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14. ABSTRACT

Campylobacter species are the predominant cause of diarrhea in military travelers to Thailand accounting for approximately 40-50% of evaluated cases. The clinical presentation and subsequent time to resolution for Campylobacter-associated cases differs from other etiologies in this setting evidenced by frequent systemic toxicity increased diarrhea severity at presentation, delayed recovery, and higher 72-hr clinical failure rates [associated with use of fluoroquinolone (FQ) antibiotics]. These findings were observed during a period of time when the rates of FQ-resistant C. jejuni exceed 85% and the most common therapy prescribed was a FQ antibiotic. Diagnostic tests were evaluated in U.S. soldiers presenting with acute diarrhea during deployment in Thailand. Bedside and field laboratory diagnostic tests were compared to stool microbiology findings in 182 enrolled patients. Clinical findings, inflammatory screening tests [stool hemoccult, fecal leukocytes, fecal lactoferrin (LFLA), plasma C-reactive protein], or Campylobacter-specific peripheral blood antibody-secreting cells failed to increase posttest probability above 90% in this Campylobacter hyperendemic setting. A Campylobacter-specific commercial EIA, and less so a research PCR, were strong positive predictors. Negative predictive value (reducing post-test probability less than 10%) was similarly observed with these Campylobacter-specific stool-based tests as well the fecal LFLA. A randomized, active drug-controlled, double-blinded study definitively demonstrated azithromycin to be the preferred antibiotic for traveler's diarrhea empiric treatment in Thailand. Clinical cure by 72 hours was highest at 96% with single dose azithromycin compared to 85% with 3-day azithromycin and 71% with levofloxacin (P = .002). Microbiologic eradication was significantly better for azithromycin-based regimens, 96-100%, as compared to levofloxacin at 38% (P = .001). Higher rate of posttreatment nausea in the 30 minutes after first dose (14 vs.< 6%, P = 0.06) were observed as a mild self-limited complaint with single dose azithromycin. Single-dose azithromycin is recommended for empiric therapy of travelers' diarrhea acquired in Thailand and should be further investigated for broader application in areas with more diverse enteropathogens.

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TRAVELERS' DIARRHEA DIAGNOSIS AND THERAPY STUDY IN UNITED STATES MILITARY PERSONNEL ON SHORT-TERM DEPLOYMENT IN THAILAND

by

David R. Tribble, M.D., M.P.H.

Thesis submitted to the Faculty of the Department of Preventive Medicine and Biometrics Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of Doctor of Public Health 2004

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TABLE OF CONTENTS

INTRODUCTION **1**

INFECTIOUS DIARRHEA EPIDEMIOLOGY 2

Relative impact between developing and developed regions *2*

Pathogen regional distribution *6*

Military relevance *9*

INFECTIOUS DIARRHEA CLINICAL ISSUES 12

Clinical features *12*

Practice Guidelines *18*

Diagnosis *24*

Management *29*

THAILAND-SPECIFIC DIARRHEA EPIDEMIOLOGY 33

Background *33*

Civilian populations *36*

Thai population 36

Civilian expatriates and travelers 42

U.S. military populations *44*

RESEARCH PROPOSAL 50

Proposal objectives *50*

Summary proposal design *50*

Retrospective analysis of clinical features and outcome by bacterial etiology *52*

Rationale 52

Approach 53

Clinical definitions 53
Clinical evaluation 54
Microbiology assessment 55
Data management and analysis 56
Limitations 58
Diarrhea diagnostic evaluation *58*
Rationale 58
Methods 59
Clinical and laboratory evaluations 59
Reference standard microbiology 60
Analysis 60
Limitations 62
Randomized controlled trial *62*
Rationale 62
Antibiotic selection 63
Sample size determination 68
Trial design 69
Entry criteria 70
Randomization and treatment assignment 71
Study treatment 71
Clinical monitoring 73
Microbiological monitoring 74
Study outcomes 75

Analysis 75
Limitations 77
REFERENCES 78

ACUTE DIARRHEA IN U.S. MILITARY PERSONNEL ON SHORT TERM DEPLOYMENT IN THAILAND: ROLE OF *CAMPYLOBACTER* IN DISEASE SEVERITY AND CLINICAL OUTCOME 106

ABSTRACT 108
INTRODUCTION 109
METHODS 110
Study population and inclusion criteria 110
Clinical evaluation and monitoring 111
Laboratory analysis 111
Statistical analysis 112
RESULTS 114
Study population characteristics 114
Clinical presentation 115
Distribution of bacterial enteric pathogens 116
Campylobacter-associated findings 116
Initial Presentation and Prediction of *Campylobacter*-associated illness 116
Clinical outcomes 118
DISCUSSION 120
REFERENCES 124
LIST OF TABLES 129

Table 1. Population characteristics among U.S. military presenting with acute diarrhea during training exercises in Thailand *129*

Table 2. Distribution of bacterial enteric pathogens at initial presentation in U.S. military personnel with acute diarrhea by Thai region *130*

Table 3. Clinical manifestations (%) at initial presentation in U.S. military personnel with acute diarrhea by bacterial isolate *131*

Table 5. Clinical outcomes in U.S. military personnel with acute diarrhea based on stool microbiology isolation of Campylobacter jejuni *133*

Figure 1. Time to cure (following first antibiotic dose) by Campylobacter species isolation in pretreatment stool culture *134*

Table 6. Prediction of Campylobacter isolation in U.S. military personnel with acute diarrhea by clinical and laboratory measures available at presentation *135*

DIAGNOSTIC APPROACH TO ACUTE DIARRHEAL ILLNESS IN A MILITARY POPULATION ON TRAINING EXERCISES IN THAILAND, A *CAMPYLOBACTER* HYPERENDEMIC REGION 137

ABSTRACT 139

INTRODUCTION 140

METHODS 140

Study population and enrollment criteria *140*

Clinical evaluation and specimen collection *141*

Stool microbiology (reference standard) *141*

Diagnostic tests *142*

Stool-based 142

Blood-based 143

Statistical analysis *144*

RESULTS 145

DISCUSSION 147

REFERENCES 151

TABLES AND FIGURES 156

TABLE 1. CHARACTERISTICS OF U.S. MILITARY PERSONNEL PRESENTING WITH ACUTE DIARRHEA ON DEPLOYMENT IN THAILAND 156

TABLE 2. CLINICAL AND LABORATORY FINDINGS AS DIAGNOSTIC MODALITIES FOR INVASIVE ENTEROPATHOGENS 157

TABLE 3. CLINICAL AND BEDSIDE STOOL CHARACTERISTICS AS CAMPYLOBACTER DIAGNOSTIC MODALITIES 158

TABLE 4. FIELD LABORATORY TESTS AS CAMPYLOBACTER DIAGNOSTIC MODALITIES. 159

FIGURE 1. POST-TEST PROBABILITY OF CAMPYLOBACTER-ASSOCIATED ILLNESS BASED ON DIAGNOSTIC APPROACH (POSITIVE TEST) IN VARIOUS ENDEMIC SETTINGS 160

FIGURE 2. POST-TEST PROBABILITY OF CAMPYLOBACTER-ASSOCIATED ILLNESS BASED ON DIAGNOSTIC APPROACH (NEGATIVE TEST) IN VARIOUS ENDEMIC SETTINGS 160

TRAVELERS' DIARRHEA IN THAILAND: RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL COMPARING AZITHROMYCIN-BASED REGIMENS (SINGLE DOSE AND 3-DAY) VS. LEVOFLOXACIN (3-DAY) 162

ABSTRACT 164

INTRODUCTION 166

METHODS 167

Participants and Subject Eligibility 167

Treatment Assignment, Randomization, and Blinding Procedures 168

Clinical Monitoring 168

Stool Microbiology 169

Outcome Measures 171

Statistical Analysis 171

RESULTS 173

Subject Enrollment and Characteristics 173

Distribution of Enteric Pathogens 175

Clinical Outcomes 176

Microbiological Outcome 178

Assessment of Confounding Factors 179

Adverse Events 180

DISCUSSION 181

REFERENCES 187

TABLES AND FIGURES 195

Figure 1. Trial enrollment profile 195

Table 1. Baseline patient characteristics prior to treatment assignment 196

Table 2. Clinical manifestations, laboratory findings, and management at presentation by treatment group 197

Table 3. Enteric pathogen distribution at presentation by treatment group..... 198

Table 4. Clinical and microbiological outcomes by treatment group..... 199

Figure 2. Time to cure (following first antibiotic dose) by treatment group 200

Figure 3. Time to cure (following first antibiotic dose) by treatment group stratified by Campylobacter diagnosis 201

Table 5. Surveillance for posttreatment nausea and vomiting (%)..... 202

Table 6. Evaluation of non-treatment related factors on clinical outcome 203

Figure 4. Time to cure (following first antibiotic dose) by pretreatment stool bacteriology result stratified by antibiotic used..... 204

CONCLUSION 206

SUMMARY OF MAJOR FINDINGS 206

LIMITATIONS 210

PUBLIC HEALTH RELEVANCE 211

RECOMMENDATIONS 216

REFERENCES..... 221

APPENDIX..... 228

RESEARCH PROTOCOL.....

INFORMED CONSENTS .....

STUDY FORMS.....

INSTITUTIONAL APPROVAL DOCUMENTS .....

Introduction

Acute bacterial diarrhea caused primarily by enterotoxigenic *E. coli* (ETEC), *Shigella* spp., *Campylobacter jejuni*, and nontyphoidal *Salmonella* spp. remain major infectious disease threats to deployed military forces. These bacterial enteropathogens may affect significant proportions of a deployed unit as an explosive outbreak or as a steady stream of sporadic cases. Patterns of diarrheal disease lead to short-term disability frequently removing the active duty member temporarily from his or her job and taxing unit medical resources. Most cases, in previously healthy persons, are self-limited and rarely life threatening. Strategic health issues for a deployed force are the following:

1) A significant proportion of the unit or key personnel sustain a self-limited illness, up to one-week duration, with intermittent ability to work effectively.

2) Medical therapy (nonspecific anti-diarrheal or pathogen-specific directed therapy) and on-site medical resources are required due to illnesses frequently associated with distressing symptoms (20-40%), such as abdominal cramps, nausea, vomiting, fever, and/or gross blood in stools partially or completely limiting activities.

"Normal" circumstances often do not exist in a deployed setting where harsh environmental conditions, marginal medical access, and intermittent capability to reduce foodborne threats require effective rapid diagnostic and therapeutic approaches. Primary preventive measures such as diarrhea vaccines are currently lacking. The DOD infectious diseases research initiatives prioritize ETEC, *Campylobacter* and *Shigella* as three of the top five infectious disease threats requiring targeted vaccine development.

Primary preventive strategies such as good hygiene practices, maintenance of safe water supply, and threat reduction by troop activity restriction can be very effective in decreasing diarrhea incidence, but can be difficult to sustain. This introductory review provides information on recent epidemiology, as well as, diagnostic and therapeutic strategies with particular focus on Thailand and specifically *Campylobacter*, the predominant pathogen affecting the U.S. military deployed to this country. A critically important aspect of diarrhea diagnostics and application of appropriate empiric therapy is an understanding of the regional variability of diarrheal pathogens and their associated antimicrobial susceptibility patterns. These issues will be discussed leading into the thesis objectives, public health concerns being addressed, and the summary project design.

Infectious diarrhea epidemiology

Relative impact between developing and developed regions

Global diarrheal disease burden most affects children in the developing world. Despite declining trends over the past 50 years in diarrhea-specific mortality rates in children under age 5, morbidity measures remain relatively unchanged [1]. Early childhood (age less than 5 years) estimated mortality rates (per 1000 children per year) for diarrheal disease for the period 1955-1979, 1980-1989, and 1992-2000 have decreased from 13.6 to 5.6 to 4.9 [1]. In contrast, median incidence as a measure of morbidity has not shown a decline with similar rates of 3.2 episodes per child-year for under 5-year-olds. In addition to stable early childhood diarrhea incidence, updated estimates of disability adjusted life years (DALYs) approximate a doubling of the 100

million estimate from the late 1990s [2]. The 100 million DALY estimate was calculated based primarily on diarrhea-specific mortality [3, 4]. The DALY estimate is a means to quantitate premature mortality and ongoing disability in order to compare the impact of diseases or conditions among populations. The summation of potential years lost in fatal conditions and years lost to disability in nonfatal conditions yield the DALY estimate. The earlier estimates for global diarrhea impact did not account for more recent evidence of impairments in child fitness, growth shortfalls, cognitive impairment, and delayed age for school entry leading to ongoing disability [2].

In contrast to the childhood mortality and morbidity observed in developing regions, the major impact of diarrheal disease in industrialized countries such as the U.S. is in short-term morbidity and economic concerns. Diarrhea-related mortality typically is limited to populations at the extremes of age, particularly the elderly. Overall case fatality rates in the U.S. are far less than rates seen within the most at-risk population (< 5 years old) in developing countries, 0.014 vs. 4.9 (deaths/1000 persons/year) [5]. A full appreciation of diarrheal disease in the U.S. is lacking due to the usual self-limited nature of the illness frequently with no medical evaluation, no diagnostic work-up, incomplete reporting and passive surveillance. The CDC has formed a foodborne disease evaluation program called FoodNet as part of their Emerging Infections Program [6]. The program's objectives are to more precisely assess burden of foodborne disease, the relative proportion of specific etiologic agents and develop the infrastructure to respond to emerging foodborne threats. The program is a population-based active surveillance among clinical microbiology laboratories in selected states. The FoodNet group performed a population survey in 1997 of 10,000 residents within the surveillance area.

The respondents reported an 11% diarrhea rate within the preceding month yielding a 1.4 episode/person/year rate. Of these affected persons, only 8% sought medical advice and when they did only 20% of practitioners obtained a stool specimen for analysis. It is somewhat intuitive and certainly clear through this survey that active surveillance based in clinical microbiology labs represents a small fraction of the diarrheal illness and likely represents the more severe and/or prolonged cases. Surveillance data and projects originating from this valuable resource will greatly expand knowledge of etiologic agents/distribution, risk factors and potential control measures. This information is important for military health care planners both within the U.S. and within deployed units since the majority of those pathogens have a cosmopolitan distribution.

The intersection of a vulnerable host, individuals from industrialized regions with low endemic rates of bacterial diarrhea, with exposures in hyperendemic less developed areas led to the syndrome of traveler's diarrhea. This syndrome was first studied in detail by Kean and colleagues in U.S. travelers to Mexico [7]. These landmark studies led to a well-described clinical syndrome prior to etiologic determination. The syndrome occurred in 25-33% of travelers to Mexico. The clinical picture consisted of acute nonbloody diarrhea, mild fever, abdominal cramps, nausea, chills, and malaise. Illness onset was on average 14 days from arrival in country with duration of 1-3 days. An extensive evaluation of etiologies, infectious and noninfectious, led Kean and colleagues to propose pathogenic *E. coli* as the likely major cause. This was later shown to be correct with enterotoxigenic *E. coli* identified as the most common etiology of traveler's diarrhea in Mexico. In addition, evidence from early antibiotic prophylaxis studies further supported the hypothesis that *turista* or traveler's diarrhea was largely due to bacterial

etiologies. Steffen and colleagues further explored this syndrome on a global scale through evaluation of 16,568 passengers returning to Switzerland or Germany following visits in regions throughout the world [8]. Two-week cumulative incidence ranged from a high of 48.5% in Tunisia to a low of 3.7% in North America. Of particular interest for this proposal, travel to Thailand (N = 1838) yielded a 2-week incidence of 21%. The majority (62%) experienced onset of illness within the first week with a mean duration of illness in travelers to the tropics of 3.6 days. The mean maximal daily diarrhea stool output was 4.6 in travelers to the tropics. Use of prophylactic or therapeutic drugs was very common (42%). Thailand was included in the tropics category and accounted for 17% of the travelers. No significant difference in diarrhea chronology was detected between tropical destinations. More recent large surveys of diarrhea risk at various tourist destinations (N = 67,231) continue to demonstrate high but variable attack rates as follows: India 32%, Kenya 31%, Jamaica 12%, and Brazil 5.4% [9].

Disease-specific seasonality may affect the predominance of particular pathogens during times of the year (such as ETEC and Shigella during summer months) and changing behaviors related to potential exposures [10, 11]. However, the 28-day attack rate in newly arrived U.S. medical students in Mexico was similar in January (29%) and August (34%) [10]. U.S. students previously residing in Mexico and returning from a 4-8 week U.S. vacation experienced lower 28-day attack rates (6%) in January as compared to a similar scenario in August (21%). In part the drop during wintertime in the established students compared with the newly arrived students appears to be due to less risky behaviors such as tap water and unsafe ice consumption. ETEC was only observed during the summer months. The overall pathogen isolation rates for this study are low,

August (32%) and January (20%) limiting some of the conclusions. The primary risk determinants for traveler's diarrhea include point of origin, destination, host factors, and exposure to contaminated food and beverages [12, 13].

Pathogen regional distribution

Traveler populations, civilian or military, provide the most direct information for regional threat assessment. The naive or semi-immune traveler leaving their relatively low risk environment to an area hyperendemic for bacterial enteropathogens has an approximately 40% diarrhea risk [14, 15]. A review of enteropathogens affecting travelers was compiled by Black in 1990 [16]. In this review a total of 34 studies were included involving travelers to Latin America (N = 24 with Mexico representing 79%), Asia (N = 8), and Africa (N = 3). The median attack rates by region were as follows: Latin America 53% (21-100%), Asia 54% (21-57%), and Africa 54% (36-62%). ETEC was the most commonly identified pathogen and demonstrated regional variability with median isolation rates of 42% in Latin America, 16% in Asia, and 36% in Africa. *Shigella* and *Salmonella* species also demonstrated significant regional variation as detailed below.

Table 1. Stool culture isolation of enteric bacteria commonly included in surveys

Bacterial pathogen	Median isolation percentages (range)		
	Latin America	Asia	Africa
ETEC	42 (26-72)	16 (0-37)	36 (33-71)
Shigella	8 (0-30)	0 (0-13)	0 (0-15)
Salmonella	1 (0-16)	4 (0-33)	0 (0)

Adapted from [16].

Information on *Campylobacter* is incomplete in this summary and limited to surveys out of Asia (Thailand and Bangladesh) and Mexico [17-19]. Higher rates of

Campylobacter isolation were observed in the Asian countries, Thailand (17%) and Bangladesh (15%), as compared to 1% in Mexico. Of viral etiologies only Rotavirus was included and was inconsistently surveyed for across the studies (Latin America 29%, Asia 50%, and Africa 100%). Despite these concerns it appears that Rotavirus is an uncommon etiology of traveler's diarrhea accounting for $\leq$ 5% in all studies with the exception of 3 studies in Latin America with rates of 21-36%. Parasitic etiologies evaluated included *Giardia* and *E. histolytica*. These pathogens were identified in < 6% of affected travelers across the studies. The failure to detect a pathogen was quite common occurring in 48% (22-83%) in Latin America, 68% (43-94%) in Asia, and 53% (29-64%) in Africa.

A more recent survey (1996-98) undertaken by DuPont, Steffen, and colleagues compiled pathogen data from travelers experiencing acute diarrhea during visits to Jamaica, India, or Kenya [20]. This series utilized current laboratory standards to identify the primary infectious etiologies. As previously observed in Black's summary, bacterial etiologies remain the most common causes for acute diarrhea. ETEC were again the most commonly identified pathogen occurring in 25% of all cases. Table 2 provides a summary of the pathogens identified as these geographically distinct sites.

Table 2. Percent isolation of enteric pathogens in international travelers

Pathogen	Mombassa (*n* = 464)	Goa, India (*n* =293)	Montego Bay, Jamaica (*n* = 322)
ETEC	35	24	12
Campylobacter	5	3	5
Salmonella	3	10	8
Shigella	9	10	< 1
Vibrio species	3	5	< 1
Aeromonas	2	3	0
Plesiomonas	2	7	0
Rotavirus	6	5	8
Adenovirus	3	2	3
Giardia	0	2	< 1
E. histolytica	0	5	< 1
Cryptosporidium	0	2	< 1
Mixed infection	6	11	5
No pathogen detected	47	45	68

Adapted from [20].

In summary, ETEC is the most common identified agent on a global basis representing between 5-40% of cases [16]. *Campylobacter jejuni* (3-45%), *Shigella* spp. (2-10%), and nontyphoidal *Salmonella* spp. (2-10%) are other commonly identified etiologic agents. Significant regional and seasonal variability affects the relative distribution of etiologic agents (i.e. ETEC more common in summer vs. *Campylobacter* more common in winter in semi-tropical regions [11]; ETEC and *Shigella* predominate in Southwest Asia vs. *Campylobacter* and *Salmonella* being more common in Thailand [16, 21, 22]. Other bacterial enteropathogens, as well as viral (Rotavirus and Norwalk virus - 5-10%) and parasitic agents (*Giardia*, *Cryptosporidium*, *Cyclospora,* and *Entamoeba histolytica* - < 5%) account for additional cases. *Aeromonas*, *Vibrio cholerae*, non-cholera vibrios, and *Plesiomonas* typically represent no more than 5% of identified etiologies in most series. The continuously evolving spectrum of *E. coli*-related diarrheal diseases, such as enteroinvasive *E. coli* (EIEC), causing febrile dysentery and enteroadherent *E. coli*, causing watery diarrhea, are increasingly being shown to account

for 5-10% of the previously undiagnosed cases [23]. Enterohemorrhagic *E. coli* (most commonly *E. coli* 0157:H7), although now recognized as a major foodborne threat in industrialized countries as the most common cause of bloody diarrhea and the associated complication of hemolytic uremic syndrome, has not been identified as a traveler's diarrhea threat [24]. Bacterial etiologies represent > 80% of identifiable causes in traveler's diarrhea surveys; however, a substantial percentage (~ 50%) of cases have no pathogen detected.

Military relevance

Diarrheal diseases have complicated military operations for centuries. Mortality associated with diarrhea for U.S. troops is extremely rare; however, cumulative incidence of approximately 30% remains common in both short-term peacetime deployments and during wartime [21, 25]. The epidemiology of militarily relevant diarrheal disease is most akin to traveler's diarrhea seen in tourists to developing regions.

Operational Scenarios

Diarrheal diseases, commonly caused by bacterial enteropathogens, have consistently been a major medical threat to military operations. During the pre-antibiotic era, admission rates as high as 400-750 per 1000 troops were observed with case fatality rates (CFR) of 1-3% [26-28]. With the advent of improved sanitary practices the admission case rates have greatly decreased, but diarrhea attack rates from 5-50% are still observed [22, 25, 28]. Antibiotic therapy and recognition of the importance of rehydration therapy have reduced CFR to less than 0.05%; therefore diarrhea-related mortality in troops is now rare [28]. Diverse military operational scenarios create varying types and levels of bacterial enteropathogen risk. The rapid movement of greater than

200,000 personnel to Saudi Arabia during Operation Desert Shield (ODS) coupled with utilization of locally grown produce and variable food preparation conditions led to reported diarrhea rates of approx. 50% in initially deployed units [21, 29]. Bacterial enteropathogens were identified in half of the cases (n = 432) evaluated, with ETEC and *Shigella sonnei* most common. During Operation Restore Hope in Somalia, avoidance of local food and limited off-duty mobility due to security threat combined to reduce enteropathogen exposure [30]. The mean weekly incidence of gastroenteritis (0.8% of troops) was much reduced as compared to ODS. Despite this overall reduction, 16% of all hospital admissions (61/381) were due to gastroenteritis. *Shigella* (33%) and ETEC (16%) were the most common isolated pathogens. Disease transmission may have occurred through a mechanical fly-borne route, occasional exposures to local food and person-to-person spread.

Peacetime operational deployments are also greatly impacted by diarrheal disease. Naval exercises may be seriously disrupted by transient crew disability from acute diarrhea following a port call. This was observed when the USS *John F. Kennedy* had a 21% diarrhea rate in the evaluated crew (N = 2747), 155 sick-call visits and at least 110 lost man-days following an Alexandria, Egypt port visit in 1988 [31]. Annual joint military exercises in Thailand, Operations Cobra Gold and Balance Torch, have seen diarrhea attack rates ranging from 5-35% with *C. jejuni* the most common identified etiology [22, 25, 32-34].

Foreign Military and Civilian Counterparts

Foreign military experience with diarrheal disease emphasizes the even greater risk to troops during humanitarian efforts. Dutch military troops serving in Rwandan

refugee camps in Zaire experienced high diarrhea attack rates of 21%, 19%, and 47% during the first, second, and third 3-week period of their deployment [35]. The higher attack rate during the final deployment period appeared to be related to a point source exposure at a local restaurant with *S. sonnei* as the probable etiology. British and Australian troops working in Iranian refugee camps during Operation Safe Haven had 69% and 36% diarrhea rates, respectively [36]. Military members, government officials and civilian counterparts residing as expatriates in enteropathogen hyperendemic regions often maintain a persistent risk increased by younger age, shorter duration of stay and eating out in restaurants [37]. Rates of 49% (95% C.I., 37-61%) per month were estimated for the first two years for these expatriates living in Nepal. ETEC, *Campylobacter* and *Shigella* were the most common isolates documented in 64% of the diarrhea cases. High asymptomatic colonization rates were seen in a control group for ETEC, *Shigella* and *Campylobacter* at 11%, 8% and 3%, respectively. Deployed military living for lengthy periods in a developing region could experience similar high exposures as evidenced through persistent diarrhea and high asymptomatic colonization rates.

The civilian population most often used for military risk assessment and to provide guidance for diagnostic and therapeutic approaches is travelers. Some important differences in behaviors and activities exist between military and civilian traveler populations including the level of activity control and command policies [i.e. restriction regarding local food exposure, military pre-packaged rations such as U.S. military Meals-Ready-to-Eat (MRE) use, etc.], availability and use of medical resources and preventive medicine infrastructure supporting desirable behavioral modifications. Despite these differences, traveler population data provides valuable threat estimate information and

has been the driving force for current therapeutic approaches. The CDC used data from the Vessel Sanitation Program to analyze diarrhea outbreak incidence rates (1989-1993) on cruise ships [38]. This study demonstrated an overall outbreak incidence of 1.4 per 1000 cruises with an estimated probability of 0.2% that an outbreak would occur. When an outbreak did happen, the crew and passengers were affected on average 9% and 31% of the time, respectively. This rate resulted in an average of 246 ill persons per outbreak. The outbreak incidence rates seen during this study are reduced from past years, felt largely due to improved water sanitation. Contaminated food served as the vehicle of transmission in the majority of outbreaks with identified etiologic agents including ETEC, nontyphoidal *Salmonella*, *Shigella sonnei* and Norwalk-like virus. Similar outbreaks have occurred on military vessels with comparable requirements for case management, outbreak investigation and subsequent hazard reduction to prevent future outbreaks. Data from these sources assists planners' awareness of potential threats to allow institution of preventive measures.

Infectious diarrhea clinical issues

Clinical features

Traveler's diarrhea represents a spectrum of illness from a fleeting mild diarrhea without associated symptoms or activity limitation to a serious dehydrating and/or febrile dysentery requiring hospitalization. Most commonly, traveler's diarrhea consists of a self-limited diarrheal illness lasting 3-5 days [12, 13, 39]. Usual number of loose or liquid bowel movements occurring daily during illness is 1-2 (approx. 20%), 3-5 (approx. 25%) and $\geq$ 6 (approx. 55%) [40]. Associated symptoms are not uncommon, including

abdominal cramps (70%), nausea (50%), fever (20-25%), blood in stools (10-20%) and vomiting (10-15%). Overall, approximately one-fifth of affected persons have evidence (fever and/or bloody stools) of inflammatory disease. This does not take into account further laboratory evidence of fecal leukocytes that frequently increase the proportion of patients with inflammatory enteritis up to approx. 50%. The mean duration of symptoms without treatment is approx. 4 days (median 2 days).

Acute bacterial diarrhea is categorized clinically as watery diarrhea or dysentery (bloody diarrhea). Considerable syndrome overlap occurs for various bacterial enteropathogens. Pathogens such as ETEC, known to predominately cause a watery, non-inflammatory diarrhea, demonstrate little overlap into the inflammatory diarrheas. On the other hand, *Campylobacter*, *Shigella* and non-typhoidal *Salmonella* all may present anywhere along the spectrum of illness.

Watery (often noninflammatory) Diarrhea

Toxigenic diarrhea is a similar term describing watery noninflammatory diarrhea best represented by cholera and ETEC. ETEC causes intestinal secretion of fluids and electrolytes by production of one or more enterotoxins that overwhelm the intestine's absorptive capacity, leading to watery diarrhea [41, 42]. In addition to ETEC toxin activity, pathogenic strains also have fimbrial attachments that are important for the initial colonization step preceding toxin elaboration [23]. Anatomically, ETEC, like many causes of watery non-inflammatory diarrhea, has its primary site of action in the small intestine [23, 42]. In addition to the bacterial causes of watery diarrhea, it is important to consider enteric viruses and parasites (such as *Giardia*).

A well-characterized ETEC [elaborating a heat-stable (ST) enterotoxin] outbreak in Wisconsin provided useful information concerning clinical and epidemiological characteristics [43]. The outbreak occurred in 1994 with pan-fried potatoes being the likely foodborne common source vehicle. An estimated attack rate between 30-52% (N = 372-645) was observed with a mean and median incubation period of 33 hours (95% CI, 30-36) and 36 hours (range, 5-69 hours), respectively. Clinical symptoms reported included diarrhea (100%), cramps (83%), body aches (57%), headaches (48%), nausea (44%), chills (34%), fever (19%), vomiting (13%), and bloody stools (6%). The median duration of symptoms emphasized the operational concerns for the military if a significant number of troops were affected, diarrhea (6 days), cramps (5 days) and generalized complaints (approx. 3 days). The diarrhea and associated symptoms resulted in 42% unable to perform usual activities and 15% missing a total of 94 days of work.

Dysentery

The term dysentery is used to describe a more severe clinical form of an inflammatory diarrhea specifically manifesting with blood in the stools. The inflammatory process is marked by clinical symptoms and signs such as fever, chills, tenesmus and gross blood in stools with stool inflammatory markers including fecal leukocytes, stool lactoferrin and occult blood [44]. The colon is the anatomic location most associated with dysentery; however, the small intestine (particularly the ileum) also can be involved [44]. A wide range of bacterial enteropathogens is associated with this syndrome, with *Shigella* being the prototype for bacillary dysentery. In order to cause dysentery, the pathogen must locally invade (often limited to the mucosal layer), multiply intracellular and spread cell-to-cell and/or produce a cytotoxin. In etiologic studies of

acute diarrhea involving both travelers and indigenous populations from developing regions, evidence of invasive bacteria was seen in approx. 20-60% of isolates [40].

Shigellosis is classically described as a triphasic illness with the first phase presenting as systemic symptoms ("flu-like") with moderate to high fever followed by a phase of large volume watery stools with upper abdominal cramping and finally by small volume, bloody stools with lower abdominal cramps and tenesmus [45, 46]. The presence of each phase and the specific order is often not observed. *C. jejuni* also can present in a manner similar to shigellosis [44, 47, 48]. Symptom frequencies observed in a *C. jejuni* enteritis outbreak in Oklahoma in 1996 linked to lettuce contaminated from raw chicken demonstrate the range of inflammatory diarrhea [49]. Diarrhea occurred in all affected persons (N=25) with fever (93%), abdominal cramps (93%), vomiting (36%), and gross blood in stools (21%). Without therapy, relapses can occur in *C. jejuni* enteritis in as many as 20% of cases [50].

Persistent Diarrhea

Persistent travelers' diarrhea ($\geq$ 14 days) is not a common problem with overall estimates of 3% [51]. Parasitic etiologies (*Giardia*, *Cryptosporidium*, *Cyclospora*, *Isospora belli*) are more commonly represented causes. ETEC is not a usual cause of persistent diarrhea; however, *Shigella*, non-typhoidal *Salmonella* and *Campylobacter* all have been reported in the range of 5-20% of persistent diarrhea cases [51, 52]. Management algorithms for persistent diarrhea emphasize an early search for the parasitic etiologies and consideration of bacterial causes, possibly empiric anti-parasitic therapy and gastroenterology consultation for both infectious and non-infectious etiologies [51, 53, 54]. Prognostic factors at the time of initial presentation that correlate

with duration of diarrhea include the presence of fever (odds ratio (OR) = 0.34; 95% CI 0.2-0.9), positive culture for an invasive pathogen (*Shigella, Salmonella, C. jejuni*) (OR = 0.7;0.6-1.0), severe abdominal cramps (OR = 0.5;0.3-0.9), and > 5 watery stools per 24 hours (OR = 0.6; 0.4-0.8) [55]. These clinical features do not necessarily correlate with the patient developing persistent diarrhea meeting the $\geq$ 14 day criteria. However, these features do assist the clinician in decisions regarding observation or therapy, since more than 50% of patients with these predictors required greater than 5 days to fully recover [55, 56].

Complications

Acute complications from bacterial diarrhea can be divided into adverse events occurring during the diarrheal illness and post-infectious sequelae. Adverse events during the diarrheal episode are typically related to dehydration with subsequent water loss, electrolyte disorder, and/or base deficit. Less commonly, an individual may suffer from a direct intestinal complication such as toxic megacolon, perforation, or protein-losing enteropathy or bacteremic spread of the pathogen resulting in sepsis and/or metastatic infection. The post-infectious complications involving rheumatic and neurologic autoimmune diseases seem to occur due to either a molecular mimicry of bacterial components with a self antigen or as bystander activation with self-tolerance disrupted as a result of the infection-induced response [57]. Based on epidemiological studies involving both serology and culture results, approximately 30-40% of patients with Guillain-Barré syndrome (GBS) have had a *C. jejuni* infection in the preceding 10-21 days [58-60]. *C. jejuni*-associated GBS appears to be associated with a more severe clinical presentation and benefits from intravenous immunoglobulin therapy vice plasma

exchange [61]. The *C. jejuni*-GBS association appears to be associated with certain Penner serotypes (such as O19 and O41) related to the lipooligosaccharide (LOS) outer membrane structure on the bacterium [60, 62-64]. The unique LOS structure, sialylated polysaccharides, of *C. jejuni* differs from the *Enterobacteriaceae* and resembles some components of mammalian tissue gangliosides (such as GM_1, GD_{1a} and GQ_{1b}) present on human nerves. A genetic predisposition to acquiring a seronegative spondyloarthropathy after a bacterial enteric infection (approximate risk gradient: *Yersinia* spp. > *Shigella* spp. (*S. flexneri*) > non-typhoidal *Salmonella* > *C. jejuni*) has been observed in individuals with the human leukocyte antigen HLA-B27 [58]. An overall estimated 18-fold increased risk of reactive arthritis exists for HLA-B27 positive as compared to negative individuals following one of these infections [58]. Reiter's syndrome (arthritis, conjunctivitis, and urethritis triad) and ankylosing spondylitis have estimated increased risks of 37- and 126-fold, respectively. The hemolytic uremic syndrome (HUS) is another serious life-threatening post-infectious complication that has been observed with enterohemorrhagic *E. coli* producing Shiga toxins, particularly strain O157:H7, and Shiga-toxin producing *S. dysenteriae*. HUS presents as acute renal failure, thrombocytopenia and microangiopathic hemolytic anemia. Toxin-mediated renal pathogenesis appears to be related to receptors, glycosphingolipid globotriaosylceramide (Gb3) that bind toxin primarily found on the glomerular endothelial cells [65-67]. The clinical signs of HUS typically manifest during convalescence following the inflammatory, often bloody, diarrhea.

Practice Guidelines

Several reviews in the past decade have provided recommendations for the clinical management of infectious diarrhea [12, 39, 68-72]. In addition to expert reviews, medical societies or organizations have proposed practice guidelines [73-78]. Most of the guidelines have focused on the developed world and are divided by pediatric or adult population. The World Health Organization has provided practice guidance for developing world populations [79]. WHO guidance is provided as a component of the Integrated Management of Childhood Illnesses. In this algorithm a child with diarrhea is immediately assessed for signs of dehydration with management focused on correcting the fluid deficit. Additionally, the syndrome is classified based on illness duration and presence of gross blood in stools in order to initiate nutritional therapy or antibiotics effective against regional *Shigella* isolates, respectively. Given the high incidence of diarrhea in children and age-related risk of dehydration, it is not surprising that practice guidelines for acute diarrhea in children were formulated prior to those in adults. The American Academy of Pediatrics released a "practice parameter" focusing on methods of rehydration, refeeding after rehydration, and the use of antidiarrheal agents [78]. The focus of this review was on the clinical syndrome of gastroenteritis. The etiologic agents most commonly anticipated would be viral pathogens therefore antibiotics were not recommended. Antidiarrheal agents, other than antibiotics, were also not recommended.

Evaluations of health care provider management strategies for acute diarrhea in adults have demonstrated considerable variability emphasizing the potential need for practice guidance [80]. In an attitude survey of practice patterns in 542 British and Scottish healthcare providers, between 24-30% of providers would take no action for 24

hours and 12-18% would wait 48 hours before proceeding with any attempts at therapy [81]. The higher percentages of inaction were reported when the diarrhea was not associated with recent travel. Antidiarrheal medication or antibiotic use was reported in 16-23% and 2-5%, respectively. The higher percentage reported for both types of medicines were in persons with recent travel. The greatest benefits in reduction of short-term morbidity for antidiarrheal therapy (particularly antibiotics) occur when instituted early in the disease course.

The American College of Gastroenterology (ACG) provided guidance in 1997 for acute diarrhea management in adults [73]. The guidelines were derived from an extensive literature review performed by the ACG Practice Parameters Guidelines Committee led by Dr. DuPont, a noted expert in the field of infectious diarrhea. The recommendations were divided into the areas of patient evaluation, laboratory tests, and management. Patient evaluation should be focused on the subset with more severe illness defined as profuse watery diarrhea with dehydration, dysentery, fever, high-output diarrhea (> 6 loose stools/24 hr period), duration > 48 h, associated severe abdominal pain in patient > 50 yr-olds, elderly ($\geq$ 70 yrs of age), or immunocompromised patient. The patient evaluation should also pay particular attention to clinical and epidemiologic clues that may assist in defining the appropriate differential diagnosis. Empiric management using antimicrobials was divided into two potential scenarios where it would be considered appropriate. The first scenario is in a patient determined to likely have a bacterial diarrhea based on clinical features and/or laboratory evidence of intestinal (probably colonic) inflammation. The recommended treatment regimen was a fluoroquinolone antibiotic for 3-5 days. The second scenario is in a patient with persistent

(> 14 d) diarrhea with suspect *Giardia* infection. Laboratory evaluation listed microscopy for fecal leukocyte, fecal lactoferrin assay, and stool hemoccult all as useful screening tests to employ in patients with moderate to severe acute diarrhea. Stool cultures were recommended for patients with fevers ($\geq$ 38.5^{o}C), dysentery, stools positive for any of the inflammatory markers (fecal leukocytes, lactoferrin, hemoccult), or persistent diarrhea. Patients with persistent diarrhea not treated empirically for *Giardia* should also have laboratory tests to assess for parasitic etiologies. Epidemiologic aspects of the patient's history also should prompt specific requests for added lab tests such as rule out for *Vibrio cholerae* in someone presenting with profuse watery diarrhea during or after visit to cholera endemic region. Standard management for all patients includes fluid therapy and dietary alteration (as emphasized in the pediatric guidelines). Nonspecific therapy is also important for symptomatic relief with loperamide recommended as the drug of choice for diarrhea complaints and bismuth subsalicylate when vomiting is the predominant symptom. Specific to traveler's diarrhea, the guideline recommended against chemoprophylaxis; however, the guideline encouraged provision of loperamide and a fluoroquinolone antibiotic for self-therapy. The self-treatment approach is as follows: if vomiting is the major manifestation use bismuth subsalicylate and no antibiotics whereas if diarrhea is the major manifestation base therapy on clinical severity [mild – no treatment or loperamide alone; moderate-severe – based on presence of fever or dysentery (absent – fluoroquinolone plus loperamide; present – fluoroquinolone alone)].

The Infectious Diseases Society of America provided a guideline in 2001, which integrated clinical management issues with public health considerations [74]. These

recommendations follow more structured evidence-based criteria than the ACG guidelines based on the quality of evidence [I - $\geq$ 1 properly conducted RCT, II - $\geq$ 1 nonrandomized trial, cohort, case-control, or dramatic results in uncontrolled experiments, III – expert opinions] and the degree of certainty [A – evidence supports recommendation for use, B – moderate evidence to support use, C – poor evidence to support for or against use, D – moderate evidence against use, and E – good evidence against use] for the given recommendation. These guidelines emphasize the importance of organism-specific diagnosis from both a clinical and epidemiologic perspective given the increasing rates of antimicrobial resistance, misuse of empiric antibiotics, potential for harm with antibiotic therapy [i.e. *C. difficile* antibiotic-associated colitis, prolongation of *Salmonella* carriage, potential increase in HUS with antibiotic therapy for Shiga toxin-producing *E. coli* (STEC) infections]. A lack of suspicion or specific diagnosis can result in higher rates of secondary transmission, failure to initiate control measures, limit value of surveillance efforts (such as outbreak detection), and fail to best direct therapy for the individual patient. The summary of the group's recommendations with the evidence-based rank are shown below:

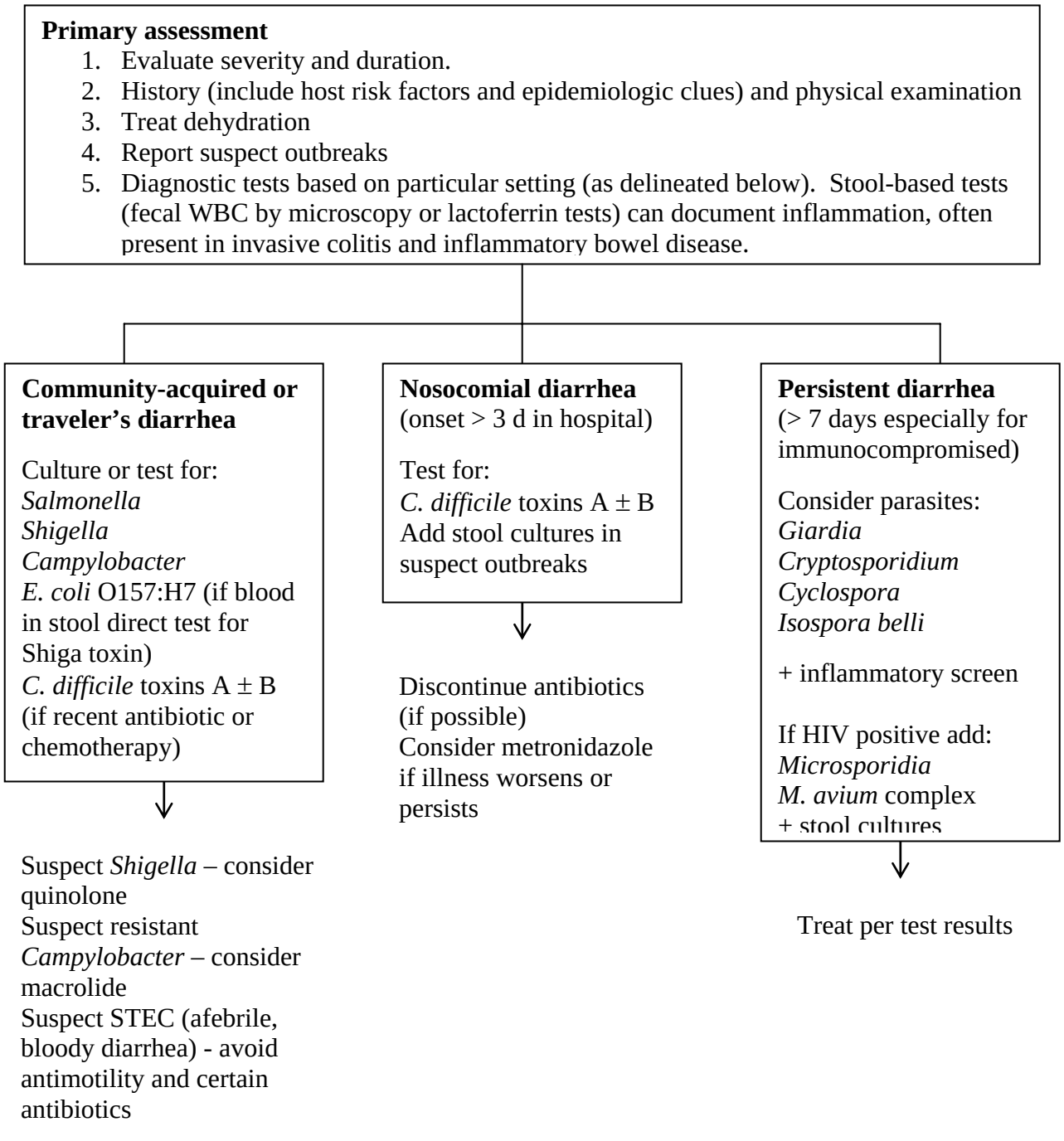
Table 3. IDSA summary recommendations for managing infectious diarrhea

Recommendation	**Score**
Initiate rehydration (oral whenever possible)	A-I
Perform a thorough clinical and epidemiological history for any patient with significant diarrheal illness [significance may be based on disease features (such as dysentery, profuse watery dehydrating diarrhea), host factors (such as infants, elderly or immunocompromised patients), or epidemiologic setting (such as outbreaks, high-risk secondary transmission).	A-II
Perform selective fecal studies (refer to management approach flow diagram)	B-II
Institute selective therapy for	
Traveler's diarrhea	A-I
Shigellosis	A-I
Campylobacter infection	B-II
Avoid antimotility agents with bloody diarrhea or suspect Shiga toxin-producing *E. coli* infections	E-I
Selective administration of typhoid vaccines for traveler (or residents) exposed in endemic settings	B-II

Adapted from [74].

A diagram outlining the recommended approach to specific laboratory testing is also included. The decision to proceed with particular laboratory tests is dependent upon the clinical categorization of the infectious diarrheal syndrome using both clinical and epidemiological criteria.

Figure 1. IDSA Recommendations for the diagnosis of infectious diarrhea.



Adapted from [74].

The IDSA guidelines, as previously emphasized by the AGA, highlight the importance of clinical evaluation with particular attention to disease severity, etiologic clues, and early treatment of dehydration followed by categorizing the syndrome. In 2002, an international working group formulated a practice guideline taking into account specific endemic concerns in their algorithm while attempting to preserve simplicity and feasibility [75]. Unique differences of this algorithm from the earlier ones discussed is the particular reference to cholera and amebiasis. Patients found to have clinical evidence of dehydration in a cholera-endemic area are evaluated with microscopy for characteristic vibriod bacteria and dysentery cases are evaluated for *Entamoeba histolytica* trophozoites. Antibiotics are recommended for use under selective circumstances including watery diarrhea with dehydration, dysentery, and unresolved diarrhea with therapy based on antibacterial sensitivity of isolated pathogen.

Diagnosis

Overview

The ability to implicate an enteropathogen in a clinical case has greatly increased since the 1970s due to the discovery of *Rotavirus* and *Campylobacter* spp., availability of diagnostic tests for ETEC (research purposes only) and several newly identified or newly implicated pathogens such as Caliciviruses, *Cryptosporidium*, *Cyclospora* and various enteropathogenic *E. coli*. Obtaining a diagnosis may primarily support clinical care or may be applied toward epidemiological surveillance. Typically, the clinical presentation is not sufficiently characteristic to allow prediction of the specific pathogen. The potential benefit of using the clinical presentation to predict the pathogen has been

investigated [40, 82]. One study assessed the clinical predictors of *Shigella* infection in a group of Bolivian children presenting with bloody diarrhea [83]. Having at least two of the clinical findings (crying with defecation, fever, or observed bloody stools) had a sensitivity, specificity and positive predictive value (PPV) of 84%, 54% and 43%, respectively. The addition of basic screening lab (positive fecal leukocytes) was able to improve the specificity and PPV to 84% and 64%, respectively, without losing much sensitivity (71%). This assessment of the value of clinical predictors is in a very select patient group, developing world children presenting with bloody diarrhea, and is not readily extrapolated to a military population. Another study in Finnish travelers to Morocco demonstrated a more severe clinical illness with *C. jejuni* as compared to ETEC; however, most of the differences were on the follow-up visits (2^{nd} and 3^{rd} days) [82]. Differentiation of diagnoses can also go beyond discriminating enteropathogens, as seen during Operation Restore Hope in Somalia [30]. Shigellosis cases were observed to present with an acute febrile illness often indistinguishable from malaria or dengue fever. An assessment of the total white blood cell count provided some assistance since shigellosis cases usually exceeded 8,500 cells/ml unlike the other diagnoses.

Approach

The options available in the laboratory include nonspecific screening tests focusing on the differentiation of inflammatory from non-inflammatory diarrhea, specific pathogen identification with stool microbiology ($\pm$ antimicrobial susceptibility testing), and a few commercially available (and many as yet unproven investigational) “rapid” pathogen-specific assays [84, 85]. When the health care provider first evaluates a patient with acute diarrhea there are several reasonable approaches to management. The

diagnosis may be solely based on clinical grounds without laboratory analysis. Alternative (other than standard stool microbiology) diagnostic tests may be used to complement clinical findings without further confirmation, as a decision point for the need for stool microbiology, or simply an additional piece of data with microbiology. Rapid diagnostic assays can be non-specific or pathogen-specific. Several important questions should be considered when deciding to proceed with laboratory analysis including:

1) Does the patient meet some "established" criteria for proceeding with lab diagnostic work-up?
2) What result from a diagnostic evaluation is important to affect therapeutic decisions and subsequent clinical outcomes? In other words, which diagnosis is sufficient - inflammatory diarrhea or campylobacterioses or quinolone-resistant *C. jejuni*?
3) From a logistics standpoint, will the turn-around time in receiving the result be adequate to impact therapeutic decision-making (i.e. bedside diagnostic vs. satellite lab vs. mailout)?

Previously published recommendations for proceeding to stool microbiology in acute diarrhea include cases with severe diarrhea (> 6 loose stools/24 hr period and/or disabling associated symptoms), febrile and/or dysenteric disease, persistent diarrhea ($\geq$ 14 days), bloody diarrhea, and fecal leukocyte-positive diarrhea [86]. The fecal screening tests, identification of fecal leukocytes, fecal occult blood or fecal lactoferrin, are used to try and differentiate between inflammatory and noninflammatory diarrhea [85, 87-89]. The reason to use these tests is to provide the clinician with evidence of an

inflammatory diarrhea case (more likely due to *Shigella*, *Campylobacter* or non-typhoidal *Salmonella* and less likely due to ETEC or viral etiologies). The clinician would then target further diagnostics (such as proceeding with stool microbiology) and/or certain therapy (such as limiting antibiotic use to inflammatory cases) based on these results. Patients with inflammatory diarrhea (caused by such pathogens as *Shigella* spp. and *Campylobacter* spp.) do not benefit as greatly from rehydration therapy as non-inflammatory watery diarrhea and often require antibiotic therapy for clinical resolution [44]. This dichotomy is problematic due to the overlap in clinical presentation among bacterial enteropathogens. A recent analysis of fecal screening tests demonstrated improved accuracy of fecal lactoferrin latex agglutination (marker for fecal leukocytes), as compared to the standard hemoccult (Guaiac test for microscopic blood) and fecal leukocyte staining (methylene blue) [85]. Assessment of the diagnostic accuracy (ability to discriminate the inflammatory diarrhea pathogens) of these various tests demonstrated the best results with the fecal lactoferrin assay; however, this assay has been evaluated in relatively small studies.

A more recent meta-analysis evaluated the diagnostic accuracy of stool assays for inflammatory bacterial gastroenteritis [90]. This analysis considered important differences in diagnostic test performance based on level of disease prevalence at each study site. The surrogate measure for hyperendemic bacterial enteropathogen levels used was developed versus resource-poor country. The primary outcome measure was the summary receiver operating curve (SROC) yielding an area under the curve (AUC). An AUC/SROC value of 1.0 defines a “perfect” test and 0.5 is a “useless” test. The AUC/SROC for the commonly used inflammatory enteritis screening tests was as follows

for developed versus resource-poor countries: fecal leukocytes (0.89 vs. 0.72), stool hemoccult (0.81 vs. 0.63), and fecal lactoferrin (0.79 in resource-poor with no comparison due to single study only in developed countries). In addition to the AUC/SROC values, summary likelihood ratios (LR), positive and negative, were calculated from pooled sensitivities and specificities. In general, a minimum threshold for test usefulness is a positive LR of 2.0 (to rule in a diagnosis) and a negative LR of 0.5 (to rule out a diagnosis). The summary below provides a comparison and relative utility, based on prevalence region, of the inflammatory enteritis screening tests.

Developed countries

Rule in (LR+): FWBC (4.6) = FLFLA (4.3) > Heme (3.4)

Rule out (LR-): FLFLA (0.1) >> Heme (0.3) > FWBC (0.4)

Resource-poor countries

Rule in (LR+): FWBC (1.6) > Heme (2.9) > FLFLA (1.3)

Rule out (LR-): FLFLA (0.2) >>> FWBC (0.6) > Heme (0.8)

Abbreviations: Microscopy for fecal leukocytes (FWBC), fecal lactoferrin latex agglutination test (FLFA), and stool hemoccult (Heme)

The importance of assessing any diagnostic test in relation to the target pathogen prevalence is emphasized by the above analysis. Clinicians utilizing these screening tests in deployed military would need to use knowledge of regional threats, prior surveillance in similar populations and clinical predictors. Laboratory techniques available for each pathogen focus on isolation, identification or characterization. Rapid diagnostic tests

range from the inexpensive Gram stain for presumptive identification of *Campylobacter* spp. (sensitivity, 60-90%) to the more technically complex and more expensive polymerase chain reaction (PCR) [91-94]. At present, none of the rapid diagnostic assays have achieved clinical utility due to a variety of concerns such as questionable impact on clinical outcomes, lack of commercial availability, cost, and variable test performance.

Management

Overview

The management approach in acute bacterial diarrhea involves a primary clinical assessment, consideration and potential application of laboratory analysis, and a plan for assessing therapeutic response. Table 4 lists several considerations at each stage of the evaluation and potential actions that may be required. Immediate assessment of fluid status with timely rehydration therapy is the cornerstone of diarrheal management. The decision to treat with medications, non-specific anti-diarrheal and/or an antimicrobial agent is based on illness severity assessment, results of screening or pathogen-specific lab tests, and pre-treatment anticipated benefit. Empiric antibiotic therapy is the usual approach given the typical lack of a definitive etiologic agent at the time of primary assessment. Diarrhea management algorithms have been proposed to assist the clinician in targeting antibiotic therapy toward patients that would most benefit and limiting antibiotic-related risks and costs in patients with a probable brief self-limited mild illness [13, 39]. Clinicians responsible for caring for patients with diarrhea should become familiar with the potential pathogen exposures in their target patient population, natural history of these enteropathogens with or without antibiotic therapy, availability and test performance characteristics of relevant lab tests, and antimicrobial resistance patterns of

the primary enteropathogen threats. Special circumstances such as outbreaks and refugee medicine require an even greater structured approach to surveillance, triage, and attention to comorbid illnesses and resource allocation.

Table 4. Management approach to acute bacterial diarrhea

Decision points	Consideration	Potential actions
Primary assessment	- Clinical features - Inflammatory vs. non-inflammatory - Regional threats/Antimicrobial resistance - Availability of bedside/rapid turn-around diagnostic assays - Relative costs of diagnostic and therapeutic strategies	- Rehydration - Empiric therapy ± antibiotics - Obtain screening lab - Directed use of pathogen-specific assays - Determine follow-up requirement
Assessment of therapeutic response	- Knowledge of expected response time - Availability of pathogen-specific diagnostics	- If lack of clinical response, broaden diagnostic work-up and/or modify therapy
Special circumstances (i.e. mass treatment scenario, refugee camp)	- Surveillance for outbreaks w/i population (need diagnostics) - Co-morbid illnesses (malnutrition, malaria, bacteremia)	- Triage patients (prioritize patients with special diagnostic (i.e. blood cultures, malaria preps) or therapeutic (nutritional, vitamin supplementation) needs using variable levels of care - Utilization of care givers and allied personal for treatment

Table 5 provides an overview of therapeutic options that can be integrated into a management algorithm as discussed above. Rehydration, oral and/or intravenous, is used in all cases to varying extent based on the primary clinical fluid status assessment. The non-specific anti-diarrheal medications fall into one of the three classes (adsorbents, anti-secretory or anti-motility) based on mechanism of action [95]. These agents are often all that is necessary for mild to moderate acute diarrhea. Antibiotic therapy is efficacious in moderate to severe acute bacterial diarrhea, shigellosis, and early treatment of campylobacterioses (< 72 hours) [95, 96]. Antibiotics have also been demonstrated to reduce symptom duration in travelers' diarrhea from an average of 50-93 to 16-30 hours [13, 39, 40, 95].

Table 5. Therapeutic options in the management of acute bacterial diarrhea

OPTION (REF)	Indication	Comments
Rehydration Therapy [95, 97]	All cases to varying extent Use clinical assessment of volume status (vital signs, level of consciousness, urine output, skin turgor) and ability to take oral (no vomiting)	**Oral:** Use irrespective of etiology. Based on co-transport of water/sodium with glucose (or other molecule/polymer). Replace what is lost. World health organization (WHO) formulation (higher osmolarity) favored in developing regions with lower sodium content used in industrialized nations. Alternatives such as rice-based ORS may further speed recovery. **Intravenous:** Must first provide replacement therapy over a brief period (acceptable solutions - LR/NS) before proceeding with maintenance. Begin ORS as soon as feasible.
Non-specific Therapy [98-103]	Mild to moderate diarrhea Generally avoid with severe cases (febrile dysentery /bloody diarrhea)	**Adsorbents:** inert, non-absorbed that adsorbs 8x its weight in water; Attapulgitte - 1.2 g (2 tbsp) initially and repeat every 2 h up to maximum of 14 tbsp; Mean time to last unformed stool - 19.5 h **Anti-secretory:** Bismuth subsalicylate and zaldaride (calmodulin inhibitor) **Anti-motility:** recommended agent - loperamide mechanism of action: increase segmental intestinal contractions slowing fluid column allowing increased absorption; anti-secretory effect (inhibit calmodulin) Reduce diarrhea by 80%
Antimicrobial Therapy [12, 68, 96, 104, 105]	Moderate to severe Efficacy demonstrated for early Tx of TD (< 72 h), *Shigella* and *C. jejuni*	Empiric therapy standard; drug of choice - fluoroquinolone Single dose therapy often efficacious (reasonable strategy of assessing therapeutic response at 12-24 h post-dose to assist in determining need for repeated doses); 3 versus 5 day therapy equivalent Combination of antibiotic plus loperamide shown beneficial in ETEC-endemic areas

Important considerations in the selection of an empiric antibiotic include probable target pathogens, antimicrobial resistance regional patterns, safety and tolerance profile of the antibiotic, effectiveness of the dosing regimen (patient compliance), and cost. Antibiotic treatment trials in military and civilian travelers to developing regions fulfilling criteria for travelers' diarrhea have provided much of the current knowledge required to design empiric therapeutic regimens. An important observation when considering empiric TD therapy is the self-limited placebo cure rates by 72 hours of

approximately 50-60% [106]. Early trials were frequently performed using a placebo group in order to assure the active drug led to therapeutic benefit. This finding has now been repeatedly demonstrated that antimicrobial therapy provides approximately 34-63h reduction in duration of illness and placebo controlled trials are no longer justified. Not unlike many other infectious disease syndromes, etiologic agents of TD have demonstrated an ability to acquire resistance to antibiotics commonly used in empiric therapy. An extensive series of clinical trial investigations have been undertaken by DuPont, Ericsson and colleagues in students traveling to Mexico [101, 104, 107-120] and Department of Defense investigators among military personnel on deployment to either Thailand or Egypt [32, 34, 121]. These trials have provided evidence of the diminished efficacy of empiric regimens, such as ampicillin and TMP/SMX, as the bacteria develop resistance [109]. Initial studies investigating empiric regimens evaluated longer treatment durations of five days to initially establish efficacy [107]. Early trials also demonstrated the efficacy of nonabsorbable antibiotics and have been more recently revived with the antibiotic rifaximin [108, 114, 119, 122]. Multiple daily doses or longer courses of therapy add to a regimen's inconvenience and increase likelihood of noncompliance. Given these concerns, studies have been undertaken to establish efficacy with shorter less cumbersome regimens [104, 113, 116, 118, 123]. Shorter duration of antibiotic therapy has been demonstrated to be equally effective for 3 vs. 5-day regimens, as well as, good results with single dose therapy [104, 113, 116, 118, 123]. Progressive antimicrobial resistance led to investigations to establish alternative regimens. Fluoroquinolone antibiotics were initially evaluated in the late 1980s and have now demonstrated efficacy across regimen ranges (5-, 3-, and 1-day) [111, 112, 116, 118,

124]. Due to increasing fluoroquinolone resistance among *Campylobacter* species, the macrolide antibiotic, azithromycin, has also been evaluated and demonstrated equal efficacy to ciprofloxacin [34, 120].

Important findings derive from the extensive series of prior traveler's diarrhea treatment trials including: 1) self-limited placebo cure rates by 72 hours are typically as high as 50-60%, 2) regional antimicrobial resistance can decrease cure rate (as evidenced by ETEC and *Shigella* ampicillin resistance leading to 49% cure rate in Mexico), 3) shorter duration of antibiotic therapy often equally effective (as evidenced by 3 vs. 5-day regimens, as well as, good results with single dose therapy), and 4) fluoroquinolone antibiotics currently are the first-line agents. Important caveats when considering short course or even single dose fluoroquinolone therapy for travelers' diarrhea include the frequent finding of quinolone-resistant *C. jejuni* and the greatly reduced cure rates in *S. dysenteriae* using short course therapy [125]. The search for alternative regimens remains important, as it was when fluoroquinolones replaced TMP-SMX, ampicillin and tetracycline.

Thailand-specific diarrhea epidemiology

Background

Thailand is a constitutional monarchy located on the Indochina peninsula in South East Asia. Thailand's topography is divided into three regions: plains (mostly central including the Chao Phraya River which transects Bangkok), highlands (mostly northeast including the Khorat Plateau), and mountainous regions (mostly in the north and southeast). Three types of climate occur in Thailand: tropical rain (year-round heavy

rainfalls in eastern coast areas in the South), tropical monsoon (southwestern and southeastern areas), and seasonal tropical grassland [Central, North, and Northeast regions with periods of heavy rainfall during southwest monsoon (mid-May-Oct) and dry winters]. The population of Thailand is approximately 64 million with a population growth rate of 0.9% (source: Thailand: The World Fact Book, CIA). The majority of the population is Thai (75%) with the other largest group being Chinese (14%). Buddhism is practiced by 95% of the populace. Literacy rates are high at 96%. Thailand's economy was the fastest growing in the world in the early 1990s then declined in mid-90s and is now in recovery. A 1998 estimate of 12.5% of the population lives below the poverty line with an unemployment rate of 2.9% (2002 estimate). The infant mortality rate is 22 per 1000 live births with an average life expectancy of 71 years. The first four leading causes of death of all ages were: (1) infectious diseases (HIV infection the major contributor in working age adults); (2) cardiovascular and cerebrovascular disease; (3) cancer; (4) respiratory disease, mainly obstructive pulmonary disease and asthma. Rates of malnutrition in preschool children have steadily decreased over the past 10-15 years currently with 91% within normal weight-height parameters. The following map of Thailand is provided as a reference when considering cities and regions discussed throughout the thesis.

Source: Perry-Casteñada Library Collection produced by the U.S. Central Intelligence Agency; Courtesy of the University of Texas Libraries, The University of Texas at Austin

http://www.lib.utexas.edu/maps/cia04/thailand_sm04.gif

Civilian populations

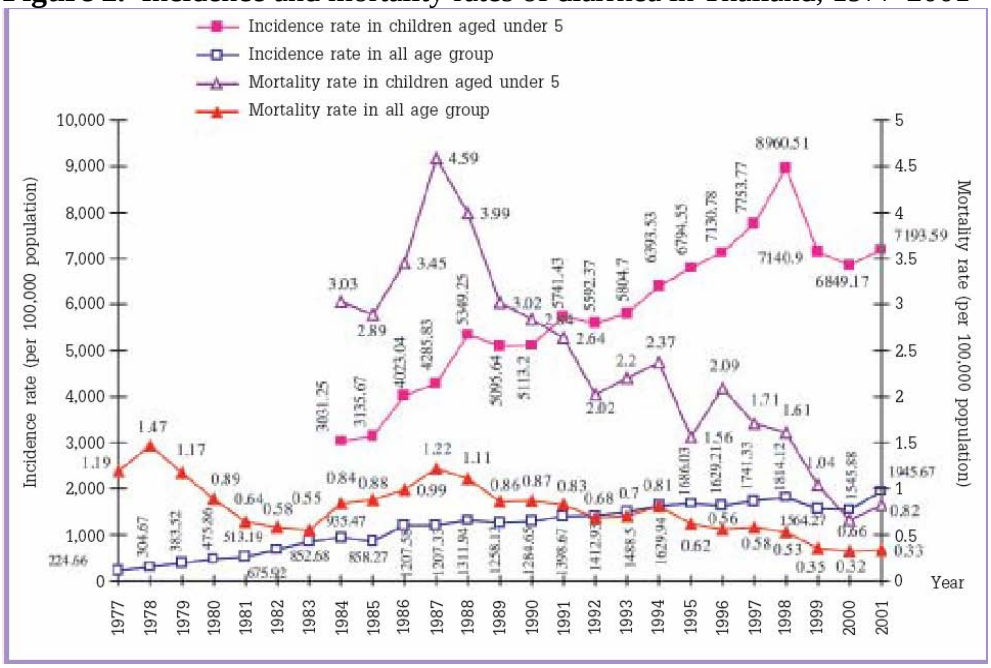
Thai population

The primary aim of this proposal is the development of diagnostic and therapeutic approaches for the management of acute diarrhea in deployed U.S. personnel in Thailand with potential for expanded applicability beyond this geographic region. The regional pathogen distribution in diarrheal diseases occurring in the indigenous population is an important consideration for threat assessment. The use of case series data from developing world children in a particular region is commonly used as a component of overall diarrhea threat assessment for the military. This is justifiable based on this age group representing the most non-immune or semi-immune among the indigenous population. The illness-to-infection ratio in young children for specific bacterial enteropathogens is high enough to allow a reasonable understanding of pathogen distribution and potential exposure frequency.

Diarrheal disease is a major health problem in Thailand with most deleterious impact upon children under 5 years of age [126, 127]. Based on Thai Ministry of Public Health estimates between 1978-1983, average diarrhea incidence was 694 per 100,000 population [126]. These numbers are based on an aggregate sum of cholera, enteric fever, food poisoning, dysentery, and acute diarrhea cases. Acute diarrhea cases account for approximately 80% of the total and peak in the under 5 years of age group (1,609 per 100,000). Peak diarrhea incidence occurs in January and between May-July. Thailand's seasons can be divided into a hot, rainy, and cool periods due to the influence of seasonal monsoons [128]. Hot season, mid-February until mid-May, has high temperatures, low rainfall, and low humidity. The rainy season begins in mid-May through mid-October

and is followed by a cooler dry period. The wintertime peak is primarily related to viral diarrhea (especially Rotavirus); whereas, bacterial etiologies are more common in hot and wet months [128]. A community-based 1-year cohort in a low-income urban community in Bangkok revealed an annual incidence of 2.3 episodes per infant and 0.9 episodes per child (under 5 years) [127]. Recent trends from the Thai Ministry of Public Health (Thai Health Profile 1999-2000, Southeast Asia Regional Office (SEARO), WHO; http://w3.whosea.org/eip/thaiSlides.htm) document the continued decline of diarrhea-specific mortality but persistently high incidence in children less than 5 years of age.

Figure 2. Incidence and mortality rates of diarrhea in Thailand, 1977-2001



Source: Division of Epidemiology, Thailand Ministry of Public Health (MOPH)
http://www.moph.go.th/ops/thealth_44/

Enteropathogen distribution in this community cohort demonstrated the most common identified agents as follows: Rotavirus (9%), *Salmonella* (9%), *Campylobacter* (8.7%), ETEC (7.2%), and *Shigella* (4.9%). Etiologic agent distribution varied by age range with the more common pathogens as follows: infants (Rotavirus and *Salmonella*), 1-2 years of age (ETEC, *Campylobacter*, and *Shigella*), and 2-5 years of age (ETEC and

Shigella). Echeverria and colleagues at the Armed Forces Research Institute of Medical Sciences in Bangkok have intensively investigated enteropathogen distribution within Thailand. Table 6 provides the results of pathogen distribution among hospitalized Thai patients obtained through Medline review of all English language publications which included at minimum reports of bacterial culture results [33, 129-138]. Rotavirus, when included in surveys of diarrheal disease not restricted to dysentery, is the most common etiology in hospitalized children accounting for approximately 27-34% [139]. In contrast, *Shigella* accounted for the majority (45-50%) of dysentery cases [132, 135, 140]. A more recent hospital-based survey demonstrates a decline in shigellosis as the etiology of dysentery in Bangkok with *C. jejuni* most common at 28% [138].

Multiple pathogens were identified in 11-41% of the cases across series. Pathogens of uncertain clinical significance, such as *Plesiomonas* were isolated as frequently as 30-47% [130]. Additionally, probable coincidental colonization of known pathogens in clinical settings, such as 27% of adult dysentery case with Rotavirus [140], further complicates discrimination. It is important to evaluate pathogen distribution in the context of case-control studies. Table 7 summarizes studies that include asymptomatic controls as part of the evaluation [129, 131, 133, 134]. Consistent findings supporting clinical significance were observed for Rotavirus, non-LT ETEC, *Shigella*, and *Cryptosporidium*. Variable findings supporting disease association were seen with *Campylobacter*, *Salmonella*, *Aeromonas*, and *Vibrio parahaemolyticus*. No consistent association with illness was observed with *Plesiomonas*, LT-ETEC, and *Giardia*. Enteropathogenic *E. coli* expressing the EPEC adherence factor was also found to be associated with disease among Thai children less than 6 months of age [134, 141].

Enteroinvasive *E. coli* have also been isolated in 5% of cases of childhood dysentery in Bangkok [132]. This summary does not account for the effect of age which likely impacts greatly on disease association due to acquired immunity leading to asymptomatic infection or possibly sterile immunity. *C. jejuni* infection in Thailand provides a good example for age effect and acquired immunity. A hospital-based study of acute diarrhea in children in 1985 documented 18% of the 586 cases had *C. jejuni* or *C. coli* infection [142]. The serotype distribution was similar to series in industrialized countries. Peak age of *Campylobacter* infection was less than 2 years of age. Duration of excretion varied based on child's age with a mean of 14 ± 2 versus 8 ± 2 days for children less than 1 compared to 1-5 years of age, respectively. The hyperendemic nature of *Campylobacter* in Thailand was well documented in this study with a 34% reinfection (with a different strain) rate in the 12-week monitoring period. Symptomatic illness was limited to children under 2 years of age. Cross-sectional studies of the *C. jejuni*-specific serum antibody responses have demonstrated intense and continued exposure early in childhood with progressive rise in IgA levels, peak IgG during second year of life, and continued increase in IgM until teen years [143]. The *Campylobacter*-specific immune responses are inversely related to fecal excretion in Thai children demonstrating evidence of acquired immunity [144].

Table 6. Distribution of pathogens isolated from hospitalized Thai patients presenting with diarrhea

					Pathogen isolation (%)										
					Bacterial										
Ref	Site	Study period	Age	N	ETEC	Campy	Shig	Salm	Aero	Ples	Vpara	Vchol	NonO1	Rota	No pathogen identified
All diarrhea cases															
[129]	BCH	May-July 1979	Children	105	15	2	4	6	7	3	0	< 1	0	22	39
[130]	NBH	Apr-Jun 1980 Oct-Sep 1981	> 15 y/o	660	5	1	27	3	47	30	19	4	3	ND	42[a]
[131]	SH	1982-83	All	299	17	ND	9	ND	9	0	5	0	2	ND	59
[133]	BCH	Jan-Jun 1985	Children	410	9	5	23	10	4	6	5[b]	NR	NR	10	38
[134]	BCH	1985-86	< 5 y/o	1230	9	13	13	12	2	3	< 1	0	< 1	20	37
[136]	SPH	Mar-Nov 1991	All	363	7	5	16	8	2	7	4	< 1	1	19	NR
[137]	NBH	May-Dec 1996	Non-HIV HIV+	350 350	2 < 1	0 0	2 2	4 6	8 5	12 5	23 1	7 0	2 < 1	ND	57 84
Restricted to dysentery cases (mucoid heme positive or bloody stools)															
[132]	NBH	Jan-May 1984	1-10 y/o	200	16	12	44[c]	10	16	22	2	< 1	2	ND	16
[135]	BCH	Jan-Jun 1989 1989-90	3-14 y/o	306	6	3	49	7	< 1	5	4	< 1	1	ND	12
[140]	NBH	1990-92[d]	Adults	88	2	2	50	7	5	18	16	0	3	27	7
[138]	NBH QS	1998-00	< 12 y/o	623	6	28	9	18	0	1	< 1[b]	NR	NR	ND	45

Note: Not done as part of surveillance (ND). Not reported (NR) refers to surveillance efforts where the result is potentially available but not reported. Bacterial etiologies included in table are as follows: enterotoxigenic *E. coli* (ETEC), *Campylobacter jejuni/coli* (Campy), *Shigella* species (Shig), nontyphoidal *Salmonella* species (Salm), *Aeromonas* (Aero), *Plesiomonas* (Ples), *V. parahaemolyticus* (Vpara). Sites include: Bangkok Children's Hospital (BCH), Nonthaburi Bamrasnaradura Hospital near Bangkok (NBH), Soongnern Hospital (Northeast Thailand), Suan Phung Hospital (Western Thailand near Burmese border), and Queen Sirikit National Institute of Child Health, Bangkok (QS). Date of study recorded as year (if year-long) or by months for a given year.

[a] Excludes *Aeromonas* and *Plesiomonas* due to uncertain enteropathogenic potential. [b] *Vibrio* species reported without further differentiation.

[c] *Shigella* was the only bacteria isolated as a solitary pathogen in $\geq$ 10% of cases. This study also documented a 5% prevalence of enteroinvasive *E. coli* (EIEC). [d] Pathogen distribution reported from randomized controlled trial (pretreatment cultures).

Table 7. Distribution of pathogens isolated from Thai patients with acute diarrhea versus asymptomatic controls

Ref	Source		Pathogen (% case:% controls)											
	Case	Control	Rota	LT-ETEC	ST-ETEC	Campy	Shig	Salm	Aero	Ples	Vpara	Giardia	Crypto	No pathogen
[129]	Hospital	Clinic (age-match)	22:1[a]	5:3	11:3	2:0	4:0	6:0[a]	7:9	3:1	0:0	ND	ND	31:13[a]
[131]	Hospital	Community	ND	NR	17:4[a, b]	ND	9: < 1[a]	ND	9:2[a]	NR	5: < 1[a]	ND	ND	41: < 5[a]
[133]	Hospital	Clinic (age-match)	10: < 1[a]	3:3	6: < 1[a]	5:3	23:3[a]	10:10	4:4	6:5	NR	4:2	3: < 1[a]	73:38[a]
[134]	Hospital	Clinic (age-match)	20: < 1[a]	4:3	6:2[a]	13:11[a]	13: < 1[a]	12:9[a]	2:2	3:2	< 1: <1	2:1	2: < 1[a]	37:69[a]

Note: Not done as part of surveillance (ND). Not reported (NR) refers to surveillance efforts where the result is potentially available but not reported. Bacterial etiologies included in table are as follows: enterotoxigenic *E. coli* (LT-ETEC refers to LT+ strains only and ST-ETEC refers to either ST only or LTST+ strains), *Campylobacter jejuni/coli* (Campy), *Shigella* species (Shig), nontyphoidal *Salmonella* species (Salm), *Aeromonas* (Aero), *Plesiomonas* (Ples), *V. parahaemolyticus* (Vpara).

[a] $P < 0.05$

[b] Refers to all ETEC isolates.

Civilian expatriates and travelers

Various studies have evaluated diarrhea risk and pathogen distribution among non-military foreign visitors to Thailand including U.S. Peace Corps volunteers (PCV), expatriates, and tourists. A series of studies in PCV were undertaken in 1979, 1980, and 1983 [145-147]. In 1979, 35 PCV were followed for 5 weeks in rural Thailand [145]. These individuals experienced a 57% diarrhea attack rate with bacterial etiologies being identified in 47%. *Aeromonas* was most commonly isolated in 31%; however, it was the solitary pathogen in only 2 individuals. Other pathogens identified include ETEC (26%), *Shigella* (13%) and *Campylobacter* (3%). A doxycycline chemoprophylaxis trial for traveler's diarrhea was undertaken in PCV in 1980. The investigators had difficulty demonstrating prophylactic efficacy due to the unexpectedly low rates of ETEC. A 24% diarrhea attack rate was observed in the placebo recipients during the 3-week monitoring period. Another observational study in PCV in 1983 again documented high cumulative 6-week attack rates of 57% with low rates of *Campylobacter*, ETEC (17%), *Salmonella* (33%), *Plesiomonas* (13%), *Aeromonas* (10%), and no *Shigella* [147]. Acute diarrhea affecting U.S. expatriates residing in Bangkok was evaluated between 1989-1994 to determine etiologic agent [148]. A total of 105 cases with a mean age of 34 and median duration of residence of 14 months were enrolled. A relatively high "no pathogen isolated" rate of 66% was observed. The most common etiologies included ETEC (17%), *Campylobacter* (10%), *Shigella* (8%), *Salmonella* (8%), and *Vibrio parahaemolyticus* (3%).

Thailand is a popular tourist destination providing many opportunities to investigate diarrhea in travelers. One of the first such studies during 1978-79 surveyed tourists staying at a Bangkok hotel [149]. A total of 146 guests presented with diarrhea (no denominator was provided so no rate was determined). *Vibrio parahaemolyticus* was the most common etiology occurring in 31% of cases with highest rates during June-July and associated with eating seafood. There was no work-up for ETEC or *Campylobacter* in this series and no etiology was determined in 66%. Diarrhea attack rates have been determined in short-term Finnish (25%) and Dutch (41%) travelers to Thailand [150, 151]. Japanese travelers returning from Southeast Asia destinations with diarrhea were found to have highest rates of ETEC (31%) followed by *V. parahaemolyticus* (16%), *Salmonella* (12%), *Campylobacter* (3%), and *Shigella* (2%) [147]. In a series of Austrian tourists (N = 322) returning with diarrhea, *Campylobacter* species were the most common single etiology at 14%; however, the cases series tended toward longer duration diarrhea (mean of 11 days) than often observed with a much greater percentage being due to parasitic etiologies (34%) [152]. Acquisition of fluoroquinolone resistant *C. jejuni/coli* while in Thailand with importation to country of residence has also been observed as a public health concern. In Finland, *Campylobacter* strains acquired abroad comprise approximately 25% of isolates with 49% ciprofloxacin resistant as compared to 9% resistance among domestic strains [153, 154]. Travel to Thailand has specifically been stated as an increased risk of acquiring a fluoroquinolone resistant *Campylobacter* infection with estimates for Finnish travelers of 0.44 infections per 1000 trips [153]. Despite the majority of domestic *Campylobacter* isolates being susceptible to fluoroquinolones in Finland, this antibiotic class has limited utility to treat this infection

due to 80% of isolates being acquired abroad in more recent surveys [154, 155]. In addition to antibiotic resistance concerns with *Campylobacter*, decreasing fluoroquinolone susceptibility (though not resistant) in nontyphoidal *Salmonella* isolates has been recently observed in travelers returning from Southeast Asia with diarrhea [156]. Thailand-specific *Salmonella* isolates have demonstrated an increase from 5.6% in 1995 to 50% in 1999 for a decreased ciprofloxacin susceptibility profile. This worrisome trend does not, as yet, equate to therapeutic failures with the fluoroquinolones for *Salmonella* infections; however, single point mutations with the chromosomal *gyr*a gene has already occurred with resistance to the first generation quinolone, nalidixic acid, setting the stage for another point mutation causing fluoroquinolone resistance as in *Campylobacter* [157].

U.S. military populations

Thailand has been a strategically important region for U.S. military forces since World War II. No detailed accounts of diarrhea disease incidence and pathogen distribution are available prior to serial surveillance efforts during the annual Cobra Gold training exercises. Cobra Gold is the most recent evolution of earlier U.S.-Thai joint military exercises beginning with the battalion-sized joint amphibious training code named “PHIBRAEX” which began in 1956. In 1982, “PHIBRAEX” was combined with “SEA SIAM”, “UNDERSEAL”, and “MINEX/EODEX” and transformed into Cobra Gold 1982. The Cobra Gold exercise has been occurring each year since 1982 during the period of April through early June with the most activity in May. Each year the Army component rotates between the four Thai Army regions. The naval and amphibious component occurs yearly in the Sattahip region along the Gulf of Thailand south of

Bangkok. An earlier investigation predating the Cobra Gold exercise in September 1962 provided some information on diarrhea incidence among the U.S. Army personnel deployed to Khorat, Thailand [158]. A very low rate of diarrhea (< 5%) was observed during a period of 3 months surveillance of approximately 1500 soldiers. The period of surveillance was outside of the observed seasonal peaks for diarrhea disease in the Thai population. In addition, the report describes more restrictive command policies on allowable Thai eating establishments in the vicinity of the base. Surveillance during training deployments (July-August) in 1987 and 1988 evaluated the effect doxycycline malaria chemoprophylaxis has on diarrhea incidence with specific concerns of increasing *Campylobacter* isolation rates [159, 160]. An observational study in 1987 documented low rates of diarrhea, 2.4%, based on clinic-based reporting [159]. However, 17% of soldiers reported diarrhea based on post-deployment survey. In this series of 28 diarrhea cases, 50% were attributable to tetracycline-resistant *C. jejuni*. These findings prompted a double blind randomized controlled trial to assess doxycycline malaria chemoprophylaxis effect on diarrheal incidence and pathogen distribution [160]. Active surveillance (N = 253) documented diarrhea in 48% of participants during the 5-week monitoring period. There was no difference in the occurrence of diarrhea or pathogen isolation rates in soldiers receiving doxycycline or mefloquine for malaria chemoprophylaxis. Interestingly, *Campylobacter* isolation rates were quite low (2-3%) compared to the 40-60% rates observed during similar exercises throughout the 1990s in the same region (Khorat). ETEC isolation rates were lower in the doxycycline group (3%) compared to mefloquine (8%). Tetracycline resistance was more common for *Campylobacter* (90%) than ETEC (21-24%) isolates.

During Cobra Gold 1990, an overall 30% diarrheal incidence in surveyed troops was observed with 25% of affected individuals seeking care [25]. This significant diarrheal attack rate resulted in a weekly incidence of 1.5% (peak 2.5% 3rd wk), 13% of all clinic visits, and 12% of all hospitalizations/sick-in quarters (SIQ). *Campylobacter* species (*C. jejuni* and *C. coli*) were the most common etiologic agents (41%) with 100% susceptibility to the fluoroquinolone antibiotic, ciprofloxacin [32]. Two earlier post-deployment surveys following the 1986 and 1987 exercises in single battalions documented diarrhea rates of 20 and 25%, respectively [25]. Also during Cobra Gold 1990, DoD investigators evaluated the efficacy of single dose ciprofloxacin (750 mg) therapy with or without loperamide compared to the standard of ciprofloxacin (500 mg twice daily for 3 days) with loperamide [32]. In this trial, comparable 24 h cure rates of 36-38% were observed in all groups without the previously observed early additive benefit of loperamide when used in combination with antibiotics [104, 121]. This study was the first to document the pathogen distribution pattern now repeatedly observed in subsequent years. *Campylobacter* was the predominant isolate accounting for 41% of the enrolled cases. Nontyphoidal Salmonella species were the second most common etiology (18%) with 50% having dual infections with *Campylobacter*. ETEC (5%) and Shigella (4%) were less common. Pretreatment *C. jejuni/coli* revealed no resistance to ciprofloxacin; however, in the 2 clinical relapse cases posttreatment cultures were positive for ciprofloxacin-resistant *C. jejuni* with the same serotype as pretreatment.

In Cobra Gold 1993, Army and Navy researchers observed the regional emergence of ciprofloxacin-resistant *C. jejuni* in ~ 50% of initial isolates [34]. In Cobra Gold 1994 and 1995, increasing rates of ciprofloxacin-resistant *C. jejuni* (65-85%), as

well as, azithromycin resistance (7-15%) were observed [33]. A total of 171 diarrheal cases in Cobra Gold 1995 were evaluated and cared for at medical treatment facilities by the research team. In this series, *C. jejuni* was again the most common pathogen (33%); however, other pathogens included non-typhoidal *Salmonella* spp. (18%), enterotoxigenic *E. coli* (11%), *Plesiomonas shigelloides* (11%), and *Shigella* spp. (8%). In Cobra Gold 1998 and 1999, observational clinic-based studies were undertaken to provide ongoing diarrheal threat assessment data and further investigate the effect that the emergence of quinolone-resistant bacterial enteropathogens, predominately *Campylobacter* spp., has on the empiric use of quinolone antibiotics for first-line travelers' diarrhea management [161]. As observed in past exercises, *Campylobacter* spp. remained the predominant cause of diarrhea in personnel reporting for medical care; however, a spectrum of other bacterial enteropathogens was observed in as many as 25-40% of the cases. The research teams in 1998 and 1999 provided clinical assessment and care for 171 and 110 personnel with acute diarrhea, respectively. Ciprofloxacin resistance was observed in > 90% of *Campylobacter* isolates and none of the non-*Campylobacter* isolates. Sub-optimal treatment response, defined as a lack of complete resolution by 72 h, was observed in approximately 10-20% of the *Campylobacter*-associated cases receiving ciprofloxacin. These results highlight the importance of investigating alternative therapies for the empiric management of travelers' diarrhea, particularly in Southeast Asia.

Table 8. Traveler's diarrhea rates and pathogen distribution in U.S. military during short-term deployment in Thailand

Ref	Year (months)	Region (Thai city)	Diarrhea rate (%)	No. cases surveyed in clinic	Clinic-based pathogen isolation (%)							
					Campy	Salm	ETEC	Shig	Other Bacteria	Viral	Parasitic	No pathogen identified
[158]	1962 (Sep-Nov)	Khorat	3.2	48	ND	2.1	ND	4.2	19	ND	ND	75
[159]	1987 (Jul-Aug)	Khorat Lopburi	17	28	50	NR	NR	NR	NR	NR	NR	NR
[160]	1988 (Jul-Aug)	Khorat	49 (doxy) 48 (meflo)	77	27	6.5	38	12	< 1	10	2.6	49
[25, 32]	1990 (Apr-Jun)	Chonburi Utapao	25	137	41	18	6	4	2	1	2	42
[22]	1993 (Feb)	Ubonratch-atthani	14 (USA) 36 (USAF)	24	25	8	0	0	13	4	0	50
[34]	1993 (May)	Phitsanulok Utapao	ND	72	58	17	4.2	1.4	13	13	ND	19
[33]	1994 (Feb)	Hat Yai	ND	48	60	13	2.1	0	0	ND	0	25
[33]	1994 (May)	Cholburi	ND	56	50	20	8.9	1.8	3.6	ND	0	16
[162]	1995 (May)	Khorat Sattahip	35	95 75	43 20	24 10	7.4 15	2.1 13	15 19	3.3 15	ND	34
[163]	1996 (Mar)	Utapao	40	16	19	5.3	0	0	10	0	ND	62
[161]	1998 (Apr-Jun)	Utapao Kanchana-buri	ND	169	14	18	14	< 1	28	4.1	ND	17

Note: Not done as part of surveillance (ND). Not reported in publication (NR). Diarrhea rate is based on % of personnel reporting illness during post-deployment survey with the exception of the 1962 cohort study [158] and a diarrhea chemoprophylaxis study using doxycycline or mefloquine [160]. Bacterial etiologies included in table are as follows: *Campylobacter jejuni/coli* (Campy), nontyphoidal *Salmonella* species (Salm), enterotoxigenic *E. coli* (ETEC), *Shigella* species (Shig), and 'Other Bacteria' including *A. hydrophila*, *P. shigelloides*, *V. parahaemolyticus*, and non-O1 *V. cholerae*. Viral etiologies investigated include Rotavirus and Noroviruses.

Table 8 provides the published data on diarrhea rates and enteropathogen distribution available for U.S. military short-term deployment to Thailand. Postdeployment surveys have documented diarrhea occurrence in 17-40%. Active surveillance during the doxycycline chemoprophylaxis study demonstrated higher rates of approximately 50% of soldiers during the 3-week monitoring period. *Campylobacter* isolation rates from clinic-based surveys have been higher than observed in other traveler's diarrhea series. There is evidence of regional variation, although still at relatively high rates, between certain areas in Thailand. The Utapao/Sattahip Naval Base region approximately 2 hours south of Bangkok near Phattaya (Gulf of Thailand) has rates of approximately 15-20% whereas the Khorat region, on the central Isaan plateau, has had rates of 27-50%. Nontyphoidal *Salmonella* infections frequently account for the second most common isolate behind *Campylobacter* occasionally as high as 24%. ETEC rates have been lower in U.S. military personnel than documented in Japanese tourists or among Thai children [134, 147]. The relative contribution of ETEC to diarrhea cases, for most years, has been in the 4-15% range. The rates of *Shigella* infection have declined since 1990 with single or no isolates observed during several years. A similar decline in cases of *Shigella* among children presenting with dysentery in Bangkok was also observed during the late 1990s to the present [138]. Periodic regional surveillance for diarrheal rates and pathogen distribution is important to monitor for emergent threats and changing pathogen trends. Changing trends in antimicrobial resistance create added challenges for patient management. Increasing antimicrobial resistance is a common feature of all the major bacterial enteropathogens. As previously discussed, fluoroquinolone-resistant *Campylobacter* have been reported in numerous locations [33,

164-168]. Thailand, specifically, has observed major shifts toward quinolone-resistant *C. jejuni/coli* have increased from 0% to >85% as well as decreased fluoroquinolone susceptibility [32-34]. Macrolide-resistant (erythromycin and azithromycin) organisms also were observed in 9/54 (31%) cases during Cobra Gold 1996 [33, 169]. Azithromycin resistance has also been observed in a few ETEC (15%) and nontyphoidal *Salmonella* (3%) in Thailand [167]. Ongoing efforts to assist clinicians in diagnostic and therapeutic management are needed and are the focus of this proposal.

Research proposal

Proposal objectives

- Evaluate relative differences in clinical presentation and outcome of acute diarrhea based on stool microbiology findings in order to assist health care providers at initial clinical presentation and assess treatment approaches [Clinic-based surveillance]
- Determine therapeutic efficacy of azithromycin, single-dose or 3-day, versus a standard 3-day fluoroquinolone (levofloxacin) as empiric therapy for travelers' diarrhea. [Randomized active drug-controlled double-blinded study]
- Determine effectiveness of bedside and field laboratory-based rapid diagnostic assays in the management of acute infectious diarrhea.

Summary proposal design

The project has three components: 1) retrospective analysis of clinical and microbiological data (Cobra Gold exercises in 1995, 1998, and 1999), 2) a randomized clinical trial (Cobra Gold exercises 2000 and 2001), and 3) performance evaluations of

diarrhea diagnostic tests (Cobra Gold exercises 2000 and 2001). Each study component originates from a clinic-based surveillance system for acute diarrhea. This passive clinic-based system is a specific Joint Task Force (JTF) tasking for the Armed Forces Research Institute of Medical Sciences (AFRIMS)/Naval Medical Research Center (NMRC) and does not constitute research. Personnel presenting with acute diarrhea at survey clinics receive appropriate clinical evaluation and care. A major resource provided as part of the research study, in addition to infectious disease clinical expertise, is the field microbiology laboratory. The presence of the field laboratory allows the inclusion of diagnostic stool microbiology during routine clinical care.

This study population for the prospective component of the thesis, treatment trial and diagnostic test assessment, consists of U.S. military personnel deployed to Thailand during annual Cobra Gold exercises during May 2000 (Nakhon Sri Thammarat) and 2001 (Phitsanulok). Volunteers must be at least 18 years old. There are no gender or race/ethnicity restrictions. Women who are known to be pregnant or found to be pregnant on pre-treatment urine pregnancy testing will be excluded from the treatment study (but are eligible for the case-control study) due to the contraindication for fluoroquinolone use in pregnancy. Patients presenting with acute diarrhea may participate in the case-control study (CG 2000 only), as well as, the randomized controlled trial (if meeting eligibility criteria). Asymptomatic personnel, often from within the same units as cases, may participate as control volunteers. Cases and controls complete a questionnaire, provide stool specimens for microbiology evaluation, and undergo phlebotomy (40 ml) for pathogen-specific immunology. Control volunteers will be evaluated at one time point. Cases volunteering for the treatment study will be

evaluated in follow-up based on the trial procedures. Incentive payments will be provided to volunteers for each blood draw ($25 per bleed).

Retrospective analysis of clinical features and outcome by bacterial etiology

Rationale

Acute diarrhea clinic-based surveillance in U.S. military personnel deployed to Thailand provides important information on etiologies, clinical presentation, and treatment of travelers' diarrhea in Southeast Asia for military and civilian populations. Increasing prevalence of fluoroquinolone (FQ)-resistant *Campylobacter* species during the decade of the 1990's has raised concerns regarding appropriate management [167]. Progressive FQ resistance among *C. jejuni* has been observed from none pretreatment in 1990, 50% in 1993, and 85% in 1998 [32, 34, 161]. In contrast, increasing macrolide resistance has not been observed with the exception of a 31% azithromycin resistance among 20 isolates from one Thai region in the Malay peninsula in 1994 [33] and 7% in 1995 [167]. Coincident with rising FQ resistance sub-optimal treatment response was observed in 1998 in approximately 10-20% of the *Campylobacter*-associated cases receiving ciprofloxacin although the number of cases was small. This study will evaluate relative differences in clinical presentation and outcome of acute diarrhea based on stool microbiology findings using available data from three exercise years (1995, 1998, and 1999) in order to assist health care providers at initial clinical presentation and assess treatment approaches.

Approach

Standardized methods of obtaining clinical and microbiological data from patients presenting for care due to acute diarrhea during Cobra Gold exercises has been undertaken over the past several years through collaborative efforts of The Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand and the Naval Medical Research Center (NMRC) in Silver Spring, MD. The common methodology provides an opportunity to investigate important questions with larger sample size for study populations utilizing merged data across exercise years. Clinic-based surveillance admittedly restricts the assessment to the subset of patients with "clinically-relevant" illness. This subset is certainly not complete since some patients will self-medicate or have variable levels of symptom tolerance. However, a clinic-based system will capture important information on the patients with more severe illness and will provide data important for medical planners in regards to resource requirements. In addition these data provides important information on regional pathogen distribution and antimicrobial susceptibility patterns.

Clinical definitions

The following definitions are used throughout the thesis project (and are standard definitions during each exercise year referred to).

Diarrhea = $\geq$ 3 loose or liquid stools in 24 hour period OR $\geq$ 2 loose or liquid stools in 24 hr period plus $\geq$ 1 associated symptoms

Fever = oral temperature $\geq$ 100.0^{o}F (also collected information on reported but undocumented fever)

Diarrhea-associated signs/symptoms = abdominal pain or cramps, nausea, vomiting, fever, tenesmus, and gross blood in stools temporally related to the diarrheal episode

Functional status (in regard to ability to work or recreate): categorized as normal, decreased, or unable.

Stool characterization based on the following grading scheme [also each initial diarrhea specimen assessed for hemoccult reaction (positive or negative) and presence/absence of gross blood]

Grade 1 - hard (normal)

Grade 2 - soft (normal)

Grade 3 - thick liquid

Grade 4 - opaque watery liquid

Grade 5 - clear watery

Clinical evaluation

Patients were evaluated and cared for as per standard clinical practice during the 1995, 1998, and 1999 exercises. Initial evaluation was documented on a standardized clinic visit form ["Cobra Gold Initial Clinic Visit (Diarrhea Surveillance)"]. These forms are included as attachments with the clinical protocol in the Appendix. The standardized format includes character and onset of diarrheal illness, symptom survey (selected symptoms consistently recorded), effect on functional capacity, prior use of self-medication, physical examination, stool characterization, and eligibility criteria for enrollment as volunteer.

Microbiology assessment

Patients were requested to submit a stool sample/rectal swab pretreatment. The study physician will send the specimen to the field laboratory after stool characterization and hemoccult (limited to 1999 exercise). In the field laboratory, routine microbiology and rapid assays (non-specific and pathogen-specific) will be completed (as per attached SOP in Appendix). Stool specimens are initially cultured at the field location with final identification of all isolates at the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok [22, 34, 167]. Stool microbiology primary plating is onto MacConkey, Hektoen Enteric (HEA), thiosulfate citrate bile salts sucrose (TCBS), sorbitol MacConkey, and Brucella with 5% sheep blood (BA) agars for overnight incubation. Samples are also inoculated into Selenite F broth, alkaline peptone water, and Doyle's enrichment broth. Following overnight incubation, suspicious colonies are subcultured, refrigerated at 4^{o}C, and then transported to the AFRIMS laboratory in Bangkok for definitive identification. *Campylobacter* isolation is undertaken using a membrane filter method on non-selective BA before and after enrichment [170].

Enteric pathogens are identified using standard morphologic and biochemical profiles, followed by appropriate specific antisera. Samples of *E. coli* will be obtained for further analysis. Five colonies will be examined with specific DNA probes for genes encoding heat-labile toxin (LT), heat-stable toxin (ST), EPEC adherence factor, *E. coli* attachment-effacement using the intimin gene (*eae*), and Shiga toxins (Stx1 and Stx2) [22, 141, 171]. Stool specimens are examined for the presence of Rotavirus by a commercially available ELISA (Rotazyme; Abbott Laboratories, North Chicago, IL) and

Caliciviruses by a non-commercially available ELISA [172]. Wet prep examinations of fresh stool specimens are used to assess for parasitology.

Data management and analysis

Surveillance efforts during exercise years 1995 [162] and 1998 [161] involved cross-sectional enrollment of patients presenting for care at designated clinics fulfilling the diarrhea definition (as stated in preceding section). In 1999 a similarly designed clinic-based study investigated host immune responses was undertaken with the additional requirement that enrolled individuals must provide a pretreatment stool specimen for culture. Inclusion criteria for this analysis include the following: acute diarrhea of $\leq$ 120 hours, onset of illness $\geq$ 24 hours after arrival in Thailand, illness conforming to the diarrhea definition, and a pretreatment stool culture. Rationale for each restriction follows. The illness duration at presentation was restricted to $\leq$ 120 hours (5 days) since study focus is on potential predictive clinical symptomology and clinical outcome comparison based on pathogen isolation in a self-limited disease known to have placebo cure rates at 72 hours of approximately 50-60% [106]. In order to avoid being overly restrictive and assess the range of presenting features a period $\leq$ 120 hours was selected. The study is targeting regional pathogen distribution in Thailand, so it was necessary to restrict time of illness onset to occur after arrival in country with a 24-hour interval to account for usual lower range of incubation periods for common bacterial etiologies. The diarrhea definition was not modified from the prospective field surveillance and was consistently applied. The study aims to evaluate clinical presentation and outcome by bacterial pathogen recovered from stool microbiology; therefore, a pretreatment stool culture was required.

Clinical and microbiological data obtained during the Cobra Gold exercise years of 1995, 1998, and 1999 will be merged for analysis. Abstracted data from patient surveys, symptom diaries, clinical records, and microbiology results will be entered into an EpiInfo version 6.04 database. Statistical analysis will be performed using SPSS for Windows (version 10.1). Differences in clinical findings at presentation and illness outcome by *Campylobacter* isolation rates will be evaluated using χ^2 testing for categorical variables or nonparametric tests to compare continuous variables. Differences in recovery times were evaluated using Kaplan-Meier analyses (time to last diarrheal stool after first antibiotic dose), log-rank (overall differences in response curves), and generalized Wilcoxon tests (response curve differences emphasizing early failures) [106]. All tests were 2-tailed and $\alpha = .05$ will be used as the level of significance.

Logistic regression modeling will be used to determine important predictors of *Campylobacter*-associated illness using independent variables available to the health-care provider at the time of initial clinical presentation. The dependent variable for modeling will be the pre-treatment stool culture isolation of *C. jejuni* or *C. coli*. Variable selection for inclusion in model building will be based on exploratory analysis, two-way contingency table analysis, and Mantel Haenszel chi-square stratified analysis. Selected variables for logistic regression analysis must have a p value < .25 in bivariate analysis for subsequent inclusion. A forced entry method of regression analysis will be used to evaluate all covariates using likelihood ratio testing. The odds ratio for each predictor variable will be calculated as the exponent of the regression coefficient with 95% confidence intervals. Assessment for interaction and confounding variables will be

undertaken using likelihood ratio testing. Homogeneity of odds ratios across strata and potential multicollinearity will be assessed. The overall goodness of fit for the model will be determined using the Hosmer and Lemeshow method. Each individual component of the model will be numerically and graphically evaluated, using residual diagnostics and assessments of influence and/or leverage, before acceptance of the model.

Limitations

The surveillance site(s) selected were based on the logistical capabilities of the research team and the clinic locations expected to receive the majority of patient visits for acute diarrhea. A complete coverage of all medical treatment sites (i.e. every battalion aid station) was not feasible; therefore, the numerator of cases will be incomplete. Since this is a clinic-based observational study the clinical presentation and subsequent outcome ascertainment will be biased toward personnel with a propensity to seek care and likely with more severe disease.

Diarrhea diagnostic evaluation

Rationale

An additional objective, other than formulating the best approach to empiric therapy, relates to optimizing diagnostic test strategies for acute diarrhea management. This project will evaluate both bedside (stool characterization and hemoccult) and field laboratory rapid diagnostic assay (fecal leukocyte smear, lactoferrin latex agglutination, *Campylobacter*-specific rapid assay, and plasma C-reactive protein) effectiveness as components in the overall management strategy. Study physicians will perform bedside diagnostics whereas study team laboratory personnel will perform the field lab rapid

assays. Field applicability of diagnostic tests is particularly relevant for the military. During military operations, the availability of a field laboratory with microbiologic capability is quite variable. Rapid, technically simple diagnostic tests need to be evaluated to determine accuracy and acceptability in field settings. Empiric therapy without supplemental laboratory is a feasible option; however, refinement of management strategy using laboratory testing may increase cost-effectiveness and allow specific adjustments in antibiotic selection based on regional susceptibility patterns.

In addition to the stool-based screening test, this study will evaluate a plasma-based test of inflammatory disease, C-reactive protein (an acute phase protein produced by the liver during infectious and non-infectious inflammatory disease). The *Campylobacter*-specific test under evaluation is the commercially available, visually read, solid phase immunoassay for the detection of *Campylobacter*-specific antigens (ProSpecT® *Campylobacter* Microplate Assay, Alexon-Trend, Inc., Ramsey, MN). This assay has been assessed previously in low *Campylobacter* prevalence regions in hospital-based settings in industrialized countries with reported sensitivity approximating 90 % and specificity of 100 % [173]. No studies have evaluated the test performance in a field setting or in high prevalence regions. All tests will be compared with the "gold standard" stool microbiology results.

Methods

Clinical and laboratory evaluations

As previously discussed, there will be a standardized approach to both clinical and laboratory measurements. The stool specimen will be evaluated and graded by the

research physician during the initial evaluation (refer to study definitions for grading scheme). The research physician will also perform a hemoccult test on the stool specimen (refer to Appendix for test procedure). The specimen will then be sent to a field microbiology lab. The blood samples will be forwarded to the field laboratory for processing.

Reference standard microbiology

The specimen will be processed in a field microbiology lab where it will be examined for fecal leukocytes, fecal lactoferrin latex agglutination (LFLA), processed for culture, and undergoes rapid *Campylobacter* EIA testing (as discussed in the "Surveillance" section). Refer to the Appendix for diagnostic test procedures and interpretations. Primary culture work-up will be performed in the field (as per the attached AFRIMS SOP), and then the samples will be forwarded to the AFRIMS in Bangkok, Thailand for final identification and determination of antibiotic susceptibilities. Stool specimens will be cultured for bacterial diarrheal pathogens and presumptive identification provided in the field laboratory (refer to "Cobra Gold Field Laboratory Data Abstraction Form"). Further evaluation will be completed at the AFRIMS in Bangkok. This includes final species identification, serotyping and susceptibility testing of all isolates. All isolates will be archived and transported to NMRC. Laboratory specimens will be also evaluated for viral or parasitic etiologies of acute diarrhea.

Analysis

The physician-performed bedside diagnostic assays (stool characterization and hemoccult) and laboratory technician-performed rapid diagnostic assays (fecal leukocyte

smear, lactoferrin latex agglutination, *Campylobacter*-specific EIA, and plasma C-reactive protein) will be compared with the gold standard stool microbiology results. Test performance characteristics will be assessed for each assay. Test performance characteristics (sensitivity, specificity, predictive values, and likelihood ratios) will be evaluated for each clinical finding (such as fever, abdominal cramps, and severe diarrhea) and diagnostic assay with 95% confidence intervals. These results will be further assessed using receiver operating characteristic (ROC) analysis. This analysis plots true positive rates (based on the reference standard of stool microbiology) against the false positive rate for the different possible cutpoints of a diagnostic test. The ROC curve demonstrates the tradeoff between sensitivity and specificity (any increase in sensitivity will be accompanied by a decrease in specificity) [174, 175]. The measure that will be used to compare the diagnostic tests is the area under the ROC curve (AUC). A curve most closely following the y-axis (true positive rate) and then across the top border or x-axis (false positive rate or 1- specificity) represents an optimal test. This optimal curve would have an AUC approximating 1. The slope of the tangent line at a cutpoint gives the likelihood ratio (LR) for that value of the test. An adjustment for AUC is necessary since these tests are being compared using the same cases [176]. The adjustment corrects for the correlation between the areas created by paired data. The likelihood ratio will be evaluated using pre- and post-test probabilities for different scenarios (low versus high prevalence region). In addition, clinical findings and diagnostic assays will be evaluated singly and in series using likelihood ratios in order to determine the most accurate and efficient diagnostic algorithm.

Limitations

The *Campylobacter* regional predominance previously documented in Thailand will limit to some extent the application of the derived diagnostic algorithm across operational platforms in various regions. These results coupled with analyses from an area of ETEC predominance (with some contribution from *Shigella* species) will better permit generalization. In addition, there is a limited attempt in this study to broadly survey for viral and parasitic etiologies of acute diarrhea. Past surveys during Cobra Gold deployments have not documented these agents as significant therefore due to logistical and resource issues there is a limited effort placed on their detection.

Randomized controlled trial

Rationale

Cobra Gold diarrhea surveillance since 1990, by the Armed Forces Research Institute of Medical Sciences (AFRIMS, Bangkok) in collaboration with NMRC and Army/Navy Preventive Medicine commands, has shown diarrheal illness to be the primary health threat to deployed troops with *Campylobacter* spp. as the predominant cause. Increasing rates of antimicrobial resistance among *Campylobacter* isolates and observational studies demonstrating sub-optimal therapeutic responses (defined as failure to resolve within 72 hours of initiation of treatment) in 10-25% of cases highlight the need for evaluating alternative treatment regimens.

Antibiotic selection

Previous clinical experience

The recommended standard empiric antibiotic therapy for travelers' diarrhea is a 3-day course with a fluoroquinolone [39, 95]. The activity of the fluoroquinolone, ofloxacin, against common enteric pathogens is well established, and is commonly used for traveler's diarrhea [105, 177]. Levofloxacin is the optical *S*- (-) isomer of ofloxacin [178]. Ofloxacin is a racemic mixture, but the *S*-isomer has antibacterial activity 32- to 128- fold more potent than the *R*-isomer. Therefore, most of the antibacterial activity of ofloxacin is due to the *S*-isomer, and levofloxacin has been developed to take advantage of this antibacterial potency allowing much smaller doses with an improved toxicity profile [179]. *In vitro* studies suggest that levofloxacin is 2-8 fold more active than ofloxacin against the most common enteric pathogens, equally efficacious as ciprofloxacin against the most common enteric pathogens, and 2-fold more potent than ciprofloxacin against *Campylobacter jejuni* [180]. A Japanese study using levofloxacin 200-300 mg/day for 5-7 days in 114 patients with bacterial enteritis showed clinical cure rates of 97% in 72 hours [179]. Based on this data, recent reviews of the prevention and treatment of traveler's diarrhea include levofloxacin with ofloxacin and ciprofloxacin as a first line treatment option [105, 177].

Alternative approaches to empiric travelers' diarrhea therapy have primarily evaluated single-dose regimens and non-fluoroquinolone antibiotic agents. Single-dose fluoroquinolone therapy has demonstrated equal effectiveness to 3- or 5-day regimens for travelers' diarrhea, as well as, specific therapy for shigellosis (not *S. dysenteriae*) [32, 116, 123, 125, 181, 182]. Non-fluoroquinolone-based empiric therapy has been studied

using a relatively new macrolide antibiotic, azithromycin, with greater *in vitro* activity against many gram-negative bacteria than erythromycin. As previously stated, azithromycin 500 mg daily was compared with ciprofloxacin 500 mg daily (each 3-day regimens) for diarrhea in U.S. service personnel during Cobra Gold 1993 and was found to have comparable efficacy [34]. This study was limited by the small sample size with minimal ability to detect moderate effect differences of the azithromycin regimen (statistical power < 25%). In fact, there were only 2 clinical failures in the entire study group, both being ciprofloxacin-treated *Campylobacter* cases. Significant differences in improved microbiologic eradication of *Campylobacter* were demonstrated with azithromycin; however, this did not translate into statistically significant clinical differences. Importantly, the only statistically significant clinical findings on subgroup analysis were a reduced duration of illness in non-*Campylobacter* cases with ciprofloxacin. Given the observations, non-*Campylobacter* bacterial etiologies represent as many as 40% of cases and azithromycin was not clearly superior to ciprofloxacin (even in *Campylobacter* cases), empiric therapy with a fluoroquinolone remained the standard recommendation.

The drug of choice for treating a known *Campylobacter* infection remains a macrolide antibiotic (typically erythromycin) [47, 50]. The drug of choice for empiric treatment of traveler's diarrhea when the etiology is unknown has not been a macrolide but rather a fluoroquinolone. In order to significantly shorten illness duration it is important to treat most *Campylobacter* infections within the first 72 hours of symptoms [183, 184]. Pathogen identification, as in the other bacterial enteropathogens, rarely occurs near the time of presentation. Therefore, empiric therapy with a quinolone

antibiotic has become the primary management approach since it provides coverage for *Shigella*, non-typhoidal *Salmonella*, ETEC and is a good alternative for *Campylobacter* [13, 39, 86, 185].

More recently, it was noted that patients receiving either 1000 mg of azithromycin weekly or 250 mg of azithromycin daily for a malaria prophylaxis trial were protected during an outbreak of dysentery [186]. A trial was conducted comparing azithromycin (500 mg initially then 250 mg daily over 5 days - total 1.5 gm) with ciprofloxacin 500 mg twice daily for 3 days in patients treated with shigellosis, and found the regimens comparable [187]. A single 1 gm dose of azithromycin was also compared with a three-day course of ciprofloxacin in patients with shigellosis, and again the results were comparable [188]. Azithromycin has been proposed as an alternative therapy for patients unable to take quinolones or travelers to areas with known high *Campylobacter* endemicity [105, 177].

Safety profile

Levofloxacin is generally well tolerated, with most adverse effects being the mild and transient gastrointestinal or central nervous system side effects shared by all quinolones [189, 190]. In 5 comparative trials with ofloxacin involving 918 patients, a lower incidence of gastrointestinal symptoms (1.2 vs. 5.2%) and CNS symptoms (0.8 vs. 2.2%) was seen in the levofloxacin recipients. The incidence of abnormal laboratory findings (mild transient elevation of liver enzymes, eosinophilia, or leukopenia) was similar in levofloxacin (2.4-15.5%) as compared with ofloxacin (4.3-18.2%). In two of the largest non-comparative trials of levofloxacin involving 984 patients, the following side effects were noted: abdominal discomfort (1%), anorexia (0.4%), diarrhea (0.4%),

insomnia (0.5%), headache (0.3%), dizziness (0.2%), oral effects, such as mouth irritation, loss of taste, tongue numbness, or dry mouth, (0.5%), and rash (0.2%). As with the other quinolones, levofloxacin has been shown to cause articular damage in animal studies at high doses, and the phototoxic potential of levofloxacin in mice appears similar to that with ofloxacin and ciprofloxacin [179]. The subjects will be informed of the potential side effects of this medicine and specifically asked about the development of these symptoms during their clinical evaluations at 24 and 72 hours, and these results will be noted on a standardized questionnaire. If any of these symptoms, or other previously undescribed side effects, is deemed to be severe by the subject or the physician, the patient will be removed from the study, the code broken, and the patient treated with alternative therapy.

Azithromycin is generally well tolerated with minimal side effects consisting mainly of gastrointestinal complaints [191-196]. In a study of 3,995 patients receiving azithromycin, 5-day regimen (total 1.5 gm) or single dose (1 gm), were less likely to report side-effects, 12% vs. 14%, as compared to 3,108 patients receiving one of 12 other antibiotics (such as penicillin, amoxicillin, erythromycin, doxycycline, cephalexin, and cefaclor) [197]. The most common symptoms were diarrhea (3.6%), abdominal pain (2.5%), nausea (2.6%), vomiting (0.8%), and headaches and dizziness (1.3%), all of which occurred less frequently than with the comparison antibiotics. The only side effects occurring more commonly than the standard comparison antibiotics were vaginitis (0.4%) and rash (0.6%). The only laboratory abnormality noted was a mild, transient increase in the hepatic transaminases in 1.7% of patients. Only 0.7% of patients receiving the 5-day course discontinued the drug due to side effects. Single-dose azithromycin

(1250 mg weekly) for MAC prophylaxis in AIDS patients is discontinued in approximately 6% due to gastrointestinal (GI) side effects [198]. Further suggestion of azithromycin dose-related GI side effect relationship is the 34% GI complaint rate observed in a study assessing gonorrhea therapy using a particularly large single dose of 2 gm [193]. Table 9 summarizes the most commonly reported adverse symptoms (and frequency of occurrence) divided by this study's treatment regimens.

Table 9. Most commonly reported side effects for study medications

Reported symptom	Azithromycin (3-day)	Azithromycin (single dose)	Levofloxacin (3-day)
Nausea	3 %	5 %	3 %
Vomiting	< 1 %	2 %	< 1 %
Diarrhea	5 %	7 %	2 %
Abdominal pain	3 %	5 %	< 1 %
Rash	< 1 %	< 1 %	< 1 %
Dizziness	< 1 %	< 1 %	< 1 %
Headache	< 1 %	< 1 %	< 1 %
Vaginitis (yeast infection)	< 1 %	1 %	< 1 %

Note: Above rates derive from the following references[189-193, 196, 199].

There have been no significant drug-drug interactions reported with either levofloxacin or azithromycin. Co-administration with magnesium- or aluminum-containing anti-acids or ferrous sulfate reduces the bioavailability of levofloxacin by 15-52% (no effect on azithromycin). Therefore, patients will be instructed to separate the ingestion of any anti-acids by at least 1-hour prior and 2 hours after the ingestion of their assigned study medication. Women using oral contraceptives (OCP) will also be advised of the potential for decreased OCP efficacy, so they may consider alternative forms of birth control while receiving the study medication. While no interactions have been noted with theophylline, digoxin, or warfarin, cautious clinical practice dictates close monitoring of drug level or INR during co-administration. Given our inability to adequately monitor levels in the field, subjects who are currently taking any of these

three medicines will be excluded from the study. Furthermore, any subject reporting prior hypersensitivity to any of the macrolides or any of the fluoroquinolones or nalidixic acid will be excluded from the study.

Azithromycin is generally considered safe in children and during pregnancy. Due to concerns over the possibility of cartilage/articular damage with fluoroquinolones noted in animal studies, this class of antibiotics is currently not approved in children or in pregnancy. Therefore, pregnancy tests (urine hCG) will be performed on female subjects prior to enrolling them into the study. Any subject found to be pregnant or unwilling/unable to submit a urine specimen for a pregnancy test will be excluded from the clinical trial.

Sample size determination

The estimated sample size requirements for each treatment group are 60 patients. The primary clinical outcome used to estimate study size is the proportion of patients meeting the clinical cure definition (complete resolution of diarrhea-associated symptoms by 72 hours). Clinical cure rate comparisons can be made with both historical placebo cure rates (approximately 60%) and rate differences between study medications. The assumptions used for calculations are as follows:

Null hypothesis: No difference between historical placebo rate of 60% and observed clinical cure study medication rate (90%).

Assumptions: $\alpha = .05$; Power = 80%; effect size = .30

Number needed per group: 38

Null hypothesis: No difference between highest and lowest observed clinical cure study medication rates.

Assumptions: α = .05; Power = 80%; effect size = .20

Number needed per group: 59

Based on previous Cobra Gold research experience, an estimated number of enrollments during a single exercise are approximately 100. In order to reach a total enrollment of 180 volunteers (also accounting for dropouts) it will be necessary to extend the study over two exercise periods. If one of the treatment regimens were to demonstrate an intermediate clinical cure rate (an effect size of .10 as compared to the most efficacious treatment) then the study size available will not be able to discriminate if the difference is statistically significant. However, other outcomes, such as time to events, total numbers of loose stools, and microbiologic cures, may contribute supporting evidence of a meaningful treatment difference.

Trial design

This project aims to study three active drug treatment regimens [levofloxacin (500 mg once daily x 3 days), azithromycin (500 mg once daily x 3 days), and azithromycin (1000 mg as a single dose)] using a randomized, double blind study. Volunteer enrollment will occur at the field support hospital in Thung Song and the battalion aid station (BAS) in Nakhon Sri Thammarat during the period of the Cobra Gold 2000 exercise and at similar treatment facilities during Cobra Gold 2001 in Phitsanulok. The required number of patients volunteering to participate in the treatment study (approx. 60 per treatment regimen) necessitates enrollment during Cobra Gold 2000 and 2001.

Entry criteria

Any active duty member presenting to a survey clinic with acute diarrhea meeting all entry criteria is eligible for study enrollment. If the subject agrees to enter the study, the study physician will complete the informed consent process with the patient. Female patients will be asked to submit a urine sample for a pregnancy test at presentation. The study physician will use a standard urine hCG pregnancy test kit at time of presentation (refer to Appendix for hCG procedure). The urine hCG has a test sensitivity > 99% with a detection limit of 20 mIU/ml for urine specimens. Volunteers will be assigned the next sequential "Treatment Number". Study medication (labeled with the appropriate "Treatment Number") will be dispensed in a "combi bottle" (described further in "Study medications" section in the clinical protocol) to the volunteer by the study physician. The study physician will administer the 1st study medication dose and document time on the SF600 Cobra Gold Initial Clinic Visit form. The patient will be observed for a 30-minute period in order to monitor for immediate adverse reactions.

Inclusion Criteria

- Patient meets diarrhea definition with diarrheal symptoms of $\leq$ 96 hours duration
- Patient will be managed on an ambulatory basis and can comply with follow-up procedures

Exclusion Criteria

- Female patient with positive urine pregnancy test at presentation (urine hCG) [contraindicated with fluoroquinolone therapy]
- Patient with history of allergy to macrolide or quinolone antibiotics (does not include limited gastrointestinal upset)

- Patient receiving antibiotics (excluding malaria prophylaxis with either mefloquine or doxycycline) in the 72 hr prior to presentation
- Patient taking medications known to have drug-drug interaction with either study drug (includes theophylline, digoxin, and warfarin)
- Patient with history of seizures (relative contraindication for fluoroquinolone therapy)

Randomization and treatment assignment

Volunteers consenting to participate in the randomized clinical trial will be assigned the next available treatment code number. The treatment code assignment schedule will be created using block randomization (block size of 6). Allocation ratio of treatment assignments will be equal for the three study regimens (1: 1: 1). The study will use a double-blinding procedure during the clinical and laboratory phases of the study.

Study treatment

There will be two medications, azithromycin and levofloxacin, used during the clinical trial. Pfizer Pharmaceuticals Clinical Research Division in Groton, CT will supply both study medications and their respective placebo formulation. Pfizer pharmacy representatives will also supply the randomization schedule using a blocked randomization (block size = 6). The individually packaged "combi bottles" will have each bottle labeled with the study identification number and the appropriate medication day as per the randomization schedule. The "combi bottle" will be also identified using a two-panel label. Panel 1 is permanently affixed to the bottle and contains the randomization number. Panel 2 of the label will be removed from the container and

affixed to the dosing record section of the "Cobra Gold Study Medication/Specimen Log". A "blinded envelope" will be provided from Pfizer for each treatment assignment. In the event of a medical emergency (such as serious medication-related allergic and/or adverse reaction or disenrollment due to hospitalizing subject due to disease progression) it will be necessary to break the double-blind code for that individual. Detailed information concerning the dosage regimen and potential risks is provided in the "Risks/Benefits" and the "Medical care" sections of the protocol. The study medications will both have an identical appearing placebo form so as to appear indistinguishable. The azithromycin will be in the form of 500-mg tablets and will be dispensed as either 500 mg daily for 3 days or 1000 mg in a single dose. Levofloxacin will be in the form of 250-mg tablets and will be dispensed as 500 mg daily for 3 days. To keep the patients and researchers blinded, each patient will receive tablets from each study medication (active drug or placebo) as detailed in Table x. The medicines will be dispensed in a three-day "combi bottle" with a separate bottle for each treatment day of study. Each of the medicines is heat stable and can be maintained at room temperature during the study. Unused doses of the medication will be returned to the manufacturer at the completion of the study.

Subjects participating in the treatment trial portion of the study will be randomly assigned to one of three treatment regimens using one of the two antibiotics, levofloxacin and azithromycin. Any one of the regimens may prove to be more or equally effective at treating acute infectious diarrhea acquired in Thailand. The treating physician will manage diarrheal patients declining participation using standard of care practices. The potential benefit to the subject is a more rapid resolution of symptoms that may occur

with one of the study regimens as might be expected with standard therapy. The potential risks of the study involve either sub-optimal efficacy of the study drug or toxicity from the drug. The use of non-antibiotic antidiarrheal medications, such as loperamide, will not be allowed during the study given the significant confounding of all diarrhea-related clinical outcomes if used. Loperamide therapy when given as a single agent has demonstrated efficacy in the management of acute diarrhea [100]. The additive efficacy of loperamide to empiric antibiotic therapy has been variable in clinical trials. Prior studies performed in military personnel have not demonstrated a significant reduction in illness duration with the inclusion of loperamide in the antibiotic treatment regimen and comparable recovery rates were demonstrated with antibiotic therapy alone [32, 34, 121].

Clinical monitoring

Efficacy determination

The follow-up evaluations at 24 and 72 hours are designed to measure both disease progression/resolution and potential drug toxicity. The patient will also be given a symptom diary card during the initial evaluation on which they will be asked to record symptoms, including number of loose stools, nausea, vomiting, abdominal cramps, fever, and bloody stools over the following 72 hours. The patient is to return with the diary card at the 24 and 72-hour follow-up. A final follow-up (5-7 days after 1st antibiotic dose) will be completed on a standardized form to assure clinical response and obtain a stool specimen to assess microbiologic eradication. This follow-up visit may be done in the clinic or through contact with study team personnel. It is anticipated that the majority of eligible volunteers will volunteer for both the case-control and the treatment trial. A single consent form is to be used for both project components incorporating each

component's eligibility criteria and sections for volunteer to opt for one or both components.

Study Days

Day 0 = day of initial clinical presentation

Day 1 = 24 hr (allowable out to 36 hr) after 1st study medication dose

Day 3 = $\geq$ 72 hr (allowable out to 120 hr) after 1st study medication dose

Day 5-7 = $\geq$ 120 hr (allowable out to 240 hr) after 1st study medication dose

Treatment-associated adverse event

The subjects will be informed of the potential side effects of this medicine and specifically asked about the development of these symptoms during their clinical evaluations at 24 and 72 hours, and these results will be noted on a standardized questionnaire. If any of these symptoms, or other side effects, is deemed to be severe by the subject or the physician, the patient will be removed from the study, the code broken for that individual, and the patient treated with alternative therapy. Illnesses present at enrollment to the study are considered pre-existing conditions and will be documented on the initial clinic visit form.

Microbiological monitoring

The stool microbiology procedures are as described in the "Surveillance" section. Enrolled patients will have a stool specimen obtained at initial presentation representing the pretreatment stool microbiology. Post-treatment stool specimens (study days 5-9) will be cultured to assess for eradication.

Study outcomes

Clinical cure = complete resolution of diarrhea and diarrhea-associated signs/symptoms within 72 hours of first dose of study medication

Last diarrheal stool = last Grade 3-5 stool occurring in a 24-hr period meeting the diarrhea definition

Last unformed stool = last Grade 3-5 stool produced by subject followed by a 24-hr period with no diarrhea-associated symptoms

Microbiologic cure = eradication of the patient's isolate, previously detected on the pre-treatment stool culture, at follow-up approx. 48-72 hours (inclusive period is study days 5-9) after last dose of study medication

Evaluable subject in clinical trial = patient receiving follow-up 2-3 days after last antibiotic dose with no use of concomitant medications likely to affect the clinical course; additional analysis will evaluate patients that have follow-up limited to the clinical visit 72 hours after 1st antibiotic dose

Analysis

Therapeutic response will be evaluated for clinical measures [clinical cure (resolution of all diarrhea-associated symptoms by 72 hr after initial treatment); abatement of symptoms each 24-hr interval; time to symptom resolution (survival analysis)], microbiologic measures [eradication rates], and frequency of adverse events for each drug regimen. Efficacy evaluation will include evaluable subjects as defined by patients receiving follow-up 2-3 days after last antibiotic dose with no use of concomitant medications likely to affect the clinical course. This criterion allows complete assessment of initial clinical response combined with adequate time to survey for clinical relapse.

This same criterion will be used for comparison of microbiologic eradication rates. In addition a comparison of clinical cure rates will evaluate patients that have follow-up limited to the clinical visit 72 hours after 1st antibiotic dose. Intention to treat analysis is defined as an analytic strategy for randomized controlled trials that compares patients in the groups in which they were originally randomly assigned [200]. Recommendations for randomized controlled trial analysis for the evaluation of acute infectious diarrhea therapy include an intention to treat analysis [106]. The primary consideration for this aspect of the analysis is an assessment of the potential effect of missing responses due to loss to follow-up. As a conservative approach the losses to follow-up will be coded as treatment failures. An overall interpretation of regimen comparative efficacy will take into account the clinical and microbiological efficacy determinations. Statistical testing will use $\alpha = .05$ level of significance.

Subject baseline characteristics and summary follow-up findings will be compared using analysis of variance, Kruskal-Wallis tests, and chi-square tests. Differences in the frequencies of clinical cures and microbiologic eradication rates between study regimens will be tested for significance with Mantel-Haenszel procedures [106]. Rates of adverse reactions will be similarly compared between study regimens. A determination of the last unformed stool will be sought for each volunteer with the respective date/time information recorded. Differences in recovery times will be evaluated using Kaplan-Meier analyses (time to last diarrheal stool), log-rank (overall differences in response curves), and generalized Wilcoxon tests (response curve differences emphasizing early failures) [106]. Stratified analyses to assess for confounding variables will be used for both qualitative and time to event outcomes.

Limitations

The potential for a broad application of these results in diverse settings is limited by the *Campylobacter* predominance that is unlike other regional travelers' diarrhea surveys. However, given the therapeutic challenges inherent in fluoroquinolone-resistant *Campylobacter*, these data will be particularly important to combine with results of antibiotic treatment trials from other regions in order to formulate empiric management recommendations. The potential loss to follow-up given the periodic high tempo military operation is a possible limitation as well as the possibility that patients who quickly resolve their symptoms may opt not to return for further follow-up. Timing of specimen collection to determine microbiological cure is not optimal given the variable times when specimens will be submitted in a field environment. Precision of clinical endpoints is also limited by the inability to accurately pinpoint an exact time for symptom resolution. In order to enhance precision to some degree, the self-reported quantitation of loose/liquid stools is based on 6-hour intervals as well as a specific date and time for the last diarrheal stool. The improved delineation of the primary symptom, diarrhea, will provide better discrimination of treatment efficacy differences.

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Acute diarrhea in U.S. military personnel on short term deployment in Thailand: role of *Campylobacter* in disease severity and clinical outcome

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Abstract

Clinic-based diarrhea surveillance was undertaken in United States military personnel during short-term deployments to Thailand in the late spring of 1995, 1998, and 1999. Patients (n = 401) were predominantly male (93%) with median age 27 (IQR 22-34). Stool bacteriology revealed a pathogen in 68% [*C. jejuni* (34%), nontyphoidal *Salmonella* (21%), enterotoxigenic *E. coli* 12%]. *Campylobacter* ciprofloxacin resistance was 86% in 1995 and 95% in the other years. *Campylobacter* cases presented more often with fever (71 vs. 29%) and other systemic complaints, higher output diarrhea (30 vs. 14%), and decreased functional ability (81 vs. 63%) than other etiologies. Recovery time was also longer for *Campylobacter* cases, 43 vs. 4 hr ($P < .001$) after first antibiotic dose frequently associated with fluoroquinolone empiric therapy. *Campylobacter* infection among military personnel in Thailand presents as a more severe form of traveler's diarrhea than other etiologies with greater adverse effects on soldier's activities.

Introduction

Diarrhea is a frequent illness affecting military and civilian travelers during overseas visits [1, 2]. Based on therapeutic response to antibacterial therapy, it is estimated that approximately 80% of cases are due to bacterial enteropathogens [3]. Among bacterial etiologies, enterotoxigenic *Escherichia coli* (ETEC) is commonly observed as the major cause on a global basis; however, important regional and seasonal differences exist [3-7]. In Thailand, several studies among deployed U.S. military personnel have shown enteropathogenic *Campylobacter* species, *C. jejuni* and *C. coli*, to account for as high as 60% of diarrheal cases [8-13].

Acute diarrhea clinic-based surveillance in U.S. military personnel deployed to Thailand provides important information on etiologies, clinical presentation, and treatment of travelers' diarrhea in Southeast Asia for military and civilian populations. Increasing prevalence of fluoroquinolone (FQ)-resistant *Campylobacter* species during the decade of the 1990's has raised concerns regarding appropriate management [14]. The present study was undertaken to evaluate relative differences in clinical presentation and outcome of acute diarrhea based on stool microbiology findings in order to assist health care providers at initial clinical presentation and assess treatment approaches.

Methods

Study population and inclusion criteria

Cobra Gold is an annual joint military training exercise conducted in the Kingdom of Thailand each May. Temporary military medical units operate during the period of the exercise. Surveillance efforts during exercise years 1995 [15], 1998 [16], and 1999 involved cross-sectional enrollment of any individual presenting for care at designated clinics fulfilling the diarrhea definition (see below). This study combines data from 1995, 1998, and 1999. Inclusion criteria for this analysis of predictive clinical symptomology and outcome comparison based on pathogen isolation are the following: acute diarrhea of $\leq$ 120 hours pretreatment, onset of illness $\geq$ 24 hours after arrival in Thailand, illness conforming to the diarrhea definition, and a pretreatment stool culture. The diarrhea definition was not modified from the prospective field surveillance and was consistently applied. Diarrhea was defined as three or more loose stools in a 24-hour period or two or more loose stools in a 24-hour period with one or more associated complaints, including abdominal cramps, nausea, vomiting, or fever (temperature of $\geq$ 38^{O}C).

After applying these restrictions to the existing data (N = 501), 80% of the available records fulfilled the inclusion criteria (N = 401). The basis for exclusion was lack of pretreatment stool culture in approximately 85% and illness duration at presentation exceeding 120 hours in approximately 15% during the 1995 and 1998 exercises (none excluded from 1999).

Clinical evaluation and monitoring

During each exercise year a team of physicians accompanied by a field microbiology laboratory supplemented the organic military medical personnel in order to conduct clinic-based surveillance for diarrheal disease. Patients were asked to provide a stool specimen prior to initiating antibiotic therapy. Bedside testing for occult blood in the stool (Hemoccult, Beckman Coulter, Inc., Fullerton, CA) was undertaken during the 1999 exercise. Prescribed therapy was based on individual clinical assessment; therefore, was not standardized and not amenable to comparative analysis. The standard practice consisted of either a fluoroquinolone antibiotic (ciprofloxacin 500 mg orally twice daily for three days) or azithromycin 500 mg orally once daily for three days, with or without loperamide 2 mg capsules after each loose stool.

Patients were provided a diary card to record the number of loose stools (each 6-h period), daily symptoms (abdominal cramps, nausea, vomiting, fever, or bloody stools), and an assessment of their functional ability for each 24-hour interval. Patients were asked to return for follow-up in three days. Clinical cure was defined as resolution of diarrhea and associated gastrointestinal symptoms and fever within 72 hours of initiating therapy. Failure of treatment was defined as persistence of these symptoms for more than 72 hours.

Laboratory analysis

A field laboratory was available during each exercise period for primary microbiological evaluation [15, 16]. Isolates were transported to the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok for species identification and susceptibility testing. Fecal leukocytes were semi-quantitatively determined using

methylene blue-stained fecal smears examined under microscopy. Fecal lactoferrin was detected using the commercial Leuko-Test® kit (TechLab, Blacksburg, VA) following manufacturer's instructions. The presence of lactoferrin was detected by a visually read positive agglutination of $\geq$ 1+ as defined by manufacturer. Fecal lactoferrin testing was undertaken during the 1999 exercise. Primary media for stool microbiology included MacConkey, Hektoen Enteric, thiosulfate citrate bile salts sucrose, and Brucella agar with 5% sheep blood for overnight incubation. *Campylobacter* species were isolated using a membrane filter method on non-selective blood agar before and after enrichment [17]. *C. jejuni* refers to both *C. jejuni* and *C. coli* throughout this report. Enrichment media included Selenite F broth, alkaline peptone water, and Doyle's broth. Enteric pathogens were identified using standard morphologic and biochemical profiles. Five lactose-fermenting and 5 non-lactose-fermenting *E. coli* colonies per specimen were tested using DNA probes for detection of ETEC toxins, enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), locally adherent enteropathogenic *E. coli* (EPEC), and attaching and effacing E. coli (*eae+ E. coli)* [18, 19]. Antibiotic susceptibility testing of presumptive pathogenic bacterial isolates was determined by the disk diffusion method of Bauer and colleagues [20], with use of commercially prepared antibiotic disks as previously described [14].

Statistical analysis

Clinical records and field microbiology results were reviewed for each case with results recorded on a standardized data abstraction form. Abstracted data from patient surveys, symptom diaries, clinical records, and microbiology results were entered into an EpiInfo version 6.04 database. Statistical analysis was performed using SPSS for

Windows (version 10.1). Differences in clinical findings at presentation and illness outcome by *Campylobacter* isolation were evaluated using χ^2 testing for categorical variables or nonparametric tests to compare continuous variables. Differences in recovery times were evaluated using Kaplan-Meier analyses (time to last diarrheal stool after first antibiotic dose), log-rank (overall differences in response curves), and generalized Wilcoxon tests (response curve differences emphasizing early failures) [21]. All tests were 2-tailed, and *P* values < .05 were considered statistically significant.

Logistic regression modeling was used to determine predictors of *Campylobacter*-associated illness using independent variables available to the health-care provider at the time of initial clinical presentation. The dependent variable used for modeling was pre-treatment stool culture isolation of *C. jejuni*. Independent variable selection for inclusion in model building was undertaken using exploratory analysis, two-way contingency table analysis, and Mantel Haenszel chi-square stratified analysis. For the purpose of the regression analysis, variable reclassification was undertaken as follows. Regional sites in Thailand were grouped into "high" and "low" *Campylobacter* endemic regions based on an apparent breakpoint in the proportion of cases at the sites. Presenting systemic features such as fever, arthralgias, and myalgias were commonly associated in a given patient; therefore, an ordinal variable was created to semi-quantitatively summarize these findings. The presence of systemic features was coded based on the number of these symptoms present ranging from zero (no symptoms) to three (all systemic complaints). Accurate reporting on number of pretreatment diarrheal stools is accompanied by potential recall bias in illnesses of longer duration or imprecision with reporting high output diarrhea. To improve precision and more accurately reflect most current illness

status, the number of diarrheal stools in the 24 hours preceding presentation was analyzed. These data include a minority of cases with daily diarrhea frequency exceeding 25. An ordinal variable was created for regression modeling by categorizing daily diarrhea total for the 24 hour period preceding initial presentation by the following groups: $\leq$ 3, 4-9, and $\geq$ 10. Oral temperature at initial visit was coded as < 38^{o}C, 38-38.3^{o}C, or > 38.3^{o}C. Other independent variables evaluated by regression modeling were not reclassified. Selected variables for logistic regression analysis must have shown a *P* value < .25 in bivariate analysis for subsequent inclusion. A forced entry method of regression analysis evaluated all covariates using likelihood ratio testing. The odds ratio for each predictor variable was calculated as the exponent of the regression coefficient with 95% confidence intervals.

Results

Study population characteristics

The characteristics of the study population (n = 401) available for analysis from the merged clinical and microbiological data for each exercise year are detailed in Table 1. Study population was predominantly male (93%) with median age 27 (IQR 22-34) in the U.S. Army (45%) and Marine Corps (33%). The exercise sites during the 3 years of surveillance included Nakhon Sri Ratchasima (Khorat), a city in the central Isaan plateau, Utapao, a Thai Naval base in southern Thailand, and Kanchanaburi, an inland city northwest of Bangkok. During the 1995 and 1998 exercises, surveillance was undertaken at two sites whereas in 1999 a single site was investigated. The Utapao site, the primary training area for U.S. Marines, was not included in the 1999 surveillance. During the

period of surveillance included in this analysis, the U.S. Army trained in the regions of Khorat and Kanchanaburi. The decision to use malaria prophylaxis is dependent upon training region (not used in troops stationed in Utapao) and is the basis for the observed variation across exercise years, low of 16% (1998) to high of 56% (1999).

Clinical presentation

Patients presented with acute diarrheal illness with a 1-day median duration (range 0-5). Median number of diarrheal stools in the 24 hours prior to presentation was five (range 1-30). High frequency diarrhea (defined as $\geq$ 10 loose/liquid stools in 24 hours) was observed in 19% of the patients. The time for symptom onset from arrival in country was a median of 10 days (range 1-34). Based on inclusion criteria all patients had diarrhea with the most commonly associated symptom of abdominal cramps in 81%. Less commonly, patients reported fever (43%), nausea (56%), myalgias (35%), joint aches (24%), vomiting (20%), and gross blood in stools (5%). Table 3 provides a comparison of clinical manifestations observed at presentation among patients with commonly identified pathogens. Included cases are restricted to patients where a single pathogen was identified in the pretreatment stool culture. Notable from this table is the higher rates of fevers (approximately 70%) observed in *Campylobacter*- and *Shigella*-associated cases but much lower, 29%, in patients with *Salmonella*-associated diarrhea, another common etiology for inflammatory enteritis. The *Campylobacter*-associated cases also reported higher rates of myalgias and arthralgias, 42-55%. Nausea and vomiting was most common among *Shigella*-associated cases however the number of these cases (n = 10) is small. A higher proportion, 33%, of *Campylobacter* and *Plesiomonas* cases reported severe diarrhea (> 10 diarrheal stools per day) in the 24-hour

period preceding initial clinic visit. The duration of symptoms prior to presentation was not significantly different based on the pathogen. A complete inability to work or recreate was observed more often in *Shigella* (70%), *Plesiomonas* (50%), and *Campylobacter* (35%).

Distribution of bacterial enteric pathogens

Stool bacteriology (Table 2) most commonly revealed *C. jejuni/coli* [137 (34%)], nontyphoidal *Salmonella* spp. [84 (21%)], and enterotoxigenic *E. coli* [46 (11%)]. The data was evaluated by region, which demonstrated *Campylobacter* isolation rates as follows: Khorat (Nakhon Sri Ratchasima) 51%, Kanchanaburi 18%, and Utapao 17%. ETEC isolation rates were higher (21%) in Kanchanaburi; however, the number of cases was only 15. Multiple bacterial isolates were found in 86 cases (21%) with various combinations of *Campylobacter*, *Salmonella*, and *eae*$^{+}$ *E. coli* accounting for the majority of the multiple pathogens. No pathogen was isolated or identified in 127 (32%) of the cases.

***Campylobacter*-associated findings**

Initial Presentation and Prediction of *Campylobacter*-associated illness

There were no differences in *Campylobacter* isolation rates by age, gender, or service affiliation of the affected personnel. The isolation rate differences observed by exercise year were primarily related to regional distribution rather than temporal variability. The data was evaluated by region with the following *Campylobacter* isolation rates: Khorat 51%, Kanchanaburi 18%, and Utapao 17%. Significant differences in *Campylobacter* infection were observed among individuals receiving malaria

chemoprophylaxis, 47 (no prophylaxis) vs. 75% (prophylaxis). There were also significant differences between malaria prophylaxis and study site: Khorat (85%), Kanchanaburi (56%), and Utapao (16%). When controlling for study site there were no statistically significant differences between malaria prophylaxis use and rate of *Campylobacter* isolation.

Campylobacter-associated cases were more likely to present with fever (71 vs. 29%) and other systemic complaints (headaches, arthralgias, and myalgias), abdominal cramps (88 vs. 77%), and decreased ability to work/recreate (81 vs. 63%) as compared to non-*Campylobacter* cases (Tables 4 and 5). Fever was the most commonly observed systemic symptom in 43%, as compared to myalgias, 35%, and joint aches, 24%. Patients reporting myalgias or joint aches were much more commonly febrile, 77 and 84%, respectively. The average number of loose or liquid stools during the entire illness pre-treatment was slightly higher for *Campylobacter*-associated illness than in cases without isolation of *C. jejuni*, 13 vs. 11 diarrheal stools ($p = .02$). The number of diarrheal stools in the 24 hours preceding presentation also demonstrated a higher mean number for *Campylobacter*-associated illness, 7.2 vs. 5.8 ($p < .001$).

Examination of the stool specimens consistently demonstrated evidence of an inflammatory enteritis more commonly in *Campylobacter*-associated cases. Stool hemoccult testing was not performed in 1995 and 1998; however, clear differences were demonstrated during the 1999 exercise. In *Campylobacter*-associated cases, hemoccult positivity was observed in 53 vs. 14% of cases associated with other enteropathogens ($P < .01$). Visible blood in the stool specimen was much less common however still more often observed in *Campylobacter*-associated cases, 19 vs. 3%. Microscopic evaluation

for fecal leukocytes as a marker for inflammatory diarrhea was undertaken each exercise year. *Campylobacter*-associated cases were more commonly positive for fecal leukocytes and fecal lactoferrin, 47 vs. 18% and 97 vs. 45%, respectively ($P < .01$).

Based on the preceding analysis, the following variables were selected for inclusion in logistic regression modeling: *Campylobacter* regional endemicity, frequency of diarrhea in 24 hours prior to presentation, oral temperature at initial visit, presence of systemic features, functional capacity at presentation (patient report), stool hemoccult result, and fecal leukocyte result. Table 6 summarizes the results of the analysis detailing both unadjusted and adjusted odds ratios for potential predictors. Adjusted analysis was performed with and without hemoccult results due to hemoccult testing in only 32% of cases.

Following adjustment, important predictors of *Campylobacter* isolation from initial stool culture included high regional *Campylobacter* endemicity, increased frequency of diarrhea (past 24 hours), systemic features at presentation, and a nonspecific stool laboratory marker of inflammatory diarrhea (hemoccult or fecal leukocytes). The analysis was also undertaken following exclusion of *Campylobacter*-associated cases that also had another enteropathogen isolated (data not shown). This analysis eliminated 69 cases from the *Campylobacter*-associated group; however, results again demonstrated the same predictor variables as important following adjustment. Overall, the model is able to accurately predict the isolation of *Campylobacter* in 77% of the cases.

Clinical outcomes

Initial empiric therapy did not differ between *Campylobacter* and non-*Campylobacter* cases: intravenous fluids (19 vs. 16%), loperamide (68 vs. 54%), or

antibiotic (91 vs. 96%). Initial antibiotic use in 1995 and 1998 was a fluoroquinolone (FQ) in $\geq$ 98% of cases, whereas in 1999 azithromycin was used in 27%. FQ-resistance in *C. jejuni* was $\geq$ 85% during all study years. Outcomes were initially evaluated after restricting analysis to cases without multiple isolates (data not shown). No difference in the findings was observed irrespective of restricting analyses based on multiple pathogen isolation; therefore, complete data is presented (Table 5). Time to diarrhea resolution was delayed for *Campylobacter* cases with median recovery period of 43 vs. 4 hr ($P <$ .001) after first antibiotic dose. This analysis was restricted to the 1998 and 1999 exercises due to the lack of recorded specific illness onset times in 1995. As demonstrated on Figure 1, differences in clinical resolution are evident within the first 24 hours and extend out to 72 hours. After three days there is no appreciable difference in clinical response based on pathogen isolation. This finding is not affected by use of antimotility agents, which were used in 65% of these cases. No difference in clinical response was observed based on loperamide use when controlling for *Campylobacter* isolation. In addition to the delayed recovery, the total number of diarrheal stools was greater in *Campylobacter* cases, 20 vs. 7 ($P <$ .001). Cure rates (defined as complete symptom resolution by 72 hr) were 94% for non-*Campylobacter* (n = 190) receiving FQ therapy vs. 81% for *Campylobacter* cases (n = 74) (P = .002). FQ susceptible isolates were observed in only 2.5% (n = 10) of the cases with *Campylobacter*. Of these, 4 cases had follow-up data on clinical response. The three individuals receiving fluoroquinolone therapy were all cured by 72 hours with an average duration of diarrhea of 32 hours (4.5 – 59) after first antibiotic dose. An evaluation of azithromycin efficacy is limited in the non-*Campylobacter* cases due to the very small number (n = 4) receiving this antibiotic.

Azithromycin was not used in 1995 and received very limited use (n = 3) in 1998. In 1999 azithromycin was used in 27% of the cases and was more commonly used in cases with documented fevers, dysenteric stools, and positive stool hemoccult results leading to a bias toward *Campylobacter*-associated cases (87% of cases receiving azithromycin had documented *C. jejuni*). Azithromycin cure rate in *Campylobacter* cases by 72 hours was 92% with a median time to last diarrheal stool of 49 hours.

Discussion

This study investigated travelers' diarrhea among a rather select population (generally healthy young male U.S. military personnel frequently using doxycycline malaria prophylaxis) traveling to Thailand during April-May timeframe. A unique aspect of the diarrheal threat for this population has been the overwhelming predominance of *Campylobacter* over the past 15 years of surveillance coupled with increasing rates of FQ resistance [8-13, 16]. This is in contrast to most travelers' diarrhea series demonstrating ETEC as a predominant etiology, as well as, typically higher rates of undetermined causes [3, 4]. Previous Cobra Gold exercise-based clinical research over the past decade highlight the impact of diarrhea, particularly *Campylobacter*-associated, for deployed military personnel in this region. During Cobra Gold 1990, a 30% cumulative incidence of diarrhea was observed with 25% of affected individuals seeking care [1]. Progressive FQ resistance among *C. jejuni* has been observed from none pretreatment in 1990, 50% in 1993, and 85% in 1998 [10, 12, 16]. In contrast, increasing macrolide resistance has not been observed with the exception of a 31% azithromycin resistance among 20 isolates from one Thai region in the Malay peninsula in 1994 [9] and 7% in 1995 [14].

Coincident with rising FQ resistance sub-optimal treatment response was observed in 1998 in approximately 10-20% of the *Campylobacter*-associated cases receiving ciprofloxacin although the number of cases was small.

This analysis demonstrates *Campylobacter*-associated diarrhea in Thailand to be more severe than other etiologies as reflected by more frequent systemic toxicity and increased diarrhea severity at presentation with delayed recovery. The observed rates of systemic symptoms, 30-50%, and rates of severe diarrhea, dysentery or high output, are comparable to other clinical case series of campylobacterioses and were predictors of *Campylobacter*-associated disease in the logistic regression modeling [22-25]. Notable in our series is the absence of *Shigella* species, which would be expected to present with a similar clinical spectrum as *Campylobacter* enteritis. The potential benefit of using the clinical presentation to predict the pathogen has been investigated [26-28]. Typically, the clinical presentation is not sufficiently characteristic to allow prediction of the specific pathogen. In addition, a clinical syndrome (i.e. fever plus dysentery) that has high specificity for an etiologic agent causing inflammatory enteritis, such as *Shigella*, have low sensitivity limiting application in a treatment algorithm if goal is to restrict antibiotic therapy to individuals with an inflammatory bacterial enteritis [26, 28]. A study in Finnish travelers to Morocco demonstrated a more severe clinical illness with *C. jejuni* as compared to ETEC; however, most of the differences were on the follow-up visits (second and third days) [27]. The observed predictors of *Campylobacter*-associated disease, high-output diarrhea, systemic complaints, and laboratory marker of inflammatory diarrhea (hemoccult or fecal leukocytes), all contributed less than regional *Campylobacter* endemicity which led to a pretest probability of approximately 50% given

the hyperendemic setting for this population. Given the high pretest probability due to *Campylobacter* regional prevalence, there is only marginal gain in ruling in this diagnosis for each of the other predictors determined from the regression model with posttest probability no higher than 80%. Prospective evaluation of standardized rapid diagnostic methods should be undertaken in this setting to assess their contribution and utility for clinical decision-making.

This analysis provides evidence of less than optimal empiric diarrhea management during the Cobra Gold exercise. Time to recovery was delayed for *Campylobacter* cases. Cure rates at 72 hours were 94% for non-*Campylobacter*-associated cases receiving FQ therapy vs. 81% for *Campylobacter* cases ($P = .002$). The recommended standard empiric antibiotic therapy for travelers' diarrhea is a 3-day course with a fluoroquinolone although Thailand-specific recommendations for use of the macrolide antibiotic azithromycin have been proposed [29, 30]. Azithromycin 500 mg daily was compared with ciprofloxacin 500 mg daily (3-day regimens) for diarrhea in U.S. service personnel during Cobra Gold 1993 and was found to have comparable efficacy [10]. This study was limited by the small sample size with minimal ability to detect moderate effect differences of the azithromycin regimen. There were only two clinical failures in the study group, both being ciprofloxacin-treated *Campylobacter* cases. Significant differences in improved microbiologic eradication of *Campylobacter* were demonstrated with azithromycin; however, this did not translate into statistically significant clinical differences. Importantly, the only statistically significant clinical findings on subgroup analysis were a reduced duration of illness in non-*Campylobacter* cases, representing as many as 40% of cases, with ciprofloxacin. Changing provider

practices are evident from this series with azithromycin first-line empiric therapy in approximately one-third of cases in 1999 as compared to no use in 1995. These changes are likely based on knowledge of increasing *C. jejuni* FQ-resistance coupled with additional studies supportive of azithromycin efficacy in other settings involving bacterial enteritis [31-33].

In conclusion, *Campylobacter* species are the predominant etiologic agents causing traveler's diarrhea in military travelers to Thailand. The clinical presentation and subsequent time to resolution for *Campylobacter*-associated cases differs from other etiologies in this setting evidenced by frequent systemic toxicity, increased diarrhea severity at presentation, delayed recovery, and higher 72-hr clinical failure rates (associated with use of FQ antibiotics). These findings have been observed during a period of time when the rates of FQ-resistant *C. jejuni* exceed 85% and the most common therapy prescribed was a FQ antibiotic. Continued surveillance is needed with observational studies to assess pathogen distribution, antibiotic susceptibility trends, and clinical response to therapy. In addition, randomized controlled trials to evaluate alternative management strategies and optimal dosing schedules are needed.

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List of Tables

Table 1. Population characteristics among U.S. military presenting with acute diarrhea during training exercises in Thailand

	Cobra Gold exercise year			
	1995	1998	1999	Total
Site	Khorat Utapao	Kanchanaburi Utapao	Khorat	All
Approx. troop size	5000	3200	2300	10,500
No. cases included in analysis	155	136	110	401
Service (%)				
Navy	7	13	5	9
Army	34	33	75	45
Air Force	21	5	8	12
USMC	38	49	7	33
Median age (IQR)	25 (22-33)	27 (23-36)	28 (22-33)	27 (22-34)
Gender (% male)	94	93	89	93
Malaria prophylaxis (%)	59	32[1]	84	56
Campylobacter isolation rate (%)	34	16[1]	56	34

Interquartile range (IQR)
[1] Signifies differences in a given characteristic between exercise years ($P < .001$).

Table 2. Distribution of bacterial enteric pathogens at initial presentation in U.S. military personnel with acute diarrhea by Thai region

Stool microbiologic finding	Khorat (n = 195)	Kanchanaburi (n = 72)	Utapao (n = 134)	Total (n = 401)
Campylobacter jejuni	100 (51)[1]	13 (18)	24 (18)	137 (34)
Nontyphoidal *Salmonella*	48 (25)	16 (22)	20 (15)	84 (21)
Enterotoxigenic *E. coli*	12 (6.2)	15 (21)[1]	19 (14)	46 (12)
eae^{+} *E. coli*	16 (8.2)	9 (13)	18 (13)	43 (11)
Plesiomonas shigelloides	8 (4.1)	6 (8.3)	14 (10)[1]	28 (7.0)
Shigella species	1 (0.5)	1 (1.4)	10 (7.5)	12 (3.0)
Vibrio parahaemolyticus	0	1 (1.4)	6 (4.5)	7 (1.7)
Non-01 *V. cholerae*	2 (1.0)	1 (1.4)	2 (1.5)	5 (1.2)
Multiple pathogens, n (%)	47 (24)	13 (18)	26 (19)	86 (21)
No pathogen identified, n (%)	50 (26)[1]	25 (35)	52 (39)	127 (32)

Note: Represents pooled data for Khorat (1995 and 1999) and Utapao (1995 and 1998).
[1] Signifies differences in pathogen isolation rates between sites ($P < .05$)

Table 3. Clinical manifestations (%) at initial presentation in U.S. military personnel with acute diarrhea by bacterial isolate

Presenting symptom or sign	*Campylobacter* (n = 89)	*Salmonella* (n = 31)	ETEC (n = 25)	*eae+ E. coli* (n = 23)	*Shigella* (n = 10)	*Plesiomonas* (n = 6)
Fever	71 (61, 80)	29 (15, 47)	16 (5.3, 34)	26 (11, 47)	70 (38, 92)	33 (6.0, 74)
Abdominal cramps	89 (81, 94)	94 (80, 99)	92 (76, 99)	78 (58, 92)	90 (60, 100)	67 (26, 94)
Diarrheal frequency in 24h before presentation						
≤ 3	15 (8.4, 23)	29 (15, 47)	40 (22, 60)	30 (14, 51)	30 (8.3, 62)	17 (0.8, 59)
3-9	52 (41, 62)	52 (34, 69)	52 (33, 71)	61 (40, 79)	60 (29, 86)	50 (15, 85)
≥ 10	33 (24, 43)	19 (8.2, 36)	8 (1.4, 24)	9 (1.5, 26)	10 (0.5, 40)	33 (6.0, 74)
Gross blood in stools	6.1 (2.1, 12)	3.4 (0.2, 15)	0 (0, 11)	4.3 (0.2, 20)	11 (0.5, 40)	0 (0, 39)
Nausea	58 (48, 68)	42 (26, 60)	52 (33, 71)	57 (36, 75)	80 (48, 97)	67 (26, 94)
Vomiting	16 (9.2, 24)	13 (4.2, 28)	12 (3.2, 29)	8.7 (1.5, 26)	50 (21, 79)	33 (6.0, 74)
Myalgias	55 (45, 65)	32 (18, 50)	16 (5.3, 34)	22 (8.4, 42)	40 (14, 71)	33 (6.0, 74)
Arthralgias	42 (32, 52)	19 (8.2, 36)	8.0 (1.4, 24)	17 (5.8, 37)	10 (0.5, 40)	0 (0, 39)
Activity limitation						
None	19 (12, 28)	36 (20, 53)	24 (10, 43)	48 (28, 68)	10 (0.5, 40)	17 (0.8, 59)
Reduced	46 (36, 57)	39 (23, 57)	60 (40, 78)	22 (8.4, 42)	20 (3.5, 52)	33 (6.0, 74)
Unable	35 (26, 45)	26 (13, 43)	16 (5.3, 34)	30 (14, 51)	70 (38, 92)	50 (15, 85)

Note: Numbers in parentheses are the 95% confidence intervals for the estimated percentage. Data are restricted to cases with a single pathogen identified.

Table 4. Comparison of percentage with presenting features in U.S. military personnel with acute diarrhea based on stool microbiology isolation of *Campylobacter jejuni*

Clinical finding	*Campylobacter jejuni/coli* isolation	
	Positive (%) (n = 137)	Negative (%) (n = 264)
Malaria prophylaxis (doxycycline)	75[1]	47
High frequency diarrhea (≥ 10 stools in 24h preceding presentation)	30[1]	14
Fever (by report)	71[1]	29
Oral temperature > 100°F (documented)	42[1]	16
Myalgias	56[1]	25
Arthralgias	40[1]	16
Systemic illness symptoms	56[1]	22
Abdominal cramps	88	77
Nausea	56	56
Vomiting	14	22
Gross blood in stools	6	5
Fecal leukocyte positive	52[1]	19
Fecal lactoferrin positive	97[1]	45
Hemoccult positive	53[1]	14

Note: Systemic illness symptoms defined as a patient reporting at least 2 of the following: fever, myalgias, or arthralgias. Stool hemoccult (n = 80) and lactoferrin (n = 97) testing was limited to 1999 exercise year.
[1] Differences between *Campylobacter* positive (all cases) and non-*Campylobacter* cases ($P < .01$). There were no differences in rates between *Campylobacter* positive cases with or without copathogens.

Table 5. Clinical outcomes in U.S. military personnel with acute diarrhea based on stool microbiology isolation of *Campylobacter jejuni*

Clinical outcome measures	Pretreatment stool microbiology	
	Campylobacter (N = 106)	Non-*Campylobacter* (N = 210)
Activity limitation (% patients with reduced function)		
At initial presentation	81[1]	63
At 24h	85[1]	62
At 48h	52[1]	28
At 72h	21[2]	9
At 96h	8.4	7
Median no. diarrheal stools during entire episode (IQR)	20 (13-31)[1]	9 (5-17)
Median illness duration in hours (IQR)	72 (57-94)[1]	38 (21-65)
Median illness duration in hours after 1st antibiotic (IQR)	43 (28-57)[1]	4 (0-24)
Overall clinical cure rate (%)	82[1]	92
Clinical cure rates (%) by antibiotic received		
Ciprofloxacin (n = 199)	83	92
Ofloxacin (n = 61)	69[1]	97
Any fluoroquinolone (n = 260)	77[1]	92
Azithromycin (n = 30)[3]	92	75

Interquartile range (IQR)

Clinical cure defined as complete resolution of diarrhea and associated symptoms within 72 hours from first antibiotic dose received. Differences between *Campylobacter* positive (all cases) and non-*Campylobacter* cases at [1]$P < .001$ and [2]$P = .01$. There were no differences in rates between *Campylobacter* positive cases with or without copathogens.

[3]The majority (87%) of patients receiving azithromycin had *Campylobacter* isolated from stool cultures.

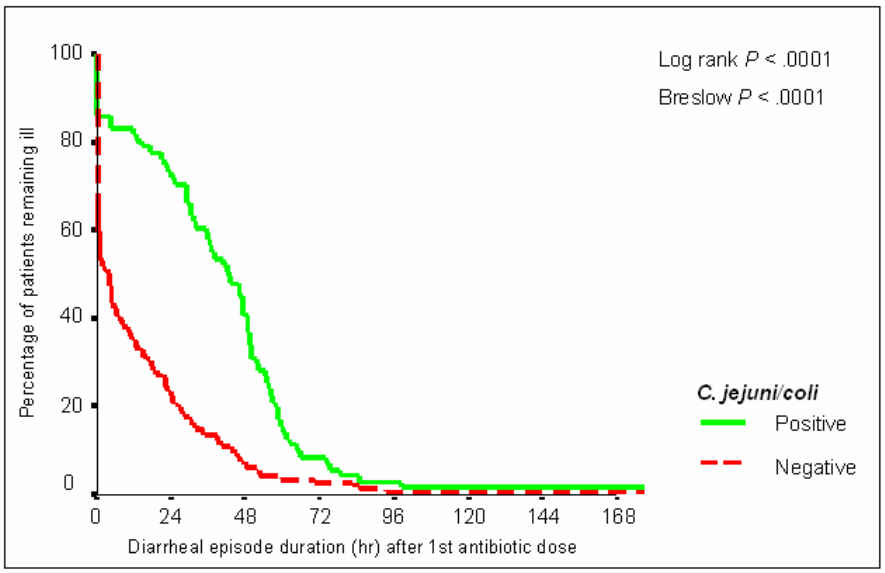

Figure 1. Time to cure (following first antibiotic dose) by *Campylobacter* species isolation in pretreatment stool culture

Table 6. Prediction of *Campylobacter* isolation in U.S. military personnel with acute diarrhea by clinical and laboratory measures available at presentation

Predictor variable	Odds ratio (95% C.I.)		
		Adjusted	
	Unadjusted	With Hemoccult	Without hemoccult
Campylobacter regional endemicity	4.8 (3.1, 7.6)	NA	5.1 (3.0, 8.9)
Frequency of diarrhea in past 24 h	2.6 (1.6, 4.4)	11 (1.0, 115)	1.9 (1.0, 3.8)
Oral temperature at initial visit	3.6 (2.2, 5.9)	8.5 (0.8, 88)	1.5 (0.8, 2.8)
Presence of systemic features	4.8 (3.0, 7.5)	4.9 (1.2, 21)	3.0 (1.7, 5.5)
Functional capacity at presentation	2.5 (1.5, 4.1)	2.1 (0.5, 9.6)	1.0 (0.5, 2.0)
Stool hemoccult result	7.0 (2.6, 19)	5.7 (1.2, 27)	NA
Fecal leukocyte result	4.7 (2.9, 7.5)	4.6 (0.7, 30)	3.6 (2.1, 6.5)

Note: NA signifies not applicable variable for the regression model under evaluation.

Diagnostic approach to acute diarrheal illness in a military population on training exercises in Thailand, a *Campylobacter* hyperendemic region

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Abstract

High rates of *Campylobacter* fluoroquinolone resistance highlight the need to evaluate diagnostic strategies to assist in clinical management. Diagnostic tests were evaluated in U.S. soldiers presenting with acute diarrhea during deployment in Thailand. Bedside and field laboratory diagnostic tests were compared to stool microbiology findings in 182 enrolled patients. *C. jejuni* was isolated in 62% of cases. Clinical findings, inflammatory screening tests [stool hemoccult, fecal leukocytes, fecal lactoferrin (LFLA), plasma C-reactive protein], or *Campylobacter*-specific peripheral blood antibody-secreting cells failed to increase post-test probability above 90% in this *Campylobacter* hyperendemic setting. A *C. jejuni/coli*-specific commercial EIA, and less so a research PCR, were strong positive predictors. Negative predictive value (reducing post-test probability less than 10%) was similarly observed with these *Campylobacter*-specific stool-based tests as well the fecal LFLA.

Introduction

Military personnel are frequently affected by short-term morbidity related to diarrheal diseases with potential adverse impact on the operational mission [1, 2]. Empiric therapy without supplemental laboratory data is a feasible option; however, refinement of management strategy using laboratory testing may increase cost-effectiveness and allow specific adjustments in antibiotic selection based on regional susceptibility patterns. During military operations, the availability of a field laboratory with microbiologic capability is variable. Rapid, technically simple diagnostic tests need to be evaluated to determine accuracy and acceptability in field settings. In Thailand, numerous surveys among deployed U.S. military personnel have shown enteropathogenic *Campylobacter* species, *C. jejuni* and *C. coli*, to account for as high as 60% of diarrheal cases [3-8]. Based on this observation, pathogen-specific diagnostic tests for this study focus on *Campylobacter*. This study of military personnel presenting with acute diarrhea during deployment in Thailand evaluates clinical findings in concert with bedside stool characterization and field laboratory rapid diagnostic tests as components of an overall diagnostic approach.

Methods

Study population and enrollment criteria

Annual U.S. military training exercises were conducted in the Kingdom of Thailand in May 2000 and 2001. Temporary medical units for evaluation and management of personnel are in operation during the period of the exercise. Personnel

presenting with acute diarrhea are requested to volunteer for participation. Enrollment criteria include the following: acute diarrhea of $\leq$ 96 hours, onset of illness $\geq$ 24 hours after arrival in Thailand, illness conforming to the diarrhea definition, no antibiotic treatment (with the exception of doxycycline used for malaria prophylaxis) in the previous 7 days, and a pretreatment stool culture. Diarrhea was defined as three or more loose stools in a 24-hour period or two or more loose stools in a 24-hour period with one or more associated complaints, including abdominal cramps, nausea, vomiting, or fever. A fever was defined as a temperature of $\geq$ 38^{O}C (100.4^{O}F).

Clinical evaluation and specimen collection

A standardized questionnaire and medical examination was used. Patients were asked to provide a stool specimen prior to first antibiotic dose. Stool characterization and bedside occult blood testing (Hemoccult, Beckman Coulter, Inc., Fullerton, CA) was completed by the study physician prior to transporting specimen to field laboratory. Stool specimens were graded on a scale of 1-5 [1 – hard (normal), 2 – soft (normal), 3 – thick liquid, 4 – opaque watery liquid, and 5 – clear watery]. Peripheral blood was collected directly into a Vacutainer® containing ethylene diamine tetraacetic acid (Beckton Dickinson Vacutainer Systems, Rutherford, NJ). Stool and blood specimens were transported to the field laboratory for immediate processing.

Stool microbiology (reference standard)

Primary plating of stool specimens was undertaken at an onsite field laboratory as previously described [9]. *Campylobacter* species were isolated using a membrane filter method on non-selective blood agar before and after enrichment [10]. *Campylobacter*

refers to both *C. jejuni* and *C. coli* throughout this report. Isolates were transported to the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok for species identification and susceptibility testing as previously described [11-13]. Five lactose-fermenting and 5 non-lactose-fermenting *E. coli* colonies per specimen were tested using DNA probes for detection of ETEC toxins (LT and ST), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), and enteropathogenic *E. coli* (EPEC; *eae* and EAF plasmid) [14, 15]. Microscopy evaluation of fresh stool specimens was used to evaluate for stool parasites. Stool specimens were examined for Rotavirus and Calicivirus antigens using a commercially available ELISA (Rotazyme; Abbott Laboratories, North Chicago, IL) and a non-commercial antigen-capture Calicivirus ELISA [16].

Diagnostic tests

Stool-based

Fecal leukocytes were semi-quantitatively determined using methylene blue-stained fecal smears examined under microscopy. Presence of fecal leukocytes per high power field (HPF) was categorized as follows: none, rare, 1-5, 6-10, and > 10. Fecal lactoferrin was detected using the commercial Leuko-Test® kit (TechLab, Blacksburg, VA) following manufacturer's instructions. The presence of lactoferrin in a 1:50 diluted stool specimen was detected by a visually read positive agglutination of $\geq$ 1+ as defined by manufacturer. Presence of *C. jejuni* or *C. coli* was detected using the commercial ProSpecT® *Campylobacter* Microplate Assay (Alexon-Trend, Inc., Ramsey, MN) following manufacturer's instructions. *Campylobacter*-specific antigens are detected by a visually read color development of $\geq$ 1+ as defined by manufacturer. A multiplex PCR

for the detection of *C. jejuni* and *C. coli* from stool specimens was included during the first year of the exercise as previously described [17]. The PCR assay detects *ceu*E genes present in *C. jejuni* and *C. coli* useful for primary detection and species differentiation. DNA templates from stool suspensions of 1:5 10% stool in TE (10 mM Tris-HCL, 1 mM Na_2EDTA, pH 8.0) were prepared using silicon dioxide extraction [17, 18]. Oligonucleotide primer sequences derived from *ceu*E genes and PCR amplification conditions have previously been reported [17]. All PCR tests were conducted in the presence of gold standards of positive and negative controls. PCR tested results were considered valid only if all gold standards of positive and negative controls were proven to be accurate, i.e., positive controls as PCR positive and negative controls as PCR negative.

Blood-based

Plasma C-reactive protein, an acute phase protein produced by the liver during infectious and non-infectious inflammatory disease, was evaluated at initial clinic presentation [19]. Fresh plasma was evaluated semi-quantitatively using the commercial RapiTex® CRP test (Dade Behring, Marburg, Germany) following manufacturer's instructions. Mononuclear cells (MNC) were isolated by ficoll-hypaque density gradient (Organon Teknika Corp., Durham, NC) and cryopreserved in the field laboratory [20]. ASC assays were performed at the Naval Medical Research Center, Silver Spring, MD as previously described [20]. *Campylobacter*-specific IgA antibody secreting cell (ASC) responses were evaluated at initial presentation and at 72-hour clinical follow-up using ELISPOT methodology [20]. Specific antigens used include *C. jejuni* strain 81-176 glycine extract, *C. jejuni* strain 81-176 whole cell, and a common Thai *C. jejuni* strain

(Lior 36) whole cell lysate preparation [21]. The numbers of spots found in comparable wells were summed and adjusted to number per 10^6 MNC. A positive ASC response was defined as $\geq$ 5 *Campylobacter*-antigen-specific spots per 10^6 MNC.

Statistical analysis

The physician-performed bedside diagnostic assays (stool characterization and hemoccult) and laboratory technician-performed rapid diagnostic assays (fecal leukocyte smear, lactoferrin latex agglutination, *Campylobacter*-specific EIA, and plasma C-reactive protein) were compared with the gold standard stool microbiology results. Test performance characteristics will be assessed for each assay. Test performance characteristics (sensitivity, specificity, predictive values, and likelihood ratios) were evaluated for each clinical finding (such as fever, abdominal cramps, and severe diarrhea) and diagnostic assay with 95% confidence intervals. The probability of accurately rating a test result as positive or negative is also quantified using the area under the receiver operating characteristic (ROC) curve [22]. In order to compare areas from various diagnostic tests derived from the same cases an adjustment was made to account for correlations between areas [23]. The likelihood ratio was evaluated in context with pre- and post-test probabilities for different scenarios (low versus high prevalence region). In addition, clinical findings and diagnostic assays were evaluated singly and in series using likelihood ratios in order to determine the most accurate and efficient diagnostic algorithm. Results of the patient surveys, symptom diaries, physician findings, and microbiologic results were entered into an EpiInfo version 6.04 databases. Statistical analysis was performed using SPSS for Windows (version 10.1). All tests were 2-tailed, and P values $< .05$ were considered statistically significant.

Results

A total of 182 U.S. military personnel presenting with acute diarrhea were enrolled. Characteristics of the study population are provided in Table 1. Cases enrolled during the two exercise years had similar age and gender distributions. A higher rate of malaria prophylaxis occurred during the first exercise year; however, no difference in time to illness presentation, characteristics of illness, or pathogen distribution was observed. *Campylobacter* was identified in initial stool cultures in 62% of all cases with 96% speciated as *C. jejuni* with *C. coli* accounting for the remainder. *Salmonella* and *Plesiomonas* were isolated in an additional 10-20% of cases. The high isolation rates of invasive bacterial pathogens are supported by frequent clinical features of inflammatory enteritis in > 50% of enrolled cases. A notable exception to this pattern is the relatively low rate of positive fecal leukocytes observed in the first exercise year. This observation was not consistent with concurrent fecal lactoferrin testing, which was consistent between exercise years, and likely represents variability in technician interpretation of fecal leukocyte stains.

Clinical and laboratory findings were evaluated to assess their potential as modalities for the diagnosis of invasive enteropathogens, as well as, the diagnosis of *Campylobacter* infection. Test performance characteristics for the prediction of invasive enteropathogens are detailed in Table 2. In general, clinical findings were not sensitive with the exception of abdominal cramping; however, this symptom had a specificity of less than 15%. Less frequent clinical findings such as high-volume diarrhea, gross blood in stools, documented fever at presentation, and hemoccult positive stools did yield greater specificity. However, the overall accuracy of these findings as represented by the

area under the ROC curve was low (< 0.65). The discordant findings in fecal leukocyte results between exercise years led to significant differences in test performance determination (Table 2). Despite these differences, the fecal leukocyte test sensitivity was less than 50% in both years. The lactoferrin latex agglutination and plasma C-reactive protein tests provided reasonable sensitivity but lacked specificity. Both tests yielded negative likelihood ratios amenable to ruling out the presence of an invasive enteropathogen.

Clinical findings and bedside evaluation of the patient's stool specimen were evaluated for their ability to support a diagnosis of *Campylobacter* enteritis (Table 3). Comparable, relatively poor, test performance as seen with invasive enteropathogens was observed. Given the predominance of *Campylobacter* in this case series, it is not surprising that the findings are similar. Table 4 provides an assessment of field laboratory tests of systemic or intestinal inflammation, as well as, *Campylobacter*-specific rapid diagnostic tests. Fecal leukocyte stains and measurement of circulating lymphocytes producing antibodies against *Campylobacter*-specific antigens in the ELISPOT assay produced poor results in all measures of test performance. The lactoferrin latex agglutination test demonstrated high sensitivity and negative predictive value with low specificity. The overall accuracy of this test was comparable to the plasma C-reactive protein findings.

The two stool-based *Campylobacter*-specific tests, PCR and EIA, yielded the highest specificity and positive likelihood ratios of all tests under evaluation. More cases were evaluated using the EIA than the PCR, which had evaluation limited to the second exercise year. False negative results in the PCR assay led to lower sensitivity than

observed in the EIA. A total of 7 culture positive *Campylobacter* cases had negative results on the PCR test. Simultaneous positive controls to detect DNA in the stool specimen were positive in all but one of the specimens ruling out nonspecific inhibitors as the primary explanation for false negative results. Of these 7 culture-positive PCR-negative specimens, 3 were also negative for the *Campylobacter* EIA test. The remaining 4 specimens had 4+ reactions in the EIA test. There were 3 culture-negative PCR-positive specimens. Two of these specimens were 4+ positive by the EIA. A total of 6 culture-positive EIA-negative specimens were observed. All of these cases had follow-up EIA tests undertaken on stools collected at either 3 or 7 days after initial treatment. None of the follow-up EIA tests were positive nor were any of the follow-up stool cultures. Four culture-negative EIA-positive specimens were observed. Two of these cases had follow-up stool specimens post-treatment in which one was culture and EIA negative and the other was culture and EIA positive. Post-treatment stool cultures and EIA tests at 3 and 7 days after first antibiotic dose detected no new positive EIA cases. Among initially EIA positive cases there continued to be positive responses in 31% at day 3 and 23% at day 7. The semi-quantitative result trended toward a higher proportion of 3-4+ results in 80% of pretreatment specimens as compared to 57% in post-treatment positive tests.

Discussion

Rapid diagnostic tests range from the inexpensive Gram stain for presumptive identification of *Campylobacter* spp. (sensitivity, 60-90%) to the more technically complex and more expensive polymerase chain reaction (PCR) [24-27]. Utility of a diagnostic test is dependent upon the prevalence of the disease in the population. In figures 1 and 2 post-test probability of *Campylobacter*-associated illness is presented in

various endemic settings. The presence of certain clinical features including gross blood in stools or documented fever at presentation is very specific (≥ 93%) for *Campylobacter* infection; however, this diagnosis will be missed in 70-80% of cases. The inability of the clinical presentation to guide therapy for travelers' diarrhea was documented by Ericsson and coworkers [28]. Reliance on specific yet insensitive clinical features would lead to withholding of therapy in individuals that may benefit from early treatment. The ability of positive test findings to alter the probability of *Campylobacter* infection is most effective using a pathogen-specific stool based test such as the EIA. In a hyperendemic setting such as Thailand with prevalence estimates of 50% a positive EIA yields a 94% post-test probability of disease. Concurrent findings of dysentery or fever further increase the post-test probability of a positive EIA to 98%. The impact of a positive EIA in a region with lower *Campylobacter* prevalence is less dramatic. An estimated prevalence of 5%, as may be observed in U.S. clinics, has a post-test probability of 46% in the event of a positive EIA. Unlike the setting in Thailand, bedside clinical findings of dysentery or fever provide additive benefit in increasing the probability to 72% in this setting.

The *Campylobacter* EIA has been previously evaluated using frozen stool sample collections and in clinic-based series in the United States and Europe [29-31]. Positive EIA results have been documented out to five years from *Campylobacter*-positive stool specimens stored at -20^{o}C with a detection threshold of 3×10^{6} CFU/g of stool [32]. Real-time assessment under routine conditions has documented sensitivity of 89% and specificity 98-99% in settings with prevalence of *Campylobacter* ranging between 3 and 8% [29, 31]. The positive likelihood ratio exceeded 30 with negative likelihood ratio less than 0.15 in both previous evaluations. These findings are comparable to the likelihood

ratios observed in this study, which is the first evaluation of this test under field conditions outside of an established hospital. The *ceu*E-based multiplex PCR evaluated in this study had previously been evaluated using frozen stool specimens with comparisons between culture and PCR result based on microbiologic recovery from stored specimens [17]. This previous study documented much higher positivity rates in PCR than culture, 77 versus 56%. Based on the observed test performance in this study using fresh specimens, the earlier observation was likely due to nonviable organisms in frozen specimens rather than a significant difference in higher PCR sensitivity. Higher rates of PCR false negative specimens were observed in this study, 18 vs. 8%, then from frozen specimens. Further development of this test is needed before it may gain clinical utility. The *Campylobacter*-specific antibody-secreting cell assay lacked test performance parameters supportive of its clinical use. Potential improvements in this test may derive from use of more purified antigens that are broadly cross-reactive in *Campylobacter* species yet not cross-reactive with other bacterial enteropathogens; however, the kinetics of the transient circulation of these lymphocytes following mucosal infection may limit the diagnostic potential of this test at time of clinical presentation [33].

Rapid diagnostic tests based on detection of an inflammatory state rather then a specific pathogen were unable to increase post-test probability beyond 75% in the event of a positive result (Figure 1). However, both the blood-based C-reactive protein and the stool-based lactoferrin test greatly reduced the likelihood of *Campylobacter* infection with a negative result from a pretest probability of 50% to 14% for C-reactive protein and 7% for lactoferrin (Figure 2). Given the improved ability to rule out *Campylobacter* infection and infrequency of obtaining blood specimens for the clinical management of

diarrhea, the fecal lactoferrin assay was the preferable inflammatory enteritis screening test. These findings are consistent with a systematic analysis of fecal screening tests [34]. A more recent meta-analysis stratified fecal screening test performance based on population studied, resource-poor regions and developed countries, in order to account for pathogen prevalence differences and disease spectrum [35]. In developing countries, the rapid stool-based markers of inflammatory enteritis performed poorly to rule-in disease possibly due to high endemicity of enteropathogens, asymptomatic carriage, frequent findings of inflammatory markers, and comorbid noninfectious conditions that may lead to positive findings as postulated by the authors [35]. The fecal lactoferrin assay has demonstrated value as a negative predictor of invasive enteropathogens in developing world children with acute diarrhea as also shown in this deployed population [36, 37]. In this study the LFLA test would have failed to identify 9 invasive pathogens (7%) or 4 *Campylobacter* cases (4%).

The *Campylobacter* regional predominance previously documented in Thailand limits the broad application of these results across operational platforms in various regions. These results coupled with analyses from an area of ETEC predominance (with some contribution from *Shigella* species) will better permit generalization. The *Campylobacter* EIA provided desirable test performance to rule-in or rule-out infection under field conditions with results available within 2 hours. Given the prevalence of *Campylobacter* in this setting and the high rates of fluoroquinolone resistance this test most aids the clinician in determining management.

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Tables and Figures

Table 1. Characteristics of U.S. military personnel presenting with acute diarrhea on deployment in Thailand

	Cobra Gold exercise year	
	2000	2001
Thai city (base for training exercise)	Nakhon Sri Thammarat	Phitsanulok
Demographics		
Enrolled # cases	109	73
Median age (IQR)	26 (22-33)	26 (23-32)
Gender (% male)	90	90
Malaria prophylaxis (%)[1]	91	75
Median days in-country pre-illness (IQR)	11 (7-16)	12 (8-15)
Clinical presentation		
Median days of illness duration (IQR)	1 (1-3)	1 (1-2)
Median no. diarrheal stools previous 24h (IQR)	5 (4-10)	5 (3-8)
Fever, by report (%)	51	49
Vomiting (%)[1]	27	11
Abdominal cramps (%)	89	85
Bedside evaluation		
Oral temperature, $\geq 100^{o}$F (%)	20	28
Stool character, watery liquid (%)	37	41
Visible gross blood (%)	13	14
Stool hemoccult positive (%)	35	36
Field laboratory evaluation		
Fecal WBC positive (%)[1]	28	58
Fecal lactoferrin positive (%)	79	80
Serum C-reactive protein positive (%)	70	ND
Campylobacter EIA positive (%)	67	54
Campylobacter PCR positive (%)	ND	51
Campylobacter ASC positive (%)	44	ND
Pathogen isolation (%)		
Campylobacter	67	55
Nontyphoidal *Salmonella*	15	25
Plesiomonas shigelloides	9	6
Noninvasive bacteria (ETEC, eae^{+} *E. coli*)	11	14
Viral (Rotavirus, Calicivirus)	6	7
None identified	17	26

Note: ND signifies assay not done during exercise year. IQR signifies interquartile range.
[1] Signifies differences in a given characteristic between exercise years ($P < .05$).

Table 2. Clinical and laboratory findings as diagnostic modalities for invasive enteropathogens

Finding (N)	Sensitivity	Specificity	NPV	PPV	LR^+	LR^-	AUC
Diarrhea frequency (past 24h)							
6-9 loose stools	52 (43, 60)	70 (55, 82)	36 (27, 46)	82 (71, 89)	1.7 (1.1, 2.7)	0.7 (0.5, 0.9)	.61 (.52, .70)
$\geq$ 10 loose stools	28 (21, 37)	86 (73, 94)	32 (24, 40)	84 (69, 93)	2.0 (1.0, 4.3)	0.8 (0.7, 1.0)	.57 (.48, .66)
Gross blood in stools	17 (11, 25)	96 (84, 99)	29 (23, 37)	92 (72, 99)	4.0 (1.0, 16)	0.9 (0.8, 1.0)	.56 (.47, .65)
Abdominal cramps	88 (81, 93)	14 (6, 28)	30 (14, 53)	73 (66, 80)	1.0 (0.9, 1.2)	0.9 (0.4, 2.0)	.51 (.42, .61)
Vomiting (absence of finding)	24 (17, 33)	90 (77, 96)	30 (23, 38)	87 (70, 95)	2.3 (1.0, 5.6)	0.9 (0.7, 1.0)	.57 (.48, .66)
Fever (by report)	58 (49, 66)	69 (54, 81)	38 (28, 49)	84 (74, 90)	1.9 (1.2, 2.9)	0.6 (0.5, 0.8)	.64 (.55, .73)
Oral temperature $\geq 100^oF$	29 (21, 37)	92 (80, 97)	33 (25, 41)	91 (77, 97)	3.6 (1.4, 10)	0.8 (0.7, 0.9)	.60 (.52, .69)
Hemoccult positive	42 (33, 51)	84 (70, 93)	34 (25, 43)	89 (77, 95)	2.7 (1.3, 5.5)	0.7 (0.6, 0.8)	.63 (.54, .72)
Opaque/watery liquid stool	42 (33, 51)	69 (54, 81)	31 (23, 41)	78 (66, 87)	1.4 (0.9, 2.2)	0.8 (0.7, 1.1)	.56 (.46, .65)
Dysentery/documented fever	42 (33, 51)	87 (74, 95)	35 (27, 45)	90 (79, 96)	3.3 (1.5, 7.1)	0.7 (0.6, 0.8)	.64 (.56, .73)
Dysentery/documented fever or hemoccult positive	57 (48, 65)	78 (63, 88)	39 (29, 49)	88 (79, 94)	2.6 (1.4, 4.5)	0.6 (0.5, 0.7)	.67 (.58, .76)
Fecal leukocytes[1]							
Any positive	44 (35, 54)	67 (52, 80)	32 (23, 43)	78 (66, 87)	1.4 (0.9, 2.2)	0.8 (0.6, 0.2)	.58 (.49, .68)
$\geq$ 1-5/HPF	33 (24, 42)	85 (71, 93)	33 (25, 42)	84 (70, 93)	2.1 (1.0, 4.4)	0.8 (07, 1.0)	
Lactoferrin latex agglutination							
Any positive	93 (87, 97)	56 (41, 70)	75 (58, 87)	85 (78, 90)	2.1 (1.5, 2.9)	0.1 (0.1, 0.2)	.77 (.68, .86)
$\geq$ 2+	86 (79, 91)	65 (49, 78)	63 (48, 76)	87 (80, 92)	2.4 (1.7, 3.6)	0.2 (0.1, 0.4)	
Serum C-reactive protein	83 (73, 90)	75 (53, 89)	56 (38, 73)	92 (83, 97)	3.3 (1.7, 6.7)	0.2 (0.1, 0.4)	.81 (.71, .91)

Note: The following abbreviations are used: negative predictive value (NPV), positive predictive value (PPV), positive likelihood ratio (LR^+), negative likelihood ratio (LR^-), area under the curve (AUC), and high power field (HPF).

[1] Fecal leukocyte test performance differed significantly between exercise years as follows for the '$\geq$ 1-5/HPF' classification (year 1 versus year 2): sensitivity (28 vs. 40%), specificity (96 vs. 75%), NPV (30 vs. 38%), PPV (95 vs. 76%), LR^+ (6.1 vs. 1.6), and LR^- (both 0.8).

Table 3. Clinical and bedside stool characteristics as *Campylobacter* diagnostic modalities

Finding (N)	Sensitivity	Specificity	NPV	PPV	LR^+	LR^-	AUC
Diarrhea frequency (past 24h)							
6-9 loose stools	53 (44, 63)	67 (54, 77)	47 (37, 57)	72 (61, 81)	1.6 (1.1, 2.3)	0.7 (0.5, 0.9)	.60 (.51, .68)
$\geq$ 10 loose stools	32 (23, 41)	87 (76, 94)	44 (36, 53)	80 (64, 90)	2.4 (1.2, 4.7)	0.8 (0.7, 0.9)	.59 (.51, .68)
Gross blood in stools	20 (13, 29)	97 (89, 100)	42 (34, 50)	92 (72, 99)	6.5 (1.6, 27)	0.8 (0.8, 0.9)	.58 (.50, .67)
Abdominal cramps	89 (82, 94)	16 (9, 28)	48 (27, 69)	64 (56, 71)	1.1 (0.9, 1.2)	0.7 (0.3, 1.4)	.53 (.44, .62)
Vomiting (absence of finding)	27 (19, 36)	90 (79, 95)	42 (34, 51)	81 (64, 91)	2.5 (1.2, 5.5)	0.8 (0.7, 0.9)	.58 (.50, .67)
Fever (by report)	64 (54, 72)	72 (60, 82)	54 (44, 65)	79 (69, 87)	2.3 (1.5, 3.4)	0.5 (0.4, 0.7)	.68 (.60, .76)
Oral temperature $\geq$ 100^{o}F	33 (24, 42)	93 (83, 97)	46 (37, 54)	88 (74, 96)	4.5 (1.9, 11)	0.7 (0.6, 0.8)	.63 (.55, .71)
Hemoccult positive	47 (37, 57)	86 (74, 93)	48 (38, 57)	85 (73, 93)	3.3 (1.7, 6.2)	0.6 (0.5, 0.8)	.66 (.58, .74)
Opaque/watery liquid stool	45 (36, 55)	72 (60, 82)	45 (35, 54)	73 (60, 82)	1.6 (1.1, 2.5)	0.8 (0.6, 1.0)	.59 (.50, .67)
Dysentery/documented fever	48 (38, 57)	89 (79, 95)	50 (41, 60)	88 (77, 95)	4.5 (2.2, 9.3)	0.6 (0.5, 0.7)	.69 (.61, .76)
Dysentery/documented fever or hemoccult positive	63 (53, 72)	79 (67, 88)	55 (44, 65)	84 (74, 91)	3.1 (1.9, 5.1)	0.5 (0.4, 0.6)	.71 (.65, .79)

Note: The following abbreviations are used: negative predictive value (NPV), positive predictive value (PPV), positive likelihood ratio (LR^+), negative likelihood ratio (LR^-), and area under the curve (AUC).

Table 4. Field laboratory tests as *Campylobacter* diagnostic modalities

Finding (N)	Sensitivity	Specificity	NPV	PPV	LR^+	LR^-	AUC
Fecal leukocytes							
Any positive	45 (35, 55)	65 (52, 76)	44 (34, 54)	66 (53, 77)	1.3 (0.9, 1.9)	0.9 (0.7, 1.1)	.57 (.48, .66)
$\geq$ 1-5/HPF	34 (25, 44)	82 (70, 90)	45 (36, 54)	73 (58, 85)	1.8 (1.0, 3.3)	0.8 (0.7, 1.0)	
Lactoferrin latex agglutination							
Any positive	96 (91, 99)	48 (36, 60)	89 (73, 96)	75 (67, 82)	1.9 (1.5, 2.3)	0.1 (0.1, 0.2)	.80 (.73, .87)
$\geq$ 2+	91 (84, 95)	58 (46, 70)	80 (65, 89)	78 (70, 85)	2.2 (1.6, 2.9)	0.2 (0.1, 0.3)	
Serum C-reactive protein	89 (79, 95)	69 (51, 83)	75 (56, 88)	85 (75, 92)	2.8 (1.7, 4.6)	0.1 (0.1, 0.2)	.83 (.75, .91)
Campylobacter EIA	95 (88, 98)	94 (84, 98)	91 (81, 96)	96 (90, 99)	16 (6.0, 40)	0.1 (0.1, 0.2)	.94 (.89, .98)
Campylobacter PCR	82 (65, 92)	90 (72, 97)	79 (61, 90)	91 (75, 98)	7.9 (2.7, 23)	0.2 (0.1, 0.4)	.86 (.76, .95)
Campylobacter-specific ASC	46 (55, 74)	60 (42, 76)	38 (25, 52)	68 (52, 81)	1.2 (0.7, 1.9)	0.9 (0.6, 1.3)	.53 (.41, .65)

Note: The following abbreviations are used: negative predictive value (NPV), positive predictive value (PPV), positive likelihood ratio (LR^+), negative likelihood ratio (LR^-), area under the curve (AUC), high power field (HPF), enzyme immunoassay (EIA), polymerase chain reaction (PCR), and antibody-secreting cell assay (ASC

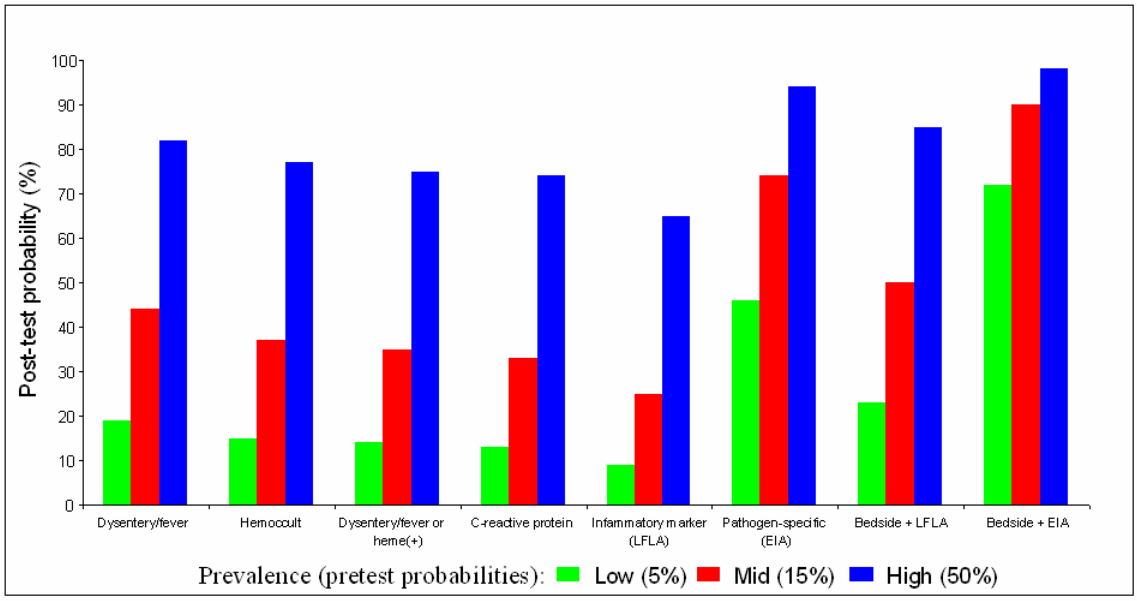

Figure 1. Post-test probability of *Campylobacter*-associated illness based on diagnostic approach (positive test findings) in various endemic settings (% prevalence)

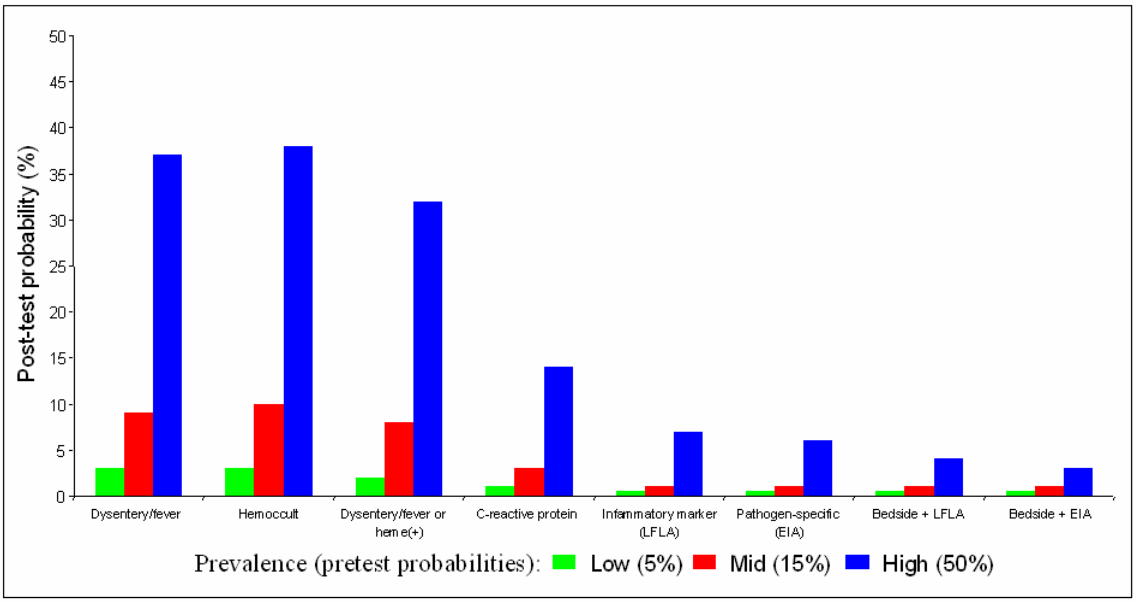

Figure 2. Post-test probability of *Campylobacter*-associated illness based on diagnostic approach (negative test findings) in various endemic settings (% prevalence)

Travelers' Diarrhea in Thailand: Randomized, Double-Blind, Placebo-Controlled Trial Comparing Azithromycin-Based Regimens (Single Dose and 3-Day) versus Levofloxacin (3-Day)

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This study was approved by ethical review committees from the Naval Medical Research Center (Protocol # 31528), the Walter Reed Army Institute of Research (WRAIR # 792), and Uniformed Services University of the Health Sciences (G187MT) in compliance with all Federal regulations governing the protection of human subjects.

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Running Title: Traveler's diarrhea treatment with azithromycin in Thailand.

Word count (text only): 5,333

Abstract

Background: Traveler's diarrhea in Thailand is frequently caused by *Campylobacter jejuni*. Fluoroquinolone-resistance rates in *C. jejuni* have exceeded 85% in recent years and reduced fluoroquinolone therapeutic efficacy has been observed.

Objective: To evaluate different azithromycin regimens in comparison to 3-day fluoroquinolone for empiric treatment of traveler's diarrhea in Thailand.

Design: Randomly assigned, double-blind, placebo-controlled trial.

Setting: Military field clinics in Thailand.

Patients: 156 patients with acute diarrhea.

Intervention: Single dose of azithromycin (1 gram), 3 days of azithromycin (500 mg), or 3 days of levofloxacin (500 mg).

Measurements: Outcome based on clinical observations and stool microbiology. Efficacy was assessed daily for 3 days and at 1 week.

Results: *C. jejuni* was the predominant pathogen accounting for 59-71% of cases across treatment groups with levofloxacin resistance in 50% and none with azithromycin. Clinical cure by 72 hours was highest at 96% with single dose azithromycin compared to 85% with 3-day azithromycin and 71% with levofloxacin (P = .002). Time to last diarrheal stool was less for single dose azithromycin, 35 h, than 49 h for the other groups (log rank, P = 0.03). Levofloxacin was inferior except in the first 24 hours among cases without an identified pathogen. Microbiologic eradication was significantly better for azithromycin-based regimens, 96-100%, as compared to levofloxacin at 38% (P = .001). Higher rate of post-treatment nausea in the 30 minutes after first dose (14 vs.< 6%, P = 0.06) were observed as a mild self-limited complaint with single dose azithromycin.

Conclusions: Single-dose azithromycin is recommended for empiric therapy of travelers' diarrhea acquired in Thailand and should be further investigated for broader application in areas with more diverse enteropathogens.

Introduction

Diarrhea is an extremely common illness reported by civilian and military travelers from industrialized countries visiting lesser developed countries (1, 2). The recommended empiric antibiotic therapy, when indicated, for travelers' diarrhea has been a 3-day course with a fluoroquinolone (FQ) (3, 4). In Thailand, numerous surveys among deployed U.S. military personnel have shown enteropathogenic *Campylobacter* species, *C. jejuni* and *C. coli*, to account for 20-60% of diarrheal cases (5-10). FQ resistance among *Campylobacter* is observed in $\geq$ 85% of isolates from Thailand in recent years (9, 11).

Concerns over increasing FQ-resistance led U.S. Department of Defense researchers to investigate azithromycin as an alternative for empiric management of travelers' diarrhea in Thailand in 1993 (7). Azithromycin 500 mg daily was found to have comparable efficacy to ciprofloxacin 500 mg daily with both regimens delivered over 3 days. The statistical power of the study limited detection of moderate effect differences of the azithromycin regimen. In fact, there were only two clinical failures in the study, both in ciprofloxacin-treated *Campylobacter* cases. Significant differences in improved microbiologic eradication of *Campylobacter* were demonstrated with azithromycin; however, this did not translate into statistically significant overall clinical differences. The only statistically significant clinical finding on subgroup analysis was a reduced duration of illness in non-*Campylobacter* cases with ciprofloxacin. The observations that non-*Campylobacter* bacterial etiologies represent as many as 40% of cases and azithromycin was not clearly superior for clinical outcomes to ciprofloxacin (even in

Campylobacter cases) led experts to cautiously recommend continued first-line FQ therapy (12).

The FQ-resistance rate among the *C. jejuni/coli* isolates in the previous trial was approximately 65%. This rate continued to rise to approximately 80% by 1999 with increasing data from observational studies during Cobra Gold exercises suggesting decreasing therapeutic effectiveness for FQ first-line therapy (Tribble, unpublished data). In addition, clinical studies demonstrating azithromycin efficacy using a 5-day, as well as, a single dose regimen, in dysenteric illness caused by *Shigella* species and traveler's diarrhea, in an enterotoxigenic *E. coli* predominant region, provided further evidence supporting azithromycin first-line therapy in bacterial enteritis (13-15). The objective of this study is to compare the standard 3-day FQ regimen with two azithromycin-based regimens, a 3-day multi-dose and a single-dose.

Methods

Participants and Subject Eligibility

The trial was conducted during the training periods, May 2000 in Nakhon Sri Thammarat and May 2001 in Phitsanulok, Thailand. Temporary military medical units operate during the period of the exercise. Active duty personnel presenting to the clinic with acute diarrhea were evaluated for enrollment following informed consent. Diarrhea was defined as three or more loose stools or two or more loose stools with one or more associated complaints, including abdominal cramps, nausea, vomiting, or fever in a 24-hour period. Inclusion criteria included illness consistent with the diarrhea definition, symptoms of $\leq$ 96 hours duration, illness compatible with ambulatory management, and

ability to comply with follow-up procedures. Exclusion criteria included positive urine pregnancy test, history of allergy to macrolide or quinolone antibiotics, use of antibiotics (excluding malaria prophylaxis with either mefloquine or doxycycline) in the 72 hr prior to presentation, medications known to have drug-drug interaction with either study drug, and history of seizures (relative contraindication for FQ therapy). The use of non-antibiotic antidiarrheal medications post-enrollment, such as loperamide, was not allowed during the study.

Treatment Assignment, Randomization, and Blinding Procedures

Azithromycin (dispensed as either 500 mg daily for 3 days or 1000 mg in a single dose) and levofloxacin (500 mg daily for 3 days) were compared during the clinical trial. Pfizer Pharmaceuticals Clinical Research Division (Groton, CT) supplied study medications and their respective placebo formulations at no cost. Pfizer pharmacy representatives supplied the computer-generated random-number code with a block size of 6. The study medications had an identical appearing placebo. To maintain blinding of the patients and researchers, each patient received tablets from each study medication (active drug or placebo). The medicines were dispensed in a three-day package with a separate bottle for each treatment day of study. Volunteers consenting to participate were sequentially assigned the next available treatment code number. The blinding procedure was maintained during the laboratory and analysis phases of the study.

Clinical Monitoring

A standardized questionnaire and medical examination form was used. Fluid therapy was provided as necessary. Patients were asked to provide a stool specimen prior

to first antibiotic dose. Stool characterization and bedside occult blood testing (Hemoccult, Beckman Coulter, Inc., Fullerton, CA) was completed by the study physician prior to transporting specimen to field laboratory. The study physician administered the first antibiotic dose under direct observation. The patient was observed for 30 minutes to monitor for immediate adverse reactions. Patients were provided a diary card to record the number of loose stools (each 6-hour period), daily symptoms (abdominal cramps, nausea, vomiting, fever, or bloody stools), and an assessment of their functional ability for each 24-hour interval.

Follow-up evaluations at 24 and 72 hours monitored for clinical outcomes and potential drug toxicity. The subjects were informed of potential side effects of either study medicine and specifically asked about the development of these symptoms during clinical evaluations. Final follow-up (5-7 days after first antibiotic dose) was completed to assess clinical response post-treatment and obtain a stool specimen for determination of microbiologic eradication.

Stool Microbiology

A stool specimen obtained at initial presentation determined pretreatment stool microbiology. Post-treatment stool specimens for eradication were obtained between study days 5-9. Primary plating of stool specimens was undertaken in an onsite field laboratory as previously described (16). *Campylobacter* species were isolated using a membrane filter method on non-selective blood agar before and after enrichment (17). Isolates were transported to the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok for species identification and susceptibility testing as previously described (18-20). Five lactose-fermenting and 5 non-lactose-fermenting *E. coli* colonies

per specimen were tested using DNA probes for detection of ETEC toxins (LT and ST), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), and enteropathogenic *E. coli* (EPEC; *eae* and EAF plasmid) (21, 22).

Fecal lactoferrin was detected using the commercial Leuko-Test® kit (TechLab, Blacksburg, VA) following manufacturer's instructions. The presence of lactoferrin is detected by a visually read positive agglutination of $\geq$ 1+ as defined by manufacturer. Fecal leukocytes were semi-quantitatively determined using Methylene blue-stained fecal smears examined under microscopy. Microscopy evaluation of fresh stool specimens was used to evaluate for stool parasites. Stool specimens were examined for Rotavirus and Calicivirus antigens using a commercially available ELISA (Rotazyme; Abbott Laboratories, North Chicago, IL) and a non-commercial antigen-capture Calicivirus ELISA (23).

Antibiotic susceptibility testing for *Campylobacter* species were performed using the E-test strip (AB Biodisk, Piscataway, N.J.) method for obtaining minimum inhibitory concentrations (MIC) for azithromycin, levofloxacin, and ciprofloxacin (24). The MIC, using the E-test strip, provides an intersection point for bacterial growth across the antibiotic-impregnated strip that represents the minimum amount of antibiotic needed to inhibit microbial growth on the agar plate. This MIC value is assessed relative to previously determined cutoff criteria designating the bacterial isolate as either susceptible or resistant. Isolates were tested on Mueller-Hinton blood agar medium with incubation at 37^{o}C in microaerobic conditions. Interpretative criteria for Enterobacteriaceae and quality control guidelines established by the National Committee for Clinical Laboratory Standards were used (25). Resistance among *C. jejuni/coli* for ciprofloxacin is defined as

a MIC $\geq$ 4 μg ml^{-1}, levofloxacin as MIC $\geq$ 8 μg ml^{-1} and azithromycin as MIC $\geq$ 8 μg ml^{-1} (26). The MIC upper limit for FQ antibiotics measured was 64 μg per milliliter. Measurements exceeding this MIC were coded as 64 μg ml^{-1} for calculation purposes. Antibiotic susceptibility testing of non-*Campylobacter* isolates was determined by the disk diffusion method of Bauer and colleagues (27), as previously described (11).

Outcome Measures

The primary outcome is diarrhea symptom abatement and shortened duration of illness. Clinical cure is defined as resolution of diarrhea and diarrhea-associated signs/symptoms within 72 hours of first dose of study medication. The time to the last diarrheal stool defined as the last Grade 3-5 stool (loose/liquid stool conforming to the shape of the collection container) occurring in a 24-hr period meeting the diarrhea definition was compared. A clinical cure was also evaluated for the 24 and 48-hour time points following first antibiotic dose, as well as, the number of unformed stools in each 24-hour period. A microbiologic cure was defined as eradication of the patient's isolate, previously detected on the pre-treatment stool culture, at follow-up approx. 48-72 hours (inclusive period is study days 5-9) after last dose of study medication.

Statistical Analysis

We estimated that a sample size of 180 cases with acute diarrhea was necessary to assess the primary clinical outcome variable (60 patients per group). The primary clinical outcome used to estimate study size was the proportion of patients meeting the clinical cure definition. The estimate was based on demonstrating a 20% effect size difference, at 80% power, between the treatments with the highest and lowest cure rates based on

previously observed FQ 72-hr cure rates approximating 75% (Tribble, unpublished data). Due to lower diarrhea rates in the second enrollment year we failed to reach the target sample size. Intention to treat analysis based on initial group assignment is presented. Patients lacking follow-up data are coded as treatment failures in the intention to treat analysis. The majority of the presented results are based on clinically evaluable patients. An evaluable subject is defined as a patient receiving follow-up 72 hours after first antibiotic dose with completion of the regimen or a patient requiring treatment modification due to clinical illness progression during the 72-h monitoring period.

Subject baseline characteristics and summary follow-up findings were compared using analysis of variance, Kruskal-Wallis tests, and chi-square tests, as appropriate. Differences in the frequencies of clinical cures and microbiologic eradication rates between study regimens were tested for significance using chi-square tests (28). Confidence intervals for primary outcomes were generated using a normal approximation to the binomial distribution. Rates of adverse reactions were similarly compared between study regimens. Differences in recovery times were evaluated using Kaplan-Meier analyses (time to last diarrheal stool), log-rank (overall differences in response curves), and generalized Wilcoxon tests (response curve differences emphasizing early failures) (28). All tests were 2-tailed, and *P* values < .05 were considered statistically significant (Bonferroni adjustments made as appropriate for multiple comparisons).

Logistic regression and Cox proportional hazard modeling were used to evaluate effects of non-treatment factors on clinical outcome independent of group assignment. The dependent variables used for logistic regression and Cox proportional hazard analyses were the cure rates at 72 hr and the time to last diarrheal stool, respectively.

Treatment group was assessed as a dichotomous variable, azithromycin-based regimen, given the limited statistical power when all study groups are included. Variable selection for inclusion in model building was undertaken using exploratory analysis, two-way contingency table analysis, and Mantel Haenszel chi-square stratified analysis. Selected variables for logistic regression analysis must have shown a *P* value < .20 in bivariate analysis for subsequent inclusion. Covariates included in analyses were dichotomous pretreatment indicators of disease severity, presence of dysentery and high frequency diarrhea (≥ 10 loose/liquid stools/24h pretreatment), and isolate antibiotic susceptibility (based on individual treatment received). A forced entry method of regression analysis evaluated all covariates using likelihood ratio testing. The risk ratio for each predictor variable was calculated as the exponent of the regression coefficient with 95% confidence intervals. Results of the patient surveys, symptom diaries, physician findings, and microbiologic results were entered into an EpiInfo version 6.04 database. Statistical analysis was performed using SPSS for Windows (version 10.1).

Results

Subject Enrollment and Characteristics

A total of 156 of 222 (70%) military personnel presenting with a chief complaint of diarrhea met entry criteria and provided informed consent for enrollment over two consecutive 1-month annual training exercises in Thailand. The results of the randomized distribution among the three treatment arms are shown in Figure 1. A larger number of troops presented to clinics in the first study year (2000) accounting for 63% of total enrolled subjects. Overall study population characteristics included median age of

26 y, predominantly male (89%) and junior enlisted rank (71%). Prior Thailand travel was reported in 27% with 71% of these individuals participating in a prior Cobra Gold exercise. Prior traveler's diarrhea episode while traveling in a developing country was relatively uncommon at 16%. Malaria prophylaxis was used in 87% of volunteers overall with doxycycline as the medication in 97%. Patient characteristics are detailed in Table 1 by treatment group assignment. Observed differences included slightly higher percentage of women in the single dose azithromycin group, 19 vs. 4-9%, and less frequently reported prior traveler's diarrhea history, 6 vs. 22-26%, and prior Thailand travel, 17 vs. 31-33%, among the volunteers randomized to receive levofloxacin.

As observed in previous exercises, the mean time in country prior to diarrhea onset, 12 days, occurred during the second week of the deployment. Volunteers typically had minimal delay in presenting to clinic from time of illness onset with mean diarrhea duration of 1.6 days. The illness was characterized with an average of 7 episodes of loose or liquid stools in the 24 hours preceding presentation, fever in 50%, abdominal cramps in 89%, vomiting in 21%, and dysentery in 14%. Table 2 provides a comparison of the clinical manifestations, stool-based markers of inflammatory diarrhea, and initial clinical management for volunteers based on treatment assignment. Volunteers randomized to receive single dose azithromycin more commonly had documented fever in the clinic prior to first antibiotic dose; however, an equal percentage of volunteers reported fever. Approximately 70% of volunteers in all groups reported a reduction in activity related to their illness with about 20% requiring intravenous hydration and 20-30% removed from work for at least 1 day. Less than 15% of volunteers reported self-treatment with a non-antibiotic medication such as loperamide or bismuth subsalicylate without differences by

treatment assignment. No difference in cure rates were observed based on pre-treatment assignment use of anti-diarrheal medication and no further use was permitted as part of eligibility criteria.

A total of 8 volunteers disenrolled for the following reasons: 4 required treatment modification due to illness progression, 3 were lost to follow-up, and 1 was noncompliant with study medication as shown on the Figure 1 trial profile. The 4 volunteers requiring treatment modification were all in the levofloxacin group with 3 being diagnosed with levofloxacin-resistant *C. jejuni* from pretreatment stool culture. All 4 received azithromycin 500-mg daily for 3 days with symptom resolution. These volunteers were censored for time to event analyses.

Distribution of Enteric Pathogens

An enteric pathogen, typically bacterial, was identified in 81% of the pre-treatment stool cultures (n = 155) from enrolled volunteers (Table 3). The most common isolate was *C. jejuni/coli* (64%) and nontyphoidal *Salmonella* spp. (17%). Multiple bacterial isolates were detected in 18% of pretreatment cultures. Among *Campylobacter*-associated cases, 22 of 99 isolates (22%) had at least one additional pathogen detected. Most common copathogens included *Salmonella* spp. (n = 12), *Plesiomonas* (n = 7), *eae*$^+$ *E. coli* (n = 6) and Rotavirus (n = 4). Speciation, by biochemical methods, of *Campylobacter* isolates identified *C. jejuni* in 95% with *C. coli* accounting for the remainder. Antibiotic susceptibility patterns for *C. jejuni/coli* using E-test methodology was as follows: no azithromycin resistance (MIC_{50} = 0.047; MIC_{90} = 0.094), 50% levofloxacin resistance (MIC_{50} = 6.0; MIC_{90} > 64.0), and 93% ciprofloxacin resistance (MIC_{50} = 16.0; MIC_{90} > 64.0). Non-*Campylobacter* bacterial isolates antibiotic

resistance rates for the study agents and commonly surveyed antibiotics are as follows: *E. coli* (*n* = 18) – levofloxacin 3.8%, azithromycin 5.6%, ampicillin 28%, tetracycline 83%, chloramphenicol 22%, TMP-SMX 44%, and nalidixic acid 17%; *Salmonella* (*n* = 28) - levofloxacin none, azithromycin 14%, ampicillin 14%, tetracycline 89%, chloramphenicol 25%, TMP-SMX 11%, and nalidixic acid 43%; and *Plesiomonas* (*n* = 11) - levofloxacin none, azithromycin none, ampicillin 18%, tetracycline 55%, chloramphenicol none, TMP-SMX 9.1%, and nalidixic acid 18%. *Campylobacter* isolates were also noted to have high tetracycline resistance rates, 86%, as observed in *E. coli* and *Salmonella* isolates compatible with selective pressure due to high rates of doxycycline use for malaria prophylaxis.

Clinical Outcomes

Complete clinical resolution was uncommon by 24 hours after first antibiotic dose irrespective of regimen with levofloxacin having the highest cure rate of 25% (Table 4). Stratified analysis within the levofloxacin demonstrated these early cures to be primarily among patients without an identified pathogen. The azithromycin-based regimens begin to demonstrate improved cure rates over the FQ as early as 48 hours, 53-65% vs. 39% (*P* = 0.02). At 72 hours, azithromycin cure rates had widened further from levofloxacin, 85-96% vs. 71% (*P* = 0.001). A trend toward improved cure rates was also observed for the single dose azithromycin group over the 3-day regimen (*P* = 0.09). Other measures of clinical outcome were notably not different between study groups including the mean number of loose stools (cumulative) by 24 or 72 hours, median time to last loose stool or first formed stool, or individual non-diarrheal associated symptom duration.

The time to cure as measured by number of hours to last diarrheal stool (Figure 2) further demonstrates significant prolongation of illness in the patients receiving levofloxacin (log rank, $P = 0.03$). The mean time to last diarrheal stool is 39 hours (95% C.I. 31-47) for single-dose azithromycin as compared to 43 hours (95% C.I. 34-51) for 3-day azithromycin and 56 hours (95% C.I. 42-71) for levofloxacin. The percentage of censored subjects is highest among the levofloxacin group (8%) due to 4 subjects requiring treatment modification prior to completion of 72-h monitoring period (azithromycin single dose – none censored and 3-day 4%). Figure 3 stratifies the time to cure by isolation of *Campylobacter* pretreatment. No significant differences in mean time to cure were evident among non-*Campylobacter* cases. Prominent differences in clinical cure among the *Campylobacter* cases follow a similar pattern as the non-stratified data. Mean time to last diarrheal stool was 41 h (32-49) for single dose azithromycin compared to 47 h (95% C.I. 35-58) for 3-day azithromycin and 65 h (50-80) for levofloxacin. Intent to treat analysis demonstrates similar clinical cure outcomes. The 72-h cure rates for single dose azithromycin were 94% (49 of 52 subjects) as compared to 80% (41 of 51) in 3-day azithromycin and 70% (37 of 53) with levofloxacin ($P = 0.006$). A direct comparison, using intent to treat analysis, for azithromycin-based regimens supports selection of the 1-gram single dose regimen ($P = 0.04$).

Illness relapse occurred in two subjects in the 3-day azithromycin group. One subject had initial cure at 54 h after first antibiotic dose followed by a 48-h symptom-free period then a 24-h episode of 7 loose stools without associated complaints. The second subject had initial cure at 72 h followed by a 72-h symptom-free period then a 24-h episode of 2-3 loose stools, mild nausea, and vomiting. Neither subject required

additional treatment and follow-up stool microbiology detected no pathogens. Both subjects had pretreatment *C. jejuni* isolates with eradication by day 3 after first antibiotic dose. No other relapses were observed.

Microbiological Outcome

Microbiologic cure rates were much higher for azithromycin-based regimens than levofloxacin primarily due to *Campylobacter* cases (Table 4). Approximately 100% eradication was observed using azithromycin as compared to 21% for levofloxacin ($P < 0.001$). Further evidence of levofloxacin lack of efficacy for microbiologic cure of *Campylobacter* species was the recovery of a new *C. jejuni/coli* strain (by Lior typing) or species on post-treatment stool cultures. A new *C. jejuni/coli* strain/species was recovered in 22% of the levofloxacin-treated cases as compared to 2-4% of the subjects who received azithromycin ($P = 0.002$). Interestingly, these new isolates were not associated with clinical illness irrespective of treatment group. Among subjects receiving levofloxacin with pretreatment *Campylobacter* isolates susceptible to levofloxacin (n = 8) 63% had in vivo resistance documented with post-treatment MIC > 32; however, in vivo resistance was not associated with therapeutic failure or relapse. In the azithromycin groups, a single highly susceptible (MIC 0.064) *C. coli*-associated case (Lior 55) treated with the 1-gram dose had recovery of a Lior nontypeable highly resistant (MIC > 256) isolate on day 3 and 7 after the antibiotic dose. The subject reported last diarrheal stool at 71 hours meeting clinical cure definition without relapse. One subject in the 3-day azithromycin group also had a highly susceptible (MIC 0.064) *C. jejuni* (Lior 36) isolate pretreatment with recovery on day 3 of a Lior nontypeable highly resistant (MIC > 256)

isolate. This subject had diarrhea resolution 4.5 hours after first dose and did not follow-up for post-treatment (day 7) stool culture.

Assessment of Confounding Factors

Stratified analyses demonstrated no confounding effect on clinical outcomes for prior travel to Southeast Asia (specifically Thailand), prior history of travelers' diarrhea, pretreatment illness duration, use of malaria prophylaxis, or year of enrollment (data not shown). Clinical outcome effect, independent of treatment received, were observed for indicators of pretreatment disease severity, as measured by presence of dysentery/documented fever or high frequency diarrhea ($\geq$ 10 diarrheal stools/24-h pretreatment period), and isolate antibiotic susceptibility. The effect was apparent for the first 48 h for the measures of disease severity. Dysentery/documented fever presence had lower 48-h cure rates (57 vs. 41%, P = 0.06) in subjects without these illness characteristics. This finding was more pronounced when comparing 24-h cure rates, 8 vs. 27%, P = 0.008. Lower cure rates at 48 and 24 hours were also observed among subjects with high-frequency diarrhea in the 24-h pretreatment period, 36 vs. 57%, P = 0.03 and 5 vs. 27%, P = 0.003, respectively. The presence of a bacterial isolate resistant to the antibiotic the subject was prescribed, most typically due to levofloxacin-resistant *C. jejuni/coli*, was associated with lower cure rates out to 72 hours, 61 vs. 88%, P = 0.002.

Multivariate analyses were undertaken to evaluate the effect of an azithromycin-based regimen, adjusted for the effects of disease severity and isolate susceptibility, on clinical outcome (Table 6). Following adjustment the azithromycin regimen was 1.3 to 3.7 times (based on clinical outcome measure) more likely associated with clinical cure at 72 hours as levofloxacin treatment. The measures of disease severity were no longer

significant predictors of clinical outcome following adjustment. However, the presence of an isolate resistant to the antibiotic prescribed continued to predict an approximately 60% lower clinical cure rate at 72 hours. Figure 4 displays the time to cure based on antibiotic received stratified by pretreatment stool bacteriology. The azithromycin-treated subjects demonstrate equal rates of cure by 72 hours; however, *susceptible C. jejuni/coli* isolates appear to respond slower as compared to non-*Campylobacter* cases. Levofloxacin-treated subjects respond very rapidly (first 24 hours) for cases without an identified bacterial pathogen. Non-*Campylobacter* and susceptible *C. jejuni/coli* have similar response curves between 48-72 hours although *Campylobacter* cases appear less responsive in first 24 hours. The resistant *C. jejuni/coli* have a much-delayed response, accounting for the majority of levofloxacin lack of therapeutic efficacy.

Adverse Events

Surveillance during the 72-h period after receipt of first antibiotic dose demonstrated no severe antibiotic-related side effects and no requirement for treatment modification due to non-illness related symptoms. Single dose azithromycin did appear to have an increased rate of reported nausea as compared to the other treatments (Table 5). The nausea was of mild-moderate severity typically not associated with vomiting and lasting approximately 1 day. The complaint was relatively uncommon with 14% of subjects reporting nausea during the 30-minute first dose monitoring period with 1 episode associated with vomiting (without pill contents) and 17% of subjects reporting nausea as a new symptom over the next 3-day monitoring period. Self-limited vaginal pruritus not requiring medication was reported in 2 subjects in levofloxacin (accounting for 29% of female subjects). Transient rash most consistent with heat rash was observed

in 1 subject in each of the azithromycin groups. Headaches were reported in 22% of single dose azithromycin subjects, 32% 3-day azithromycin group, and 35% of levofloxacin group; although, in approximately 85% of these individuals the headache preceded the treatment with duration of approximately 0.5-0.9 days for all groups. Dizziness was reported equally across groups in the range of 8-12% and was of short duration (< 0.5 days).

Discussion

In this trial, azithromycin was definitively demonstrated to be the preferred antibiotic for traveler's diarrhea empiric treatment in Thailand. The single 1-gram dose was the optimal regimen. It is unclear if a 1 gram dose is required or if a similar therapeutic effect may be achieved with a lower dose. Azithromycin, an azalide antibiotic, has favorable pharmacokinetics for single-dose use with an average half-life of 11-14 hours after a single 500 mg dose achieving high tissue concentrations (29, 30). Oral bioavailability of azithromycin (500 mg dose) is approximately 37%; however, on average 46% of active drug is nonabsorbed and passed in the feces (31). The elimination dynamics provide theoretic advantage in the treatment of bacterial enteritis. Estimated gastrointestinal lumenal levels of azithromycin exceed 200 μg/ml in the 6-hour period after a 500-mg dose (31). These levels far exceed the MIC_{90} of common enteric pathogens (32). The lower dose, if proven effective, would be desirable given the observed dose-related nausea and vomiting observed with azithromycin. The rates of new-onset nausea, 8-17%, observed among azithromycin recipients in this study are higher than reported nausea rates for both 500-mg (3-day) and 1-gm single dose regimens

in azithromycin treatment trials of non-gastrointestinal infections, 3% and < 1%, respectively (33-35). The higher rates are likely due to the exacerbating effect of the primary illness that presents similarly as azithromycin GI side effects. The higher rate of GI side effects observed in the 1-gram dose group than the 500-mg dose is consistent with previously observed dose-related symptoms (36). The post treatment symptoms were mild, of brief duration, and affected less than 20 percent of volunteers. Given the improved efficacy, likely improved compliance, and ease of dosing schedule the mild side effects would seem to be outweighed toward selection of the single-dose regimen. A similar single dose azithromycin regimen was recently reported efficacious in travelers to Mexico (15). In this study, azithromycin recipients had ETEC recovered in 52%, *Shigella* in 5%, no *Campylobacter*, time to last unformed stool < 24 hours, no apparent increase in nausea/vomiting over levofloxacin comparator group, microbiologic eradication in 58%, and 9.5% treatment failures. The more rapid abatement of diarrhea compared to our study is consistent with therapeutic responses observed in trials with the majority of cases infected with ETEC as compared to *Campylobacter* (7, 9, 37, 38).

Efficacy determination in travelers' diarrhea treatment trials is complicated by the relatively short duration of the acute infection, self-limited nature of the illness, and potential confounding effects of pre-treatment illness severity and isolates susceptibility. Azithromycin superiority, even when adjusting for illness severity and isolate susceptibility (Table 6), provides convincing evidence for superiority over levofloxacin (Fig. 3). *Campylobacter* predominance in this study directly affects clinical outcomes given the delayed treatment responses compared to the non-*Campylobacter* cases irrespective of isolate susceptibility. A more rapid recovery in travelers' diarrhea

treatment trials has been observed in a larger percentage of subjects in ETEC predominant areas with 24-h cures in 40-70% of subjects even without antimotility agents (38, 39). A previous trial in Thailand with 41% *Campylobacter*-associated cases had lower 24-h cure rates, 36-38%, more comparable though still higher than the approximately 20% cure rates observed in this trial (9). The current trial differed by enrolling a broader clinical spectrum of illness whereas the earlier study excluded patients with dysentery and fever > 38.3^{o}C likely accounting for higher 24-h cure rates (9). Non-antibiotic antidiarrheal therapy with antimotility agents, such as loperamide, lead to more rapid clinical recovery when used as an adjunct to antibiotics. This observation has been demonstrated in ETEC predominant areas; however, an earlier study in Thailand did not demonstrate added benefit with loperamide plus ciprofloxacin (9). The combination of azithromycin and loperamide has not been evaluated and may provide more rapid recovery. A limitation of the study is the concurrent use of doxycycline for malaria prophylaxis in a majority of study subjects. Stratified analysis failed to demonstrate a confounding effect on outcomes although the treatment comparison is admittedly not as controlled as desired. In addition, the bacteriological assessment did not include measures to identify enteroaggregative *E. coli* (EAEC) increasingly being identified as a common etiology for travelers' diarrhea (40). The rapid (< 24 hr) clinical cures observed in the "no pathogen isolated" group (Fig. 3) in the levofloxacin group and the slight advantage observed for the FQ in the initial 24 hours appears to be a real antimicrobial effect possibly due to unrecognized EAEC or other pathogenic *E. coli*.

Potential concerns for selection of an azithromycin dose less than 1-gram include primary therapeutic failure or the potential development of in vivo resistance. *Campylobacter* eradication was near 100% and typically evident as early as 3 days after beginning azithromycin consistent with the mean duration of *C. jejuni* excretion of 1.1 days after first dose of erythromycin (41). Of concern, although an uncommon event, was the occurrence of high-level azithromycin resistant *Campylobacter* species in two subjects treated with azithromycin. Azithromycin has become a widely used antibiotic particularly for empiric treatment of acute respiratory infections (42). A concern for broadening azithromycin indications to include acute bacterial enteritis is the development of resistance as observed with FQ antibiotics. *C. jejuni* macrolide resistance has been relatively stable worldwide with rates of 0-11% (typically higher rates with *C. coli*) in contrast to progressively rising FQ-resistance observed in many countries (43). In the mid-1990s an increase of azithromycin resistance (7-15%) was documented in *Campylobacter* cases occurring among military personnel (11). In addition, surveys in 1996-99, predominantly in Thai children with diarrhea, documented *C. jejuni/coli* azithromycin rates of 6% as well as demonstrating dual resistance of azithromycin and ciprofloxacin in all of the macrolide-resistant isolates with greater likelihood in *C. coli* isolates (44). The current report demonstrated no azithromycin resistance among pretreatment *C. jejuni/coli* isolates.

In addition to *Campylobacter* species, emergence of macrolide resistance for pathogenic *E. coli*, *Salmonella*, and *Shigella* requires consideration and ongoing surveillance. A recent survey of azithromycin susceptibility in 284 enteropathogen isolates (predominantly ETEC, EAEC, *Salmonella*, and *Shigella*) from travelers' diarrhea

cases in India, Jamaica, Mexico, and Kenya demonstrated MIC_{90} of 0.0625 μg/ml providing greater confidence for broader clinical use (45). Nontyphoidal *Salmonella* isolates with reduced FQ susceptibility have been documented in travelers returning from Southeast Asia (most commonly Thailand) as well as increasing nalidixic acid resistance in Thailand (11, 46, 47). FQ-resistant *Salmonella* were not observed in this trial although approximately 4% of the *E. coli* had levofloxacin resistance with azithromycin resistance between 6-14% for non-*Campylobacter* bacterial pathogens. Also of concern, given the association between nalidixic acid (NA) resistance and decreased FQ susceptibility (48), were NA-resistance rates of 43%, 17%, and 18% in *Salmonella*, *E. coli*, and *Plesiomonas* isolates, respectively.

Alternative antibiotic agents for empiric management of acute bacterial enteritis continue to be needed given the progressive emergence of resistance. FQ-resistant travel-associated and domestic *Campylobacter* cases in industrialized countries have been increasingly reported and not restricted to countries such as Thailand and Spain (26, 49, 50). One alternative agent under development is the nonabsorbable antibiotic rifaximin currently unlicensed in the U.S. (51). This antibiotic has equal efficacy to ciprofloxacin in ETEC prominent regions but very limited data for treatment of acute *Campylobacter* or *Salmonella* bacterial enteritis (52, 53). In addition, it is not clear if the lack of systemic absorption will adversely affect therapeutic efficacy against invasive enteropathogens. A recent review emphasizes two key strategies to maintain FQ class efficacy; limit use to situations where benefit has been documented and use antibiotic with optimal activity against likely pathogens (54). This is a sound strategy that applies to most antibiotics; however, in the case of *Campylobacter* and *Salmonella* species the complicating concern

of antibiotic usage in animal husbandry must also be considered when developing public health strategies to control antibiotic resistance (43). Antibiotic therapy should be restricted to patients with moderate to severe illness, individuals at risk for poor clinical outcomes based on comorbid illnesses, or high tempo settings with complicating issues such as risk of heat-associated illness (frequently the case in deployed military personnel). In addition, given current azithromycin use in children and during pregnancy, these data in acute bacterial enteritis can likely be reasonably extrapolated for clinical application in these populations where concerns exist for FQ use and alternative antibiotics are lacking. In conclusion, single-dose (1-gram) azithromycin is recommended for empiric therapy of travelers' diarrhea acquired in Thailand and should be further investigated for broader application in areas with more diverse enteropathogen distribution.

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Tables and Figures

Figure 1. Trial enrollment profile

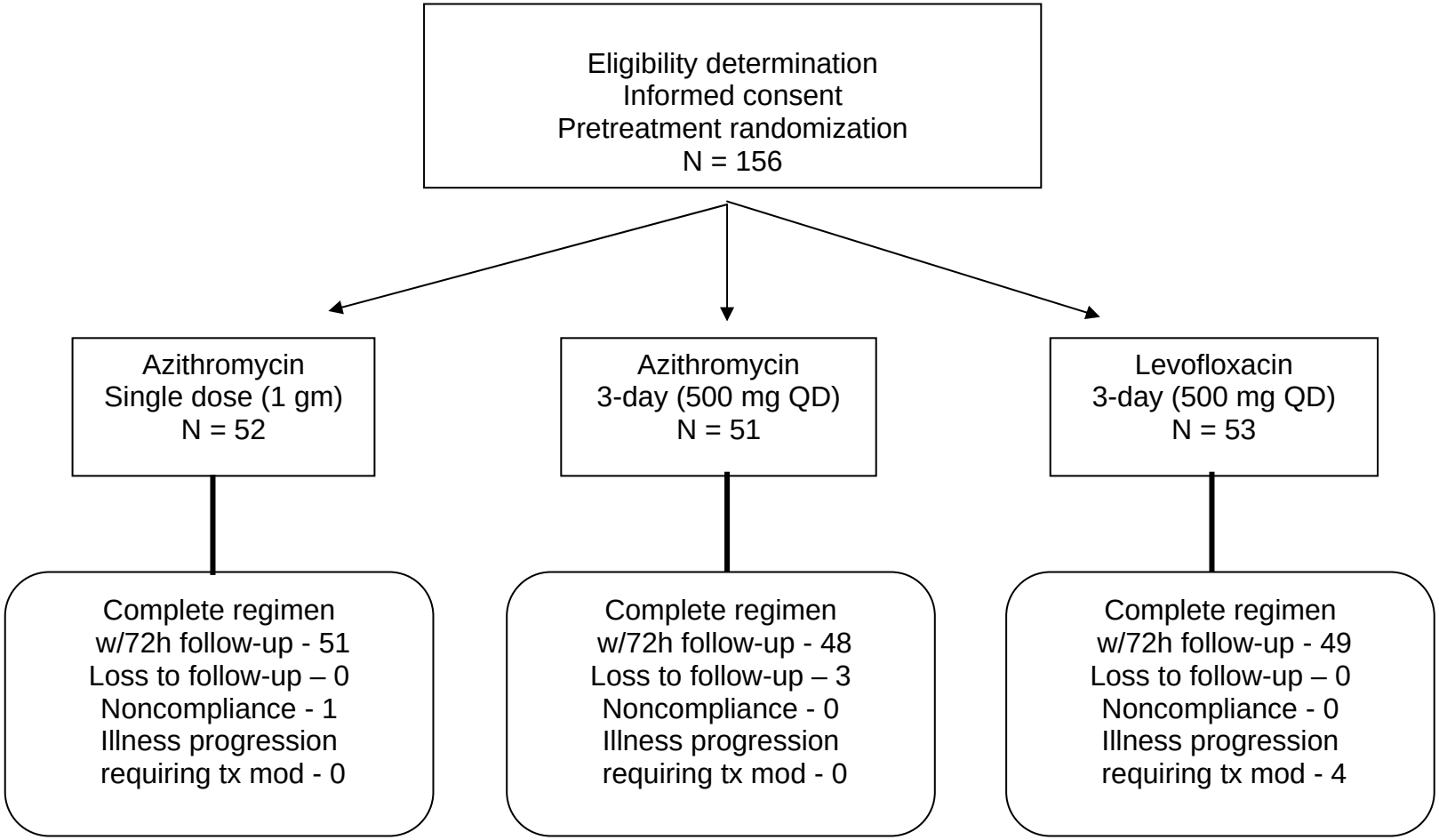


Table 1. Baseline patient characteristics prior to treatment assignment

Variable	Treatment Groups		
	Azithromycin		Levofloxacin
	Single dose (n = 52)	3-day (n = 51)	3-day (n = 53)
Age, y (mean ± SD)	28 ± 7.6	29 ± 7.0	27 ± 7.3
Men, n (%)	42 (81)*	49 (96)	48 (91)
Military rank, n (%)			
Junior enlisted	37 (71)	33 (66)	41 (77)
Senior enlisted/officers	15 (29)	17 (34)	12 (23)
Site/year, n (%)			
Nakhon Sri Thammarat, 2000	33 (64)	32 (63)	33 (62)
Phitsanulok, 2001	19 (36)	19 (37)	20 (38)
Malaria prophylaxis (doxycycline), n (%)	44 (85)	44 (86)	48 (91)
History of traveler's diarrhea, n (%)	8 (15)	14 (28)	3 (5.7)†
Prior Cobra Gold deployment, n (%)	13 (26)	11 (22)	5 (9.4)
Prior Thailand travel, n (%)	16 (31)	17 (33)	9 (17)
Time in country before illness, days	11 ± 6.3	12 ± 7.3	12 ± 6.1

* $P < .05$
† $P < .01$

Table 2. Clinical manifestations, laboratory findings, and management at presentation by treatment group

Variable	Treatment Groups		
	Azithromycin		Levofloxacin
	Single dose (n = 52)	3-day (n = 51)	3-day (n = 53)
Clinical manifestation			
Duration of illness pre-treatment, days (mean ± SD)	1.6 ± 0.8	1.7 ± 1.0	1.6 ± 0.8
Diarrhea frequency 24-h pre-treat (mean ± SD)	7.5 ± 6.4	6.7 ± 4.9	7.1 ± 4.3
Subjective fever, n (%)	28 (54)	24 (47)	26 (49)
Documented fever $\geq$ 100.0°F, n (%)	16 (31)	8 (16)	9 (17)
Abdominal cramps, n (%)	47 (90)	45 (88)	47 (89)
Gross blood in stools, n (%)	8 (16)	6 (12)	8 (15)
Nausea, n (%)	36 (69)	27 (53)	36 (68)
Vomiting, n (%)	15 (29)	7 (14)	10 (19)
Myalgias, n (%)	27 (52)	22 (43)	20 (38)
Arthralgias, n (%)	10 (19)	7 (14)	8 (15)
Headache, n (%)	29 (56)	28 (55)	29 (55)
Orthostatic hypotension, n (%)	13 (26)	15 (30)	14 (26)
Laboratory findings			
Hemoccult positive, n (%)	17 (33)	20 (40)	21 (41)
Fecal leukocytes present, n (%)	17 (39)	22 (48)	15 (31)
Fecal lactoferrin positive, n (%)	42 (81)	38 (78)	42 (81)
Patient assessment and management			
Activity limitation, n (%)			
None	14 (27)	12 (25)	15 (28)
Reduced	26 (51)	27 (55)	27 (51)
Unable	11 (22)	10 (20)	11 (21)
Pre-enrollment non-antibiotic therapy, n (%)			
Loperamide	8 (15)	5 (10)	7 (13)
Bismuth subsalicylate	1 (1.9)	2 (3.9)	4 (7.5)
Intravenous fluids, n (%)	11 (23)	8 (18)	9 (18)
Initial disposition, n (%)			
Return to duty	35 (67)	40 (78)	42 (79)
Sick in quarters	17 (33)	11 (22)	11 (21)

Note: No statistically significant differences between treatment groups.

Table 3. Enteric pathogen distribution at presentation by treatment group

Stool microbiology finding	Treatment Groups		
	Azithromycin		Levofloxacin
	Single dose (n = 52)	3-day (n = 51)	3-day (n = 53)
Any pathogen identified, n (%)	42 (81)	42 (82)	39 (75)
No pathogen identified, n (%)	10 (19)	9 (18)	13 (25)
Multiple pathogens, n (%)	12 (23)	7 (14)	9 (17)
Selected pathogen isolation rates			
Campylobacter jejuni/coli	37 (71)	30 (59)	32 (62)
Nontyphoidal *Salmonella*	11 (21)	8 (16)	7 (14)
Enterotoxigenic *E. coli*	1 (2.0)	2 (4.0)	2 (3.8)
Enteropathogenic *E. coli*	3 (5.9)	4 (8.0)	6 (11.5)
Plesiomonas shigelloides	3 (5.8)	5 (9.8)	3 (5.8)
Rotavirus	2 (4.3)	2 (4.4)	1 (2.0)
Norwalk virus	2 (4.5)	1 (2.2)	1 (2.0)

Note: Not identified – *Shigella* species, enterohemorrhagic *E. coli*, enteroinvasive *E. coli*, or parasitic etiologies. Enteropathogenic *E. coli* designation based on *eae*+ probe results (all EAF and SLT probes were negative classifying these as atypical EPEC although no O serogrouping done to verify serotype) (55). No statistically significant differences between treatment groups.

Table 4. Clinical and microbiological outcomes by treatment group

Outcome measure	Treatment Groups		
	Azithromycin		Levofloxacin
	Single dose (n = 52)	3-day (n = 51)	3-day (n = 53)
Clinical cure, % (95% C.I.)			
By 24 hours	20 (9.8-33.1)	18 (8.6-31.4)	25 (13.8-38.3)
By 48 hours	65 (50.1-77.6)	53 (38.3-67.5)	38 (25.3-53.0)*
By 72 hours	96 (86.5-99.5)	85 (72.2-93.9)	70 (56.9-82.9)†
Time to event, hours (median, IQR)			
Last febrile episode	0.5 (0.5-12.0)	4.0 (0.5-12.0)	12.0 (0.5-24.0)
Last diarrheal stool	35 (19.5-52.5)	45 (19.7-54.6)	50 (8.8-69.1)
Number of loose stools (mean ± SD)			
Pretreatment 24 hours	7.5 (6.4)	6.7 (4.9)	7.1 (4.3)
1st 24 hours	4.5 (4.2)	3.2 (2.8)	3.7 (3.6)
2nd 24 hours	2.7 (2.8)	2.4 (2.1)	4.0 (4.4)
3rd 24 hours	1.1 (1.5)	1.6 (2.0)	2.3 (2.6)
4th 24 hours	0.6 (1.2)	0.7 (1.8)	1.1 (2.0)
Microbiologic cure, % (95% C.I.)			
Overall	96 (81.0-99.9)	100 (87.2-100)	38 (18.8-59.4)†
Campylobacter-associated cases	96 (80.0-99.9)	100 (81.5-100)	21 (6.1-45.6)†

* $P = .02$
† $P = .001$

Figure 2. Time to cure (following first antibiotic dose) by treatment group

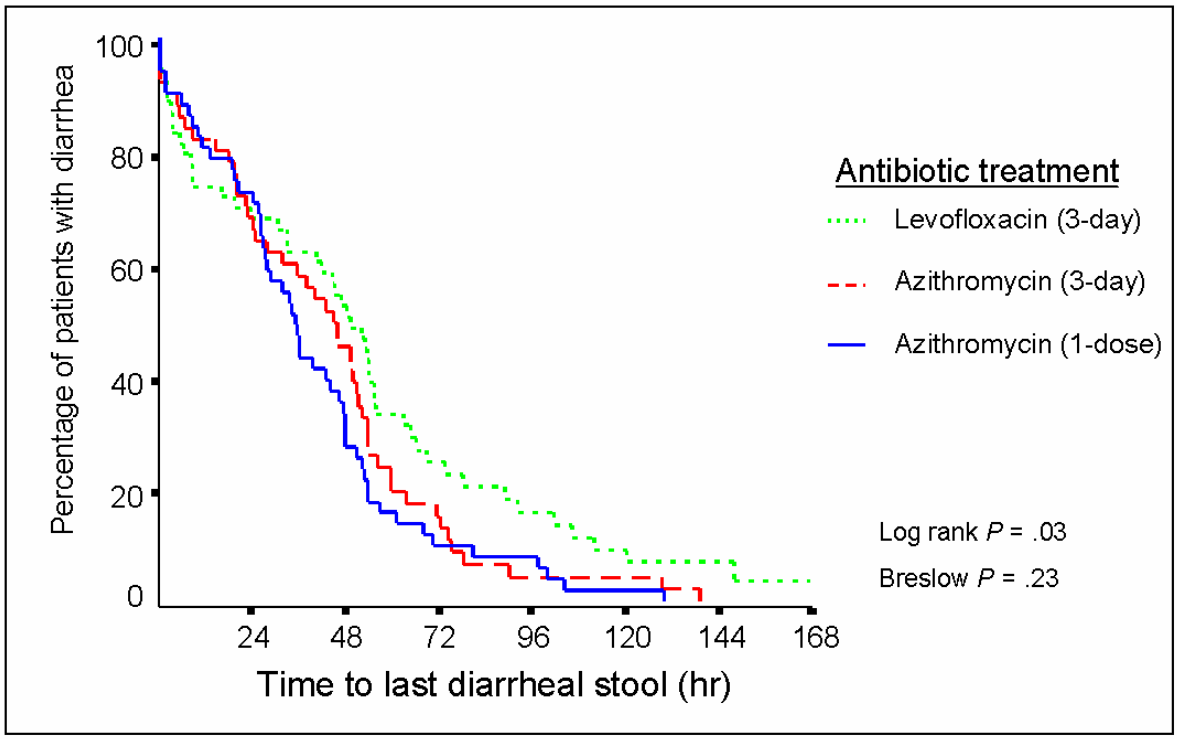


Figure 3. Time to cure (following first antibiotic dose) by treatment group stratified by *Campylobacter* diagnosis

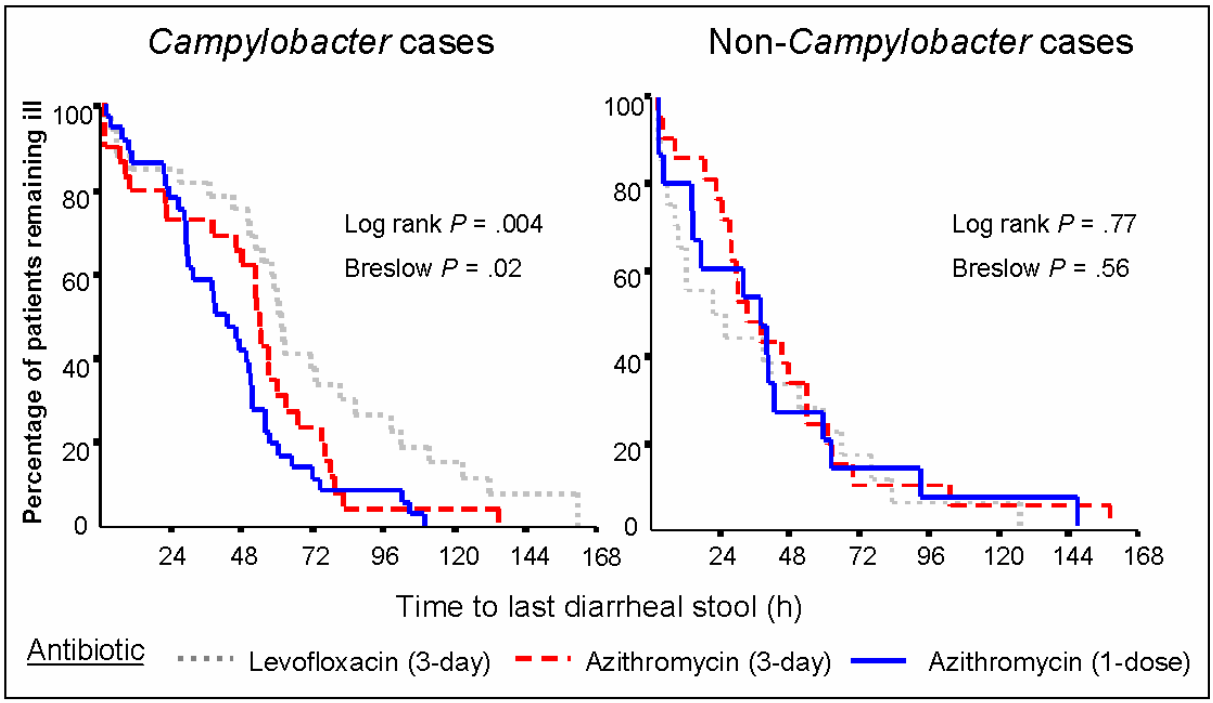


Table 5. Surveillance for posttreatment nausea and vomiting (%)

Symptom	Treatment Groups		
	Azithromycin		Levofloxacin
	Single dose (n = 52)	3-day (n = 51)	3-day (n = 53)
Immediate after 1st dose (30 min)			
Nausea	14*	6	2
Vomiting	2	0	0
During remainder of 3-day observation period			
Nausea			
Present pretreatment	35	16	32
Limited to posttreatment	17†	8	6
Vomiting			
Present pretreatment	26	14	19
Limited to posttreatment	8	2	4

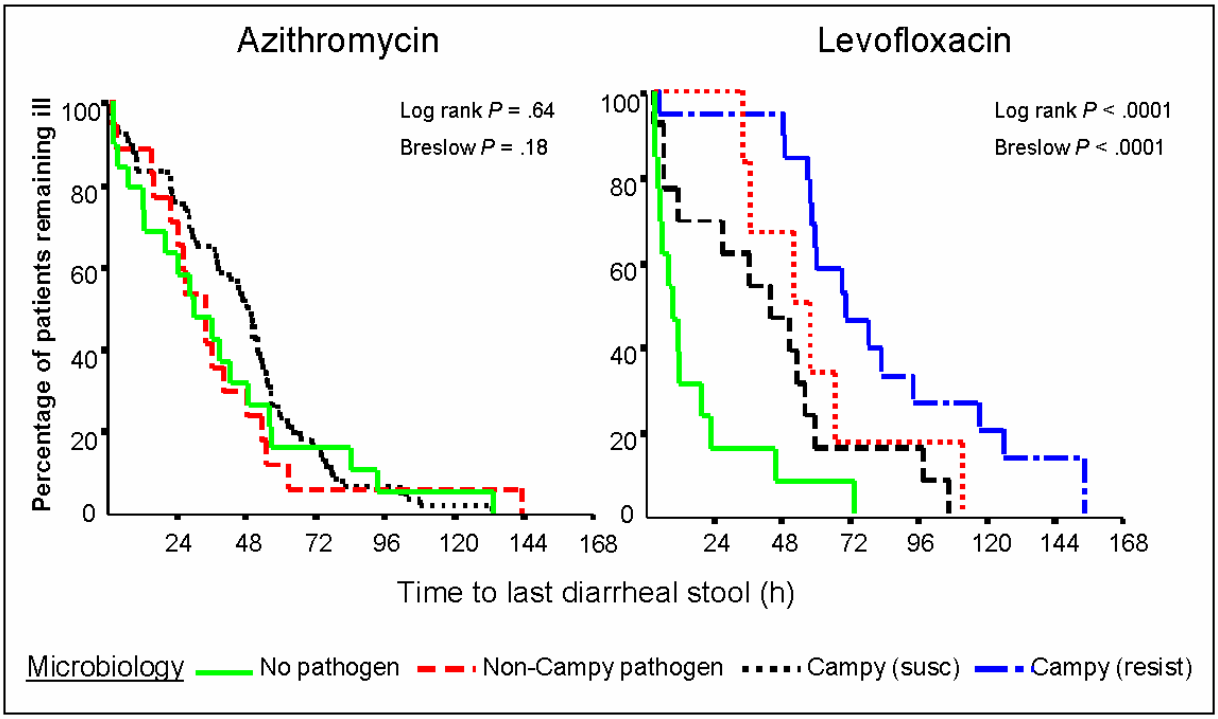
* $P = .06$
† $P = .03$

Table 6. Evaluation of non-treatment related factors on clinical outcome

Factor	Clinical cure measure			
	Complete symptom resolution by 72h		Time to last diarrheal stool	
	Crude RR (95% CI)	Adjusted RR (95% CI)	Crude RR (95% CI)	Adjusted RR (95% CI)
Azithromycin-based regimen	4.05 (1.63-10.08)	3.65 (1.09-12.21)	1.59 (1.10-2.29)	1.32 (0.80-2.19)
High frequency diarrhea ($\geq$ 10 episodes/day)	0.83 (0.31-2.18)	1.40 (0.44-4.47)	0.66 (0.44-0.97)	0.87 (0.57-1.32)
Dysentery and/or documented fever	0.74 (0.30-1.83)	1.58 (0.51-4.90)	0.69 (0.48-0.99)	0.97 (0.65-1.44)
Resistant isolate (based on treatment received)	0.20 (0.07-0.58)	0.39 (0.11-1.36)	0.41 (0.25-0.67)	0.50 (0.27-0.92)

Note: Dependent variable for clinical outcome measured by proportion with cure at 72 h (logistic regression model) and by time to last diarrheal stool (Cox proportional hazard model). Presence of dysentery defined as gross blood in diarrheal stool specimen pretreatment. Presence of resistant isolate is based on the antibiotic susceptibility pattern of the pretreatment stool culture and specific for the antibiotic subject received.

Figure 4. Time to cure (following first antibiotic dose) by pretreatment stool bacteriology result stratified by antibiotic used



Note: No pathogen designation based on the failure to identify a bacterial pathogen based on the microbiological techniques described in methods section. No "Campy (resist)" curve is seen in the azithromycin figure given the lack of any azithromycin-resistant *C. jejuni/coli* pretreatment isolates.

Conclusion

Summary of major findings

The primary objectives of this work are as follows: evaluate relative differences in clinical presentation and outcome of acute diarrhea based on stool microbiology findings, assess bedside and field laboratory-based diagnostic strategies, and determine therapeutic efficacy of azithromycin, single-dose or 3-day, as empiric therapy for travelers' diarrhea. The retrospective analysis of clinical records from Cobra Gold exercises in 1995, 1998, and 1999 demonstrated *Campylobacter*-associated diarrhea to be accompanied more often by systemic toxicity and increased diarrhea severity at presentation with delayed recovery. These observations of systemic symptoms in 30-50% and either dysentery or high output diarrhea in approximately 35% of affected cases are consistent with clinical case series of campylobacterioses [1-4]. The findings of *Campylobacter*-associated disease, high-output diarrhea, systemic complaints, and laboratory markers of inflammatory diarrhea (hemoccult or fecal leukocytes), all contributed less than regional *Campylobacter* endemicity to disease prediction. The epidemiologic setting led to a pretest probability of approximately 50% given the hyperendemic setting for this population. Retrospective evaluation of predictor's effects, if applied as a diagnostic test, was unable to yield posttest probability higher than 80% emphasizing the need to assess standardized rapid diagnostic methods.

The application of a diagnostic test relies on several factors such as test performance, appropriate target population, reliability, ease of use, and cost. In this research, the primary focus was to assess test performance of various diagnostic

approaches amenable to either bedside use by a clinician or performed at a field laboratory during overseas deployment of U.S. military personnel. Specimen sources included both stool and blood with stool microbiology serving as the reference standard. Blood specimens were included due to the frequent occurrence of patient hesitancy or inability to provide a stool specimen in real-time during initial clinic presentation. In addition to evaluating different specimen sources, we also evaluated tests based on identification of either pathogen (*Campylobacter* in this case) or inflammatory enteritis, not specific to pathogen.

As observed in the retrospective analysis, certain clinical features such as gross blood in stools or documented fever at presentation were very specific (≥ 93%) for *Campylobacter* infection; however, clinical features alone were not sensitive leading to a missed diagnosis in 70-80% of cases. Reliance on specific yet insensitive clinical features would lead to withholding of therapy in individuals that may benefit from early treatment [5]. The blood-based C-reactive protein, which would detect systemic inflammation, had similar test performance to the stool-based lactoferrin latex agglutination assay (LFLA). Both tests lacked specificity leading to inadequate positive predictive value with positive likelihood ratios of 2-3. However, both yielded excellent negative predictive value based on negative likelihood ratios of 0.1 (reducing post-test probability less than 10%). The ability of positive test findings to alter the probability of *Campylobacter* infection is most effective using a pathogen-specific stool based test. A *C. jejuni/coli*-specific commercial EIA, and less so a research PCR, were strong positive predictors. In a hyperendemic setting such as Thailand with prevalence estimates of 50% a positive EIA yields a 94% post-test probability of disease. Concurrent findings of

dysentery or fever further increase the post-test probability of a positive EIA to 98%. The test performance in this first-time field clinic assessment for the *Campylobacter* EIA and the fecal LFLA is consistent to observations in previously reported hospital-based series with much lower prevalence of *Campylobacter* [6-8].

In addition to evaluating clinical predictors, the retrospective analysis provided evidence of less than optimal empiric diarrhea management during the Cobra Gold exercises. Time to recovery was significantly delayed for *Campylobacter* cases with median time to clinical resolution after initial antibiotic dose of 43 as compared to 4 hours for non-*Campylobacter* cases. This observation occurred over a period of time that standard practice, as represented by a 3-day course with a fluoroquinolone antibiotic plus loperamide, was being reconsidered for this region of the world [9, 10]. The basis for reconsideration was the predominance of *Campylobacter,* for which a macrolide such as erythromycin or azithromycin are first-line antibiotics, and the rapidly increasing rates of fluoroquinolone-resistant *Campylobacter* [11-13]. A previous study in Cobra Gold 1993 provided evidence of azithromycin efficacy as an alternative to ciprofloxacin [11]. However, ciprofloxacin resulted in a reduced duration of illness in non-*Campylobacter* cases, accounting for 40% of affected individuals. The stated equivalency of the regimens for *Campylobacter*-associated illness is misleading since two clinical failures occurred, both being ciprofloxacin-treated *Campylobacter* cases, and likely related to limited statistical power to detect moderate treatment effect differences. This study combined with sub-optimal therapeutic responses observed in the retrospective analysis provides evidence to support reconsideration of initial empiric therapy in this region of the world. Additional studies have demonstrated azithromycin efficacy in other settings involving

bacterial enteritis as well as initial evidence of the potential of single-dose therapy [14-16]. A randomized controlled trial to evaluate azithromycin-based management strategies and optimal dosing schedules was needed.

We designed a randomized, active drug-controlled, double-blinded study to determine the therapeutic efficacy of azithromycin-based regimen as empiric therapy for travelers' diarrhea in Thailand. In this study, azithromycin was provided as either a single-dose (1 gram) or 3-day (500 mg once daily) regimen. The 3-day regimen is the same as used in the Cobra Gold 1993 trial [11]. Both regimens were compared to a standard 3-day fluoroquinolone (levofloxacin at 500 mg once daily) regimen. *C. jejuni* was the predominant pathogen accounting for 59-71% of cases across treatment groups with levofloxacin resistance in 50% and none with azithromycin. In this trial, azithromycin was definitively demonstrated to be the preferred antibiotic for traveler's diarrhea empiric treatment in Thailand. The single 1-gram dose was the optimal regimen. Clinical cure by 72 hours was highest at 96% with single dose azithromycin compared to 85% with 3-day azithromycin and 71% with levofloxacin (P = .002). Time to last diarrheal stool was less for single dose azithromycin, 35 h, than 49 h for the other groups (log rank, P = 0.03). Microbiologic eradication was significantly better for azithromycin-based regimens, 96-100%, as compared to levofloxacin at 38% (P = .001). Higher rate of post-treatment nausea in the 30 minutes after first dose (14 vs.< 6%, P = 0.06) were observed as a mild self-limited complaint with single dose azithromycin. The higher rate of GI side effects observed in the 1-gram dose group than the 500-mg dose is consistent with previously observed dose-related symptoms [17]. Given the improved efficacy, likely improved compliance, and ease of dosing schedule, the mild side effects would

seem to be outweighed toward selection of the single-dose regimen. Based on these findings combined with a recent study reporting efficacy of a single dose (1 gram) azithromycin regimen in travelers to Mexico, with ETEC in 52%, *Shigella* in 5%, and no *Campylobacter*, a recommendation for broader use of azithromycin as empiric therapy for traveler's diarrhea can be made [14].

Limitations

This work investigated travelers' diarrhea among a rather select population (generally healthy young male U.S. military personnel frequently using doxycycline malaria prophylaxis) traveling to Thailand during April-May timeframe. A unique aspect of the diarrheal threat for this population has been the overwhelming predominance of *Campylobacter* over the past 15 years of surveillance coupled with increasing rates of FQ resistance [11-13, 18-21]. This is in contrast to most travelers' diarrhea series demonstrating ETEC as a predominant etiology, as well as, typically higher rates of undetermined causes [22, 23]. The epidemiologic setting in Thailand limits the broad application of these results across operational platforms in various regions. These results must be combined with analyses from an area of ETEC predominance (with some contribution from *Shigella* species) to permit generalization.

An additional limitation of the study is the concurrent use of doxycycline for malaria prophylaxis in a majority of study subjects. Stratified analysis failed to demonstrate a confounding effect on outcomes although the treatment comparison is admittedly not as controlled as desired. A double blind randomized controlled trial evaluated doxycycline malaria chemoprophylaxis effect on diarrheal incidence and pathogen distribution [21]. Active surveillance (N = 253) documented diarrhea in 48% of

participants during the 5-week monitoring period. There was no difference in the occurrence of diarrhea or pathogen isolation rates in soldiers receiving doxycycline or mefloquine for malaria chemoprophylaxis. *Campylobacter* isolation rates during this study were quite low (2-3%) compared to the 40-60% rates observed during similar exercises throughout the 1990s in the same region (Khorat) in which doxycycline continued to be used. ETEC isolation rates were lower in the doxycycline group (3%) compared to mefloquine (8%). Tetracycline resistance was more common for *Campylobacter* (90%) than ETEC (21-24%) isolates. It seems likely that the frequent use of doxycycline reduces likelihood of ETEC infection but has minimal impact on risk of *Campylobacter*.

In addition, the bacteriological assessment did not include measures to identify enteroaggregative *E. coli* (EAEC) increasingly being identified as a common etiology for travelers' diarrhea [24]. The rapid (< 24 hr) clinical cures observed in the group with no pathogen isolated in the levofloxacin group and the slight advantage observed for the FQ in the initial 24 hours appears to be a real antimicrobial effect possibly due to unrecognized EAEC or other pathogenic *E. coli*.

Public health relevance

The relevance of this work for public health relates to three areas: *Campylobacter* diagnostics, treatment of FQ-resistant *Campylobacter*-associated illness, and approach to the clinical management of traveler's diarrhea. The global impact of *Campylobacter* infection must be considered to provide a perspective on the extent of this public health concern. The World Health Organization (WHO) has recently invested significant effort in furthering the understanding of human campylobacterioses [25]. The WHO partnered

with the Food and Agriculture Organization of the United Nations (FAO) to promote risk assessment and eventual control measures to lessen the global risk of *Campylobacter* infection through exposure to contaminated poultry [26]. In the United States, an even more aggressive public health surveillance effort has been underway since 1996 through the Center for Disease Control and Prevention (CDC) Foodborne Diseases Active Surveillance Network (FoodNet) to determine the impact of *Campylobacter* infection, implement strategies to reduce incidence, and monitor effectiveness of control efforts [27]. The recent attention on this pathogen's public health effect stems from three major concerns: increasingly recognized high incidence in developed and developing countries exceeding most bacterial enteropathogens, rapid global emergence of fluoroquinolone resistance, and the association of prior infection with this pathogen as the most common predisposing factor for the development of the Guillain-Barré Syndrome (GBS), the most common cause of acute neuromuscular paralysis [25, 28-32]. The WHO report recognized the uncertainty of the public health burden of campylobacteriosis; although, an increasing trend has been observed in most developed countries. Incidence estimates in developing countries are even less certain. Previous estimates of 40,000 to 60,000 infections per 100,000 children < 5 years of age annually have been proposed based on epidemiologic studies [31, 33]. Rates have risen over the past 10-20 years in several developed countries with incidence ranges of 15-350 per 100,000 persons [25]. A recent exception to this trend is the U.S. with a decreased incidence from 1996 to 1999, 23.6 to 17.5 per 100,000, respectively [34]. The basis for this reduction is not fully understood; however, the reduction occurred contemporaneous with implementation of pathogen

reduction measures in the U.S. poultry industry. Despite this reduction, the yearly estimate for *Campylobacter* infection in the U.S. is approximately 2 million cases.

Given the incidence of this infection throughout the world, efforts to validate previous clinical observations as well as expand options for diagnostic strategies, as provided as a component of this work, are needed. The WHO report highlighted the need to validate antigen and molecular based methods for eventual use in diagnosis and surveillance for *Campylobacter* infections [25]. The requirement for investigation into this area has become even more urgent given the progressive development of FQ resistance [29]. The situation monitored during the Cobra Gold exercise has progressed, although at an accelerated rate of resistance emergence ($\geq$ 85% since 1998), similar to observations around the world [11-13]. Recent U.S. data from the National Antimicrobial Resistance Monitoring System (NARMS) documents the increasing FQ-resistance in *Campylobacter* species from none in 1990 to 19% in 2001 [32].

Azithromycin has become a widely used antibiotic particularly for empiric treatment of acute respiratory infections [35]. A concern for broadening azithromycin indications to include acute bacterial enteritis is the development of resistance as observed with FQ antibiotics. Fortunately, *C. jejuni* macrolide resistance has been relatively stable worldwide with rates of 0-11% (typically higher rates with *C. coli*) in contrast to progressively rising FQ-resistance observed in many countries [29]. This report demonstrated no azithromycin resistance among pretreatment *Campylobacter* isolates despite earlier reports of approximately 6-15% azithromycin resistance among military personnel and Thai children [30, 36]. The results of therapeutic efficacy of a

single-dose azithromycin regimen provide treatment options for improved clinical management.

In addition to *Campylobacter* species, emergence of macrolide resistance for pathogenic *E. coli*, *Salmonella*, and *Shigella* requires consideration and ongoing surveillance. This report provides updated regional information of value for clinicians caring for travelers to this area. Nontyphoidal *Salmonella* isolates with reduced FQ susceptibility have been documented in travelers returning from Southeast Asia (most commonly Thailand) as well as increasing nalidixic acid resistance in Thailand [30, 37, 38]. FQ-resistant *Salmonella* were not observed in this trial although approximately 4% of the *E. coli* had levofloxacin resistance with azithromycin resistance between 6-14% for non-*Campylobacter* bacterial pathogens. Also of concern, given the association between nalidixic acid (NA) resistance and decreased FQ susceptibility [39], were NA-resistance rates of 43%, 17%, and 18% in *Salmonella*, *E. coli*, and *Plesiomonas* isolates, respectively. Azithromycin efficacy was also demonstrated for non-*Campylobacter* cases.

Alternative antibiotic agents for empiric management of acute bacterial enteritis continue to be needed given the progressive emergence of resistance. A recent review emphasizes two key strategies to maintain FQ class efficacy; limit use to situations where benefit has been documented and use antibiotic with optimal activity against likely pathogens [40]. This is a sound strategy that applies to most antibiotics; however, in the case of *Campylobacter* and *Salmonella* species the complicating concern of antibiotic usage in animal husbandry must also be considered when developing public health strategies to control antibiotic resistance [29]. A recent U.S. court decision requiring

Bayer Pharmaceuticals to discontinue poultry industry use of enrofloxacin, a FQ antibiotic, based on adverse effects on human health was unique application of public health-related law [41]. This ruling was encouraging; however, widespread use of antibiotics in the poultry industry throughout the world and the well-documented importation of FQ-resistant isolates remain a major concern. Antibiotic therapy should be restricted to patients with moderate to severe illness, individuals at risk for poor clinical outcomes based on comorbid illnesses, or high tempo settings with complicating issues such as risk of heat-associated illness (frequently the case in deployed military personnel). In addition, given current azithromycin use in children and during pregnancy, these data in acute bacterial enteritis can reasonably be extrapolated for clinical application in these populations where concerns exist for FQ use and alternative antibiotics are lacking.

The number of individuals needed to treat (NNT) to benefit from the added advantage of a particular intervention provides assistance in justifying a new therapeutic approach for a given population [42]. In the target population of deployed U.S. military, a treatment failure rate at 72 hours for the FQ-based regimen approximates 25% whereas the azithromycin-based treatment is approximately 5%. The absolute risk reduction is 20% (95% C.I., 10.5-29.5) yielding a NNT of 5 (95% C.I., 3.4-9.5). This means that about one in every 5 patients will benefit from selecting azithromycin rather than levofloxacin. The government rate (based on the 2003 Federal Supply Schedule) for single-dose azithromycin regimen is $9.41 per dose compared to a less expensive, but less effective, 3-day levofloxacin regimen cost of $5.94 (a difference of $3.47 between regimens per person treated). As observed in the retrospective analysis and the

prospective randomized trial, the FQ treatment failures frequently require salvage azithromycin therapy in addition to the delay in their recovery and return of full function during the military operation. Given this situation occurring as frequently as every 5-7 persons, the recommended empiric regimen should be single-dose azithromycin.

Recommendations

General

- Diagnostic test application
    - The *Campylobacter* EIA is a sensitive and specific rapid diagnostic test that can assist in diagnostic evaluation and therapeutic decision-making. In order to be most cost-effective, the following should be considered: patient selection, epidemiologic setting, turn-around time, microbiology lab facilities and personnel resources, and ability to recover the isolate for antibiotic susceptibility testing.
    - Laboratory testing for evidence of inflammatory enteritis (such as the fecal lactoferrin latex agglutination) are not recommended in the initial management of the traveler's diarrhea syndrome. This recommendation is based on the predominance of bacterial pathogens (invasive and noninvasive) as etiologic agents in the traveler's diarrhea syndrome and the well-documented therapeutic benefit of empiric therapy provided early in the disease course.
- Treatment approach for *Campylobacter* infection

- A single-dose (1 gram) of azithromycin is the recommended regimen. The advantages in patient compliance and decreased gastrointestinal intolerance outweigh the increased cost of erythromycin. Patients with severe enteritis, comorbid illness, and bacteremia should not be managed with single-dose therapy. The appropriate regimen for these patients has not been defined; however, a 5-day course of azithromycin (500 mg daily), possibly via an intravenous route, combined with careful clinical monitoring would be a conservative approach. The dose required for the empiric treatment of traveler's diarrhea has not been clearly defined.
- The additive benefit of adjunctive therapy with the antimotility agent loperamide has not been evaluated in combination with azithromycin. In addition, a prior randomized controlled treatment trial of empiric management of traveler's diarrhea evaluating FQ-based regimens with adjunctive loperamide failed to demonstrate benefit in a *Campylobacter* predominant setting [12]. Reduced time to clinical resolution has been well documented in regimens containing either a fluoroquinolone or trimethoprim/sulfamethoxazole and should be further evaluated for azithromycin-based regimens [43-45].

- Treatment approach for traveler's diarrhea
  - Immediate assessment of fluid status with timely rehydration therapy is the cornerstone of diarrheal management. The decision to treat with medications, non-specific anti-diarrheal and/or an antimicrobial agent is based on illness severity assessment, results of screening or pathogen-

specific lab tests, and pre-treatment anticipated benefit. Empiric antibiotic therapy is the usual evidence-based approach given the typical lack of a definitive etiologic agent at the time of primary assessment. Fluoroquinolone antibiotics remain appropriate first-line therapy in ETEC-predominant settings accounting for much of the developing world. In areas with high rates of *Campylobacter*, particularly with documented FQ-resistance, then azithromycin should be considered the first-line agent.

- Additional traveler's diarrhea scenarios when azithromycin should be considered first-line include: children, pregnancy, FQ allergy or prior intolerance, FQ chemoprophylaxis failure, and treatment failure or relapse following FQ therapy. An alternative agent for a patient with watery diarrhea and low risk of an invasive pathogen is the nonabsorbable antibiotic, rifaximine [46].
- The preferred regimen for both FQ and azithromycin is single-dose. The decision to continue therapy beyond 24 hours (typically to complete a 3-day course) is based on re-evaluation at 24 hours.

Military-specific

Traveler's diarrhea clinical management during military deployment raises unique challenges, such as up-tempo high threat conditions with environmental and occupational stressors, as compared to civilian travelers. However, the military deployment also affords opportunities not available to the typical civilian traveler such as medical infrastructure, potential to promote optimal clinical practice, and deploy diagnostic resources that may assist clinical management and population health assessment.

- Diarrhea management during Southeast Asia (Thailand) deployment
    - The information provided from this work (and previous efforts) is directly applicable for medical practice as outlined above under 'General Recommendation'.
- Development of military-specific clinical practice guideline for acute infectious diarrhea

Clinical practice guidelines are systematically developed statements for a specific clinical circumstance to assist the healthcare provider about appropriate health care [47, 48]. The Infectious Diseases Society of America has proposed practice guidelines for the management of infectious diarrhea that contain important components of what could be a military-specific guidance [49]. This guidance incorporated the overlapping interests of healthcare providers (interventions that alleviate symptoms and prevent secondary transmission) and public health practitioners (timely notification of reportable pathogens through surveillance systems and prompt detection and control of outbreaks). Application of this guideline requires adaptation to the deployed military environment. Timely military public health data (battlefield medical intelligence) and effective clinical management to maintain military readiness stress the need to pursue operational medicine clinical practice guidelines.

At present, the U.S. military has developed practice guidelines through the support of the U.S. Army MEDCOM Quality Management Office (http://www.cs.amedd.army.mil/qmo/pguide.htm) with the focus on common chronic diseases managed at U.S. military treatment facilities. Guidance for

clinical practice during deployment can be found in the Navy's General Medical Officer Manual and theater-specific technical guides such as the US Army Center for Health Promotion and Preventive Medicine recent guide related to the Iraq operations for the "Diagnosis and treatment of diseases of tactical importance to U.S. Central Command" [50, 51]. These reports vary considerably. In addition, military research and development, US Army Medical Research and Materiel Command Military Infectious Diseases Research Program Task Area L (Diagnostic Systems for Infectious Diseases), is expending considerable effort to develop point of care and nucleic acid detection (real-time PCR) diagnostic assay systems for eventual deployment. Enteric pathogens causing acute diarrhea in deployed personnel have been prioritized for diagnostic assay development. The military could benefit from development and interval reassessment of practice guidelines that aim to improve quality of care, serve as educational tools for providers, integrate military public health considerations (i.e. early detection and control of outbreaks), appropriate use of new technologies (i.e. diagnostic tests), and guidance for appropriate use of pharmaceuticals in response to new evidence and changing pathogen threats (i.e. antibiotic resistance). This work will contribute to the evidence needed to formulate these important guidelines.

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Appendix

Research protocol

DoD # 31528-acj

RECEIVED 2/07/00

31 January 2000

MEMORANDUM

From: Principal Investigator
To: Chairman, Committee for the Protection of Human Subjects (CPHS), Office of Research Administration (OORA), Naval Medical Research Command (NMRC)

Subj: **SUBMISSION OF ATTACHED PROTOCOL FOR REVIEW**

1. The attached protocol entitled, "Travelers' Diarrhea Surveillance, Diagnosis and Therapy Study in United States Military Personnel on Deployment in Thailand (Cobra Gold 2000/2001)" is submitted for NMRC CPHS review. The protocol has been read by and its submission approved based on sound scientific basis and military relevance by the Chief Scientist, Infectious Diseases Directorate and the Director, Enteric Diseases Department.

2. The protocol includes a case-control study and a randomized active treatment controlled trial. The case-control component involves the collection of questionnaire information, blood [total volume: cases (80 ml) and controls (40 ml)], and stool specimens from soldiers with ("cases") and without ("controls") diarrhea. Cobra Gold is an annual U.S.-Thai joint military training exercise occurring each May in various sites in Thailand. The personnel for Cobra Gold 2000 are deployed to the Thung Song/Nakhon Si Thammarat area in Thailand during the Cobra Gold 2000 exercise (May 1-24). Deployed personnel presenting with acute diarrhea will also be assessed for their willingness to participate in the travelers' diarrhea treatment trial. Eligible personnel will be randomly assigned to one of three treatment regimens [levofloxacin (500 mg once daily x 3 days), azithromycin (500 mg once daily x 3 days), and azithromycin (1000 mg as a single dose)] using a randomized, double-blinded study design. Both medications being evaluated in this study are licensed antibiotics. The Armed Forces Institute of Medical Sciences (AFRIMS), the U.S. Army research laboratory in Bangkok, is a collaborating institution. AFRIMS investigators are responsible for in-country logistics, field microbiology, and follow-on microbiology analyses post-deployment.

3. NMRC is the lead agency for this research with responsibility for protection of human subjects and project implementation. The Walter Reed Army Institute of Research (WRAIR), as the approving authority for AFRIMS, will also be provided with the protocol. I am enrolled as a doctoral student in the Doctor of Public Health (Dr.P.H.) degree program of the Preventive Medicine and Biometrics Department at the Uniformed Services University of the Health Sciences (USUHS). This protocol is being submitted to my academic advisory committee and the USUHS Institutional Review Board as part of my degree program.

4. Documented protocol approval (when available) will be forwarded to the Cobra Gold 2000 Joint Task Force Surgeon, currently the III Marine Expeditionary Force Surgeon in Okinawa, who is responsible for medical support and activities for Cobra Gold 2000. Given the fixed time schedule of joint military training exercises, I request that consideration be given to having approval documentation available (if deemed appropriate for approval) no later than April 1, 2000. Thank you for your consideration of this protocol.

David Tribble

David Tribble, M.D., M.P.H.

Copy provided to:
Chief Scientist, Infectious Diseases Directorate, NMRC
Director, Enteric Diseases Department, NMRC
Director, Office of Research Management (ORM), WRAIR
Chairman, USUHS Institutional Review Board
Members of USUHS PMB Academic Advisory Committee (for Dr. David Tribble)
Associate investigators

Travelers' Diarrhea Surveillance, Diagnosis and Therapy Study in United States Military Personnel on Deployment in Thailand (Cobra Gold 2000/2001)

PROTOCOL NUMBER ( )

TITLE: Travelers' Diarrhea Surveillance, Diagnosis and Therapy Study in United States Military Personnel on Deployment in Thailand (Cobra Gold 2000/2001)

DATE OF SUBMISSION: 31-Jan-2000

APPROVED WORK UNIT TITLE (NUMBER): Advanced evaluation and testing of enteric vaccines and mucosal adjuvants suitable for human use (643807A..849.D.A0002)

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PRIMARY PERFORMING INSTITUTION/LEAD AGENCY: Naval Medical Research Center (NMRC), Silver Spring, MD

COLLABORATING INSTITUTION: Armed Forces Research Institute of Medical Science (AFRIMS), Bangkok, Thailand

CORPORATE SUPPORT: Pfizer Central Research, Groton, CT (limited to donation of study medications, placebo, and randomization schedule/medication labeling)

PROJECT LOCATION: Volunteer enrollment: diarrhea patients (cases) during the Cobra Gold 2000 [Thung Song field support hospital and Nakhon Si Thammarat battalion aid station (BAS)] and asymptomatic persons (controls) (deployed troops in the field in the same areas as cases) . Sites during Cobra Gold 2001 to be determined in Nov 2000. Post-deployment laboratory research performed at NMRC and AFRIMS.

TIME REQUIRED TO COMPLETE: Study planned for implementation during Cobra Gold 2000 (30-April-00 – 25-May-00) and Cobra Gold 2001 (May 2001). Final reporting and manuscript preparation estimated Oct-2001).

ESTIMATED VOLUNTEER SIZE REQUIREMENTS: Diarrhea patients – 200 (100 per exercise year); Control volunteers – 200-300 (enrolled in Cobra Gold 2000)

IND INFORMATION: Not Applicable (Levofloxacin and azithromycin are licensed products.)

CHAIN OF COMMAND SIGNATURE PAGE

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01/31/00

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AFRIMS microbiology/immunology technicians (3) and drivers (2)

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Daniel A. Scott, CAPT(sel), MC, USN MM DD YY

f. Chief Scientist, Infectious Diseases Directorate 02/03/00
Curt Hayes, Ph.D. MM DD YY

Chief Scientist's approval indicates certification of NMRC Scientific Review.

g. Director, Office of Research Administration
Dr. Edward F. Gabriele MM DD YY

Travelers' Diarrhea Surveillance, Diagnosis and Therapy Study in United States Military Personnel on Deployment in Thailand (Cobra Gold 2000/2001)

PROTOCOL PERSONNEL/ROUTING PAGE

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e. Department Head (Enteric Diseases)

Daniel A. Scott, CAPT(sel), MC, USN MM DD YY

f. Directorate Head (Infectious Diseases)

Curt Hayes, Ph.D. MM DD YY

g. Director, Office of Research Administration)

Dr. Edward F. Gabriele MM DD YY

h. Commanding Officer (pending NMRC IRB review and recommended approval)

Richard G. Hibbs, CAPT, MC, USN MM DD YY

Travelers' Diarrhea Surveillance, Diagnosis and Therapy Study in United States Military Personnel on Deployment in Thailand (Cobra Gold 2000/2001)

Table of Contents

		Page
1.	Study Coordination and Organizational Plan	4
2.	Project Synopsis	4
3.	Objectives	5
4.	Background/Rationale	6
5.	Bibliography	8
6.	Study Design	10
	A. Project Overview	10
	B. Project Flow Diagram	11
	C. General Volunteer Information	12
	i. Source of volunteers	
	ii. Age range and gender inclusions	
	iii. Subject identification	
	D. Project Definitions	12
	E. Diarrheal Surveillance	13
	i. Clinical procedures	
	ii. Laboratory procedures	
	iii. Analysis	
	F. Case-control Study	14
	i. Sample size estimates	
	ii. Entry criteria	
	iii. Procedures (with flow chart)	
	a. Clinical	
	b. Laboratory	
	iv. Risk/Benefit	
	v. Analysis	
	G. Randomized controlled trial	17
	i. Sample size estimates	
	ii. Entry criteria	
	iii. Procedures (with flow chart)	
	a. Clinical	
	b. Laboratory	
	iv. Study medications	
	v. Risk/Benefit	
	vi. Analysis	
	H. Diarrheal diagnostics evaluation	23
	i. Procedures (with test site table)	
	a. Bedside testing	
	b. Field laboratory testing	
	c. Research laboratory testing	
	ii. Specimen test schedule	
	iii. Analysis	
7.	Adverse Event Management	26
8.	Medical Care/Equipment Requirements	29
9.	Record Maintenance/Data Management	30
10.	Protocol Modification/Deviation	30
11.	Appendices	31

1. Study Coordination and Organizational Plan

This study is a Department of Defense/Department of Navy Medical Research and Development protocol with Program 6 support. The Lead Agency responsible for assurance of appropriate human subject research review is the Naval Medical Research Center (NMRC). Due to the collaborative involvement of investigators at AFRIMS, concurrent review will take place at the Walter Reed Army Institute of Research (WRAIR). The Cobra Gold exercise is an annual joint military training exercise between U.S and Thai forces occurring each May in rotating sites within Thailand. Project logistics are coordinated with the Cobra Gold exercise medical planners and the Joint Task Force (JTF) Surgeon [III Marine Expeditionary Force (III MEF) Surgeon, Okinawa, Japan for Cobra Gold 2000]. The research team has been officially placed in the exercise plan. IRB approval will be forwarded to the JTF Surgeon when available. Pfizer Central Research (Groton, CT) is providing both study medications with their respective placebos without charge. Dr. Tribble and representatives from Pfizer Central Research will coordinate the randomization schedule and the double-blinding procedures. No funds are being providing by Pfizer. Research results will be shared between NMRC and AFRIMS and provided to Pfizer. Upon direction of the Office of Technology Transfer, the appropriate required instrument clarifying work statements and responsibilities for each party will be completed in advance of project implementation.

The PI, Dr.Tribble, is enrolled in the doctoral Public Health program of the Department of Preventive Medicine and Biometrics (PMB) at the Uniformed Services University of the Health Sciences (USUHS) in Bethesda, MD and this project constitutes work toward degree completion. The USUHS PMB Dept. and the IRB will be provided the protocol for review. All approval documentation and project reporting will be provided to the PMB Dept. and the USUIIS IRB. Dr. Tribble, with the assistance of LCDR Sanders, will be involved in overall project coordination, oversee clinical trial management, abstraction and validation of clinical data, data analysis and reporting. Study Naval Medical Officers will be responsible for making appropriate entries in patient medical records as per standard patient care, as well as, proper completion of study clinical procedures. All clinically relevant results pending at the time of completion of the exercise will be forwarded to the medical unit holding the patient's medical record. LTC Pang with the assistance of Dr. Bodhidatta will coordinate the field microbiology laboratory, follow-on microbiology at AFRIMS, and in-country resources/logistics (not involved in clinical care of volunteers). LCDR Naile will be the Field Data Manager with the responsibility for study file coordination, field data abstraction (laboratory), data entry/verification, and report generation (for PI review). Dr. Baqar and LCDR(sel) Lebron will oversee the processing of clinical specimens in the field laboratory. Dr. Baqar is responsible for the post-deployment immunologic analysis at NMRC.

2. Project Synopsis

The Enteric Diseases Department at the Naval Medical Research Center (NMRC) has primary responsibility within the Department of Defense for developing an effective vaccine to protect troops against *Campylobacter*-associated diarrhea as a component of a "travelers' diarrhea" vaccine. The Cobra Gold exercise is an annual joint military training exercise between U.S and Thai forces occurring each May in rotating sites within Thailand. Cobra Gold diarrhea surveillance since 1990, by the Armed Forces Research Institute of Medical Sciences (AFRIMS, Bangkok) in collaboration with NMRC and Army/Navy Preventive Medicine commands, has shown diarrheal illness to be the primary health threat to deployed troops with *Campylobacter* spp. as the predominant cause. In addition to epidemiology and immunology studies supporting the feasibility of vaccine development, improvements in diagnostic strategies and therapeutic management for application in deployed troops are needed. Increasing rates of antimicrobial resistance and observational studies demonstrating sub-optimal therapeutic responses

(defined as failure to resolve within 72 hours of initiation of treatment) in 10-25% of cases highlight the need for evaluating alternative treatment regimens. This project aims to study three active drug treatment regimens [levofloxacin (500 mg once daily x 3 days), azithromycin (500 mg once daily x 3 days), and azithromycin (1000 mg as a single dose)] using a randomized, double-blinded study design. Volunteer enrollment will occur at the field support hospital in Thung Song and the battalion aid station (BAS) in Nakhon Si Thammarat during the period of the Cobra Gold 2000 exercise (May 1-24). In addition, the project will extend diarrhea threat assessment in Thailand, provide diarrheal pathogen-associated information on epidemiologic and immunologic factors using a case-control design, and assess bedside and field laboratory diagnostic methods.

The required number of patients volunteering to participate in the treatment study (approx. 60 per treatment regimen) necessitates enrollment during Cobra Gold 2000 and 2001. The case-control study will investigate personal characteristics, behaviors and host immunology in order to better understand risks associated with specific enteric pathogens. As part of the case-control study, volunteers will have measurements of pathogen-specific antibody and cellular immune responses. The pathogen-specific immunology will provide new information in healthy and ill persons with community exposures to enteric pathogens. This information may be particularly valuable since it originates from the target population (deployed U.S. active duty personnel) for the DoD combined travelers' diarrhea vaccine. A monetary incentive will be provided to volunteers participating in the case-control study (as undertaken in Cobra Gold 1999). Based on DoD regulations, a monetary incentive may be provided for blood draws (planned payment of $25 per bleed). There will be a total of 2 bleeds [at initial presentation (40 ml) to clinic with diarrhea and 3 days after presentation (20 ml)] for patients with diarrhea (cases) and 1 blood draw (40 ml) for the asymptomatic persons (controls). The research effort will be limited to the Nakhon Si Thammarat (Thong Song) site in southern Thailand during Cobra Gold 2000.

Following verification of clinical and microbiologic data from Cobra Gold 2000 (approx. July-Aug-2000), an interim analysis will be completed without breaking the treatment assignment code. If the overall clinical failure rate (no resolution of diarrhea-associated symptoms by 72 hours) is $\geq$ 10%, an interim analysis by treatment assignment will be undertaken. The interim analysis will determine if one of the study regimens accounts for the majority of the treatment failures. Clear evidence of inferiority of one of the treatment arms would lead to narrowing the study to a 2-arm trial for Cobra Gold 2001. A lack of significant difference between treatments would result in continuing enrollment in Cobra Gold 2001 using the 3-arm design. A report of this interim analysis will be provided to the institutional review board (IRB) with detailed plans for Cobra Gold 2001 study sites. Final analysis will commence after all clinical and microbiology data is entered and verified (approx. July-2001). The research team will supplement the exercise medical resources with additional research physicians (with infectious disease expertise) and laboratory support (real-time stool microbiology results from on-site field lab).

3. Objectives

- Surveillance of diarrheal enteropathogens affecting deployed troops in Thailand. [Clinic-based passive surveillance]

- Evaluate epidemiologic, microbiologic and immunologic factors associated with diarrheal illness. [Case-control study]

- Determine therapeutic efficacy of azithromycin, single-dose or 3-day, versus a standard 3-day fluoroquinolone (levofloxacin) as empiric therapy for travelers' diarrhea. [Randomized active drug-controlled double-blinded study]

- Determine effectiveness of bedside and field laboratory-based rapid diagnostic assays in the management of acute infectious diarrhea.

4. Background/Rationale

A selected summary of previous Cobra Gold exercise research efforts over the past decade highlights the importance of this research for deployed military personnel. During Cobra Gold 1990, an overall 30% diarrheal incidence in surveyed troops was observed with 25% of affected individuals seeking care {*Sanchez JL, et al., 1998*}. This significant diarrheal attack rate resulted in a weekly incidence of 1.5% (peak 2.5% 3rd wk), 13% of all clinic visits, and 12% of all hospitalizations/sick-in quarters (SIQ). *Campylobacter* species (*C. jejuni* and *C. coli*) were the most common etiologic agents (41%) with 100% susceptibility to the fluoroquinolone antibiotic, ciprofloxacin {*Petrucelli BP, et al., 1992*}. In Cobra Gold 1993, Army and Navy researchers evaluated an alternative antibiotic, azithromycin, as empiric therapy for travelers' diarrhea as compared to the standard ciprofloxacin regimen {*Kuschner RA, et al., 1995*}. During this study, the regional emergence of ciprofloxacin-resistant *C. jejuni* was observed (~ 50% of initial isolates). Empiric treatment with azithromycin demonstrated benefit in reducing the average duration of the diarrheal episode from 49 to 39 hours as compared to ciprofloxacin in campylobacter cases however; there was no overall difference in clinical cure rates. In contrast, ciprofloxacin was superior in reducing diarrhea duration as compared to azithromycin in non-campylobacter cases, 21 vs. 33 hours. There was no azithromycin resistance observed in this study. Based on these findings, recommendations for empiric use of fluoroquinolone antibiotics as first-line therapy did not change.

In Cobra Golds 1994 and 1995, increasing rates of ciprofloxacin-resistant *C. jejuni* (65-85%), as well as, azithromycin resistance (7-15%) were observed {*Murphy GS, et al., 1996; Walz S, personal communication*}. A total of 171 diarrheal cases in Cobra Gold 1995 were evaluated and cared for at medical treatment facilities by the research team. In this series, *C. jejuni* was again the most common pathogen (33%) however, other pathogens included non-typhoidal *Salmonella* spp. (18%), enterotoxigenic *E. coli* (11%), *Plesiomonas shigelloides* (11%), and *Shigella* spp. (8%). In Cobra Gold 1998 and 1999, observational clinic-based studies were undertaken to provide ongoing diarrheal threat assessment data and further investigate the effect that the emergence of quinolone-resistant bacterial enteropathogens, predominately *Campylobacter* spp., has on the empiric use of quinolone antibiotics for first-line travelers' diarrhea management. As observed in past exercises, *Campylobacter* spp. remained the predominant cause of diarrhea in personnel reporting for medical care however, a spectrum of other bacterial enteropathogens were observed in as many as 25-40% of the cases. The research teams in 1998 and 1999 provided clinical assessment and care for 171 and 110 personnel with acute diarrhea, respectively. *In vitro* ciprofloxacin resistance was observed in > 90% of *Campylobacter* isolates and none of the non-*Campylobacter* isolates. Sub-optimal treatment response, defined as a lack of complete resolution by 72 h, was observed in approximately 10-20% of the *Campylobacter*-associated cases receiving ciprofloxacin. These results highlight the importance of investigating alternative therapies for the empiric management of travelers' diarrhea, particularly in Southeast Asia.

The recommended standard empiric antibiotic therapy for travelers' diarrhea is a 3-day course with a fluoroquinolone {*DuPont HL and Ericsson CD, 1993; Scarpignato C and Rampal P, 1995; Ericsson CD, 1998*}. The activity of the fluoroquinolone, ofloxacin, against common enteric pathogens is well established, and is commonly used for traveler's diarrhea {*Lang, 1990; Ansdell, 1999; Juckett, 1999*}. Levofloxacin is the optical *S*- (-) isomer of ofloxacin. Ofloxacin is a racemic mixture, but the *S*-isomer has antibacterial activity 32- to 128- fold more potent than the *R*-isomer. Therefore, most of the antibacterial activity of ofloxacin is due to the *S*-isomer, and levofloxacin has been developed to take advantage of this antibacterial potency allowing much smaller doses with an improved toxicity profile {*Kucer, 1997*}. *In vitro* studies suggest that levofloxacin is 2-8 fold more active than ofloxacin against

the most common enteric pathogens, equally efficacious as ciprofloxacin against the most common enteric pathogens, and 2-fold more potent than ciprofloxacin against *Campylobacter jejuni* {*Inagaki, 1989*}. A Japanese study using levofloxacin 200-300 mg/day for 5-7 days in 114 patients with bacterial enteritis showed clinical cure rates of 97% in 72 hours {*Davis and Bryson, 1994*}. Based on this data, recent reviews of the prevention and treatment of traveler's diarrhea include levofloxacin with ofloxacin and ciprofloxacin as a first line treatment option {*Ansdell VE and Ericsson CD, 1999; Juckett G, 1999*}.

Alternative approaches to empiric travelers' diarrhea therapy have primarily evaluated single-dose regimens and non-fluoroquinolone antibiotic agents. Single-dose fluoroquinolone therapy has demonstrated equal effectiveness to 3- or 5-day regimens for travelers' diarrhea, as well as, specific therapy for shigellosis (not *S. dysenteriae*) {*Oldfield EC, et al., 1987, Gotuzzo E, et al., 1989; Salam I, et al., 1994; Petrucelli BP, et al., 1992; Bennish ML, et al., 1992; Ericsson CD et al., 1997*}. Non-fluoroquinolone-based empiric therapy has been studied using a relatively new macrolide antibiotic, azithromycin, with greater *in vitro* activity against many gram-negative bacteria than erythromycin. As previously stated, azithromycin 500 mg daily was compared with ciprofloxacin 500 mg daily (each 3-day regimens) for diarrhea in U.S. service personnel during Cobra Gold 1993 and was found to have comparable in efficacy {*Kuschner RA, et al., 1995*}. This study was limited by the small sample size with minimal ability to detect moderate effect differences of the azithromycin regimen (statistical power < 25%). In fact, there were only 2 clinical failures in the entire study group, both being ciprofloxacin-treated campylobacter cases. Significant differences in improved microbiologic eradication of campylobacter were demonstrated with azithromycin however, this did not translate into statistically significant clinical differences. Importantly, the only statistically significant clinical findings on subgroup analysis were a reduced duration of illness in non-campylobacter cases with ciprofloxacin. Given the observations, non-campylobacter bacterial etiologies represent as many as 40% of cases and azithromycin was not clearly superior to ciprofloxacin (even in campylobacter cases), empiric therapy with a fluoroquinolone remained the standard recommendation.

More recently, it was noted that patients receiving either 1000 mg of azithromycin weekly or 250 mg of azithromycin daily for a malaria prophylaxis trial were protected during an outbreak of dysentery {*Shanks GD, et al., 1996*}. A trial was conducted comparing azithromycin (500 mg initially then 250 mg daily over 5 days - total 1.5 gm) with ciprofloxacin 500 mg twice daily for 3 days in patients treated with shigellosis, and found the regimens comparable {*Khan WA, et al. 1997*}. A single 1 gm dose of azithromycin was also compared with a three day course of ciprofloxacin in patients with shigellosis, and again the results were comparable {*Shanks GD et al., 1999*}. Azithromycin has been proposed as an alternative therapy for patients unable to take quinolones or travelers to areas with known high *Campylobacter* endemicity {*Ansdell, 1999; Juckett, 1999*}. Based on this data, the increasing prevalence of quinolone-resistant *Campylobacter*, and observational studies suggesting increasing clinical failures with standard empiric therapy (10-20%), the proposed trial seeks to investigate the question of what antimicrobial regimen is most appropriate for empiric management of travelers' diarrhea in Thailand.

An additional objective, other than formulating the best approach to empiric therapy, relates to optimizing diagnostic test strategies for acute diarrhea management. This project will evaluate both bedside (stool characterization and hemoccult) and field laboratory rapid diagnostic assay (fecal leukocyte smear, lactoferrin latex agglutination, *Campylobacter*-specific rapid assay, *Shigella*-specific rapid assay, and plasma C-reactive protein) effectiveness as components in the overall management strategy. Study physicians will perform bedside diagnostics whereas study team laboratory personnel will perform the field lab rapid assays. Field applicability of diagnostic tests is particularly relevant for the military. During military operations, the availability of a field laboratory with microbiologic capability is quite variable. Rapid, technically simple diagnostic tests need to be evaluated to determine accuracy and acceptability in field settings. Empiric therapy without supplemental laboratory is a feasible option however; refinement of management strategy using laboratory testing may increase cost-effectiveness and

allow specific adjustments in antibiotic selection based on regional susceptibility patterns.

Alternative diagnostic tests may be used to complement clinical findings without further confirmation, as a decision point for the need for stool microbiology, or simply an additional piece of data with microbiology. Rapid diagnostic assays can be non-specific or pathogen-specific {*Hines J and Nachamkin I, 1996*}. Non-specific testing is directed at distinguishing non-inflammatory from inflammatory diarrhea. Patients with inflammatory diarrhea (caused by such as pathogens as *Shigella* spp. and *Campylobacter* spp.) do not benefit as greatly from rehydration therapy as non-inflammatory watery diarrhea and often require antibiotic therapy for clinical resolution {*Riley LW, 1995*}. This dichotomy is problematic due to bacterial enteropathogen clinical presentation overlap {*Hines J and Nachamkin I, 1996*}. A recent meta-analysis of fecal screening tests demonstrated improved accuracy of a newer test, fecal lactoferrin latex agglutination (marker for fecal leukocytes), as compared to the standard hemoccult (guaiac test for microscopic blood) and fecal leukocyte staining (methylene blue) {*Huicho L, et al, 1996*}. In addition to the stool-based screening test, this study will evaluate a plasma-based test of inflammatory disease, C-reactive protein (an acute phase protein produced by the liver during infectious and non-infectious inflammatory disease) {*Pepys MB, 1981*}. Pathogen-specific tests under evaluation include a commercially available, visually read, solid phase immunoassay for the detection of *Campylobacter*-specific antigens (ProSpecT® Campylobacter Microplate Assay, Alexon-Trend, Inc., Ramsey, MN) and an experimental lateral flow immunoassay for the detection of *Shigella* spp. (Shigella Reveal™, AMPCOR Diagnostics, Inc., Neogen Corp. subsidiary, Bridgeport, NJ). Neither assay has peer-reviewed published data (refer to attached package inserts for further information). All tests will be compared with the "gold standard" stool microbiology results.

The case-control study will investigate a specific enteric pathogen's risk association with various factors (demographic characteristics, developing region travel experience, past episodes of travelers' diarrhea, recent food/water exposures, and host immunology). Post-deployment questionnaire-based studies have investigated behavior-related risk factors for diarrheal disease {*Sanchez JL, et al., 1998*}. Ice consumption and visiting a nearby resort city were associated with an increased risk of diarrheal disease however; no other specific food/drink exposures were identified. Limitations of this type of survey include the lack of microbiology data, temporal spacing between the exposure and questionnaire, self-report for case ascertainment, and the lack of host immunology. The NMRC Enteric Diseases Department has conducted experimental *C. jejuni* infection studies to better understand post-infection immunologic responses. These studies have demonstrated *C. jejuni*–specific antibody secreting cell (ASC) responses (100%), fecal IgA (~90%), and serological responses (~70-100%) in experimentally infected volunteers (Tribble, DR; unpublished data). In addition, experimental challenge studies provide support for an immune correlate/surrogate role for *C. jejuni*–specific fecal IgA and *in vitro* production of interferon gamma (IFN γ) in response to *C. jejuni* whole cells. These findings need to be compared to community-acquired *Campylobacter*-associated immune responses. This project aims to better understand illness-associated risk factors and possibly provide preliminary results concerning *Campylobacter*-associated immune correlates from a population-based study. The research objectives attempt to address a range of questions concerning host behavior and susceptibility, as well as, determining the most effective diagnostic and therapeutic approaches for deployed military personnel in Southeast Asia.

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6. Study Design

A. Project Overview

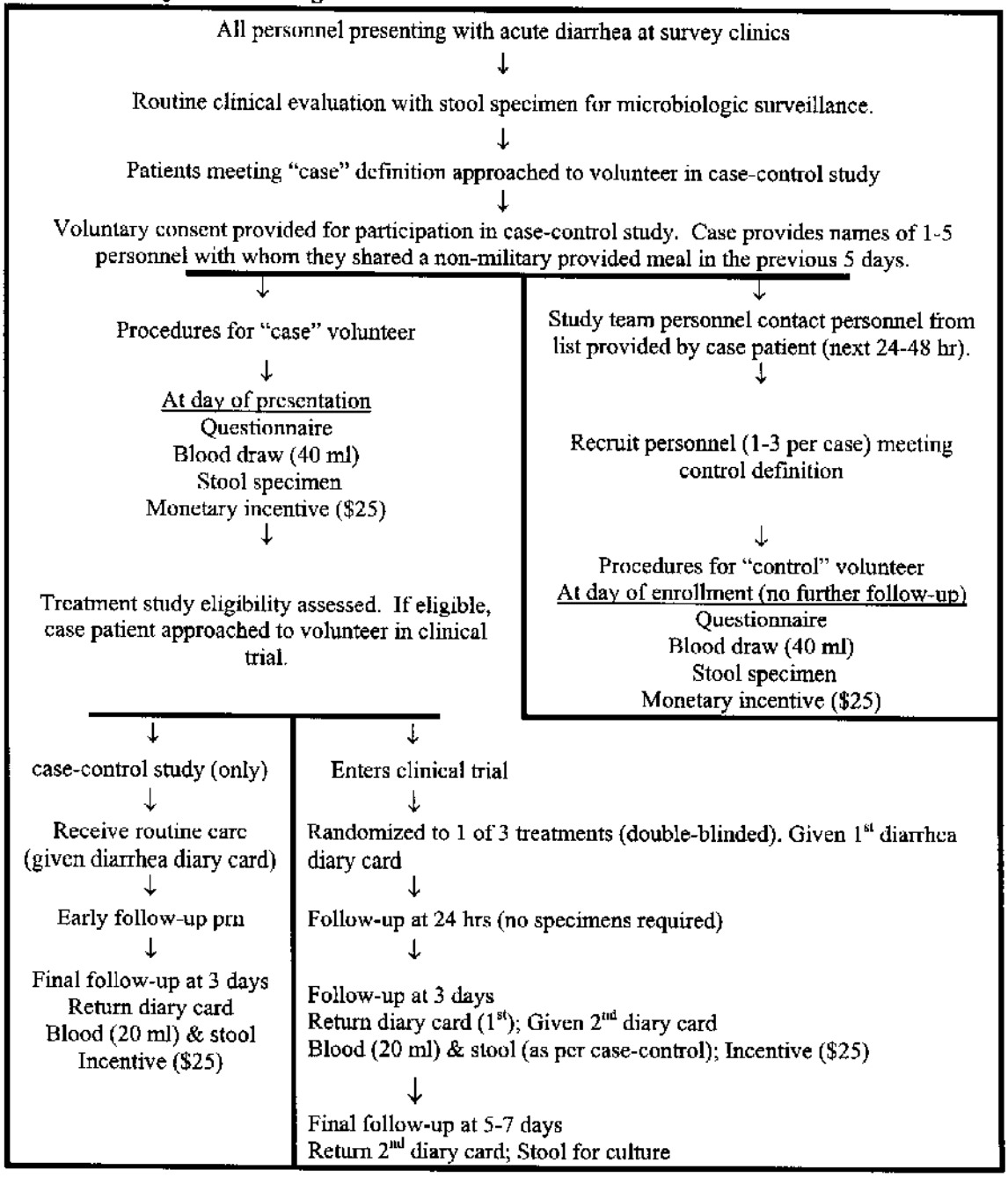
The project has three primary components: a case-control study, a randomized clinical trial, and performance evaluations of diarrheal diagnostic assays. Each study component originates from a surveillance system for acute diarrhea. This passive clinic-based system is a specific JTF tasking for AFRIMS/NMRC and does not constitute research. Personnel presenting with acute diarrhea at survey clinics will all receive appropriate clinical evaluation and care. A major resource being provided as part of the research study, in addition to infectious disease clinical expertise, is the field microbiology laboratory. The presence of the field laboratory allows the inclusion of diagnostic stool microbiology during routine clinical care.

Diarrhea patients will be offered participation in the case-control study, as well as, the randomized controlled trial (if meeting eligibility criteria). Patients consenting to the case-control study will be asked to provide 1-5 names of other active duty personnel with whom they have shared a meal in the previous 5 days (consisting of food not provided by the U.S. military). Study team personnel will attempt to contact these individuals, determine if they fulfill the asymptomatic control definition, and assess their willingness to participate as control volunteers. A total of 1-3 controls may be enrolled per diarrhea case. Cases and controls will both complete a questionnaire, provide stool specimens for microbiology evaluation, and undergo phlebotomy (40 ml) for pathogen-specific immunology. Control volunteers will be evaluated at one time point (within 2 days of the case enrollment). Cases volunteering for the treatment study will be evaluated in follow-up as determined by the trial procedures. If a diarrhea patient volunteers for the case-control study, but

not for the clinical trial (or was not eligible) then the patient would return for follow-up three days after enrollment for additional blood and stool specimens. Incentive payments will be provided to volunteers for each blood draw ($25 per bleed).

Volunteers consenting to participate in the randomized clinical trial will be assigned the next available treatment code number. The treatment code assignment schedule will be created using block randomization (block size of 6). Allocation ratio of treatment assignments will be equal for the three study regimens (1: 1: 1). The study will use a double-blinding procedure during the clinical and laboratory phases of the study. Endpoints for the treatment trial, case-control study, and the diagnostic test performance evaluation are discussed in the "Study Design" section.

B. Project Flow Diagram



C. General Volunteer Information

i. Source of volunteers

The study population is limited to U.S. military personnel participating in Cobra Gold exercises in Thailand during May 2000 and May 2001. During Cobra Gold 2000, the study will be limited to troops stationed in the Nakhon Si Thammarat area in southern Thailand. Study site(s) for the Cobra Gold 2001 exercise will be determined at the time of the planning meetings in November 2000.

ii. Age range and gender inclusions

The study population consists of U.S. military personnel deployed to Thailand during annual Cobra Gold exercises each May (2000 and 2001) (males and females at least 18 years old). There are no gender, age, or race/ethnicity restrictions.

iii. Subject identification

Confidentiality will be maintained by removing personal identifiers and assigning the volunteer a study number during laboratory specimen processing, data entry, and analysis. The clinical forms used in this study are for inclusion in the volunteer's medical record and will maintain personal identifiers. No personal identifiers will be used in publications.

D. Project Definitions

- **Diarrhea** = $\geq$ 3 loose or liquid stools in 24 hour period OR $\geq$ 2 loose or liquid stools in 24 hr period plus $\geq$ 1 associated symptoms
- **Fever** = oral temperature $\geq$ 100.0° F
- **Diarrhea-associated signs/symptoms** = abdominal pain or cramps, nausea, vomiting, fever, tenesmus, and gross blood in stools temporally related to the diarrheal episode
- **Stool character based on the following grading scheme**

  Grade 1 - hard (normal)
  Grade 2 - soft (normal)
  Grade 3 - thick liquid
  Grade 4 - opaque watery liquid
  Grade 5 - clear watery

- **Study Days**

  Day 0 = day of initial clinical presentation
  Day 1 = 24 hr (allowable out to 36 hr) after 1st study medication dose
  Day 3 = ≥ 72 hr (allowable out to 120 hr) after 1st study medication dose
  Day 5-7 = ≥ 120 hr (allowable out to 240 hr) after 1st study medication dose

- **"Case"** = military personnel presenting to one of the survey clinics with illness meeting diarrhea definition with duration of < 14 days and illness onset at least 24 hours after arrival in Thailand

- **"Control"** = military personnel not meeting the diarrhea definition during the previous 5 days and the following 2 days after enrollment; volunteers are matched (1-3 per case) with a case patient based on similar opportunity for exposure defined by sharing a meal (non-U.S. military provided) with case patient in past 5 days

- **Case-control predictor (potential) variables**: demographic characteristics (age, gender), prior Thailand/Southeast Asia travel, history of travelers' diarrhea, food/water exposures, and host immunology (pathogen-specific if isolates recovered)

- **Clinical cure** = complete resolution of diarrhea and diarrhea-associated signs/symptoms within 72 hours of first dose of study medication

- **Last diarrheal stool** = last Grade 3-5 stool occurring in a 24-hr period meeting the diarrhea definition

- **Last unformed stool** = last Grade 3-5 stool produced by subject followed by a 24-hr period with no diarrhea-associated symptoms

- **Microbiologic cure** = eradication of the patient's isolate, previously detected on the pre-treatment stool culture, at follow-up approx. 48-72 hours after last dose of study medication

- **Evaluable subject in clinical trial** = patient receiving follow-up 2-3 days after last antibiotic dose with no use of concomitant medications likely to affect the clinical course; additional analysis will evaluate patients that have follow-up limited to the clinical visit 72 hours after 1st antibiotic dose

- **Seroconversion and fecal IgA conversion** - ≥ 4-fold increase over the baseline titer against a pathogen-specific antigen.

- **Antibody secreting cell (ASC) response** - ≥ 5 pathogen-specific ASC per 10^6 peripheral mononuclear cells.

E. Diarrheal Surveillance

i. Clinical procedures

Clinic-based passive surveillance does not constitute a research component of the overall project. Patients will be evaluated and cared for as per standard clinical practice. Initial

evaluation will be documented on the standardized (SF 600) clinic visit form ["Cobra Gold Initial Clinic Visit (Diarrhea Surveillance)"].

ii. Laboratory procedures

Patients will be requested to submit a stool sample/rectal swab as per routine patient care. The specimen container will be labeled with the patient's initials and last 4 of their SSN if they are not enrolled in the research study. The date specimen collected, patient's name, and labeling used on specimen will be recorded on the "Study Site Volunteer Log" (see Appendix). The study physician will send the specimen to the field laboratory after stool characterization and hemoccult. In the field laboratory, routine microbiology and rapid assays (non-specific and pathogen-specific) will be completed (as per attached SOP).

iii. Analysis

Descriptive analysis of relative frequencies of culture-confirmed specific pathogens will be compared in relation to clinical presentation features (such as presence or absence of fever), non-culture-based tests (inflammatory vs. non-inflammatory), and temporal relationship during the exercise. The temporal relationship will explore if any differences exist concerning isolation rates of specific pathogens based on the time in country and the period of the exercise. The independent variables include the various clinical symptoms, signs, demographic characteristics, and time period. The dependent variables used will be discrete including presence or absence of specific pathogens and any isolate. Statistical tests used for bivariate analysis will vary based on whether or not the independent variable uses discrete or continuous measurement. The variable designation will be consistent throughout the analysis of all study components.

F. Case-control Study

i. Sample size estimates

The case-control study size is based on estimates of fixed numbers of cases available for enrollment (assumed 100 for year 1). Controls are being matched based on sharing a meal not provided by the U.S. military in the past 5 days from the time the case was first evaluated. Given the finite number of cases (100), a realistic number of matched controls that could be enrolled for each case in a field setting are 2 (allowable range 1-3). The exposure of interest is the presence of bacterial enteropathogens on stool culture. During Cobra Gold 1998, a convenience sample of stool cultures was obtained from asymptomatic deployed military personnel (n = 77) in Thailand. This pilot study revealed the following pathogen-specific rates: *Campylobacter* spp. (2.6%), ETEC (2.6%), *Shigella* spp. (none), and non-typhoidal *Salmonella* spp. (12%). The case-control analysis will be performed on a pathogen-specific basis to assess demographic characteristics, prior travel experience, travelers' diarrhea history, food/water exposures, and host immunology. The assumed exposure rate in the controls was 5% and the odds ratio deemed meaningful to detect was 4.0. Given these assumptions at $\alpha = .05$, the study's statistical power exceeds 90%.

ii. Entry criteria

Inclusion Criteria

- patient meets diarrhea definition with diarrheal symptoms of < 14 days duration with onset at least 24 hours after arrival in Thailand

Exclusion Criteria

- patients receiving antibiotics (excluding malaria prophylaxis with either mefloquine or doxycycline) in the 72 hr prior to presentation

iii. Procedures

a. Clinical

Refer to the "Project Flow Diagram" and the "Project Definitions" for time points and procedures for cases and controls. The study forms detailed below are all provided as Appendices.

Case enrollment and evaluation: Any active duty member presenting to a survey clinic with acute diarrhea (as defined above) will be considered for study enrollment. If the subject is eligible and agrees to enter the study, the study physician will complete the informed consent process with the patient. Eligible volunteers participating in the randomized controlled trial will follow clinical procedures as outlined in that section. The patient will be given a symptom diary card ("Diarrhea Symptom Diary") during the initial evaluation on which the patient will be asked to record symptoms, including number of loose stools, nausea, vomiting, abdominal cramps, fever, and bloody stools over the following 72 hours. The patient will be asked to return with the diary card at the 72-hour follow-up. Cases will be asked to provide the names of 1-5 personnel with whom they have shared a non-U.S. military provided meal in the previous 5 days. Study team personnel will attempt to contact these individuals as described below under "Control enrollment". The forms and procedures used at each clinical time point are listed below by study day.

Study Day	Clinical forms/procedures (in order of occurrence) for Cases
0	1. SF600 Cobra Gold Initial Clinic Visit (Diarrhea Surveillance) - Standard medical history & physical examination [Assess eligibility] 2. Bedside stool characterization & hemoccult test 3. Provide clinical care as appropriate (if not in treatment trial) 4. Volunteer Consent Form (Diarrhea cases) ***provide copy to volunteer** 5. Volunteer Registry Form 6. Cobra Gold Diarrhea Study Questionnaire 7. Collect names of 1-5 personnel with whom they have shared a non-military provided meal in the past 5 days 8. Diarrhea Symptom Diary Card (given to patient) 9. Phlebotomy (40 ml) 10. Incentive payment provided ($25) 11. Complete entry in individual volunteer study file [Study Medication, Specimen, and Clinical Visit Log] 12. Complete entry in Study Site Volunteer Log

3	1. SF600 Cobra Gold Follow-Up Clinic Visit (Diarrhea Surveillance) - Standard medical history & physical examination 2. Collect Diarrhea Symptom Diary Card 3. Bedside stool characterization & hemoccult test 4. Phlebotomy (20 ml) 5. Incentive payment provided ($25) 6. Complete entry in individual volunteer study file [Study Medication, Specimen, and Clinical Visit Log] 7. Mark attendance on Study Site Volunteer Log

Controls: Potential control subjects will be identified and recruited as previously described. Interested personnel who meet eligibility criteria will have informed consent administered by study team personnel. The forms and procedures used at each clinical time point are listed below by study day.

Study Day	Clinical forms/procedures (in order of occurrence) for Controls
Day of enrollment	1. Volunteer Consent Form (Asymptomatic controls) ***provide copy to volunteer** 2. Volunteer Registry Form 3. Cobra Gold Diarrhea Study Questionnaire 4. Collect stool specimen 5. Phlebotomy (40 ml) 6. Incentive payment provided ($25) 7. Complete entry in individual volunteer study file [Study Medication, Specimen, and Clinical Visit Log] 8. Complete entry in Study Site Volunteer Log

b. Specimen Collection/Laboratory

The case subjects and the control subjects will be asked to submit a stool and blood specimen. Stool specimens for cases will be collected twice (pre-treatment and day 3). Controls will submit a single stool specimen at the time of enrollment. Volunteers will be given "hats" that fit over the rim of the toilet to aid in the collection of stool. If the volunteer is unable to submit a stool specimen, they will be given the option to submit a rectal swab instead. They will be instructed on the use of the swab, and then they will collect the specimen. If a swab specimen is collected then an additional request for a stool specimen within the next 12 hr will be made. The research physician or a trained phlebotomist using standard phlebotomy techniques will draw the blood specimen. Forty milliliters (8 tablespoons) will be drawn at the time of the initial evaluation. The control subjects will only be asked to undergo phlebotomy at one time point, but case subjects will have blood specimens taken a second time at Day 3 (20 ml). Refer to "Diarrheal diagnostics evaluation" section for further detail concerning timing of specimens, laboratory procedures, determinations for each specimen, and test site. The date specimen collected, patient's name, assigned Vol. ID number, and specimen label number (LAN = laboratory accession number) used will be recorded on the "Study Site Volunteer Log" (see Appendix).

iv. Risk/Benefit

The case-control study would seem to be minimal since risk is limited to potential specimen collection-related adverse events. Volunteers will be asked to submit stool specimen(s) and undergo phlebotomy. No risk to the subject is anticipated for stool collection. If the subject is unable to provide a stool specimen for their initial stool culture, he/she will be given the

option of submitting a rectal swab specimen. The volunteer will be properly instructed and will collect the swab specimen. Mild transient discomfort may occur related to the swab collection but is otherwise safe. Risks associated with venipuncture include minor pain, discomfort, bleeding (hematoma), infection, or injury. Blood drawing by trained personnel will minimize risks. There are no special risks to pregnant or potentially pregnant women volunteers related to specimen collection. A minimal amount of total blood volume is being withdrawn as part of this study [cases (60 ml) and controls (40 ml)]. There are no direct benefits to volunteers through their participation in the case-control study. Subjects participating in the case-control portion of the study as either a case or a control will be given a monetary incentive of $25.00 for each blood sample. Case subjects will be asked to submit samples at presentation and at 72 hours and will therefore be offered $50.00 in total incentive payment. Control subjects will be asked to submit a blood specimen once and will therefore be offered $25.00 in total incentive. Payments will be provided in cash at the time of each phlebotomy.

v. Analysis

The presence of diarrhea with duration of < 14 days and illness onset at least 24 hours after arrival in Thailand will be used for case definition. Asymptomatic controls will have had a similar opportunity as the cases for exposure by selecting individuals who have shared a meal (non-U.S. military provided) with case patient in past 5 days. The exposure variable(s) for primary analysis is the presence/absence of bacterial enteropathogens in stool cultures at the time of enrollment. The disease association of each enteropathogen (i.e. *Campylobacter* spp., non-typhoidal *Salmonella* spp., enterotoxigenic *E. coli*, and *Shigella* spp.) will be analyzed in relation to reported demographic characteristics, prior Southeast Asia deployment, past history of travelers' diarrhea, recent dietary history, and immunology assays at the time of enrollment. Cases are individually matched to 1-3 controls. It is likely there will be a variable number of controls per case therefore a matched analysis using maximum likelihood estimates of the Mantel-Haenszel odds ratio will be evaluated on a pathogen-specific basis. The case-control pairings will also be broken in order to compare matched and unmatched analyses. Stratified analyses of exposure odds ratios (with 95% confidence intervals) will explore the potential predictor data collected through the questionnaire and host immunology testing. Pathogen-specific immune responses will be summarized with descriptive statistics. Internal comparisons of immunologic responses will be undertaken based on diarrheal diagnostic analyses (particularly culture results) with a given pathogen. Humoral immune responses, serology, ASC and fecal IgA, will be assessed both within and between individuals with similar pathogen isolates.

G. Randomized controlled trial

i. Sample size estimates

The estimated sample size requirements for each treatment group are 60 patients. The primary clinical outcome used to estimate study size is the proportion of patients meeting the clinical cure definition (complete resolution of diarrhea-associated symptoms by 72 hours). Clinical cure rate comparisons can be made with both historical placebo cure rates (approximately 60%) and rate differences between study medications. The assumptions used for calculations are as follows:

Null hypothesis: No difference between historical placebo rate of 60% and observed clinical cure study medication rate (90%).
Assumptions: $\alpha = .05$; Power = 80%; effect size = .30
Number needed per group: 38

Null hypothesis: No difference between highest and lowest observed clinical cure study medication rates.
Assumptions: $\alpha = .05$; Power = 80%; effect size = .20
Number needed per group: 59

Based on previous Cobra Gold research experience, an estimated number of enrollments during a single exercise are approximately 100. In order to reach a total enrollment of 180 volunteers (also accounting for dropouts) it will be necessary to extend the study over two exercise periods. If one of the treatment regimens were to demonstrate an intermediate clinical cure rate (an effect size of .10 as compared to the most efficacious treatment) then the study size available will not be able to discriminate if the difference is statistically significant. However, other outcomes, such as time to events, total numbers of loose stools, and microbiologic cures, may contribute supporting evidence of a meaningful treatment difference. An interim analysis (as discussed in the "Data Analysis" section) may lead to abandoning one of the treatment arms in the second enrollment period. If this were to occur then the number of enrolled subjects per group for the remaining regimens would be approximately 85 patients. This would increase the statistical power to approx. 50% that this study could detect a true difference between treatments as low as 10%.

ii. Entry criteria

Inclusion Criteria

- patient meets diarrhea definition with diarrheal symptoms of $\leq$ 96 hours duration
- patient will be managed on an ambulatory basis and can comply with follow-up procedures

Exclusion Criteria

- female patients with positive urine pregnancy test at presentation (urine hCG) [contraindicated with fluoroquinolone therapy]
- patients with history of allergy to macrolide or quinolone antibiotics (does not include limited gastrointestinal upset)
- patients receiving antibiotics (excluding malaria prophylaxis with either mefloquine or doxycycline) in the 72 hr prior to presentation
- patients taking medications known to have drug-drug interaction with either study drug (includes theophylline, digoxin, and warfarin)
- patient with history of seizures (relative contraindication for fluoroquinolone therapy)

iii. Procedures

a. Clinical

Refer to the "Project Flow Diagram" and the "Project Definitions" for time points and procedures. The study forms detailed below are all provided as Appendices.

Treatment study enrollment and evaluation: Any active duty member presenting to a survey clinic with acute diarrhea meeting all entry criteria is eligible for study enrollment. If the subject agrees to enter the study, the study physician will complete the informed consent process with the patient. Female patients will be asked to submit a urine sample for a pregnancy test at presentation. Volunteers will be assigned the next sequential "Treatment

Number". Study medication (labeled with the appropriate "Treatment Number") will be dispensed to the volunteer by the study physician in a "combi bottle" (described further in "Study medications" section). The study physician will administer the 1st study medication dose and document time on the SF600 Cobra Gold Initial Clinic Visit (Diarrhea Surveillance) form. The patient will be observed for a 30-minute period in order to monitor for immediate adverse reactions.

The follow-up evaluations at 24 and 72 hours are designed to measure both disease progression/resolution and potential drug toxicity. The patient will also be given a symptom diary card during the initial evaluation on which they will be asked to record symptoms, including number of loose stools, nausea, vomiting, abdominal cramps, fever, and bloody stools over the following 72 hours. The patient is to return with the diary card at the 24 and 72-hour follow-up. A final follow-up (5-7 days after 1st antibiotic dose) will be completed on a standardized form to assure clinical response and obtain a stool specimen to assess microbiologic eradication. This follow-up visit may be done in the clinic or through contact with study team personnel. The forms and procedures used at each clinical time point are listed below by study day. The blood collections and the incentive payments are included for completeness however; blood specimen collection is a component of the case-control study and not the clinical trial. It is anticipated that the majority of eligible volunteers will volunteer for both the case-control and the treatment trial. A single consent form is to be used for both project components incorporating each components' eligibility criteria and sections for volunteer to opt for one or both components.

Study Day	Clinical forms/procedures (in order of occurrence) for Clinical Trial
0	1. SF600 Cobra Gold Initial Clinic Visit (Diarrhea Surveillance) - Standard medical history & physical examination [Assess eligibility] 2. Bedside stool characterization & hemoccult test 3. Prospective female volunteers provide urine sample for urine hCG testing. 4. Volunteer Consent Form (Diarrhea cases) ***provide copy to volunteer** 5. Volunteer Registry Form 6. Cobra Gold Diarrhea Study Questionnaire 7. Collect names of 1-5 personnel with whom they have shared a non-military provided meal in the past 5 days (potential controls) 8. 1st Diarrhea Symptom Diary Card (given to patient) 9. Phlebotomy (40 ml) 10. Incentive payment provided ($25) 11. Complete entry in individual volunteer study file [Study Medication, Specimen, and Clinical Visit Log] 12. Complete entry in Study Site Volunteer Log
1	1. SF600 Cobra Gold Follow-Up Clinic Visit (Diarrhea Surveillance) - Standard medical history & physical examination 2. Check compliance with 1st Diarrhea Symptom Diary Card 3. Complete entry in individual volunteer study file [Study Medication, Specimen, and Clinical Visit Log] 4. Mark attendance on Study Site Volunteer Log
3	1. SF600 Cobra Gold Follow-Up Clinic Visit (Diarrhea Surveillance) - Standard medical history & physical examination 2. Collect 1st Diarrhea Symptom Diary Card 3. Provide 2nd Diarrhea Symptom Diary Card 4. Bedside stool characterization & hemoccult test 5. Phlebotomy (20 ml) 6. Incentive payment provided ($25)

	7. Complete entry in individual volunteer study file [Study Medication, Specimen, and Clinical Visit Log] 8. Mark attendance on Study Site Volunteer Log
5-7	1. Cobra Gold Post-Treatment Follow-up (Diarrhea Surveillance) 2. Collect 2nd Diarrhea Symptom Diary Card 3. Collect stool specimen 4. Complete entry in individual volunteer study file [Study Medication, Specimen, and Clinical Visit Log] 5. Mark attendance on Study Site Volunteer Log

b. Specimen Collection/Laboratory

Specimen collection is limited to stool samples and a pre-treatment urine sample for hCG in female volunteers. As previously stated, most, if not all, of the treatment study volunteers are anticipated to be volunteers in the case-control study. Given this, refer to the case-control specimen collection schedule and determinations for blood and stool immunology. Stool specimen collection will be done at three time points for microbiology (day 0, day 3, and day 5-7). Collection procedures are similar to methods previously described in the case-control study. Refer to "Diarrheal diagnostics evaluation" section for further detail concerning timing of specimens, laboratory procedures, determinations for each specimen, and test site. The date specimen collected, patient's name, assigned Vol. ID number, and specimen label number (LAN = laboratory accession number) used will be recorded on the "Study Site Volunteer Log" (see Appendix).

iv. Study medications

There will be two medications, azithromycin and levofloxacin, used during the clinical trial. Pfizer Pharmaceuticals Clinical Research Division in Groton, CT will supply both study medications and their respective placebo formulation. Pfizer pharmacy representatives will also supply the randomization schedule using a blocked randomization (block size = 6). The individually packaged "combi bottles" will have each bottle labeled with the study identification number and the appropriate medication day as per the randomization schedule. The "combi bottle" will be also identified using a two-panel label. Panel 1 of the label is permanently affixed to the bottle and will contain the randomization number. Panel 2 of the label will be removed from the container and affixed to the dosing record section of the "Cobra Gold Study Medication/Specimen Log". A "blinded envelope" will be provided from Pfizer for each treatment assignment. In the event of a medical emergency (such as serious medication-related allergic and/or adverse reaction or disenrollment due to hospitalizing subject due to disease progression) it will be necessary to break the double-blind code for that individual. Detailed information concerning the dosage regimen and potential risks is provided in the "Risks/Benefits" and the "Medical care" sections of the protocol. The study medications will both have an identical appearing placebo form so as to appear indistinguishable. The azithromycin will be in the form of 500-mg tablets and will be dispensed as either 500 mg daily for 3 days or 1000 mg in a single dose. The levofloxacin will be in the form of 250-mg tablets and will be dispensed as 500 mg daily for 3 days. To keep the patients and researchers blinded, each patient will receive tablets from each study medication (active drug or placebo) as detailed in Table 2. The medicines will be dispensed in a three-day "combi bottle" with a separate bottle for each treatment day of study. Each of the medicines is heat stable and can be maintained at room temperature during the study. Unused doses of the medication will be returned to the manufacturer at the completion of the study.

Table 2. "Combi bottle" components (A = active drug; P = placebo) for each study regimen

Study regimen	Day 1				Day 2				Day 3			
	Azithro		Levo		Azithro		Levo		Azithro		Levo	
	A	P	A	P	A	P	A	P	A	P	A	P
Azithro (1 gm x 1)	2	0	0	2	0	1	0	2	0	1	0	2
Azithro (500 mg/d x 3 d)	1	1	0	2	1	0	0	2	1	0	0	2
Levoflox (500 mg/d x 3d)	0	2	2	0	0	1	2	0	0	1	2	0

* Azithromycin supplied as 500 mg tablets; Levofloxacin supplied as 250 mg tablets

v. Risk/Benefit

Specimen collection-related risks are provided under the case-control section. Subjects participating in the treatment trial portion of the study will be randomly assigned to one of three treatment regimens using one of the two antibiotics, levofloxacin and azithromycin. Any one of the regimens may prove to be more or equally effective at treating acute infectious diarrhea acquired in Thailand. The treating physician will manage diarrheal patients declining participation using standard of care practices. The potential benefit to the subject is a more rapid resolution of symptoms that may occur with one of the study regimens as might be expected with standard therapy. The potential risks of the study involve either sub-optimal efficacy of the study drug or toxicity from the drug. The use of non-antibiotic antidiarrheal medications, such as loperamide, will not be allowed during the study given the significant confounding of all diarrhea-related clinical outcomes if used. Loperamide therapy when given as a single agent has demonstrated efficacy in the management of acute diarrhea {*Ericsson CD and Johnson PC, 1990*}. The additive efficacy of loperamide to empiric antibiotic therapy has been variable in clinical trials. Prior studies performed in military personnel have not demonstrated a significant reduction in illness duration with the inclusion of loperamide in the antibiotic treatment regimen and comparable recovery rates were demonstrated with antibiotic therapy alone {*Taylor DN, et al. 1991, Petrucelli BP, et al., 1992, Kuschner RA, et al., 1995*}.

Levofloxacin is generally well tolerated, with most adverse effects being the mild and transient gastrointestinal or central nervous system side effects shared by all quinolones. In 5 comparative trials with ofloxacin involving 918 patients, a lower incidence of gastrointestinal symptoms (1.2 vs. 5.2%) and CNS symptoms (0.8 vs. 2.2%) was seen in the levofloxacin recipients. The incidence of abnormal laboratory findings (mild transient elevation of liver enzymes, eosinophilia, or leukopenia) was similar in levofloxacin (2.4-15.5%) as compared with ofloxacin (4.3-18.2%). In two of the largest non-comparative trials of levofloxacin involving 984 patients, the following side effects were noted: abdominal discomfort (1%), anorexia (0.4%), diarrhea (0.4%), insomnia (0.5%), headache (0.3%), dizziness (0.2%), oral effects, such as mouth irritation, loss of taste, tongue numbness, or dry mouth, (0.5%), and rash (0.2%). As with the other quinolones, levofloxacin has been shown to cause articular damage in animal studies at high doses, and the phototoxic potential of levofloxacin in mice appears similar to that with ofloxacin and ciprofloxacin. {*Davis and Bryson, 1994*} The subjects will be informed of the potential side-effects of this medicine and specifically asked about the development of these symptoms during their clinical evaluations at 24 and 72 hours, and these results will be noted on a standardized questionnaire. If any of these symptoms, or other previously undescribed side-effects, are deemed to be severe by the subject or the physician, the patient will be removed from the study, the code broken, and the patient treated with alternative therapy.

Azithromycin is generally well tolerated with minimal side effects consisting mainly of gastrointestinal complaints {*Kucer, 1997*}. In a study of 3,995 patients receiving azithromycin, 5-day regimen (total 1.5 gm) or single dose (1 gm), were less likely to report side-effects, 12% vs. 14%, as compared to 3,108 patients receiving one of 12 other antibiotics (such as penicillin, amoxicillin, erythromycin, doxycycline, cephalexin, and cefaclor){*Hopkins, 1991*}. The most common symptoms were diarrhea (3.6%), abdominal pain (2.5%), nausea (2.6%), vomiting (0.8%), and headaches and dizziness (1.3%), all of which occurred less frequently than with the comparison antibiotics. The only side effects occurring more commonly than the standard comparison antibiotics were vaginitis (0.4%) and rash (0.6%). The only laboratory abnormality noted was a mild, transient increase in the hepatic transaminases in 1.7% of patients. Only 0.7% of patients receiving the 5 day course discontinued the drug due to side effects. Single-dose azithromycin (1250 mg weekly) for MAC prophylaxis in AIDS patients is discontinued in approximately 6% due to gastrointestinal (GI) side effects {*Bartlett, 1998*}. Further suggestive of azithromycin's dose-related GI side effect relationship is the 34% GI complaint rate observed in a study assessing gonorrhea therapy using a particularly large single dose of 2 gm {*Drew and Gallis, 1992*}. A summary table of the most commonly reported adverse symptoms (and frequency of occurrence) divided by this study's treatment regimen is provided below.

Most commonly reported side effects for each study medication

Reported symptom	Azithromycin (3-day treatment)	Azithromycin (single dose treatment)	Levofloxacin (3-day treatment)
Nausea	3 %	5 %	3 %
Vomiting	< 1 %	2 %	< 1 %
Diarrhea	5 %	7 %	2 %
Abdominal pain	3 %	5 %	< 1 %
Rash	< 1 %	< 1 %	< 1 %
Dizziness	< 1 %	< 1 %	< 1 %
Headache	< 1 %	< 1 %	< 1 %
Vaginitis (yeast infection)	< 1 %	1 %	< 1 %

There have been no significant drug-drug interactions reported with either levofloxacin or azithromycin. Co-administration with magnesium- or aluminum-containing anti-acids or ferrous sulfate reduces the bioavailability of levofloxacin by 15-52% (no effect on azithromycin). Therefore, patients will be instructed to separate the ingestion of any anti-acids by at least 1-hour prior and 2 hours after the ingestion of their assigned study medication. Women using oral contraceptives (OCP) will also be advised of the potential for decreased OCP efficacy, so they may consider alternative forms of birth control while receiving the study medication. While no interactions have been noted with theophylline, digoxin, or warfarin, cautious clinical practice dictates close monitoring of drug level or INR during co-administration. Given our inability to adequately monitor levels in the field, subjects who are currently taking any of these three medicines will be excluded from the study. Furthermore, any subject reporting prior hypersensitivity to any of the macrolides or any of the fluoroquinolones or nalidixic acid will be excluded from the study.

Azithromycin is generally considered safe in children and during pregnancy. Due to concerns over the possibility of cartilage/articular damage with fluoroquinolones noted in animal studies, this class of antibiotics is currently not approved in children or in pregnancy {*Kucer, 1997*}. Therefore, pregnancy tests (urine hCG) will be performed on female subjects prior to enrolling them into the study. Any subject found to be pregnant or unwilling/unable to submit a urine specimen for a pregnancy test will be excluded from the

clinical trial.

vi. Analysis

Therapeutic response will be evaluated for clinical measures [clinical cure (resolution of all diarrhea-associated symptoms by 72 hr after initial treatment); abatement of symptoms each 24-hr interval; time to symptom resolution (survival analysis)], microbiologic measures [eradication rates], and frequency of adverse events for each drug regimen. Intent-to-treat and standard (subjects meeting evaluable criteria) analysis will be performed. Statistical testing will use a p-value of $< .05$ as significant. An interim analysis is planned after the first year if the overall treatment failure rate $\geq 10\%$. In this case, data analysis will use a significance level of $p < .02$ in order to reduce the likelihood of increasing Type I error. Given this decision, a $p < .03$ will be used for the final analysis after year 2 giving a cumulative $p < .05$.

Subject baseline characteristics and summary follow-up findings will be compared using analysis of variance, Kruskall-Wallis tests, and chi-square tests. Differences in the frequencies of clinical cures and microbiologic eradication rates between study regimens will be tested for significance with Mantel-Haenszel procedures. Rates of adverse reactions will be similarly compared between study regimens. A determination of the last unformed stool will be sought for each volunteer with the respective date/time information recorded. Differences in recovery times will be evaluated using life-table analyses (daily number and frequency of patients remaining ill), log-rank (overall differences in response curves), and generalized Wilcoxon tests (response curve differences emphasizing early failures).

H. Diarrheal diagnostics evaluation

i. Procedures

a. Bedside testing

The stool specimen will be evaluated and graded by the research physician during the initial evaluation (refer to study definitions for grading scheme). The research physician will also perform a hemoccult test on the stool specimen (refer to Appendix for test procedure). The specimen will then be sent to a field microbiology lab. The study physician will use a standard urine hCG pregnancy test kit at time of presentation (refer to Appendix for hCG procedure). The urine hCG has a test sensitivity > 99% with a detection limit of 20 mIU/ml for urine specimens. The blood samples will be forwarded to the field laboratory for processing for future immunologic studies to be performed later at the Naval Medical Research Center.

b. Field laboratory testing/processing

1. Microbiology laboratory

The specimen will then be sent to a field microbiology lab where it will be examined for fecal leukocytes, fecal lactoferrin latex agglutination (LFLA), processed for culture, undergo rapid *Campylobacter* EIA and *Shigella* EIA testing (as discussed in the "Background/Rationale" section), and processed for future immunologic testing. Refer to the Appendix for diagnostic test procedures and interpretations. Primary culture work-up will be performed in the field (as per the attached AFRIMS SOP), then the samples will be forwarded to the Armed Forces Research Institute for Medical Sciences in Bangkok,

Thailand for final identification and determination of antibiotic susceptibilities. Stool specimens will be cultured for bacterial diarrheal pathogens and presumptive identification provided in the field laboratory (refer to "Cobra Gold Field Laboratory Data Abstraction Form").

2. Immunology laboratory

Stool and blood will be collected throughout the course of the study to measure the immune response to *C. jejuni* infection, as well as other bacterial enteric pathogens (enterotoxigenic *Escherichia coli* (ETEC), *Shigella*, and *Salmonella*). In the field laboratory, blood samples will be separated into plasma and mononuclear cell fractions using a ficoll-hypaque gradient technique. Plasma C-reactive protein tests will be performed as per the attached SOP. Upon completion of the study, clinical specimens will be shipped to the Naval Medical Research Center for laboratory analysis. The samples (plasma, mononuclear cells, and stool) will be stored at -70° C and transported in liquid nitrogen and dry ice.

c. Research laboratory testing

1. Microbiology

Further evaluation will be completed at the AFRIMS in Bangkok. This includes, but is not limited to, final species identification, serotyping and susceptibility testing of all isolates. All isolates will be archived and transported to NMRC. Laboratory specimens will be further evaluated for viral or parasitic etiologies of acute diarrhea. Samples of *E. coli* will be obtained for further analysis. Five colonies will be examined with specific DNA probes for genes encoding heat-labile toxin (LT), heat-stable toxin (ST), EPEC adherence factor, *E. coli* attachment effacement (eae), shiga-like toxin (SLT), I and II, enteroaggregative *E. coli* heat stable enterotoxin 1 (EAST-1), cytolethal distending toxin (CDT), cytotoxic necrotizing factor (CNF), and enteroinvasion determinants. These results will be used along with samples obtained from asymptomatic controls to correlate clinical symptoms with the presence of potentially pathogenic strains of *E. coli*.

2. Immunology

Immunologic assays used in the assessment include serology, antibody secreting cell (ASC) responses, fecal IgA, and other cellular immune responses to be determined (such as lymphocyte proliferation). The NMRC Immunology SOP for the serologic, coproantibody, and ASC determinations is included in the Appendix. Specific antigens that will be used in assays remain to be fully determined based on the microbiologic spectrum observed. However, at a minimum will include *Campylobacter* specific antigens (*C. jejuni* strain 81-176 glycine extract and whole cell), B-subunit of the heat-labile toxin of ETEC, somatic and protein *Shigella* antigens, and *Salmonella enteritides* whole cells.

ii. Specimen test schedule

Table 1. Specimen collection schedule and laboratory determinations

Days since presentation	Specimen type (amount)	Determinations	Test site
0	Urine (female volunteers)	hCG	Bedside
	Stool (10-12 g)	Stool characterization (grading)	Bedside
		Hemoccult	Bedside
		Lactoferrin Latex Agglutination	Field lab
		Routine Microbiology	Field lab/AFRIMS
		Fecal leukocyte smear	Field lab
		Rapid Campylobacter test	Field lab
		Rapid Shigella test	Field lab
		Total and pathogen-specific secretory immunoglobulin A (sIgA)	NMRC
	EDTA-Blood (40 ml)	C-reactive protein	Field lab
		Pathogen-specific serology	NMRC
		Mononuclear cells (MNC) for cellular responses [antibody secreting cell (ASC) and T cell responses]	NMRC
3	Stool (10-12 g)	Same as above	Same as above
	EDTA-Blood (20 ml)	Pathogen-specific serology	NMRC
		Cellular responses (as above)	NMRC
5-7	Stool (10-12 g)	Routine Microbiology	Field lab/AFRIMS
		Total and pathogen-specific sIgA	NMRC

Quality Control (QC)

Laboratory technicians from AFRIMS, NMRC, NEPMU6, and NAMRU-2 will staff the field laboratory. An on-site study microbiologist and immunologist will provide oversight. The College of American Pathologists (CAP) do not certify field labs, but daily quality controls will be performed for each test (refer to each test's SOP in Appendix).

Specimen storage/labeling/disposal

Following initial processing and culturing, stool specimens and cultures will be transported and stored in the Enteric Diseases Lab at AFRIMS. A portion of each stool specimen will be transported on dry ice back to NMRC and will be stored there in a –70° C freezer. The processed blood and stool specimens will be immediately placed into a portable –70° C in the field. The specimens will be transported on dry ice to AFRIMS for temporary storage then in liquid nitrogen dry shippers and dry ice back to NMRC (permanent storage pending testing). Each specimen will be given a 5-digit numerical identifier at the time it is submitted. The patient's record and each specimen will be marked with a pre-printed adhesive label denoting the numerical identifier.

iii. Analysis

The physician-performed bedside diagnostic assays (stool characterization, hemoccult, and lactoferrin latex agglutination) and laboratory technician-performed rapid diagnostic assays (fecal leukocyte smear, *Campylobacter*-specific rapid assay, *Shigella*-specific rapid assay, and plasma C-reactive protein) will be compared with

the gold standard stool microbiology results. Test performance characteristics will be assessed for each assay. Logistic regression modeling will be undertaken to determine the most predictive diagnostic approach. Cost data will also be obtained in order to integrate cost-effectiveness into the management approach.

7. Adverse Event Management

The subjects will be informed of the potential side-effects of this medicine and specifically asked about the development of these symptoms during their clinical evaluations at 24 and 72 hours, and these results will be noted on a standardized questionnaire. If any of these symptoms, or other previously undescribed side-effects, are deemed to be severe by the subject or the physician, the patient will be removed from the study, the code broken for that individual, and the patient treated with alternative therapy. Illnesses present at enrollment to the study are considered pre-existing conditions and will be documented on the initial clinic visit form.

Adverse events possibly associated with one of the study regimens or blood draws include:

- Allergic hypersensitivity reaction
- Gastrointestinal upset [either shortly after receiving medication dose (~1-2 hrs) manifesting as nausea, vomiting., and/or new onset abdominal cramping OR a mild/moderate diarrhea typically occurring after infectious diarrhea symptoms resolved without other evidence of infectious diarrheal relapse]
- CNS effects (headaches, insomnia, dizziness, psychiatric disturbance, or seizures)
- Phototoxicity reaction
- Hematoma or infection at the site of blood draw

Duration of a symptom will be approximated by the duration reported by the volunteer. In addition, symptoms will be assessed as to whether they are continuous or intermittent. All adverse events, including intercurrent illnesses, must be reported and documented as described below.

Definitions

An adverse event (AE) is any medical occurrence in a clinical investigation subject administered a pharmaceutical product. An AE does not necessarily have a causal relationship with the study product given. An AE can therefore be any unfavorable or unintended sign (including abnormal laboratory finding), symptom, or disease associated with the use of a medicinal product, whether or not related to the medicinal product.

Assessment of Adverse Events

Volunteer observed and elicited AEs will be recorded. This includes AEs the subject reports spontaneously, those observed by the Investigator, and those elicited by the Investigator in response to questions.

The Investigator with regard to the following categories will assess each AE:

Serious / Not Serious

Federal law defines a serious adverse event as any event that suggests a significant hazard, contraindication, side effect, or precaution. Adverse events will be noted on the CRF as being serious or non-serious. With respect to human clinical experience, a serious adverse event can be described as one or more of the following:

- Fatal:

This includes deaths that appear to be completely unrelated to study therapy (e.g., car accident). If a subject dies during the study and an autopsy is performed, autopsy results will become part of this subject's case report form. Possible evidence of organ toxicity and the potential relationship of the toxicity to the study medication will be of particular interest. The autopsy report should distinguish the relationship between underlying diseases, the study medication's side effects, and the cause of death.

- Life-threatening:

A life-threatening adverse event is any adverse event during which the subject was, in the view of the Investigator, at immediate risk of death from the event as it occurred. This definition does not include an event that, had it occurred in a more serious form, might have caused death. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered a life-threatening event though drug-induced hepatitis can be fatal.

- Permanently or significantly disabling
- Requiring or prolonging in-patient hospitalization
- A congenital anomaly/birth defect

Adverse events that fall outside of these categories are not considered serious.

Intensity

Regardless of the classification of an AE as serious or not, its severity must be assessed according to the following categories:

Mild:	does not interfere with routine activities
Moderate:	Interferes with routine activities
Severe:	Unable to perform routine activities

Note that a severe AE need not be serious and that a serious adverse event need not, by definition, be severe.

Relationship to Study Medication

The Investigator will assess the relationship between the study medication and the adverse event. The following definitions are to be used to determine the relationship between the study medication and the adverse event.

Definite

This category applies to those adverse events which the Investigator feels are incontrovertibly related to the study medication.

Probable

This category applies to those events which, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to study medication.

Possible

This category applies to those adverse events which, after careful medical consideration at the time they are evaluated, are considered to be unlikely to be related but cannot be ruled out with certainty.

Unlikely

In general, this category can be considered applicable to those adverse events, which, after careful medical consideration at the time they are evaluated are considered to be unrelated to the study medication.

Not related

This category applies to those adverse events which, after careful medical consideration, are clearly and incontrovertibly due to causes other than the study medication.

Recording Adverse Events

All adverse events, regardless of relationship to study medication, must be recorded on the follow-up clinical form. All adverse event reports should contain, but are not limited to, the date the adverse event occurred, a brief description, the approximate time of onset and duration, continuous or intermittent, the intensity, treatment required, relationship to study medication, outcome, and whether the event is classified as serious.

Reporting Serious Adverse Events

Adverse experiences that are both serious and unexpected will be immediately reported by telephone to the NMRC Committee for the Protection of Human Subjects (CPHS) (301) 295-0179 **and** send information by Fax to 301-295-5938. A written report will follow the initial telephone call **within 24 hours**. Address the written report to the Office of Research Administration, U.S. Navy Medical Research Center, ATTN: Chairman, CPHS, 8901 Wisconsin Avenue, Bethesda, MD 20889.

These events should also be reported to the Medical Monitor.

An adverse event temporally related to participation in the study should be documented, whether or not related to the study medication. This definition includes intercurrent illnesses and injuries, and exacerbations of pre-existing conditions. The following will be included in all safety reports:

- subject identification number and initials
- investigator's name and medical treatment facility
- subject's date of birth, gender, and ethnicity
- study medication and date of administration
- signs/symptoms and severity

- date of onset; date of resolution or death
- relationship of the study medication (including date of last dose)
- action taken
- concomitant medication(s) including dose, route and duration of treatment

The following incidents require immediate reporting: Any event that meets the FDA definition of serious or unexpected (with the exception of subject disenrollment due to the requirement for hospitalization for progression of diarrhea; these disenrollments will be reported at the completion of the volunteer phase unless the progression is deemed life-threatening in which case immediate reporting will be done).

8. Medical Care/Equipment Requirements

All patients will receive standard medical care (including oral and/or intravenous rehydration as necessary) and appropriate follow-up. Laboratory and clinical supplies will be coordinated with the Armed Forces Research Institute of Medical Sciences in Bangkok and the responsible medical units. The study medications will be dispensed using a "combi bottle" method that will contain azithromycin – (1) 500 mg tabs (or an identical appearing placebo tab) or levofloxacin – (2) 250 mg tabs (or identical appearing placebo tabs). The volunteer will be assigned a study number based on a sequential randomized list. The volunteer will be supplied with a "combi bottle" that will supply each day's drug in a separate bottle. Study-specific personnel and supplies/equipment are as follows:

a. Personnel

1. Clinical team (4-5 physicians working 2 survey medical treatment facility sites)
   Responsibilities: evaluation and care of diarrhea patients, obtaining informed consent ("cases"), administer questionnaire, collect blood/stool specimens, bedside stool characterization, bedside hCG testing and completion of all clinical study forms
2. "Control" enrollment team (2 Preventive Medicine technicians)
   Responsibilities: track down and obtain informed consent from asymptomatic "controls", administer questionnaire, collect blood/stool specimen, and provide post-treatment clinical follow-up with collection of final stool specimen to assess microbiologic eradication (5-7 days after start of therapy)
3. Field data manager
   Responsibility: daily data entry (clinical and preliminary microbiology results), coordinate study file maintenance, generate daily results for study teams, and generate data for team leader's weekly JTF Surgeon report
4. Immunology processing team (1 immunologist and 2 laboratory technicians)
   Responsibilities: field processing and storage of all blood specimens (includes C-reactive protein testing and lymphocyte separation) as per appendix
5. Field microbiology lab team (2 AFRIMS microbiology technicians and 1 Navy lab personnel)
   Responsibilities: stool microbiology procedures as per attached AFRIMS SOP, Navy microbiologist/technician will perform fecal smear, fecal leukocytes, and all rapid diagnostic assays (refer to appendix)
6. AFRIMS drivers (2 vans)

b. Supplies/Equipment (estimates based on 100 cases and 200 controls)

1. Clinical - study forms ["cases" consent (will include optional section for treatment), "controls" consent, case-control questionnaire, initial visit, follow-up visits (24 hr, 72 hr, or additional time points), symptom diary]; stool collection containers; stool characterization (hemoccult and lactoferrin latex agglutination assay); urine hCG test kits, phlebotomy supplies (specifically 10 ml lavender top tubes); and study medications (pre-packaged in "combi bottles" with study code assignment number)

2. Immunology processing laboratory - supply list to be coordinated between NMRC and AFRIMS; freezer (-70°C), LN_2 storage (dry shippers), centrifuge (400g;hanging bucket), and portable biosafety hood (all equipment supplied by AFRIMS) - approx. space requirements of 10x12 sq. ft. with reliable electrical power

3. Field microbiology laboratory - supply list generated by AFRIMS; preferably physically separated from immunology processing lab; 1 incubator; approx. space requirements of 10x12 sq. ft. with reliable electrical power

9. Record Maintenance/Data Management

Protocol Records

All related protocol materials will be held in the NMRC Office of Research Administration (OOR). The original signed consents, Volunteer Registry forms, and related materials will be transferred to the OOR for archival at NMRC within 90 days of final report completion and stored permenantly.

Data Management

During the study, volunteer's study folders will be kept in a secure location within the medical treatment facility. Clinical and microbiologic data will be abstracted in real-time during the exercise using the attached forms ["Cobra Gold Initial Visit Abstraction Form", "Cobra Gold Follow-up Abstraction Form" and "Cobra Gold Field Data Manager Abstraction Form"]. Based on these abstraction forms, data will be entered by the study data manager into an EpiInfo 6.0 record file using a laptop computer. Daily disk backup will be completed with interim record totals for each database recorded into field data record log. Only study personnel or appropriate medical authorities will have access to the clinical study records or electronic data. At the completion of the exercise, volunteer's study folders will be stored at the NMRC in secure locations pending final transfer to OOR. Data will again be entered independent of the initial entry during the exercise. Validation of data will be performed on the double key-entered data prior to final verification. Immunology results will be entered into an electronic spreadsheet and verified by two independent observers. All of the data will be edited with standard strategies for range and consistency checks.

10. Protocol Modification/Deviation

Scientific or human use review committees that will review the modifications

Modification of the protocol will require approval by the relevant scientific review and Human Use Committees of WRAIR and the NMRC, respectively, unless necessitated by a medical emergency. This applies also to extension of the protocol. These committees will be informed of a decision to terminate the protocol prematurely. Minimal risk modifications

may be expedited by approval of the Chairman, Committee for the Protection of Human Subjects, NMRC.

Protocol deviations

Departures from the schedule will not be allowed. Protocol deviations will be reported to the IRB.

Disenrollment criteria

Volunteers that fail to comply with study procedures (particularly as it relates to appropriate clinical follow-up or study medication compliance) may be disenrolled without their consent. In addition, volunteers that clinically worsen with requirement for hospitalization or experience a serious adverse event will be disenrolled and provided with appropriate clinical care. Subjects may also voluntarily disenroll themselves from the study at any time point however, this will be done in an orderly manner to ensure the volunteer's safety. The "Subject Withdrawal/Disenrollment Form" will be completed in each case of volunteer disenrollment (refer to Appendix).

11. Appendices

a. Investigator Assurance Agreement
b. Cobra Gold Volunteer Consent Form (Diarrhea cases)
c. Cobra Gold Volunteer Consent Form (Asymptomatic controls)
d. Cobra Gold Diarrhea Study Volunteer Registry Form
e. Cobra Gold Diarrhea Study Questionnaire
f. Cobra Gold Initial Clinic Visit (Diarrhea Surveillance)
g. Cobra Gold Follow-Up Clinic Visit (Diarrhea Surveillance)
h. Cobra Gold Diarrhea Symptom Diary
i. Cobra Gold Post-Treatment Follow-up (Diarrhea Surveillance)
j. Cobra Gold Subject Withdrawal/Disenrollment Form
k. Cobra Gold Study Medication, Specimen, and Clinical Visit Log
l. Cobra Gold Initial Visit Abstraction Form
m. Cobra Gold Follow-up Abstraction Form
n. Cobra Gold Specimen Result Log (Fecal leukocytes)
o. Cobra Gold Specimen Result Log (Lactoferrin Latex Agglutination)
p. Cobra Gold Specimen Result Log (ProSpect® Campylobacter Microplate Assay)
q. Cobra Gold Specimen Result Log (Shigella Reveal™ Assay)
r. Cobra Gold Specimen Result Log (Daily Preliminary Microbiology)
s. Cobra Gold Specimen Result Log (C-reactive protein)
t. Cobra Gold Field Data Manager Abstraction Form
u. Cobra Gold Study Site Volunteer Log
v. Bedside Diagnostic Procedures
w. AFRIMS Microbiology Procedures
x. Immunology Assay Procedures

Informed consents

PRIVACY ACT STATEMENT

1. Authority. 5 U.S.C. 301
2. Purpose. Medical research information will be collected in an experimental research project entitled "(State Name of Research Protocol)" to enhance basic medical knowledge, or to develop tests, procedures, and equipment to improve the diagnosis, treatment, or prevention of illness, injury, or performance impairment.
3. Routine Uses. The Departments of the Navy and Defense, and other U.S. Government agencies will use medical research information for analysis and reports. The Navy Surgeon General following the provisions of the Freedom of Information Act or contracts and agreements may grant use of the information to non-Government agencies or individuals. I voluntarily agree to its disclosure to agencies or individuals identified above and I have been informed that failure to agree to this disclosure may make the research less useful. The "Blanket Routine Uses" that appear at the beginning of the Department of the Navy's compilation of medical data bases also apply to this system.
4. Voluntary Disclosure. Provision of information is voluntary. Failure to provide the requested information may result in failure to be accepted as a research volunteer in an experiment or removal from the program.

VOLUNTARY CONSENT TO PARTICIPATE

1. You are being asked to volunteer to participate in a research study entitled " Travelers' Diarrhea Surveillance, Diagnosis and Therapy Study in United States Military Personnel on Deployment in Thailand (Cobra Gold 2000/2001)". There are three main purposes of this study: 1) to determine the risks associated with getting diarrhea in deployed military personnel, 2) to determine the best field diagnostic tests for diarrheal disease in military operations, and 3) to determine the best antibiotic therapy for diarrhea in deployed military in Thailand.

2. You will be asked to participate in a study to determine the risk factors associated with getting diarrhea, called a case-control study, **and** a study to evaluate different antibiotic treatments for diarrhea, called a treatment study. The research study physician will first determine if your diarrheal illness meets the criteria for participating in one or both studies. This form will explain what is the purpose, who is eligible, what procedures (routine versus experimental), time involved, number of volunteers needed, what risks or discomforts may occur, potential benefits, and monetary incentives for each study, case-control and treatment study, separately. You will be asked, if you are eligible to participate, to sign the section of this form noting whether or not you consent to participate to the case-control as well as the treatment study. The diarrhea diagnostic tests are primarily routine approved tests that are part of a standard clinical assessment (not research). In addition to these routine tests, we will also evaluate some new investigational tests for diagnosing causes of diarrhea. The blood and stool specimens that you would be required to provide will be explained in a later section of this form.

3. Participation in the Case-control study

What is the purpose?

This study will determine what risks are related to getting diarrhea in deployed military personnel by comparing different factors between you and other personnel who had a similar opportunity to get sick but did not. Some of the factors to compare include where you've traveled in the past, what you've been eating and drinking lately, what bacteria are found in your bowel movement (stool) culture, and what your immune response (infection-fighting ability in your blood and stool samples) is against different diarrhea-causing bacteria.

Who is eligible?

Your diarrheal illness must consist of at least 3 loose or liquid bowel movements (stools) in a 24 hour period OR at least 2 diarrheal stools plus at least one of these symptoms (abdominal cramps, nausea, vomiting, fever, rectal urgency/pain, or blood in stools) during the episode. Your symptoms must have lasted less than 14 days and have begun at least 24 hours after arrival in Thailand. You also could not be receiving antibiotics (not counting your malaria prophylaxis with either mefloquine or doxycycline) in the past 72 hours (3 days).

What procedures (routine versus experimental)?

If you are eligible and volunteer to participate in the treatment study you will follow the clinical procedures as outlined on page 6 of this form. The case-control study includes two clinical visits (today and 3 days from now). At each visit you will need to provide a stool specimen. You will be provided with a stool collection container. If you are unable to submit a stool specimen, you have the option to submit a rectal swab instead. A study physician will instruct you on the proper collection method. This may cause mild discomfort but is otherwise safe. It is important to always use careful personal hygiene practices to prevent diarrhea (i.e. hand-washing after using the bathroom). If a swab specimen is collected then a stool specimen will still be required within the next 12 hours. You will also undergo phlebotomy (routine blood drawing through an arm vein) twice, today and 3 days from now. You will be given a symptom diary card today and will need to record your symptoms (including number of loose stools, nausea, vomiting, abdominal cramps, fever, and bloody stools over the next 72 hours). This card needs to be returned on your 3-day follow-up. You also will be asked to provide the study physician with 1-5 names of personnel with whom you have shared a non-U.S. military provided meal in the previous 5 days. Study team personnel will attempt to contact these individuals to see if they have remained well and if so, ask them to volunteer in the study. There are no alternative procedures or courses of treatment related to participation in the case-control study. The clinical evaluations and procedures at each clinic visit are listed in the following table.

Day of evaluation	Clinical evaluation or procedures	Routine vs. Experimental
Today (1st clinic visit)	Medical history & physical exam by study physician.	Routine
	Provide a stool specimen in provided container.	Routine
	Study physician determines if you are eligible for study.	
	Provide voluntary consent to participate in case-control study.	
	Provide names of 1-5 persons with whom you shared a non-military provided meal in the previous 5 days.	
	Complete "Volunteer Registry" form (your name, SSN, address, phone #, gender, race)	
	Complete Questionnaire concerning your risks of getting diarrhea.	Experimental
	Given a diarrhea diary card to keep track of your diarrhea illness.	
	Have blood drawn (40 ml – about 3 tbsp.).	
	Provided with a cash incentive of $25 after the blood draw.	
	Offered participation in treatment study (if eligible). If not in treatment study you will receive routine care. Refer to the later section for the treatment study schedule.	
3 days	Medical history & physical exam by study physician.	Routine
	Provide stool specimen.	
	Return properly completed diary card	
	Have blood drawn (20 ml – about 1 ½ tbsp.).	Experimental
	Provided with a cash incentive of $25 after the blood draw.	

Time involved?

You will be asked to participate for the 3-day period outlined above (if your participation does not include the treatment study). Clinic visits typically require approximately 1 hour to complete.

Number of volunteers needed?

There will be approximately 100 individuals with diarrhea ("cases") and about 200-300 individuals without diarrhea, from the lists provided by the diarrhea patients, ("controls") participating in this study.

What risks or discomforts may occur?

The study physicians believe that the risks or discomforts to you (related to case-control study participation) are minimal and limited to adverse events from blood specimen collection. Blood drawing by trained personnel will minimize risks. Risks associated with blood collection may include minor pain, discomfort, bleeding (hematoma), infection, or injury. No risk to you is anticipated for stool collection. If you are unable to provide a stool specimen for your initial stool culture, you have the option of submitting a rectal swab specimen. You will be properly instructed and will collect the swab specimen. Mild transient discomfort may occur related to the swab collection but is otherwise safe. There are no special risks to pregnant or potentially pregnant women volunteers related to specimen collection (although there are risks related to the treatment study – refer to that section for further information). A minimal total amount of blood is being withdrawn as part of this study [total of 60 ml – about 4 ½ tbsp.].

Potential health-related benefits?

There are no direct benefits to you through participation in the case-control study. However, the information will be used to help protect military personnel from diarrheal illness in the future.

Monetary incentives?

If you participate in the case-control study you will be given a cash incentive of $25.00 for each blood sample (total $50.00). Payments will be provided in cash following each blood draw.

Do you provide your voluntary informed consent to participate in the case-control study?

Circle one: Yes No

_____ _____

Volunteer Date (DD/MM/YY)

_____ _____

Witness Date (DD/MM/YY)

_____ _____

Investigator Date (DD/MM/YY)

4. Participation in the Treatment study

What is the purpose?

This study seeks to determine the best antibiotic therapy for diarrhea in deployed military in Thailand. The standard antibiotic therapy for travelers' diarrhea involves the use of a type of antibiotic called a fluoroquinolone such as levofloxacin. In some areas of the world such as Thailand there has been an increasing problem with bacteria developing a resistance against antibiotics. Some of the common bacteria that cause diarrhea in deployed troops in Thailand have developed a resistance against the fluoroquinolones when tested in a laboratory. It is not clear whether or not this has made these antibiotics less effective in treating deployed military with diarrhea. Recent studies in travelers with diarrhea continue to support the use of fluoroquinolones as first-line treatment. Alternative antibiotics, such as azithromycin, have shown promise for use in diarrhea treatment. The antibiotic azithromycin has also been shown to effectively treat some infections (such as some sexually transmitted diseases) when given as a single dose. A research study comparing ciprofloxacin (another fluoroquinolone) and azithromycin in deployed military with diarrhea during Cobra Gold 1993 showed the two treatments to be equal overall. This study attempts to determine the best first-line diarrhea treatment for deployed military by comparing three different treatments (all of which have scientific and medical data to support their use). The three different treatments being studied are levofloxacin [500 mg (2 tablets) given once daily for a total of 3 days, azithromycin [500 mg (1 tablet) given once daily for 3 days, and azithromycin [1000 mg given once].

Who is eligible?

Your diarrheal illness must be similar to what was already mentioned in the case-control study except your symptoms must have lasted less than or equal to 96 hours (4 days). The study physician must also determine that it is safe and reasonable to treat you as an outpatient (not admit you to the hospital). You will also have to follow the study's follow-up schedule (shown in the table below).

Some specific situations that will not allow you to participate include:

- Female patients known to be pregnant or tested and found to have a positive urine pregnancy test.
- If you have a history of allergy to either a macrolide (like erythromycin or azithromycin) or quinolone antibiotics (like ciprofloxacin or levofloxacin). This does not include if your only reaction was limited to gastrointestinal upset.
- If you are have been taking an antibiotic in the past 3 days (not counting your malaria prophylaxis with either mefloquine or doxycycline).
- If you are taking medications known to have interaction with one of the medications used for the study (includes theophylline, digoxin, and warfarin).
- If you have a history of seizures.

What procedures (routine versus experimental)?

If you are eligible and volunteer to participate in the treatment study you will follow the clinical procedures as outlined in the following table. The treatment study includes a total of four clinical visits (today, tomorrow, 3 days from now, and 5 to 7 days from now). You will need to provide a stool specimen today, 3 days and 5 to 7 days from now. The stool collection procedures are the same as described in the case-control study. No blood specimens are being collected specifically for the treatment study (you would follow the blood draw schedule form the case-control study, if you volunteered). Female patients must submit a urine sample for a pregnancy test today. You will be given the same symptom diary card and similar instructions as in the case-control study. However, you will also be given a second diary card on the day 3 visit that will need to be returned on the day 5-7 visit. The follow-up clinic visits will be used to monitor your improvement on the study medication and check for any potential medication side effects. The final follow-up visit (5 to 7 days from now) may be done in the clinic or through arrangements made with study team personnel who will check with you at your berthing or work site.

There are three different treatments being studied: levofloxacin [500 mg (2 tablets) given once daily for a total of 3 days], azithromycin [500 mg (1 tablet) given once daily for 3 days], and azithromycin [1000 mg given

once]. You will be randomly (by chance; as in flipping a coin) assigned to one of these three treatments. Neither you nor the study physician will know which one of the treatments you are receiving. Your treatment assignment will be disguised by providing you with both your assigned antibiotic and a similar appearing tablet that does not contain any medication called a placebo. **You will be treated with an active antibiotic and not only a placebo.** Therefore each day of the study you will take tablets (some will be active antibiotic and others placebo). Today you will receive 4 tablets, 3 tablets tomorrow, and 3 tablets again on the third day. You will receive a "combi bottle" that contains a separate bottle with the tablets you are to take for each of the three treatment days. The study physician will administer the first day's study medication dose. You will be observed for a 30-minute period in the clinic after the first dose.

You are advised to take the study medication on an empty stomach one hour before or two hours after meals. You should avoid taking antacids (within the two hours before or after the medication) because they can decrease the medication's effectiveness. Women using oral contraceptives for birth control should use an alternative means of contraception during the next thirty days (for an entire menstrual cycle) since some antibiotics have been known to temporarily decrease oral contraceptive effectiveness. The medication should be taken at the same time each day. You should record the time of each medication dose on your diary card. The clinical evaluations and procedures at each clinic visit are listed in the table on the next page. The table provides information on all procedures for both the case-control and treatment studies. If you choose not to participate in the case-control study you would delete the sections about providing names of potential 'control" volunteers, blood draws, and incentive payments.

Time involved?

You will be asked to participate for a total of 5 to 7 days from today as outlined on the above table. Clinic visits typically require approximately 1 hour to complete.

Number of volunteers needed?

There are anticipated to be approximately 100 volunteers participating in the treatment study during Cobra Gold 2000. The study will continue into Cobra Gold 2001 and will likely involve another 100 volunteers.

Day of evaluation	Clinical evaluation or procedures	Routine vs. Experimental
Today (1st clinic visit)	Medical history & physical exam by study physician.	Routine
	Provide a stool specimen in provided container.	Routine
	Study physician determines if you are eligible for studies.	
	Provide voluntary consent to participate in case-control and treatment studies.	
	Provide names of 1-5 persons with whom you shared a non-military provided meal in the previous 5 days.	
	Complete "Volunteer Registry" form (your name, SSN, address, phone #, gender, race)	
	Complete Questionnaire concerning your risks of getting diarrhea.	
	Given a diarrhea diary card to keep track of your diarrhea illness.	Experimental
	Have blood drawn (40 ml – about 3 tbsp.).	
	Provided with a cash incentive of $25 after the blood draw.	
	Assigned (by chance) one of the treatments. Study physician gives first antibiotic dose. Stay in clinic for at least 30 minutes after dose to be sure you tolerate it without problems. You will be given the "combi bottle" with the remainder of your antibiotic doses.	
1 day	Medical history & physical exam by study physician.	Routine
	Physician will check your diary card.	Experimental
3 days	Medical history & physical exam by study physician.	Routine
	Provide stool specimen.	
	Return properly completed 1st diary card (will be given 2nd diary card)	Experimental
	Have blood drawn (20 ml – about 1 ½ tbsp.).	
	Provided with a cash incentive of $25 after the blood draw.	
5-7 days	Brief study follow-up questionnaire by either physician or other study personnel.	
	Return properly completed 2nd diary card.	Experimental
	Provide stool specimen.	

What <u>risks or discomforts</u> may occur?

Specimen collection-related risks are discussed under the case-control section. Subjects participating in the treatment trial portion of the study will be randomly assigned to one of three treatment regimens using one of the two antibiotics, levofloxacin and azithromycin. Any one of the regimens may prove to be more or equally effective at treating acute infectious diarrhea acquired in Thailand. Alternatively, the treating physician will manage diarrheal patients declining participation using standard of care practices. The potential risks of the study involve either sub-optimal treatment or side effects from the medication.

Neither you nor your physician will know which antibiotic treatment you are receiving. Therefore the potential side effects of both levofloxacin and azithromycin will be described. Both antibiotics are licensed approved medications that have been used extensively and shown to be very safe with only rare side effects. Rare allergic reactions to these medications have been observed. One of the antibiotics, levofloxacin, is not recommended for used in pregnancy due to concerns of joint damage to the unborn child (based on studies in young animals). Because of this, all potentially eligible women must submit a urine specimen for pregnancy test prior to inclusion in the treatment study. Refer to section 9 of this form for further information regarding women volunteers. The most common side effects (occurring in about 1% or more of treated patients) with the estimated rates of occurrence are included on the following table for each medication.

Most commonly reported side effects for each study medication

Reported symptom	Azithromycin (3-day treatment)	Azithromycin (single dose treatment)	Levofloxacin (3-day treatment)
Nausea	3 %	5 %	3 %
Vomiting	< 1 %	2 %	< 1 %
Diarrhea	5 %	7 %	2 %
Abdominal pain	3 %	5 %	< 1 %
Rash	< 1 %	< 1 %	< 1 %
Dizziness	< 1 %	< 1 %	< 1 %
Headache	< 1 %	< 1 %	< 1 %
Vaginitis (yeast infection)	< 1 %	1 %	< 1 %

Potential health-related benefits?

The potential benefit to you is a more rapid resolution of symptoms that may occur with one of the study regimens than might be expected with standard therapy. In addition, the information that may be learned from this study will benefit future medical planning and management of deployed military developing diarrhea.

Do you provide your voluntary informed consent to participate in the treatment study?

Circle one: Yes No

_____ _____

Volunteer Date (DD/MM/YY)

_____ _____

Witness Date (DD/MM/YY)

_____ _____

Investigator Date (DD/MM/YY)

5. The medical records associated with this protocol are subject to the provisions of the Privacy Act of 1974, 5 U.S.C., Section 552A, SECNAVINST 3900.39B, NMRCINST 3900.6D and AR 340-21. All data and medical information obtained about you, as an individual, will be considered privileged and held in confidence. You will not be identified by name in any published report or presentation of the results. Representatives of the U.S. Navy or Army research command and the Food and Drug Administration may inspect the records of this research as part of their responsibility to oversee research and ensure protection of volunteers. **By signing this consent form, you agree to such inspection and disclosure.** Complete confidentiality cannot be promised to volunteers, particularly those who are active duty military personnel, because information bearing on your health may be required to be reported to appropriate medical or command authorities. The study results are to be published in scientific and medical journals; however, the identity of individual volunteers will not be disclosed. The study records will be stored permanently at the Naval Medical Research Center in Silver Spring, MD.

6. If you have questions about this study you should contact one or more of the following individuals:

   - Questions about research (science) aspects contact one of the Principal Investigator, **David R. Tribble, M.D.,** Enteric Diseases Division, Naval Medical Research Center (NMRC), Room 3A-26, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500 at (301) 319-7673 and/or **LCDR John Sanders, MC, USNR**, Infectious Diseases Division, National Naval Medical Center, Wisconsin Avenue, Bethesda, MD 20889-5600 at (301) 295-2982.

   - Questions about medical aspects, injury, or any health or safety questions you have about your participation, contact the study's medical monitor, MAJ Scott Miller in the U.S. Armed Forces Research

Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand at 66-2-644-xxxx or one of the Principal Investigators mentioned above.

- Questions about the ethical aspects of this study, your rights as a volunteer, or any problem related to protection of research volunteers, contact Chairperson of the Committee for the Protection of Human Subjects at (301) 319-7650 or the Office of Research Administration at the Naval Medical Research Center in Bethesda, MD at (301) 295-0179.

7. Your participation in this study is completely voluntary. If you do not want to participate, there will be no penalty and you will not lose any benefit to which you are otherwise entitled. You may discontinue your participation in this study at any time you choose. If you do stop, there will be no penalty and you will not lose any benefit to which you are otherwise entitled.

8. Should you be injured as a direct result of participating in this research project, you will be provided medical care at no cost to you for that injury. You will not receive any injury compensation, only medical care. You should also understand that this is not a waiver or release of your legal rights. You should discuss this issue thoroughly with one of the study physicians.

9. **For Women:** Your signature indicates that you have read and understand the following. You have been advised that participation in the treatment study may present a risk to an unborn child. Therefore, you must not participate in this study if you believe there is any chance you may become pregnant during the study period. You understand that a urine test for human chorionic gonadotropin (hCG, the pregnancy test) will be performed and must be negative at the time you would receive the first dose of the study medication. You will not be allowed to participate in this study if the pregnancy test is found to be positive. Except for surgical removal of the uterus, birth control methods such as the use of condoms, a diaphragm or cervical cap, hormonal contraceptives, intrauterine device (IUD), or sperm-killing products are not totally effective in preventing pregnancy. Because of a delay between conception and a positive pregnancy test, there is a small chance that you could be pregnant even though your pregnancy test is reported as negative. The only way to completely avoid pregnancy is to abstain from sexual intercourse. The only ways to completely avoid medication-associated risk to an unborn baby are (1) do not become pregnant or (2) do not participate in the treatment study.

10. The researchers or the medical monitor may, without your consent, stop your participation in this study. Reasons that would make this necessary include if you were to clinically worsen (such as requiring hospitalization), have a serious side effect to the study medication, or fail to comply with the study procedures.

11. No additional costs to you are expected as a result from your voluntary participation in this study.

12. If you decide to withdraw from further participation in this study, it is expected that clinical follow-up will still be necessary to ensure your safety. You can discontinue your participation in this study at any time you choose and without penalty. Abruptly stopping your participation in the treatment study may be harmful to you, therefore, when you inform us that you want to stop, the study physicians will determine if follow-up is required and, if deemed necessary, you must comply with the prescribed follow-up procedures.

13. Major new findings developed during the course of the research, which may relate to your willingness to continue participation, will be provided to you.

14. I have received a statement informing me about the provisions of the Privacy Act.

15. I have been informed that Drs. Tribble and Sanders, the Principal Investigators, are responsible for storage of my consent form and the research records related to my participation in this study. These records will be stored at the Naval Medical Research Center (NMRC) in Silver Springs, MD.

16. I have asked the questions on the attached paper, and the written answers provided by the researcher(s) are understandable to me and are satisfactory. I understand what has been explained in this consent form about my participation in this study. I (do / do not) need further information to make my decision whether or not I want to volunteer to participate. By my signature below, I give my voluntary informed consent to participate in the research as it has been explained to me, and acknowledge receipt of a copy of this form for my own personal records.

Volunteer	Date (DD/MM/YY)
Witness	Date (DD/MM/YY)
Investigator	Date (DD/MM/YY)

- DO NOT REMOVE -
THIS DOCUMENT REQUIRED TO BE PERMANENTLY FILED IN MEDICAL/DENTAL RECORD IN ACCORDANCE WITH SECNAVINST 3900.39B

PRIVACY ACT STATEMENT

1. Authority. 5 U.S.C. 301
2. Purpose. Medical research information will be collected in an experimental research project entitled "(State Name of Research Protocol)" to enhance basic medical knowledge, or to develop tests, procedures, and equipment to improve the diagnosis, treatment, or prevention of illness, injury, or performance impairment.
3. Routine Uses. The Departments of the Navy and Defense, and other U.S. Government agencies will use medical research information for analysis and reports. The Navy Surgeon General following the provisions of the Freedom of Information Act or contracts and agreements may grant use of the information to non-Government agencies or individuals. I voluntarily agree to its disclosure to agencies or individuals identified above and I have been informed that failure to agree to this disclosure may make the research less useful. The "Blanket Routine Uses" that appear at the beginning of the Department of the Navy's compilation of medical data bases also apply to this system.
4. Voluntary Disclosure. Provision of information is voluntary. Failure to provide the requested information may result in failure to be accepted as a research volunteer in an experiment or removal from the program.

VOLUNTARY CONSENT TO PARTICIPATE

1. You are being asked to volunteer to participate in a research study entitled " Travelers' Diarrhea Surveillance, Diagnosis and Therapy Study in United States Military Personnel on Deployment in Thailand (Cobra Gold 2000/2001)". There are three main purposes of this study: 1) to determine the risks associated with getting diarrhea in deployed military personnel, 2) to determine the best field diagnostic tests for diarrheal disease in military operations, and 3) to determine the best antibiotic therapy for diarrhea in deployed military in Thailand.

2. You will be asked to participate in a study to determine the risk factors associated with getting diarrhea, called a case-control study. The research study physician will first determine if you meet the criteria for participating in the study. This form will explain what is the purpose, who is eligible, what procedures (routine versus experimental), time involved, number of volunteers needed, what risks or discomforts may occur, potential benefits, and monetary incentives for the study. You will be asked, if you are eligible to participate, to sign this form providing your consent to participate.

3. Participation in the Case-control study as a healthy volunteer

What is the purpose? This study will determine what risks are related to getting diarrhea in deployed military personnel by comparing different factors between you and another person (who developed diarrhea) who had similar exposure to a recent non-U.S. military provided meal. Some of the factors to compare include where you've traveled in the past, what you've been eating and drinking lately, what bacteria are found in your bowel movement (stool) culture, and what your immune response (infection-fighting ability in your blood and stool samples) is against different diarrhea-causing bacteria.

Who is eligible? You must not have had a diarrheal illness in the past 5 days. The diarrheal illness is defined as at least 3 loose or liquid bowel movements (stools) in a 24 hour period OR at least 2 diarrheal stools plus at least one of these symptoms (abdominal cramps, nausea, vomiting, fever, rectal urgency/pain, or blood in stools) during the episode. You also could not be receiving antibiotics (not counting your malaria prophylaxis with either mefloquine or doxycycline) in the past 72 hours (3 days).

What procedures (routine versus experimental)? The study includes only this single encounter. You will need to provide a stool specimen either today or within the next 24 hours. You will be provided with a stool collection container. . A portion of this study will look at whether a certain group of bacteria cause diarrhea. Lab tests will be performed on your stool specimens. If these bacteria are found in your stool specimen, no treatment is necessary. It is important to always use careful personal hygiene practices to prevent diarrhea (i.e. hand-washing after using the bathroom. You will also undergo phlebotomy today (routine blood drawing through an arm vein). You will be asked to fill out a questionnaire including such topics as symptoms you might have had, medicines you may have taken, food and drink exposures during this deployment, etc. There are

no alternative procedures or courses of treatment related to participation in this study. The clinical evaluations and procedures at today's encounter are listed in the following table.

Day of evaluation	Clinical evaluation or procedures	Routine vs. Experimental
Today	Study personnel determines if you are eligible for study.	Experimental
	Provide voluntary consent to participate.	
	Complete "Volunteer Registry" form (your name, SSN, address, phone #, gender, race)	
	Complete Questionnaire concerning your risks of getting diarrhea.	
	Provide a stool specimen in provided container.	
	Have blood drawn (40 ml – about 3 tbsp.).	
	Provided with a cash incentive of $25 after the blood draw.	

Time involved? You will be asked to participate only during the time required to complete the above procedures (approx. 1 hour).

Number of volunteers needed? There will be approximately 100 individuals with diarrhea ("cases") and about 200-300 individuals, like yourself, without diarrhea, from the lists provided by the diarrhea patients, ("controls") participating in this study.

What risks or discomforts may occur? The study physicians believe that the risks or discomforts to you are minimal and limited to adverse events from blood specimen collection. Blood drawing by trained personnel will minimize risks. Risks associated with blood collection may include minor pain, discomfort, bleeding (hematoma), infection, or injury. No risk to you is anticipated for stool collection. There are no special risks to pregnant or potentially pregnant women volunteers related to specimen collection. A minimal total amount of blood is being withdrawn as part of this study [total of 40 ml – about 3 tbsp.].

Potential health-related benefits? There are no direct benefits to you through participation in the case-control study. However, the information will be used to help protect military personnel from diarrheal illness in the future.

Monetary incentives? If you participate in the case-control study you will be given a cash incentive of $25.00 for the blood sample. Payment will be provided in cash following the blood draw.

4. The medical records associated with this protocol are subject to the provisions of the Privacy Act of 1974, 5 U.S.C., Section 552A, SECNAVINST 3900.39B, NMRCINST 3900.6D and AR 340-21. All data and medical information obtained about you, as an individual, will be considered privileged and held in confidence. You will not be identified by name in any published report or presentation of the results. Representatives of the U.S. Navy or Army research command and the Food and Drug Administration may inspect the records of this research as part of their responsibility to oversee research and ensure protection of volunteers. **By signing this consent form, you agree to such inspection and disclosure.** Complete confidentiality cannot be promised to volunteers, particularly those who are active duty military personnel, because information bearing on your health may be required to be reported to appropriate medical or command authorities. The study results and data may be published in scientific and medical journals; however, the identity of individual volunteers will not be disclosed. I have been informed that Drs. Tribble and Sanders, the Principal Investigators, are responsible for storage of my consent form and the research records related to my participation in this study. These records will be stored at the Naval Medical Research Center (NMRC) in Silver Springs, MD.

5. I have received a statement informing me about the provisions of the Privacy Act. If you have questions about this study you should contact one or more of the following individuals:

- Questions about research (science) aspects contact one of the Principal Investigator, **David R. Tribble, M.D.**, Enteric Diseases Division, Naval Medical Research Center (NMRC), Room 3A-26, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500 at (301) 319-7673 and/or **LCDR John Sanders, MC, USNR**, Infectious Diseases Division, National Naval Medical Center, Wisconsin Avenue, Bethesda, MD 20889-5600 at (301) 295-2982.

- Questions about medical aspects, injury, or any health or safety questions you have about your participation, contact the study's medical monitor, MAJ Scott Miller in the U.S. Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand at 66-2-644-xxxx or one of the Principal Investigators mentioned above.

- Questions about the ethical aspects of this study, your rights as a volunteer, or any problem related to protection of research volunteers, contact Chairperson of the Committee for the Protection of Human Subjects at (301) 319-7650 or the Office of Research Administration at the Naval Medical Research Center in Silver Spring, MD at (301) 319-xxxx.

6. Your participation in this study is completely voluntary. If you do not want to participate, there will be no penalty and you will not lose any benefit to which you are otherwise entitled. You may discontinue your participation in this study at any time you choose. If you do stop, there will be no penalty and you will not lose any benefit to which you are otherwise entitled. If major new findings develop during the course of the research, which may relate to your willingness to continue participation, they will be provided to you.

7. Should you be injured as a direct result of participating in this research project, you will be provided medical care at no cost to you for that injury. You will not receive any injury compensation, only medical care. You should also understand that this is not a waiver or release of your legal rights. You should discuss this issue thoroughly with one of the study physicians. No additional costs to you are expected as a result from your voluntary participation in this study.

8. I have asked the questions on the attached paper, and the written answers provided by the researcher(s) are understandable to me and are satisfactory. I understand what has been explained in this consent form about my participation in this study. I (do / do not) need further information to make my decision whether or not I want to volunteer to participate. By my signature below, I give my voluntary informed consent to participate in the research as it has been explained to me, and acknowledge receipt of a copy of this form for my own personal records.

_____ _____
Volunteer Date (DD/MM/YY)

_____ _____
Witness Date (DD/MM/YY)

_____ _____
Investigator Date (DD/MM/YY)

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Study forms

Cobra Gold Diarrhea Study
Volunteer Registry Form

FILLED OUT BY STUDY PERSONNEL

Date of volunteer enrollment: ____/____/____
DD MM YY

Enrollment status: CASE CONTROL

IF being enrolled as a case, enrolled in treatment study? YES NO

IF enrolled in treatment study, record treatment assignment code number: _____

Please print clearly

Last Name:_____ **First Name:**_____ **Rank:** ____

Current home address:_____
(Street)

_____
(City, State, Zip code)

Current home phone: _____ **Work phone:**_____
(Include area code) (Include area code)

Age:____ **Date of Birth:** ____/____/____ **SSN:** __ __ __ - __ __ - __ __ __ __
DD MM YY

Gender (circle one): Male Female

Race (circle one): African-American Caucasian Hispanic Asian
Other: _____

IF enrolled as a CASE, record the names and unit (with suggestions for contacting them) of 1-5 personnel with whom you shared a non-U.S. military provided meal in the past 5 days:

	Name	Unit	Comments (contacting the individual)
1			
2			
3			
4			
5			

Study Investigator:_____ **Date:** _____

Cobra Gold Diarrhea Study Questionnaire

Study Number

Do not mark in box (assigned by study team personnel)

FILLED OUT BY STUDY PERSONNEL

Enrollment status: CASE CONTROL
IF being enrolled as a control who is the case (record name and Vol. ID):

Volunteer instructions: Please read the instructions carefully and answer all of the questions. When there is a choice given on a question circle only one answer. If you do not know the answer ask for assistance from the study personnel administering the questionnaire. Do not leave any questions blank in sections that apply to you. Thank you.

Your Name: Last:_____ **First:** _____ **Rank:**___

SSN: _____--_____--_____ **Gender: Male Female**

Race: African-American Caucasian Hispanic Asian Other: _____

Today's Date (mm/dd/yy): _____/_____/_____

Date you arrived in Thailand (mm/dd/yy): _____/_____/_____

Prior travel experience:

Thailand	Yes	No	
Southeast Asia	Yes	No	
Cobra Gold	Yes	No	What year(s) _____
Other developing region(s)		Yes	No

Prior episodes of traveler's diarrhea: Yes No

IF Yes, record country/year: _____

During the past 5 days, have you had diarrhea? Yes No

****Diarrhea definition** = 3 or more loose or liquid stools in a 24 hour period OR 2 or more loose or liquid stools in 24 hr period plus at least one of the following symptoms (abdominal pain or cramps, nausea, vomiting, fever, tenesmus (rectal urgency/pain), or gross blood in stools during the diarrheal episode.

Have you taken any medication to prevent/treat diarrhea? Yes No

IF Yes, what medicine? _____

Have you taken any medication to prevent malaria? Yes No

If Yes which medication: Doxycycline Mefloquine (Lariam) Other _____

During this assignment in Thailand, have you been sick? Yes No
(Do not count the diarrheal illness listed above or injuries)

If you answered yes to being sick, circle all that applies to your recent illness. Otherwise skip to the section concerning your recent food & water.

Diarrhea	Yes	No	**If diarrhea, # days** _____			
			If diarrhea, approx. total loose stools _____			
Abdominal cramps	Yes	No	**Fever**	Yes	No	
Vomiting	Yes	No	**Blood in stools**	Yes	No	**Don't know**
Nausea	Yes	No	**Muscle/joint aches**	Yes	No	
Sore throat	Yes	No	**Cough/cold symptoms**	Yes	No	

Other illness _____

Seen at clinic	Yes	No	**Able to work**	Yes	No
Received antibiotic	Yes	No	**If antibiotics, date of last dose** _____		

In the past 5 days have you been off base on liberty/pass? Yes No

Source of recent (<u>past 5 days</u>) food & drink (circle all that apply):

Military dining facility	Yes	No
Meals-ready-to-eat (MRE)	Yes	No
On-base Thai civilians	Yes	No
Off-base Thai street vendors	Yes	No
Off-base Thai "traditional" restaurant	Yes	No
Off-base Thai "non-traditional" restaurant (such as PizzaHut)	Yes	No

List names of off-base dining (if known): _____
If outside of the Nakhon Si Thammarat/Thung Song area, list city: _____

Specific food/drinks you consumed (<u>past 5 days</u>) from non-U.S. military provided source (circle all that apply):

Salads/Uncooked vegetables	Yes	No	**Fruit**	Yes	No
Chicken	Yes	No	**Cooked vegetables/rice**	Yes	No
Seafood (shellfish, shrimp)	Yes	No	**Milk**	Yes	No
Pork	Yes	No	**Non-carbonated beverage**	Yes	No
Beef	Yes	No	**Ice**	Yes	No
Duck	Yes	No	**Tap water**	Yes	No
Fish	Yes	No	**River water**	Yes	No

Other exposures you think are important: _____

<u>FILLED OUT BY STUDY PERSONNEL</u>

Questionnaire administered by: _____
Reviewed by:
Study Investigator: **Date:**

Cobra Gold Initial Clinic Visit Form (Diarrhea Surveillance)

Date: ___/___/___ **Time:** _____ **Study ID #:** _____ **Tx Code #:** _____

Name: Last:_____ **First:**_____ **Rate/Rank**_____

SSN:_____-____-_____ **Service:**_____ **Unit:**_____
Age:____ **Sex: M/F**
Barrack location_____ **Barrack Tel#**_____ **Work#**_____

Chief Complaint:

Date/Time of onset of diarrhea: ___/___/___ **Time** _____**hrs**
Date/Time of onset of fever: ___/___/___ **Time** _____**hrs**

Review of Systems:

Symptom			Duration (days)
Diarrhea	Yes	No	
# loose/liquid stools last 24 hours			
# loose/liquid stools since start of sx			
Blood in stools	Yes	No	
Nausea	Yes	No	
Vomiting	Yes	No	
# of episodes of vomiting			
Abdominal cramps	Yes	No	
Tenesmus	Yes	No	
Subjective Fever	Yes	No	
Headaches	Yes	No	
Muscle aches	Yes	No	
Joint pains	Yes	No	

Other Symptoms (record symptom/duration):

PMH/PSH:

Social/FHX:

Meds:

Allergies:

Diarrhea Self-Medication: **Any self-medication? Yes No (If no skip to next section)**

Medication	Purpose: Prevention		Purpose: Treatment		Duration (# days)	Amount (# pills)
Imodium (loperamide)	Yes	No	Yes	No		
Pepto-Bismol	Yes	No	Yes	No		
Antibiotic (state:)	Yes	No	Yes	No		
Other (state:)	Yes	No	Yes	No		

How has this illness affected your ability to work/go on liberty/go on pass? (mark one)
☐ Normal ability ☐ Decreased ability ☐ Not able

Physical Exam: Vital Signs: Temp_____^{0}F RR_____ **Sitting:** **Pulse:**_____ **BP:**_____
Standing: **Pulse:**_____ **BP:**_____

HEENT:	**normal**	**abnormal**
Oral mucosa:	**normal**	**dry**
Cardiac:	**normal**	**abnormal**
Lungs:	**normal**	**abnormal**
Abdomen:	**normal**	**abnormal**
Ext/joints	**normal**	**abnormal**
Skin:	**normal**	**dry** **describe rash:**

Other findings:

Stool characterization: (check only one box)
- ☐ **Grade 1 - hard (normal)**
- ☐ **Grade 2 - soft (normal)**
- ☐ **Grade 3 - thick liquid**
- ☐ **Grade 4 - opaque watery liquid**
- ☐ **Grade 5 - clear watery**

Gross blood in stools: ☐ **Yes** ☐ **No**

Hemoccult: ☐ **Pos** ☐ **Neg**

Urine hCG (if applicable): ☐ **Pos** ☐ **Neg** **(Internal QC – mark if valid: ☐Pos control ☐Neg control)**

Assessment/eligibility:

Illness most consistent with:
- ☐ **watery, non-inflammatory diarrhea**
- ☐ **dysentery**
- ☐ **gastroenteritis (non-inflam. diarrhea w/signif. vomiting component)**
- ☐ **other (describe)**

Eligibility determination:

Case-control study (check if eligible):
- ☐ diarrheal symptoms of < 14 days duration
- ☐ symptom onset at least 24 hours after arrival in Thailand
- ☐ not on antibiotics (excluding malaria prophylaxis) past 72 hr

Treatment study (check if eligible):
- ☐ diarrheal symptoms of ≤ 96 hours duration
- ☐ ambulatory management with planned follow-up
- ☐ negative urine hCG for prospective female volunteers
- ☐ no macrolide or quinolone allergy (not limited GI intolerance)
- ☐ not on antibiotics (excluding malaria prophylaxis) past 72 hr
- ☐ not taking theophylline, digoxin, or warfarin
- ☐ no history of seizures

Treatment:

Rehydration therapy: ☐ **Increase fluids** ☐ **ORS solution** ☐ **IV fluids (Qty: _____L)**
(check all that apply)

Enrolled in treatment study: ☐ **Yes** ☐ **No** **IF No, medication given:** _____
Tx code: _____ **Time of 1st dose:** _____

Post-dose observation (minimum 30 min) symptoms: **Nausea** ☐ **Yes** ☐ **No**
Vomiting ☐ **Yes** ☐ **No**

Other post-dosing symptoms:

Disposition: ☐ **RTD** ☐ **SIQ 24h** ☐ **SIQ _____h** ☐ **Admit**
Follow-up: ☐ **As needed** ☐ **1 day** ☐ **3 days**

Cobra Gold Follow-Up Clinic Visit Form (Diarrhea Surveillance)

Date: ___/___/___ Time: _____ Study ID #: _____
Study day: _____
Name: Last:_____ First:_____ Rate/Rank_____
SSN: ___-___-___ Last seen in clinic: ___/___/___

Summary of current illness:

Enrolled in treatment study: □ Yes □ No IF Yes, Tx code: _____
As applicable – Date/Time of 2nd dose: _____
Date/Time of 3rd dose: _____
If not in treatment study, what therapy was given: _____

Date/Time of diarrhea ceased: ___/___/___ Time _____hrs
Date/Time of fever ceased: ___/___/___ Time _____hrs

Review of Systems:

Symptom	Currently present		Present since last visit*		Duration**
Diarrhea	Yes	No	Yes	No	
Blood in stools	Yes	No	Yes	No	
Nausea	Yes	No	Yes	No	
Vomiting	Yes	No	Yes	No	
Total # of episodes of vomiting since last visit					
Abdominal cramps	Yes	No	Yes	No	
Tenesmus	Yes	No	Yes	No	
Subjective Fever	Yes	No	Yes	No	
Headaches	Yes	No	Yes	No	
Muscle aches	Yes	No	Yes	No	
Joint pains	Yes	No	Yes	No	
Rash	Yes	No	Yes	No	
Dizziness	Yes	No	Yes	No	
Vaginal discharge/pruritus	Yes	No	Yes	No	
Other Symptoms	Yes	No	Yes	No	

* To determine if symptom present since last visit, use both interview and diary card (if not surveyed on card use interview only).
** If symptom ongoing record "P" for present illness otherwise record # days.

Summary results to date directly from diary card (as available, if by interview note this):

Study day	Total # Loose/Liquid stools				Daily Functional Assessment
	0001-0600	0601-1200	1201-1800	1801-2400	
0					NL Decrease Unable
1					NL Decrease Unable
2					NL Decrease Unable
3					NL Decrease Unable
4					NL Decrease Unable
5					NL Decrease Unable
6					NL Decrease Unable
7					NL Decrease Unable

Record other symptoms or comments (include symptom description/duration):

Has the patient been self-medicating? Yes No **(If no skip to next section)**

Medication			Duration (# days)	Amount (# pills)
Imodium (loperamide)	Yes	No		
Pepto-Bismol	Yes	No		
Antibiotic (state:)	Yes	No		
Other (state:)	Yes	No		

Physical Exam: Temp_____°F
Directed exam as appropriate:

Stool characterization: (check only one box)
- ☐ Grade 1 - hard (normal)
- ☐ Grade 2 - soft (normal)
- ☐ Grade 3 - thick liquid
- ☐ Grade 4 - opaque watery liquid
- ☐ Grade 5 - clear watery

Gross blood in stools: ☐ Yes ☐ No

Hemoccult: ☐ Pos ☐ Neg

Illness Re-assessment:

Illness now most consistent with:
- ☐ watery, non-inflammatory diarrhea
- ☐ dysentery
- ☐ gastroenteritis
- ☐ other (describe)

Patient has met clinical cure definition: ☐ Yes ☐ No
Patient has had a clinical relapse: ☐ Yes ☐ No
[Relapse = met clinical cure definition with symptom recurrence following a 24-hr symptom-free period]

Additional treatment:
Rehydration therapy: ☐ Increase fluids ☐ ORS solution ☐ IV fluids (Qty: _____L)
(check all that apply)
Other therapy:

Disposition: ☐ RTD ☐ SIQ 24h ☐ SIQ _____h ☐ Admit
Follow-up: ☐ As needed ☐ 1 day ☐ 3 days

Cobra Gold: Diarrhea Symptom Diary Card #: 1 or 2

Name: Last: _____ First: _____ SSN: ____-___-____

INSTRUCTIONS: ❶ Carry the card with you and make entries each day. ❷ Mark down each time you have a bowel movement or vomit. ❸ Return the card to the clinic on the date listed. ❹ Return to the clinic on the date (approx. times) listed. **[Return sooner if you feel you need further medical care]**

VOL ID # _____ **FILLED OUT BY STUDY PERSONNEL**

	Date (mm/dd)	Time (24-hr clock)		Date (mm/dd)	Time (24-hr clock)
Card issued:	____/___	_____	**Card returned:**	____/___	_____
First (24 hr) visit:	____/___	_____	**Second (72 hr) visit :**	____/___	_____
Third (5-7 day) visit:	____/___	_____			

Place a tick mark (!) in the correct box (6-hr intervals) for each time you have diarrhea (watery or loose stool).

Write in dates→				
0001-0600				
0601-1200				
1201-1800				
1801-2400				

Place a check mark (✓) in the correct box for each symptom you experience during each day.

Write in dates→				
Nausea				
Vomiting				
Abdominal Cramps				
Fever				
Bloody Stools				

Place a check mark (✓) in the box that best describes how this illness affected your function that day.

Write in dates→				
Normal ability to work, go on pass, etc.				
Decreased ability to work, go on pass, etc.				
Not able to work, go on pass, etc.				

Record the following:	Date (mm/dd)	Time (24-hr clock)
Time of 1st antibiotic dose:	____/___	_____
Time of 2nd antibiotic dose:	____/___	_____
Time of 3rd antibiotic dose:	____/___	_____
Time of 1st formed stool:	____/___	_____

Cobra Gold Post-Treatment Follow-up (Diarrhea Surveillance)

Name:_____ **VOL ID #:**_____ **Tx Code #:**_____

Follow-up date: _____ **Follow-up time:** _____

Collected 2nd diarrhea diary card: YES NO

**** If volunteer has lost or incorrectly completed card, then attempt to complete card with volunteer. If this is necessary, record this below under comments section.**

Collected stool specimen: YES NO

****If volunteer did not provide stool specimen, make sure a container is provided to them and arrange the time you will retrieve the specimen.**

Date of the last clinic visit: _____

Since the last clinic visit, record if any of these symptoms have occurred:

Diarrhea YES NO Fever YES NO

****Diarrhea definition** = 3 or more loose or liquid stools in a 24 hour period OR 2 or more loose or liquid stools in 24 hr period plus at least one of the following symptoms (abdominal pain or cramps, nausea, vomiting, fever, tenesmus (rectal urgency/pain), or gross blood in stools during the diarrheal episode.

Other symptoms? [Record symptom description, how long present, affect on functional ability (use diary card scoring system)] Continue on back if needed.

Additional comments (if patient has symptoms encourage them to come to clinic for evaluation):

FILLED OUT BY STUDY PERSONNEL

Follow-up completed by: _____
Reviewed by:
Study Investigator:_____ **Date:** _____

Subject Withdrawal/Disenrollment Form

Name:_____ **Study #:**_____

The volunteer was enrolled in the clinical trial component of the study on _____ **(date)**

This volunteer received study agent code: _____

The volunteer did not complete the study and was withdrawn on _____ **(date) because:**

1. **Adverse Event**
2. **Disease progression necessitating hospitalization**
3. **Non-Compliance**
4. **Voluntary Withdrawal**

Signature of Volunteer (if voluntary withdrawal):_____**Date:**

Signature of Witness (if voluntary withdrawal):_____**Date:**

5. **Other:**_____

Comments (include information regarding breaking the individual code if appropriate):

Study Investigator:_____ Date: _____

Cobra Gold Study Clinic Visit, Medication, & Specimen Log

Volunteer ID #:_____ **Study:** **SRV** **C-C** **RCT**

Clinic Visits:	**Planned**		**Actual**	
	Date (mm/dd)	**Time (24-hr clock)**	**Date (mm/dd)**	**Time (24-hr clock)**
First (24 hr) visit:				
Second (72 hr) visit :				
Third (5-7 day) visit:				
Additional visit				
Additional visit				

Treatment Code # (affix label):

SPECIMEN DATE (mm/dd/yy)	**LAN** (Place sticker in shaded area)	**SPECIMEN TYPE** (Bl=blood; St=stool; Rs=rectal swab)

Cobra Gold Initial Clinic Visit Abstraction Form

Date: ___/___/___ **Time:** _____ **Study ID #:** _____

SSN (last 4): _____ **Service:** ☐ Army ☐ Marines ☐ Navy ☐ Air Force ☐ Civilian

Age:____ **Sex:** ☐ Male ☐ Female

Date/Time of onset of diarrhea: ___/___/___ **Time** _____**hrs**
Date/Time of onset of fever: ___/___/___ **Time** _____**hrs**

Review of Systems:

Symptom			Duration (days)			
Diarrhea	Yes	No	☐ 1	☐ 2	☐ 3	☐ 4+
# loose/liquid stools last 24 hours						
# loose/liquid stools since start of sx						
Blood in stools	Yes	No	☐ 1	☐ 2	☐ 3	☐ 4+
Nausea	Yes	No	☐ 1	☐ 2	☐ 3	☐ 4+
Vomiting	Yes	No	☐ 1	☐ 2	☐ 3	☐ 4+
# of episodes of vomiting						
Abdominal cramps	Yes	No	☐ 1	☐ 2	☐ 3	☐ 4+
Tenesmus	Yes	No	☐ 1	☐ 2	☐ 3	☐ 4+
Subjective Fever	Yes	No	☐ 1	☐ 2	☐ 3	☐ 4+
Headaches	Yes	No	☐ 1	☐ 2	☐ 3	☐ 4+
Muscle aches	Yes	No	☐ 1	☐ 2	☐ 3	☐ 4+
Joint pains	Yes	No	☐ 1	☐ 2	☐ 3	☐ 4+

Other Symptoms (record symptom/duration):

Diarrhea Self-Medication: **Any self-medication?** Yes No **(If no skip to next section)**

Medication	Purpose: Prevention		Purpose: Treatment		Duration (# days)	Amount (# pills)
Imodium (loperamide)	Yes	No	Yes	No		
Pepto-Bismol	Yes	No	Yes	No		
Antibiotic (state:)	Yes	No	Yes	No		
Other (state:)	Yes	No	Yes	No		

How has this illness affected your ability to work/go on liberty/go on pass? (mark one)
☐ Normal ability ☐ Decreased ability ☐ Not able

Physical Exam: Vital Signs: Temp_____^{0}**F** **Evidence of orthostasis:** ☐Yes ☐No

Stool characterization: ☐ Grade 1 - hard (normal)
(check only one box) ☐ Grade 2 - soft (normal)
☐ Grade 3 - thick liquid
☐ Grade 4 - opaque watery liquid
☐ Grade 5 - clear watery

Gross blood in stools: ☐Yes ☐No

Hemoccult: ☐Pos ☐Neg

Urine hCG (if applicable): ☐Pos ☐Neg

Primary Assessment:

Illness most consistent with:
- ☐ **watery, non-inflammatory diarrhea**
- ☐ **dysentery**
- ☐ **gastroenteritis**
- ☐ **other (describe)**

Eligibility determination (in database as yes or no answers; if checked = yes):

Case-control study (check if eligible):
- ☐ diarrheal symptoms of < 14 days duration
- ☐ symptom onset at least 24 hours after arrival in Thailand

Treatment study (check if eligible):
- ☐ diarrheal symptoms of ≤ 96 hours duration
- ☐ ambulatory management with planned follow-up
- ☐ no macrolide or quinolone allergy (not limited GI intolerance)
- ☐ not on antibiotics (excluding malaria prophylaxis) past 72 hr
- ☐ not taking theophylline, digoxin, or warfarin
- ☐ no history of seizures

Treatment (in database as yes or no answers; if checked = yes):

Rehydration therapy: ☐ **Increase fluids** ☐ **ORS solution** ☐ **IV fluids (Qty: _____L)**
(check all that apply)

Enrolled in treatment study: ☐ **Yes** ☐ **No** **Tx code:** _____

Time of 1st dose: _____

IF Not in Tx study, medication given: ☐ **FQ** ☐ **Azithro** ☐ **Other** _____

Post-dose observation (in clinic) symptoms:
Nausea ☐ **Yes** ☐ **No**
Vomiting ☐ **Yes** ☐ **No**

Other post-dosing symptoms (in clinic):

Initial Disposition: ☐ **RTD** ☐ **SIQ 24h** ☐ **SIQ _____h** ☐ **Admit**

Initial Follow-up: ☐ **As needed** ☐ **1 day** ☐ **3 days**

Abstractor's initials: _____ **Date:** _____

Cobra Gold Follow-Up Abstraction Form

Study ID #: _____ **In treatment study:** ☐ **Yes** ☐ **No** **IF Yes, Tx Code #:** _____

As applicable – Date/Time of 2nd dose: _____
Date/Time of 3rd dose: _____

	Date	Time
Last diarrheal stool:	_____	_____
Last unformed stool:	_____	_____
First formed stool:	_____	_____

Calculate TOTAL diarrhea duration: (hrs) _____

Calculate duration SINCE first dose of antibiotic: (hrs) _____

Date fever ceased: (MM/DD/YY) _____

Time fever ceased: (2400 clock) _____

Calculate fever duration: (hrs) _____

Symptom	Ever present?				Day of onset	Day of resolution	Total Duration (days)
Diarrhea	**Yes**	**No**	**NA**	**Unknown**			
Blood in stools	**Yes**	**No**	**NA**	**Unknown**			
Nausea	**Yes**	**No**	**NA**	**Unknown**			
Vomiting	**Yes**	**No**	**NA**	**Unknown**			
Total episodes of vomiting							
Abdominal cramps	**Yes**	**No**	**NA**	**Unknown**			
Tenesmus	**Yes**	**No**	**NA**	**Unknown**			
Subjective Fever	**Yes**	**No**	**NA**	**Unknown**			
Headaches	**Yes**	**No**	**NA**	**Unknown**			
Muscle aches	**Yes**	**No**	**NA**	**Unknown**			
Joint pains	**Yes**	**No**	**NA**	**Unknown**			
Rash	**Yes**	**No**	**NA**	**Unknown**			
Dizziness	**Yes**	**No**	**NA**	**Unknown**			
Vaginal discharge/pruritus	**Yes**	**No**	**NA**	**Unknown**			

- **Use the following to record "study days" → 0 = day of 1st antibiotic dose, –# = pre-treatment days (such as –1), and 1-# (last relevant follow-up day) = post-treatment days.**

Other symptoms:

Study day	Total # Loose/Liquid stools				Daily Functional Assessment
	0001-0600	0601-1200	1201-1800	1801-2400	
0					NL Decrease Unable
1					NL Decrease Unable
2					NL Decrease Unable
3					NL Decrease Unable
4					NL Decrease Unable
5					NL Decrease Unable
6					NL Decrease Unable
7					NL Decrease Unable

Did patient self-medicate? ☐ Yes ☐ No **(If no skip to next section)**

Medication			Duration (# days)	Amount (# pills)
Imodium (loperamide)	Yes	No		
Pepto-Bismol	Yes	No		
Antibiotic (state:)	Yes	No		
Other (state:)	Yes	No		

Physical Exam: **Day 1 Temp_____^{0}F Day 3 Temp_____^{0}F**
Other exam comments:

Stool characterization:

Day 1 - ☐ **Grade 1** ☐ **Grade 2** ☐ **Grade 3** ☐ **Grade 4** ☐ **Grade 5**
Day 1 - Gross blood: ☐**Yes** ☐**No** **Hemoccult:** ☐**Pos** ☐**Neg**

Day 3 - ☐ **Grade 1** ☐ **Grade 2** ☐ **Grade 3** ☐ **Grade 4** ☐ **Grade 5**
Day 3 - Gross blood: ☐**Yes** ☐**No** **Hemoccult:** ☐**Pos** ☐**Neg**

Illness Re-assessment:

Day 1 - ☐ **watery diarrhea** ☐ **dysentery** ☐ **gastroenteritis** ☐ **other** _____
Day 3 - ☐ **watery diarrhea** ☐ **dysentery** ☐ **gastroenteritis** ☐ **other** _____

Final outcomes:

Patient met clinical cure definition: ☐ **Yes** ☐ **No**
Patient had clinical relapse: ☐ **Yes** ☐ **No**
[Relapse = met clinical cure definition with symptom recurrence following a 24-hr symptom-free period]
Patient followed up post-treatment? ☐ **Yes** ☐ **No**
Patient provided post-treatment stool? ☐ **Yes** ☐ **No**
Total # missed days of work: ____
Ever required admission? ☐ **Yes** ☐ **No**
Ever required IV rehydration? ☐ **Yes** ☐ **No**
Ever required antibiotic treatment modification? ☐ **Yes** ☐ **No**
If modified, what change: ☐ **Therapy extension** ☐ **Antibiotic change** ☐ **Other** _____
Comments:

Abstractor's initials: _____ **Date:** _____

Cobra Gold Field Data Manager Abstraction Form

Volunteer ID #: _____ **Treatment Code #:** _____

Volunteer Last Name: _____ **Volunteer First Name:** _____

Study: SRV C-C RCT

Clinic Visits:	Planned		Actual	
	Date (mm/dd)	Time (24-hr clock)	Date (mm/dd)	Time (24-hr clock)
First (24 hr) visit:				
Second (72 hr) visit :				
Third (5-7 day) visit:				
Additional visit				
Additional visit				

Field Laboratory Results

Study Day	Stool LAN	Stool frozen		Blood LAN
0		Yes	No	
3		Yes	No	
5-7		Yes	No	
Additional		Yes	No	
Additional		Yes	No	

Study day	Fecal WBC	LFLA	LFLA titer	CRP
0	ND | N | 1 | 2 | 3 | 4	ND | N | 1 | 2 | 3 | 4		ND | N | 12 | 24 | 48 | 96
3	ND | N | 1 | 2 | 3 | 4	ND | N | 1 | 2 | 3 | 4		ND | N | 12 | 24 | 48 | 96
5-7	ND | N | 1 | 2 | 3 | 4	ND | N | 1 | 2 | 3 | 4		ND | N | 12 | 24 | 48 | 96
Added	ND | N | 1 | 2 | 3 | 4	ND | N | 1 | 2 | 3 | 4		ND | N | 12 | 24 | 48 | 96
Added	ND | N | 1 | 2 | 3 | 4	ND | N | 1 | 2 | 3 | 4		ND | N | 12 | 24 | 48 | 96

** Circle the correct response for each test

** ND = not done; N = NEG; P = POS

Study day	Prelim Micro	Campy EIA	Shigella EIA
0	ND | NG | C | NF | V	ND | N | 1 | 2 | 3 | 4	ND | N | P
3	ND | NG | C | NF | V	ND | N | 1 | 2 | 3 | 4	ND | N | P
5-7	ND | NG | C | NF | V	ND | N | 1 | 2 | 3 | 4	ND | N | P
Added	ND | NG | C | NF | V	ND | N | 1 | 2 | 3 | 4	ND | N | P
Added	ND | NG | C | NF | V	ND | N | 1 | 2 | 3 | 4	ND | N | P

**For Prelim Micro: ND = Not done NG = no growth to date C = Campy
NF = Non-lactose fermenter (R/O Salmonella, Shigella) V = Vibrio species

COMMENTS:

Institutional approval documents

DEPARTMENT OF THE NAVY
NAVAL MEDICAL RESEARCH CENTER
503 ROBERT GRANT AVENUE
SILVER SPRING, MARYLAND 20910-7500

IN REPLY REFER TO:
3900
Ser 00R/10042
21 Mar 00

From: Commanding Officer, Naval Medical Research Center
To: Dr. David Tribble, M.D., M.P.H.

Subj: APPROVAL OF PROTOCOL INVOLVING THE USE OF HUMAN SUBJECTS

Ref: (a) SECNAVINST 3900.39B
(b) NAVMEDRSCHCENINST 3900.6
(c) NAVMEDRSCHDEVCOM Instruction 3900.2
(d) Mtg of the Committee for the Protection of Human Subjects of 22 February 00 and Memo from Vice-Chair, NMRC CPHS via Dr. Schrot and CDR Murphy of 6 March 00
(e) CNO ltr Ser 093/2V239066 of 10 Mar 92

1. In accordance with references (a), (b), and (c), the Committee for the Protection of Human Subjects (CPHS) reviewed your protocol **DOD#: 31528** entitled **"Travlers' Diarrhea Surveillance, Diagnosis and Therapy Study in United States Military Personnel on Deployment in Thailand (Cobra Gold 2000/2001)"** during reference (d). The Committee unanimously recommended approval of this protocol at the level as minimal risk.

2. Based on the authority granted to me in reference (e), I hereby approve the protocol.

3. All human subject research protocols must be reviewed at least annually by the CPHS until the project is completed. The next continuing review must be received, accepted, approved and filed before **22 February 2001**.

4. It is your responsibility as Principal Investigator to ensure that any procedural or experimental modifications to the approved protocol, any changes in the investigators attached to the study, or any unanticipated problems which arise in the course of the study that may affect the rights and welfare of the Human Subjects are immediately reported to my office through the CPHS Chairperson.

5. My point of contact is Captain Charles Auker who may be reached at (301) 319-7480 or Dr. Edward Gabriele at (301) 295-0179

R. G. HIBBS

Copy to:
CAPT Scott
BUMED (MED-26H)

DEPARTMENT OF THE NAVY
BUREAU OF MEDICINE AND SURGERY
2300 E STREET NW
WASHINGTON DC 20372-5300

RECEIVED 5/31/00

IN REPLY REFER TO

3900
Ser 26H/00U246
11 May 00

From: Chief, Bureau of Medicine and Surgery
To: Commanding Officer, Naval Medical Research Center

Subj: REVIEW OF PROTOCOL **DoD#31528-** TRAVELS' DIARRHEA SURVEILLANCE, DIAGNOSIS AND THERAPY STUDY IN UNITED STATES MILITARY PERSONNEL ON DEPLOYMENT IN THAILAND (COBRA GOLD 2000/2001)

Ref: (a) NAVMEDRSCHCENT ltr 3900 Ser 00R/10042 of 21 Mar 00
(b) NAVMEDRSCHDEVCOMINST 3900.2 of 7 Jun 93

1. Reference (a) is acknowledged received as minimal risk research.

2. In accordance with reference (b), a continuing review of this protocol should be completed in **February 2001.** The CPHS documentation will be forwarded to MED-26 via the appropriate chain of command. Additionally, a CPHS-approved completion report should be forwarded upon the conclusion of the study.

3. If you have any questions, my point of contact is CAPT James T. Alexander, MC, USN at ((202)/DSN) 762-0477 or e-mail (jtalexander@us.med.navy.mil).

JAMES T. ALEXANDER
By direction

DEPARTMENT OF THE ARMY
WALTER REED ARMY INSTITUTE OF RESEARCH
WALTER REED ARMY MEDICAL CENTER
WASHINGTON, D.C. 20307-5100

REPLY TO
ATTENTION OF

MCMR-UWZ (5-14a)

14 March 2000

MEMORANDUM THRU Deputy Director, Walter Reed Army Institute of Research, 503 Robert Grant Ave, Silver Spring, MD 20910-7500

FOR David Tribble, M.D., M.P.H., Enteric Diseases Department, Naval Medical Research Center, 503 Robert Grant Ave, Silver Spring, MD 20910-7500

SUBJECT: Human Use Protocol: entitled "Travelers' Diarrhea Surveillance, Diagnosis and Therapy Study in United States Military Personnel on Deployment in ~~Korat,~~ Thailand (Cobra Gold 2000/2001)" (WRAIR #792)

1. The referenced human use protocol and supporting information have been submitted and reviewed in accordance with AR 70-25 and WRAIR Policy Letter 98-07.

2. This protocol proposes to: 1) initiate clinic-based passive surveillance of diarrheal enteropathogens affecting deployed troops in Thailand, 2) evaluate epidemiologic, microbiologic, and immunologic factors associated with diarrheal illness, 3) determine the therapeutic efficacy of azithromycin versus levofloxin, both standard therapies, as empiric therapy for traveler's diarrhea, and 4) determine the effectiveness of bedside and field laboratory-based rapid diagnostic assays in the management of acute infectious diarrhea. The protocol was reviewed by a WRAIR Scientific Review Committee and approved on 6 March 2000. The Chair of the WRAIR Human Use Review Committee recommended approval on 14 March 2000, as posing no greater than minimal risk to volunteers. USUHS and NMRC ethical review committees are currently reviewing this protocol.

3. Authority is, therefore, granted to implement this minimal risk protocol, pending receipt of USUHS and NMRC ethical approvals.

MARTIN H. CRUMRINE
COL, MS
Director

CF:
HSP, MRMC-RCQ-HR
Edward Gabriele, Ph.D., NMRC

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

March 29, 2000

MEMORANDUM FOR DAVID R. TRIBBLE, M.D., M.P.H., DEPARTMENT OF PREVENTIVE MEDICINE AND BIOMETRICS

SUBJECT: IRB Approval of Protocol **G187MT-01** for Human Subject Use

In accordance with 32 CFR 219.114, USUHS accepts the review and approval by the NMRC and WRAIR Committees for the Protection of Human Subjects (CHPS) for the research protocol entitled ***"Travelers' Diarrhea Surveillance, Diagnosis and Therapy Study in U.S. Military Personnel on Deployment in Thailand"*** under your direction. It is requested that NMRC and WRAIR provide this office with human subject use review updates at least annually.

The purpose of this study is to determine the risk factors associated with getting diarrhea in deployed military personnel and to evaluate different antiobiotic treatments for diarrhea. The IRB understands that this study involves the collection of blood and stool specimens from deployed military personnel presenting with diarrhea. The IRB further understands that this study also involves a treatment arm which requires the random administration and evaluation of 3 antibiotic treatments.

You are required to submit amendments to this protocol, changes to the consent form, adverse event reports, and other pertinent information relative to human subject use for this project to this office for review. It is your responsibility to maintain an accurate and accessible file of all consent forms of participating human subjects.

If you have any questions regarding human subject use, please call me at 301-295-3303.

Richard R. Levine, Ph.D.
LTC, MS, USA
Director, Research Programs and
Executive Secretary, IRB

cc: Director, Grants Administration

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UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

May 13, 2002

MEMORANDUM FOR DAVID R. TRIBBLE, M.D., M.P.H., DEPARTMENT OF PREVENTIVE MEDICINE AND BIOMETRICS

SUBJECT: IRB Approval of Protocol **G187MT** for Human Subject Use

This memorandum is to confirm the termination of IRB approval for human subject use for the following study under your direction:

- Protocol **G187MT**, *"Travelers' Diarrhea Surveillance, Diagnosis and Therapy Study in the U.S. Military"*

Kathryn H. Knudson, Ph.D.
LTC, MS, USA
Human Research Protections Program
Administrator and Executive Secretary, IRB

cc: Director, Grants Administration

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DEPARTMENT OF THE ARMY
WALTER REED ARMY INSTITUTE OF RESEARCH
WALTER REED ARMY MEDICAL CENTER
WASHINGTON, D.C. 20307-5100

REPLY TO
ATTENTION OF

MCMR-UWZ-C

22 May 2002

MEMORANDUM FOR

David Tribble, MD, MPH, Enteric Diseases Department, Naval Medical Research Center, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500

SUBJECT: Final Report to WRAIR Human Use Protocol: "Travelers' Diarrhea Surveillance, Diagnosis, and Therapy Study in United States Military Personnel on Deployment in Thailand (COBRA GOLD 2000/2001)" (WRAIR #792)

1. The final report submitted for this protocol was reviewed by the April 2002 WRAIR Human Use Review Committee.

2. The report was accepted unanimously. The study is officially closed.

for Sara W. Rothman, Ph.D.
Director
Office of Research Management

02 July 2003

MEMORANDUM FOR THE RECORD

From: IRB Vice-Chair, Naval Medical Research Center

Subj: MINIMAL RISK HUMAN USE RESEARCH PROTOCOL DOD#30596

Encl: (1) Memo from D. Tribble to IRB Executive Administrator, NMRC of 13 FEB 2003

1. The final report for the subject protocol entitled, "Cobra Gold 99 – Immune Response to Community-acquired *Campylobacter jejuni* Infection in United States Military Personnel on Deployment in Korat, Thailand" was reviewed and recommended for acceptance by the Committee for the Protection of Human Subjects on 09 May 2000 pending minor clarifications.

2. This memorandum certifies that enclosure (1), the minor clarifications of the final report, has been submitted, is accepted and has been filed with the protocol records.

Judith E. Epstein

J. E. EPSTEIN, MC, USNR

Copy to:
M2HU
D. Tribble (w/o enclosure)