Diagnostic Approach to Acute Diarrheal Illness in a Military Population on Training Exercises in Thailand, a Region of *Campylobacter* Hyperendemicity[▽]

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High rates of *Campylobacter* fluoroquinolone resistance highlight the need to evaluate diagnostic strategies that can be used to assist with clinical management. Diagnostic tests were evaluated with U.S. soldiers presenting with acute diarrhea during deployment in Thailand. The results of bedside and field laboratory diagnostic tests were compared to stool microbiology findings for 182 enrolled patients. *Campylobacter jejuni* was isolated from 62% of the cases. Clinical and laboratory findings at the time of presentation were evaluated to determine their impact on the posttest probability, defined as the likelihood of a diagnosis of *Campylobacter* infection. Clinical findings, the results of tests for inflammation (stool occult blood testing [Hemoccult], fecal leukocytes, fecal lactoferrin, plasma C-reactive protein), and the numbers of *Campylobacter*-specific antibody-secreting cells in peripheral blood failed to increase the posttest probability above 90% in this setting of *Campylobacter* hyperendemicity when these findings were present. Positive results by a *Campylobacter*-specific commercial enzyme immunoassay (EIA) and, less so, a research PCR were strong positive predictors. The negative predictive value for ruling out *Campylobacter* infection, defined as a posttest probability of less than 10%, was similarly observed with these *Campylobacter*-specific stool-based tests as well the fecal leukocyte test. Compared to the other tests evaluated, the *Campylobacter* EIA is a sensitive and specific rapid diagnostic test that may assist with diagnostic evaluation, with consideration of the epidemiological setting, logistics, and cost.

Military personnel are frequently affected by short-term morbidity related to diarrheal diseases, with a potential adverse impact on the operational mission (24, 37). The use of empirical therapy without supplemental laboratory data is a feasible option; however, refinement of the management strategy by using laboratory testing may increase the cost-effectiveness and allow specific adjustments in antibiotic selection on the basis of regional susceptibility patterns. During military operations, the availability of a field laboratory with a microbiological testing capability is variable. Rapid, technically simple diagnostic tests need to be evaluated to determine their accuracy and acceptability in field settings. In Thailand, numerous surveys among deployed U.S. military personnel have shown that enteropathogenic *Campylobacter* species, *Campylobacter jejuni* and *C. coli*, account for as many as 60% of diarrheal cases (2, 4, 8, 27, 30, 34, 42), a very high prevalence compared to the prevalence detected in similar studies conducted in other regions (35). On the basis of this observation, the pathogen-specific diagnostic tests used in this study focused on *Campylobacter*. This study of military personnel presenting with acute diarrhea during deployment in Thailand evaluated the clinical findings in concert with bedside stool characterization and the results of field laboratory rapid diagnostic tests as components of an overall diagnostic approach.

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MATERIALS AND METHODS

Study population and enrollment criteria. Annual U.S. military training exercises were conducted in the Kingdom of Thailand in May 2000 and May 2001. Temporary medical units for the evaluation and management of personnel were in operation during the period of the exercise. Personnel presenting with acute diarrhea were requested to volunteer for participation. Enrollment criteria included the following: acute diarrhea duration of ≤96 h, onset of illness ≥24 h after arrival in Thailand, illness conforming to the definition of diarrhea, no antibiotic treatment (with the exception of doxycycline, used for malaria prophylaxis) in the previous 7 days, and the availability of a pretreatment stool culture. Diarrhea was defined as three or more loose stools in a 24-h period or two or more loose stools in a 24-h period with one or more associated complaints, including abdominal cramps, nausea, vomiting, or fever (oral temperature, ≥38°C).

Clinical evaluation and specimen collection. A standardized questionnaire was used, and a medical examination was performed. The patients were asked to provide a stool specimen prior to administration of the first antibiotic dose. Stool characterization and bedside occult blood testing (Hemoccult; Beckman Coulter, Inc., Fullerton, CA) was completed by the study physician prior to transport of the specimen to the field laboratory. Stool specimens were graded on a scale from 1 to 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

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Vacutainer tube containing EDTA (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 the stool specimens was undertaken at an on-site field laboratory, as described previously (38). *Campylobacter* species were isolated by a membrane filter method on nonselective blood agar before and after enrichment (39). Throughout this report, *Campylobacter* refers to both *C. jejuni* and *C. coli*. The isolates were transported to the Armed Forces Research Institute of Medical Sciences in Bangkok, Thailand, for species identification and susceptibility testing, as described previously (7, 9, 11). Five lactose-fermenting and five non-lactose-fermenting *Escherichia coli* colonies per specimen were tested by using DNA probes for the detection of enterotoxigenic *E. coli* (ETEC) toxins (heat-labile and heat-stable toxins), enteroinvasive *E. coli* (pMR17), enterohemorrhagic *E. coli* (Shiga-like toxin I and II producing), and enteropathogenic *E. coli* (*eae* and EAF plasmid positive) (10, 40). Microscopy evaluation of fresh stool specimens was used to evaluate the specimens for parasites. Aliquots of stool samples were frozen at $-70°C$ on the day of receipt for viral antigen detection. The stool samples were evaluated for the presence of rotavirus and calicivirus antigens by a commercially available enzyme-linked immunosorbent assay (Rotazyme; Abbott Laboratories, North Chicago, IL) and a noncommercial antigen-capture calicivirus-specific enzyme-linked immunosorbent assay, respectively (26).

Diagnostic tests under evaluation (index tests). Individuals performing the index tests were trained laboratory personnel masked to the results for the reference standard and clinical findings, with the exception of the results of the stool PCR assay, which was under development.

(i) Stool-based index tests. All stool-based index tests were performed with fresh specimens on the day of receipt. Fecal leukocytes were semiquantitatively determined by examining methylene blue-stained fecal smears under a microscope. The numbers of fecal leukocytes present per high-power field were categorized as follows: none, rare, 1 to 5, 6 to 10, and >10. Fecal lactoferrin was detected with the commercial Leuko-Test kit (TechLab, Blacksburg, VA), according to the manufacturer's instructions. The presence of lactoferrin in a 1:50-diluted stool specimen was detected by a visually read positive agglutination of ≥1+, as defined by the manufacturer. The presence of *C. jejuni* or *C. coli* was detected by using the commercial ProSpecT *Campylobacter* microplate assay (Alexon-Trend, Inc., Ramsey, MN), according to the manufacturer's instructions. *Campylobacter*-specific antigens were detected by a visually read color development of ≥1+, as defined by the manufacturer. A multiplex PCR for the detection of *C. jejuni* and *C. coli* in the stool specimens was included during the first year of the exercise, as described previously (21). The PCR assay detects the *ceuE* genes in *C. jejuni* and *C. coli* and is useful for primary detection and species differentiation. DNA templates from 10% stool suspensions in TE (10 mM Tris-HCl, 1 mM disodium EDTA, pH 8.0) were prepared by silicon dioxide extraction (20, 21). Oligonucleotide primer sequences derived from the *ceuE* genes and the PCR amplification conditions have been reported previously (21). All PCR tests were conducted in the presence of appropriate controls, and the results were considered valid only if the results for the controls were proven to be accurate; i.e., positive controls were PCR positive and negative controls were PCR negative.

(ii) Blood-based index tests. Blood samples were evaluated for plasma C-reactive protein (CRP), an acute-phase protein whose level is elevated during inflammatory disease, at the time of initial presentation to the clinic (32). Fresh plasma was evaluated semiquantitatively by using the commercial RapiTex CRP test (Dade Behring, Marburg, Germany), according to the manufacturer's instructions. Mononuclear cells (MNCs) were isolated by Ficoll-Hypaque density gradient analysis (Organon Teknika Corp., Durham, NC) and were cryopreserved in the field laboratory (3). Assays for antibody-secreting cells (ASCs) were performed at the Naval Medical Research Center, Silver Spring, MD, as described previously (3). *Campylobacter*-specific immunoglobulin A ASC responses were evaluated at the initial presentation and at the 72-h clinical follow-up by using the enzyme-linked immunospot assay methodology (3). The specific antigens used included a *C. jejuni* strain 81-176 glycine extract, *C. jejuni* strain 81-176 whole cells, and a common Thai *C. jejuni* strain (Lior 36) whole-cell lysate preparation (28). The numbers of spots found in comparable wells were summed and adjusted to the number per 10^6 MNCs. A positive ASC response was defined as five or more *Campylobacter* antigen-specific spots per 10^6 MNCs.

Statistical analysis. The physician-performed bedside diagnostic assays (stool characterization and occult blood testing) and laboratory technician-performed rapid diagnostic assays (fecal leukocyte smear, lactoferrin latex agglutination assay, *Campylobacter*-specific enzyme immunoassay [EIA], and CRP test) were compared with the "gold standard" stool microbiology assay results. The performance characteristics of each assay were assessed. Test performance character-

istics (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 that a test result would be accurately rated as positive or negative was quantitated by using the area under the receiver operating characteristic curve (ROC) (16). In order to compare the areas under the ROC curves obtained from various diagnostic tests derived from the same cases, an adjustment was made to account for correlations between areas (17). The likelihood ratio was evaluated in context with pre- and posttest probabilities for different scenarios (low- versus high-prevalence region). In addition, clinical findings and diagnostic assays were evaluated singly and in series by using likelihood ratios in order to determine the most accurate and efficient diagnostic algorithm. The results of the patient surveys, symptom diaries, physician findings, and microbiological testing results were entered into EpiInfo software (version 6.04) databases. Statistical analysis was performed with SPSS for Windows (version 10.1). All tests were two tailed, and *P* values of <0.05 were considered statistically significant.

RESULTS

A total of 182 U.S. military personnel presenting with acute diarrhea were enrolled in the study, and all individuals underwent reference standard testing. All index tests were performed prior to the administration of treatment and concurrent with the reference standard testing, with the exception of testing for ASCs (as described in Materials and Methods). The characteristics of the study population are provided in Table 1. The cases enrolled during the two exercise years had similar age and gender distributions. A higher proportion of individuals received malaria prophylaxis during the first exercise year; however, no difference in the time to presentation with illness, the characteristics of the illness, or the pathogen distribution was observed. *Campylobacter* was identified in initial cultures of stools from 62% of all cases, with 96% of the organisms found to be *C. jejuni* and *C. coli* accounting for the remainder. *Salmonella* and *Plesiomonas* were isolated from an additional 10 to 20% of the cases. The high rates of isolation of invasive bacterial pathogens were supported by the frequent clinical features of inflammatory enteritis in >50% of the enrolled cases. A notable exception to this pattern was the relatively low rate of positivity for fecal leukocytes observed in the first exercise year. This observation was not consistent with the results of 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 detection of invasive enteropathogens, as well as the diagnosis of *Campylobacter* infection. The performance characteristics of the tests 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 occult blood testing-positive stools, did yield greater specificities. However, the overall accuracy of these findings, as represented by the area under the ROC curve, was low (<0.65). The discordant findings in the fecal leukocyte test 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 CRP tests provided reasonable sensitivities but lacked specificity. Both tests yielded negative likelihood

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

Characteristic	Result in Cobra Gold exercise yr:	
	2000	2001
Thai city (base for training exercise)	Nakhon Sri Thammarat	Phitsanulok
Demographics		
No. of enrolled cases	109	73
Median age (yr [IQR[a]])	26 (22–33)	26 (23–32)
Gender (% male)	90	90
Malaria prophylaxis (% of cases)[b]	91	75
Median no. of days in country preillness (IQR)	11 (7–16)	12 (8–15)
Clinical presentation		
Median no. of days of illness duration (IQR)	1 (1–3)	1 (1–2)
Median no. of diarrheal stools before 24 h (IQR)	5 (4–10)	5 (3–8)
Fever, by report (% of cases)	51	49
Vomiting (% of cases)[b]	27	11
Abdominal cramps (% of cases)	89	85
Bedside evaluation (% of cases)		
Oral temp ≥100°F	20	28
Stool character, watery liquid	37	41
Visible gross blood	13	14
Stool occult blood testing positive	35	36
Field laboratory evaluation (% of cases)		
Fecal leukocytes positive[b]	28	58
Fecal lactoferrin positive	79	80
Serum CRP positive	70	ND[c]
Campylobacter EIA positive	67	54
Campylobacter PCR positive	51	ND
Campylobacter ASC positive	44	ND
Pathogen isolation (% of cases)		
Campylobacter	67	55
Nontyphoidal *Salmonella*	15	25
Plesiomonas shigelloides	9	6
Noninvasive bacteria (ETEC, *eae*-positive *E. coli*)	11	14
Viral (rotavirus, calicivirus)	6	7
None identified	17	26

[a] IQR, interquartile range.
[b] Differences in a given characteristic between exercise years ($P < 0.05$).
[c] ND, the assay was not done during the exercise year.

ratios, amenable to ruling out the presence of an invasive enteropathogen.

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

The two stool-based *Campylobacter*-specific tests, PCR and

EIA, yielded the highest specificities and positive likelihood ratios of all tests under evaluation. More cases were evaluated by EIA than by PCR, by which the stool specimens were evaluated only in the second exercise year. False-negative results by the PCR assay led to a lower sensitivity than that observed for the EIA. A total of seven culture-positive *Campylobacter* cases had negative results by the PCR test. The simultaneously tested positive controls included for the detection of DNA in the stool specimen were positive for all but one of the specimens, ruling out the presence of nonspecific inhibitors as the primary explanation for the false-negative results. Of these seven culture-positive, PCR-negative specimens, three were also negative by the *Campylobacter*-specific EIA. The remaining four specimens had 4+ reactions by the EIA. There were three culture-negative, PCR-positive specimens. Two of these specimens were 4+ positive by the EIA. A total of six culture-positive, EIA-negative specimens were observed. Follow-up EIAs were undertaken for all of these cases by using stool specimens collected either 3 or 7 days after the initial treatment. None of the follow-up EIAs were positive, nor were any

TABLE 2. Clinical and laboratory findings as diagnostic modalities for invasive enteropathogens[a]

Finding	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	LR$^+$	LR$^-$	AUC
Diarrhea frequency (past 24 h)							
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)	0.61 (0.52, 0.70)
≥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)	0.57 (0.48, 0.66)
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)	0.56 (0.47, 0.65)
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)	0.51 (0.42, 0.61)
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)	0.57 (0.48, 0.66)
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)	0.64 (0.55, 0.73)
Oral temp ≥100°F	29 (21, 37)	92 (80, 97)	33 (25, 41)	91 (77, 97)	3.6 (1.4, 10)	0.8 (0.7, 0.9)	0.60 (0.52, 0.69)
Occult blood testing 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)	0.63 (0.54, 0.72)
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)	0.56 (0.46, 0.65)
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)	0.64 (0.56, 0.73)
Dysentery/documented fever or occult blood testing 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)	0.67 (0.58, 0.76)
Fecal leukocytes[b]							
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)	0.58 (0.49, 0.68)
≥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)	0.77 (0.68, 0.86)
≥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 CRP	83 (73, 90)	75 (53, 89)	56 (38, 73)	92 (83, 97)	3.3 (1.7, 6.7)	0.2 (0.1, 0.4)	0.81 (0.71, 0.91)

[a] Abbreviations: NPV, negative predictive value; PPV, positive predictive value; LR$^+$, positive likelihood ratio; LR$^-$, negative likelihood ratio; AUC, area under the ROC curve (AUC values represent true positive rate/true negative rate ratios); HPF, high-power field. The data in parentheses are 95% confidence intervals.

[b] Fecal leukocyte test performance differed significantly between exercise years, as follows, for the ≥1 to 5/high-power field classification (year 1 versus year 2): sensitivity, 28 versus 40%; specificity, 96 versus 75%; negative predictive value, 30 versus 38%; positive predictive value, 95 versus 76%; positive likelihood ratio, 6.1 versus 1.6; and negative likelihood ratio, both 0.8.

of the follow-up stool cultures. Four culture-negative, EIA-positive specimens were observed. Two of these cases had follow-up stool specimens posttreatment in which one was culture negative and EIA negative and the other was culture positive and EIA positive. Stool cultures and EIAs performed 3 and 7 days after administration of the first antibiotic dose detected no new EIA-positive cases. Among the cases initially EIA positive, there continued to be positive responses for 31% at day 3 and 23% at day 7. The semiquantitative test results trended toward a high proportion of 3+ and 4+ results in 80% of pretreatment specimens compared to 57% of posttreatment specimens.

DISCUSSION

Rapid diagnostic tests range from the inexpensive Gram stain for the presumptive identification of *Campylobacter* spp. (sensitivity, 60 to 90%) to the more technically complex and more expensive PCR (1, 5, 19, 29, 31). The utility of a diagnostic test is dependent upon the prevalence of the disease in the population. In Fig. 1, the posttest probability of *Campylobacter*-associated illness is presented in various settings of endemicity and is separated by the effect that a positive or a negative result has on ruling in or ruling out a diagnosis of

campylobacteriosis, respectively. 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 to 80% of cases. The inability of the clinical presentation to guide therapy for individuals with traveler's diarrhea was documented by Ericsson and coworkers (13). Reliance on specific yet insensitive clinical features would lead to the withholding of therapy in individuals who may benefit from early treatment. The ability of positive test findings to alter the probability of the presence of *Campylobacter* infection is most effective when a pathogen-specific stool-based test, such as the EIA, is used. In a setting of hyperendemicity, such as Thailand, with prevalence estimates of 50%, a positive EIA result yields a 94% posttest probability of disease. Concurrent findings of dysentery or fever further increase the posttest probability of a positive EIA result to 98%. The impact of a positive EIA result in a region with a lower prevalence of *Campylobacter* is less dramatic. An estimated prevalence of 5%, as may be observed in U.S. clinics, has a posttest probability of 46% in the event of a positive EIA result. 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*-specific EIA has previously been eval-

TABLE 3. Clinical and bedside stool characteristics as *Campylobacter* diagnostic modalities[a]

Finding	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	LR+	LR−	AUC
Diarrhea frequency (past 24 h)							
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)	0.60 (0.51, 0.68)
≥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)	0.59 (0.51, 0.68)
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)	0.58 (0.50, 0.67)
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)	0.53 (0.44, 0.62)
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)	0.58 (0.50, 0.67)
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)	0.68 (0.60, 0.76)
Oral temp ≥100°F	33 (24, 42)	93 (83, 97)	46 (37, 54)	88 (74, 96)	4.5 (1.9, 11)	0.7 (0.6, 0.8)	0.63 (0.55, 0.71)
Occult blood testing 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)	0.66 (0.58, 0.74)
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)	0.59 (0.50, 0.67)
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)	0.69 (0.61, 0.76)
Dysentery/documented fever or occult blood testing 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)	0.71 (0.65, 0.79)

[a] Abbreviations: NPV, negative predictive value; PPV, positive predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio; AUC, area under the ROC curve (AUC values represent true positive rate/true negative rate ratios). The data in parentheses are 95% confidence intervals.

uated with frozen stool sample collections and in clinic-based series in the United States and Europe (6, 18, 41). Positive EIA results have been documented out to 5 years from *Campylobacter*-positive stool specimens stored at −20°C, with a detection threshold of 3×10^6 CFU/g of stool (12). Real-time assessment under routine conditions has documented a sensitivity of 89% and a specificity of 98 to 99% in settings with a prevalence of *Campylobacter* ranging from 3 to 8% (6, 18). The positive likelihood ratio exceeded 30 and the negative likelihood ratio was 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 and outside of an established hospital. The *ceuE*-based multiplex PCR evaluated in this study had previously been evaluated by using frozen stool specimens, with comparisons of the cul-ture and PCR results based on microbiological recovery from stored specimens (21). The previous study documented much higher positivity rates for PCR than for culture (77 and 56%, respectively). On the basis of the test performance observed in this study with fresh specimens, the earlier observation was likely due to the presence of nonviable organisms in frozen specimens rather than a significant difference in the sensitivities of the PCRs. The rates of PCR false-negative results for fresh specimens observed in this study were higher than those observed for the frozen specimens in the previous study (21) (18 and 8%, respectively). This finding has been reported previously and in certain cases has been postulated to be secondary to the presence in the specimen of inhibitors that affect the PCR test (25, 43). This may also be explained by a PCR detection limit in the range of 10^5 campylobacters per ml stool (33), which may be

TABLE 4. Field laboratory tests as *Campylobacter* diagnostic modalities[a]

Finding	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)	0.57 (0.48, 0.66)
≥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)	0.80 (0.73, 0.87)
≥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 CRP	89 (79, 95)	69 (51, 83)	75 (56, 88)	85 (75, 92)	2.8 (1.7, 4.6)	0.1 (0.1, 0.2)	0.83 (0.75, 0.91)
Campylobacter EIA	95 (88, 98)	94 (84, 98)	91 (81, 96)	96 (90, 99)	16 (6.0, 40)	0.1 (0.1, 0.2)	0.94 (0.89, 0.98)
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)	0.86 (0.76, 0.95)
Campylobacter-specific ASCs	46 (55, 74)	60 (42, 76)	38 (25, 52)	68 (52, 81)	1.2 (0.7, 1.9)	0.9 (0.6, 1.3)	0.53 (0.41, 0.65)

[a] Abbreviations: NPV, negative predictive value; PPV, positive predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio; AUC, area under the ROC curve (AUC values represent true positive rate/true negative rate ratios); HPF, high-power field. The data in parentheses are 95% confidence intervals.
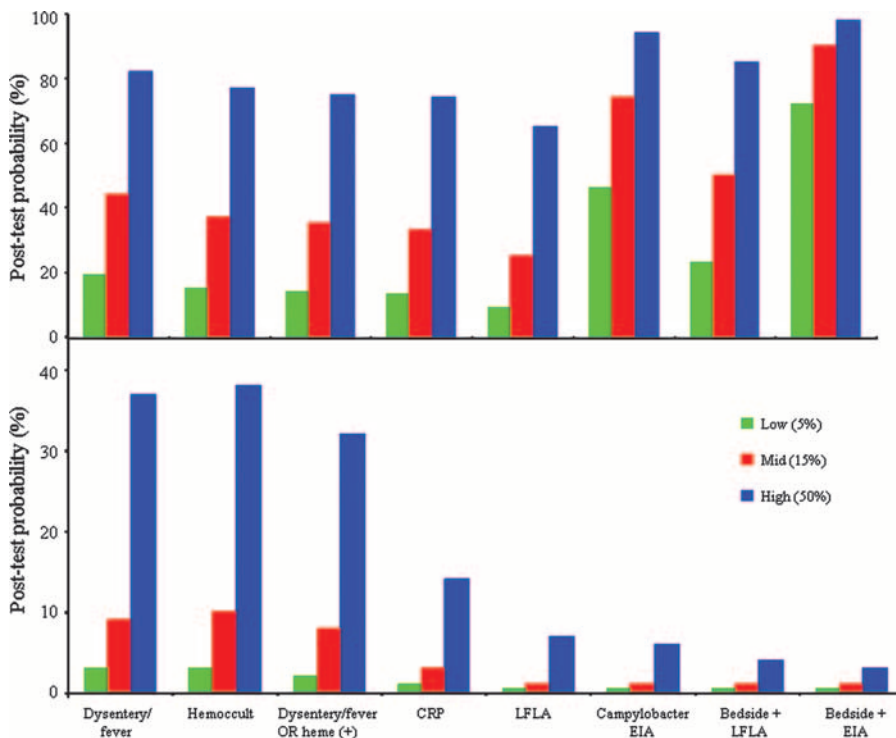
FIG. 1. Posttest probability of *Campylobacter*-associated illness based on the diagnostic approach used in various settings of endemicity (% prevalence). (Top panel) Results based on a diagnostic approach yielding a positive test finding (rule in disease); (bottom panel) results based on a negative test finding (rule out disease).

higher than the detection limits of culture methods. Further development of this test is needed before it may gain clinical utility. The *Campylobacter*-specific ASC assay lacked test performance parameters supportive of its clinical use. Potential improvements in this test may derive from the use of more purified antigens that are broadly cross-reactive with *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 the time of clinical presentation (14).

Rapid diagnostic tests based on the detection of an inflammatory state, blood-based CRP, and stool-based lactoferrin rather than on the detection of a specific pathogen were unable to increase the posttest probability beyond 75% in the event of a positive result (Fig. 1). However, the CRP and lactoferrin tests performed well at ruling out campylobacteriosis. A negative finding resulted in posttest probabilities of 14% for CRP and 7% for the lactoferrin assay (Fig. 1) in a setting of hyperendemicity, such as Thailand, which has a 50% prevalence (pretest probability). Given the improved ability to rule out *Campylobacter* infection and the infrequency with which blood specimens for the clinical management of diarrhea are obtained, the use of the fecal lactoferrin assay was preferable for screening for inflammatory enteritis. These findings are consistent with a systematic analysis of fecal screening tests (22). A meta-analysis stratified fecal screening test performance on the basis of the population studied, that is, populations from resource-poor regions and populations from developed countries, in order to account for differences in pathogen preva-

lence and disease spectrum (15). In developing countries, the rapid stool-based markers of inflammatory enteritis performed poorly in ruling in disease, possibly due to the high degree of 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 (15). The fecal lactoferrin assay has demonstrated value as a negative predictor of the presence of invasive enteropathogens in children in the developing world with acute diarrhea (23, 36), as was also shown in the deployed population in the present study. In this study, the fecal lactoferrin test would have failed to identify nine invasive pathogens (7%) or four *Campylobacter* cases (4%).

The regional predominance of *Campylobacter* previously documented in Thailand limits the broad application of these results across operational platforms in various regions. The results of the present study, coupled with analyses from an area where ETEC is predominant (with some contribution from *Shigella* species, which were not observed in this study), will better permit generalization. The *Campylobacter*-specific EIA provided desirable performance in ruling in or ruling out infection under field conditions, with results available within 2 h. Given the prevalence of *Campylobacter* in this setting and the high rates of fluoroquinolone resistance, this test most aids the clinician in determining the most appropriate type of patient management.

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