isolated from volunteers during their first five weeks in Thailand are shown in Table 2. The eleven participants were infected with ETEC of different serotypes. Nine (82%) of 11 volunteers infected with ETEC were infected with antibiotic resistant isolates. Of the *Y. enterocolitica* isolated from six volunteers after their arrival, five were infected with serotype 0-16 and one was infected with serotype 0-35. All of these non-enterotoxigenic *Y. enterocolitica* were resistant to one antibiotic, ampicillin.

This study is complete.

Table 1. Enteric pathogens isolated from 30 volunteers receiving doxycycline and 33 receiving placebo during episodes of diarrhea during and after the treatment period.

<u>Enteric pathogen isolated</u>	<u>Travelers' diarrhea</u>		<u>Loose motions</u>	
	<u>Placebo</u>	<u>Doxycycline</u>	<u>Placebo</u>	<u>Doxycycline</u>
			First three weeks (treatment period)	
	9 <sup>+</sup>	3	10	9
LT-ST+ <i>E. coli</i>	1(0)	0	1(1)	0
<i>C. fetus</i> subsp. <i>jejuni</i>	0	0	0	1(1)
<u>Possible pathogen</u>				
<i>A. hydrophila</i>	4(0)	0	7(0)	1(1)
			Final two weeks (after treatment period)	
	5	5	1	2
<i>S. sonnei</i>	1(0)	0	0	1(1)
<i>Salmonella</i>	0	0	0	1(1)
<u>Possible pathogen</u>				
<i>A. hydrophila</i>	4(0)	4(0)	0	0

+ number of episodes

( ) number of isolates resistant to doxycycline by the Kirby-Bauer method (16)

LT-ST+ *E. coli* = heat-stable enterotoxigenic *Escherichia coli*

Table 2. Antibiotic resistance and serotype of enterotoxigenic *Escherichia coli* isolated from Peace Corps volunteers in Thailand, 1980

<u>Heat-labile ETEC</u>		<u>Serotype</u>	<u>Antibiotic resistance pattern</u>
5PS-2	LT+ST-	015:K?H-	2* Ap Cm Do Sm Su Tc
2PS-3	LT+ST-	07:K?H15	1 Cm Do Sm Su Tc
PS-14	LT+ST-	017:K?H45	10 Do Km Nm Tc
PS-17	LT+ST-	0?K?H7	1 MS
PS-23	LT+ST-	071:K?H7	5 Do Tc
2PS-25	LT+ST-	08:K88:H7	1 Do Km Nm Sm Su Tc
2P-39	LT+ST-	080:K?H19	10 Ap Do Sm Su Tc
<u>Heat-stable ETEC</u>			
PS12A1	LT-ST+	0148:K?H28	6 MS
PS-27	LT-ST+	0102:K?H6	1 MS
		0 Rough:H41	2 MS
5PS-28	LT-ST+	023:K?H15	2 Cm Do Tc
		0101:K?H16	1 MS
2P30A1	LT-ST+	0126:K?H12	9 Cm Do Sm Su Tc
		0126:K?H12	1 Ap Cm Do Sm Su Tc

ETEC = enterotoxigenic *Escherichia coli*

\* = number of enterotoxigenic *E. coli* tested for susceptibility to antibiotics

LT = heat-labile enterotoxin; ST = heat-stable enterotoxin

Ap, ampicillin; Cm, chloramphenicol; Do, doxycycline;

Km, kanamycin; Nm, neomycin; Sm, streptomycin; Su, sulfisoxazole;

Tc, tetracycline

MS = multiple sensitivity

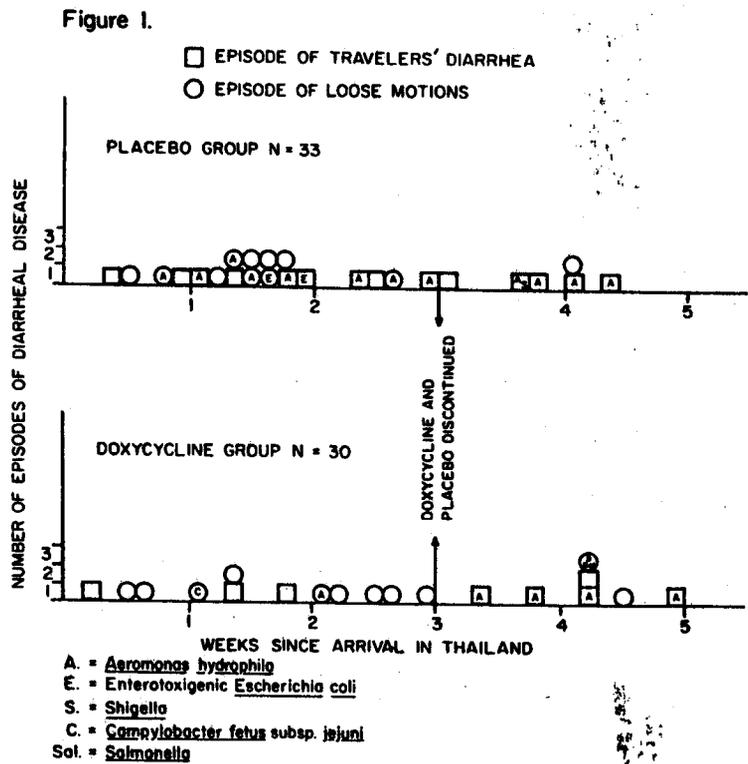
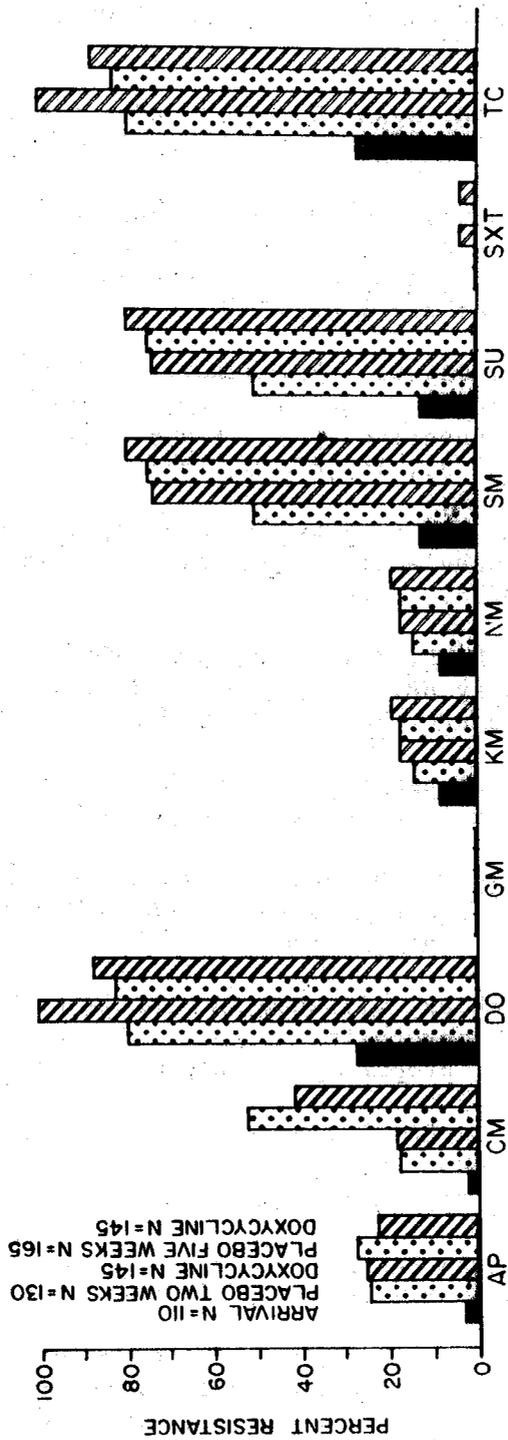


Figure 1. Time of onset of gastrointestinal symptoms and association with enteric pathogens

**Figure 2. Acquisition of Antibiotic resistant *Escherichig coli* by Volunteers during their five weeks in Thailand.**



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