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Detection of *Campylobacter* in Stool and Determination of Significance by Culture, Enzyme Immunoassay, and PCR in Developing Countries

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***Campylobacter* is a common bacterial enteropathogen that can be detected in stool by culture, enzyme immunoassay (EIA), or PCR. We compared culture for *C. jejuni/C. coli*, EIA (ProSpecT), and duplex PCR to distinguish *Campylobacter jejuni/C. coli* and non-*jejuni/coli Campylobacter* on 432 diarrheal and matched control stool samples from infants in a multisite longitudinal study of enteric infections in Tanzania, Bangladesh, and Peru. The sensitivity and specificity of culture were 8.5% and 97.6%, respectively, compared with the results of EIA and 8.7% and 98.0%, respectively, compared with the results of PCR for *C. jejuni/C. coli*. Most (71.6%) EIA-positive samples were positive by PCR for *C. jejuni/C. coli*, but 27.6% were positive for non-*jejuni/coli Campylobacter* species. Sequencing of 16S rRNA from 53 of these non-*jejuni/coli Campylobacter* samples showed that it most closely matched the 16S rRNA of *C. hyointestinalis* subsp. *lawsonii* (56%), *C. troglodytis* (33%), *C. upsaliensis* (7.7%), and *C. jejuni/C. coli* (2.6%). *Campylobacter*-negative stool spiked with each of the above-mentioned *Campylobacter* species revealed reactivity with EIA. PCR detection of *Campylobacter* species was strongly associated with diarrhea in Peru (odds ratio [OR] = 3.66, $P < 0.001$) but not in Tanzania (OR = 1.56, $P = 0.24$) or Bangladesh (OR = 1.13, $P = 0.75$). According to PCR, *Campylobacter jejuni/C. coli* infections represented less than half of all infections with *Campylobacter* species. In sum, in infants in developing country settings, the ProSpecT EIA and PCR for *Campylobacter* reveal extremely high rates of positivity. We propose the use of PCR because it retains high sensitivity, can ascertain burden, and can distinguish between *Campylobacter* infections at the species level.**

Campylobacter is a fastidious Gram-negative bacterium considered to be a common cause of acute, self-limiting gastroenteritis in the developed world (1). The majority of studies of *Campylobacter* infection have used selective culture techniques designed to improve isolation of *Campylobacter jejuni* and *C. coli*, which are thought to be the primary species associated with human disease (2). Recently, several antigen-based tests for the detection of *Campylobacter* have been developed, and in a European setting these were revealed to have excellent sensitivity and specificity compared with the results of culture (>89%) (3). Additionally, the correlation between the most commonly used antigen-based tests has been shown to be excellent (4). However, subsequent studies have documented a substantial excess detection of *Campylobacter* using these tests in comparison to the results of selective culture (5, 6). Though this is thought to be primarily a product of both false-positive enzyme immunoassay (EIA) results and the low sensitivity of *Campylobacter* culture, there is some evidence that increased detection of *Campylobacter* by antigen-based tests can represent detection of non-*jejuni/coli Campylobacter* species, specifically, *C. upsaliensis* (7). Finally, several studies have now used PCR tests to detect a diverse range of *Campylobacter* species of unclear pathogenicity in patients with and without gastroenteritis (2, 8, 9).

The majority of studies validating the performance of these varied diagnostic techniques have been performed in the developed world, where exposure to *Campylobacter* species is sporadic. The relative performance of these tests in settings where *Campylobacter* is endemic has not been well characterized. A strong association between *Campylobacter* infection and diarrhea has also been described in the developed world (1). In developing country settings, *Campylobacter* infection has been most clearly implicated as a cause of diarrhea only in the first 6 months of life (10). *Campylobacter* is often shed for extended periods following such episodes, and asymptomatic excretion is common (11). The prevalence and consequences of these infections on childhood development are unclear, though recently, not only symptomatic *Campylobacter* infection but also asymptomatic *Campylobacter* infection has been associated with poor early-childhood weight gain in Peru (12).

Due to these multiple knowledge gaps, we sought to document the performance of these diagnostic methods for the purposes of our multisite Etiology, Risk Factors and Interactions of Enteric

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TABLE 1 Primers and probes used in the study

Organism (assay)	Target	Oligonucleotide[a]	Sequence[b]	Reference
C. jejuni/C. coli (duplex PCR)	*cadF*	F	CTGCTAAACCATAGAAATAAAATTTCTCAC	15
		R	CTTTGAAGGTAATTTAGATATGGATAATCG	
		P	HEX-CATTTTGACGATTTTTGGCTTGA-BHQ2	
Campylobacter species (duplex PCR)	16S rRNA	F	GATGACACTTTTCGGAGCGTAA	This study
		R	GCTTGCACCCTCCGTATTACC	
		P	FAM-CGTGCCAGCAGCC-BHQ1-MGB	
Campylobacter species (sequencing)	16S rRNA	F	GGATGACACTTTTCGGAGC	16
		R	CATTGTAGCACGTGTGTC	

[a] F, forward primer; R, reverse primer; P, probe.
[b] HEX, hexachloro-6-carboxyfluorescein; FAM, 6-carboxyfluorescein; BHQ1 and BHQ2, black hole quenchers 1 and 2, respectively.

Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) cohort study, which is investigating the effects of nutrition and enteric infection on infant and early-child growth and development and includes sites from South America, Africa, and Asia. We chose a study design that would additionally allow a preliminary investigation of the association between *Campylobacter* infection and diarrhea across multiple tests and sites in developing countries.

MATERIALS AND METHODS

Selection of specimens. The MAL-ED study is an ongoing multisite birth cohort study with approximately 250 infants enrolled in each of eight countries. All children passed their first year of life in February 2013. According to study protocols, stool samples were collected monthly as well as for any episodes of diarrhea captured during biweekly surveillance during the first year of life. Diarrheal samples were collected during or up to 48 h after cessation of diarrhea. All samples were placed in Cary-Blair transport medium by field-workers at the time of collection.

A total of 216 diarrheal cases and 216 matched control samples (Iquitos, Peru, $n = 150$; Haydom, Tanzania, $n = 138$; Dhaka, Bangladesh, $n = 144$) were subjected to PCR testing. For Peru and Bangladesh, a random sample of 75 diarrheal episodes was selected from all diarrheal episodes for which a matched control was available. A matched control was defined as a prior monthly surveillance sample from the same subject within the prior 8 weeks for which the subject was diarrhea free for 1 week before and after collection. For Tanzania, all diarrheal episodes meeting these criteria were tested. In Bangladesh, three of the diarrheal samples did not have sufficient stool available for DNA extraction, and thus, those stools as well as the matched controls were excluded. The study was approved by the Institutional Review Board of the University of Virginia, the National Institute for Medical Research of Tanzania, the Institutional Review Board of the Johns Hopkins Bloomberg School of Public Health, the Ethics Committee of the Asociación Benéfica PRISMA, the Regional Health Department of Loreto, Peru, and the Ethical Review Committee of the International Centre for Diarrhoeal Disease Research, Bangladesh.

Testing of clinical specimens. *Campylobacter* culture was previously performed on all samples in Peru and Bangladesh by transferring stool from Cary-Blair transport medium onto solid medium and incubating at 42°C under microaerophilic conditions. In Bangladesh, a blood agar plate (Campy-BAP) was used; in Peru, *Campylobacter* blood-free selective agar base was used. Antigen-based testing for *Campylobacter* was previously performed on all samples using the ProSpecT *Campylobacter* enzyme immunoassay (EIA; Remel, Lenexa, KS). DNA extraction was performed using a QIAmp DNA stool minikit (Qiagen, Valencia, CA) following a modified protocol, including bead beating to lyse organisms (13, 14). DNA was stored at −20°C until use. A duplex PCR assay was developed using a previously described assay for *C. jejuni/C. coli* as well as a modified 16S rRNA-based assay for genus-level detection of *Campylobacter* species (Table 1) (15). Each well included a 25-μl reaction mixture with 1 μl of sample, 12.5 μl of TaqMan environmental master mix, 6.5 μl of nuclease-free water, and 5 μl of a primer-probe mix at final concentrations of 0.2 μM for *cadF* primers, 0.1 μM for the *cadF* probe and 16S rRNA primers, and 0.05 μM for the 16S rRNA probe. The cycling conditions were as follows: 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 55°C for 1 min. For assay validation, 78 Campylobacter culture-positive samples (Bangladesh, $n = 35$; Peru, $n = 43$) were selected at random from all *Campylobacter* culture-positive samples from children 0 to 12 months of age at these MAL-ED sites. All of these samples had both *cadF* and 16S rRNA gene quantification cycle (C_q) values of less than 45. On the basis of a linear regression between the quantification cycles (16s rRNA $C_q = -3.471 + 0.880 \times$ *cadF* C_q; $R^2 = 0.90$, $P < 0.001$), a sample positive for *cadF* at the limit of detection ($C_q = 45$) would be expected to have a 16S rRNA gene C_q value of 36.1. On this basis, a C_q cutoff of 36 was used for the 16S rRNA gene assay. Samples were thereby considered positive by PCR for *Campylobacter* species if tests for the 16S rRNA gene were positive, for *C. jejuni/C. coli* if tests for *cadF* were additionally positive, and for non-*jejuni/coli Campylobacter* species if tests for the 16S rRNA gene were positive and those for *cadF* were negative.

Sequencing. Selected samples were amplified using previously described 816-bp 16S rRNA *Campylobacter* genus-level PCR primers (Table 1) (16). Each well included a reaction mixture with 1 μl of sample, 12.5 μl of OneTaq Hot Start 2× master mix, 10.5 μl of water, and 1 μl of primer mix at a final concentration of 0.2 μM. The cycling conditions were as follows: 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 55°C for 1 min. Amplified DNA was then purified, sequenced (Genewiz Inc., South Plainfield, NJ), and queried, using the BLASTn program, for maximum identity in the NCBI nucleotide database.

EIA cross-reactivity testing. Reference *Campylobacter* and *Helicobacter* strains (see Table 4) were cultured on 5% sheep blood agar (Remel) for 48 to 72 h at 37°C under microaerophilic conditions. Colonies of *C. jejuni* were harvested from blood agar plates, washed with phosphate-buffered saline, and adjusted to an optical density (OD) at a wavelength of 660 nm of 1.0. Limiting dilution plating established that an OD of 1.0 was equivalent to 3×10^9 CFU/ml. Human donor feces were negative by genus-level PCR for *Helicobacter* spp. and *Campylobacter* spp. (17). Aliquots of donor fecal slurry (300 μl) were added to 600 μl of the kit bacterial specimen diluent. Samples were then spiked with 1×10^8 CFU of each *Campylobacter* species, and the sample total volume was adjusted to 1 ml using fecal slurry. Samples were then serially diluted 10-fold in fecal slurry/bacterial specimen diluents to achieve additional samples containing 10^7, 10^6, 10^5, 10^4, 10^3, and 10^2 CFU/ml for each species. Samples were assayed in duplicate using the ProSpecT *Campylobacter* microplate assay as described above.

Statistical analysis. The nonparametric Mann-Whitney test and the Kruskal-Wallis one-way analysis of variance (ANOVA) were used to compare continuous variables between sites. The Pearson chi-square test was used to compare the distribution of *Campylobacter* species between sites. To analyze the association between *Campylobacter* detection and diarrhea, generalized estimating equations were used to fit a logistic regression model for each site and diagnostic test to adjust for the potential noninde-

pendence of tests results for each subject. An independent working correlation matrix was assumed. Age and sex were considered for inclusion in each model and retained on the basis of model fit using the corrected quasilikelihood under independence model criterion. A significance level of 0.05 was used for all analyses. All statistical analysis was performed using SPSS software (version 20; IBM Corp., Armonk, NY).

RESULTS

The distribution of ages at the time of diarrheal sample collection was statistically significantly different between the sites (for Bangladesh, median age = 204 days and interquartile range [IQR] age = 141 to 291 days; for Tanzania, median age = 128 days and IQR = 92 to 216 days; for Peru, median age = 177 days and IQR = 123 to 247 days; $P < 0.001$, Kruskal-Wallis one-way ANOVA). The duration between controls and subsequent cases was statistically significantly longer for Peru (median = 27.0 days, IQR = 17.0 to 32.0 days) than for Bangladesh (median = 20.5 days, IQR = 13.5 to 29.5 days; $P = 0.02$, Mann-Whitney test) and Tanzania (median = 20.0 days, IQR = 12.0 to 30.0 days; $P = 0.04$). The time from stool production to placement in transport medium was statistically significantly shorter in Peru (median = 0.25 h, IQR = 0 to 0.75 h) than in Bangladesh (median = 0.77 h, IQR = 0.50 to 1.17 h; $P < 0.001$, Mann-Whitney test).

Comparison of culture, EIA, and PCR results. Test results by diagnostic modality and site are presented in Table 2, as are the test characteristics of culture and EIA compared to those of a PCR "gold standard." In Bangladesh and Peru, of the 12 samples (4.1%) positive by culture, 9 were classified as *C. jejuni/C. coli* by PCR, 2 were classified as non-*jejuni/coli Campylobacter*, and 1 was negative for *Campylobacter* species. Across all sites, 26.9% of samples were positive by EIA, while 31.7% of samples were positive by PCR for *C. jejuni/C. coli* and 36.3% were positive for other *Campylobacter* species. Among EIA-positive samples, 71.6% were PCR positive for *C. jejuni/C. coli* and 99.1% were PCR positive for *Campylobacter* species. The percentage of all *Campylobacter* PCR-positive samples that were *Campylobacter jejuni/C. coli* was similar across sites (Bangladesh, 47.5%; Peru, 51.1%; Tanzania, 42.1%; Pearson chi-square test, $P = 0.44$).

***Campylobacter* quantity by PCR and EIA positivity.** To determine whether the low sensitivity of EIA in comparison to that of PCR was due to detection of low-burden infection and PCR, we analyzed the association between *Campylobacter* quantity and PCR and EIA positivity. Figure 1 shows the association between the *cadF* and 16S rRNA C_q values for all samples tested, stratified by EIA result. A receiver operating characteristic (ROC) curve of the *cadF* C_q and EIA positivity had an area under the curve (AUC) of 0.80, and a 62.1% sensitivity and 90.5% specificity were obtained with a *cadF* C_q of 40. An ROC curve of the 16S rRNA C_q and EIA positivity had an AUC of 0.89, and a 80.2% sensitivity and 81.3% specificity were obtained with a 16S rRNA gene C_q of 30.

Sequencing results. To confirm that our duplex PCR assay appropriately discriminated between *C. jejuni/C. coli* and non-*jejuni/coli Campylobacter* infections, we selected 71 samples that were positive by PCR for non-*jejuni/coli Campylobacter* species, of which 53 were successfully sequenced. All but one of the samples for which no sequence data were received had a 16S rRNA C_q greater than 30. The sequencing results are shown stratified by country in Table 3. We additionally selected 20 samples that were positive by PCR for *C. jejuni/C. coli*, and 19 of these were successfully sequenced. These most closely matched *C. jejuni/C. coli*

TABLE 2 Comparison of *Campylobacter* detection by culture, EIA, and PCR

Assay	Bangladesh (n = 144)			Peru (n = 150)			Tanzania (n = 138)			Overall (n = 432)		
	No. (%) of samples positive	Sensitivity (%)	Specificity (%)	No. (%) of samples positive	Sensitivity (%)	Specificity (%)	No. (%) of samples positive	Sensitivity (%)	Specificity (%)	No. (%) of samples positive	Sensitivity (%)	Specificity (%)
Culture (vs EIA)	6 (4.2)	6.1	96.8	6 (4.0)	12.1	98.3	NA[a]	NA	NA	12 (4.1)[b]	8.5	97.6
Culture (vs PCR for *C. jejuni/C. coli*)		6.4	96.9		11.1	99.0		NA	NA		8.7	98.0
EIA (vs PCR for *C. jejuni/C. coli*)	49 (34.0)	70.2	83.5	33 (22.0)	53.3	91.4	34 (24.6)	57.8	91.4	116 (26.9)	60.6	88.8
EIA (vs PCR for *Campylobacter* spp.)		49.5	100		31.8	100		36.4	98.4		39.1	99.3
PCR for *C. jejuni/C. coli*	47 (32.6)			45 (32.8)			45 (32.8)			137 (31.7)		
PCR for non-*jejuni/coli Campylobacter*	52 (36.1)			43 (28.7)			62 (44.9)			157 (36.3)		

[a] NA, not applicable.
[b] Samples from Bangladesh and Peru only (n = 294).

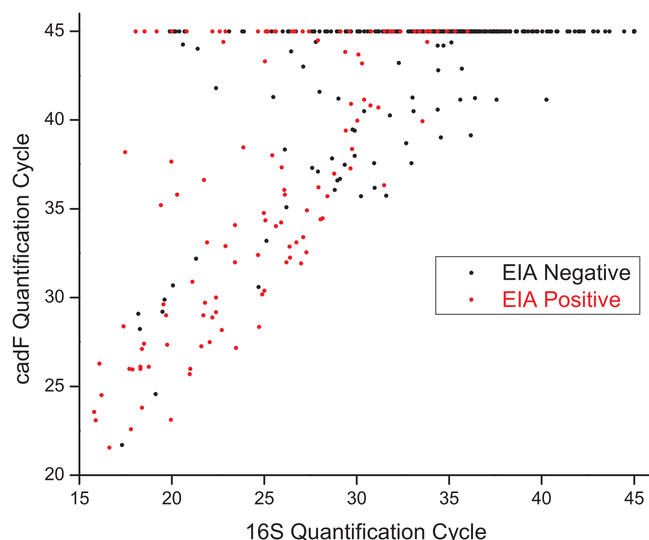

FIG 1 Quantity of *C. jejuni/C. coli* (*cadF*) and *Campylobacter* species (16S rRNA) by real-time PCR for all samples categorized by EIA result (n = 432 samples). Samples with *Campylobacter jejuni/C. coli* present would be expected to fall along the diagonal band running from the bottom left to top right, suggesting a similar level of detection of the two *Campylobacter* PCR targets.

(73.7%), *C. troglodytis* (15.8%), and *C. hyointestinalis* subsp. *lawsonii* (10.5%). The samples that sequenced as *C. jejuni/C. coli* had a lower *cadF* C_q than those that revealed other sequences, though this did not reach statistical significance (median = 29.4 and IQR = 22.9 to 34.6 versus median = 38.4 and IQR = 30.5 to 40.9; Mann-Whitney test, P = 0.06).

Detection of *Campylobacter* strains by EIA. To directly evaluate EIA reactivity to diverse *Campylobacter* species, samples were spiked with reference *Campylobacter* and *Helicobacter* strains. Table 4 shows the EIA result and the lowest spiked concentration detected and reveals broad cross-reactivity with non-*jejuni/coli* *Campylobacter* species, including all of those detected by sequencing in this study, but not with *Helicobacter* strains.

Association of *Campylobacter* infection with diarrhea. As this was a case-control study, we examined the association between *Campylobacter* infection and diarrhea stratified by diagnostic modality and site. In Peru, Campylobacter and *C. jejuni/C. coli* detection by PCR was strongly associated with diarrhea (Table 5). The result for no other modality or site yielded statistical significance. Next, we analyzed the association between total *Campylobacter* quantity and diarrhea in Peru. An ROC curve of the 16S rRNA C_q and diarrhea had an AUC of 0.71. At a C_q cutoff of 34, 16S rRNA positivity was 54.7% sensitive and 78.7% specific for

TABLE 3 16S rRNA sequencing of EIA-positive, *C. jejuni/C. coli* PCR-negative samples[a]

Site (total no. of samples)	Species of maximum identity	No. of isolates (% of total)
Bangladesh (19)	*C. troglodytis*	10 (46.7)
	C. hyointestinalis subsp. *lawsonii*	6 (26.7)
	C. concisus	2 (10.5)
	C. upsaliensis	1 (6.7)
Peru (8)	*C. hyointestinalis* subsp. *lawsonii*	5 (55.6)
	C. troglodytis	2 (22.2)
	C. upsaliensis	1 (11.1)
Tanzania (26)	*C. hyointestinalis* subsp. *lawsonii*	17 (65.4)
	C. troglodytis	6 (23.1)
	C. upsaliensis	2 (7.7)
	C. jejuni/coli	1 (3.8)

[a] Data are for 53 samples.

TABLE 4 EIA results for selected *Campylobacter* and *Helicobacter* strains

Species	Strain	EIA result	Limit of detection (CFU/ml)
Campylobacter jejuni	ATCC 33560	Positive	10^6 and 10^7[a]
Campylobacter coli	ATCC 33559	Positive	10^7
Campylobacter helveticus	ATCC 51209	Positive	10^7
Campylobacter upsaliensis	MIT 85-519	Positive	10^7
Campylobacter concisus	UNSWCD	Positive	10^8
Campylobacter troglodytis type II	MIT 05-9150	Positive	10^8
Campylobacter hyointestinalis subsp. *hyointestinalis*	MIT 10-5757	Positive	10^8
Campylobacter lanienae	MIT 11-231	Positive	10^8
Campylobacter troglodytis type I	MIT 05-9159	Negative	NA[b]
Campylobacter novel sp.	MIT 12-8780	Negative	NA
Helicobacter canadensis	ATCC 700968	Negative	NA
Helicobacter fennilliae	ATCC 35684	Negative	NA
Helicobacter canis	ATCC 51401	Negative	NA
Helicobacter cinaedi	CCUG 18818	Negative	NA
Helicobacter pullorum	MIT 98-5489	Negative	NA

[a] The sample was run in duplicate.
[b] NA, not applicable.

TABLE 5 Association between *Campylobacter* detection and diarrhea by site and diagnostic method

Site	Diagnostic method	No. (%) of subjects		OR[a]	P value
		Cases	Controls		
Bangladesh	EIA	25 (34.7)	24 (33.3)	0.89	0.71
	Culture	4 (5.6)	2 (2.8)	1.82	0.53
	PCR for *C. jejuni/ C. coli*	25 (34.7)	22 (30.6)	1.09	0.81
	PCR for non-*jejuni/coli* *Campylobacter*	27 (37.5)	25 (34.7)	1.01	0.98
	PCR for any *Campylobacter* spp.	52 (70.8)	47 (63.9)	1.13	0.75
Peru	EIA	21 (28.0)	12 (16.0)	2.04	0.12
	Culture	4 (5.3)	2 (2.7)	2.27	0.40
	PCR for *C. jejuni/ C. coli*	31 (41.3)	14 (18.7)	2.80	0.007
	PCR for non-*jejuni/coli* *Campylobacter*	25 (33.3)	18 (24.0)	1.55	0.20
	PCR for any *Campylobacter* spp.	56 (74.7)	32 (42.7)	3.66	<0.001
Tanzania	EIA	17 (24.6)	17 (24.6)	0.79	0.59
	PCR for *C. jejuni/ C. coli*	24 (34.8)	21 (30.4)	1.12	0.74
	PCR for non-*jejuni/coli* *Campylobacter*	33 (47.8)	29 (42.0)	1.19	0.62
	PCR for any *Campylobacter* spp.	57 (82.6)	50 (72.5)	1.56	0.24

[a] OR, odds ratio.

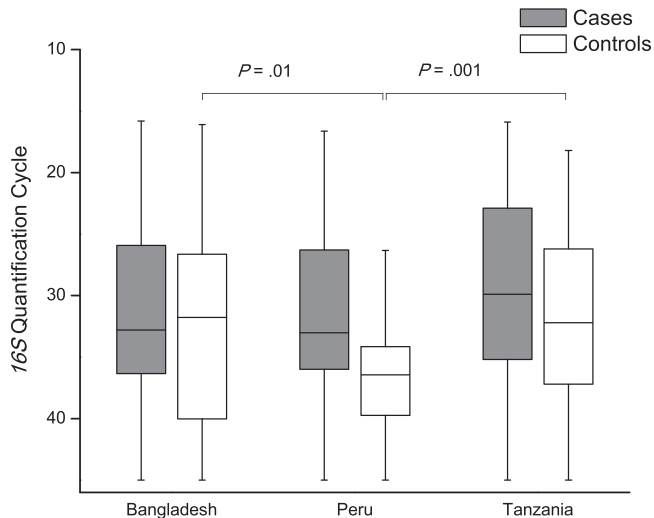

FIG 2 Quantity of *Campylobacter* species (16S rRNA) by real-time PCR for case and control samples for each site. Among *Campylobacter*-positive samples, real-time PCR C_qs are shown for each site as the median, interquartile range, and range. The burden of asymptomatic *Campylobacter* infection was statistically significantly lower in Peru than in the other two sites by the Mann-Whitney U test. No other two-way comparisons between sites were statistically significant. The difference between all sites was statistically significant (P = 0.002, Kruskal-Wallis one-way ANOVA).

diarrhea. There was a statistically significantly lower burden (C_q) of asymptomatic carriage of *Campylobacter* in samples from Peru than samples from Bangladesh or Tanzania (Fig. 2).

DISCUSSION

In this work, we compared the results of three diagnostic modalities to detect *Campylobacter* and documented substantial underdetection by selective culture in comparison to the levels of detection by both EIA and PCR. We speculate that this is due to the inherently poor sensitivity of this culture technique, the frequent exposure of these children to antibiotics, and detection by nonculture-based diagnostics of low-level infection of unclear clinical significance and viability. The high level of detection by the EIA or PCR methods is not surprising. Prior studies using less selective culture conditions have revealed a high burden of *Campylobacter* in infants in the developing world (18, 19). Similarly, PCR-based detection of *C. jejuni/C. coli* in Malawi revealed a substantially higher burden of disease than had been previously reported (20), and we recently found a 20 to 40% detection rate in a different cohort of Bangladeshi children using a PCR-based approach (21). This contrasts with the previously described comparable performance of culture in comparison to that of immunoassay and PCR in children with diarrhea in the setting of more sporadic exposure (22), as well as in military personnel in settings where *Campylobacter* is endemic (23). One possible explanation for the poor performance of culture here might be the relative difficulty of performing timely culture when capturing stools in field studies in comparison to the level of difficulty of culture in studies performed in patients presenting for care.

To our knowledge, the MAL-ED study represents the first use of an EIA for *Campylobacter* detection in an epidemiologic study outside the United States and Europe. We chose it because it was straightforward to deploy to 8 diverse laboratories, and we undertook this study to understand the high rates of positivity identified during interim analyses. We demonstrate both indirectly (in clinical specimens by sequencing) and directly (by EIA testing of strains) that the ProSpecT EIA can broadly detect *Campylobacter* species. Previous knowledge was limited to observations that a different EIA (ImmunoCard STAT CAMPY; Meridian Bioscience) could detect *C. upsaliensis* (7).

In three diverse settings where *Campylobacter* is endemic, it appears that approximately two-thirds of EIA-positive samples represent *C. jejuni/C. coli* infection, with the additional one-third likely due to non-*jejuni/coli Campylobacter* species. Our results might suggest that EIA sensitivity in comparison to that of PCR is poor; however, we show that this is primarily due to additional detection of low-burden *Campylobacter* infection by PCR. The diversity of *Campylobacter* species described by PCR alone is broader still. Indeed, our aggregated PCR results suggest that *C. jejuni/C. coli* infections make up less than half of all *Campylobacter* infections in these infants. Little is known about the additional *Campylobacter* species of highest prevalence in these sites. *C. hyointestinalis* subsp. *lawsonii* is of porcine origin, and pigs are commonly raised in homes in Haydom, where this was the most frequently detected non-*jejuni/coli Campylobacter* species. It has been associated with gastroenteritis after transmission from a pig to a human (24). *C. troglodytis* is a recently described species isolated from chimpanzees in Tanzania (25). A similar uncultured species has been described by 16S rRNA-based sequencing of stool from infants in Bangladesh (26). Neither the source of human infection nor the clinical significance of infections with this species is known.

We observed an association of *Campylobacter* PCR positivity with diarrhea in infants in Peru but not in Bangladesh and Tanzania but not with other assay methods. It is important to underscore that this is a preliminary finding in the context of the ongoing MAL-ED study. However, the results in Fig. 2 suggest that, for this set of samples, the overall burden of asymptomatic *Campylobacter* infection is lower in Peru than at the other sites. This might suggest that the force of infection is lower at that site. Also, it has long been known that the attack rate of *Campylobacter* infection declines over time, likely a marker of recurrent infection and the development of natural immunity (11, 12, 27). Thus, the older median age of children at the time of diarrheal sample collection in Bangladesh could explain the attenuated association seen in that setting. Differences in *C. jejuni/C. coli* serotypes as well as differences in the distribution of other *Campylobacter* species may also be responsible. In the Global Enteric Multicenter Study (GEMS) multicenter case-control study of moderate to severe diarrhea, *Campylobacter* infection was not significantly associated with diarrhea for any age group in the four African sites, while in Bangladesh, it was associated with diarrhea only in the first year of life (28), but as culture was the diagnostic of choice, this finding is of limited comparability with the findings of this study.

The choice of assay for *Campylobacter* detection of course depends on one's goal, for instance, whether the assay is for the etiologic diagnosis of diarrhea or other clinical syndromes or for surveillance. The strongest implication of this study is that culture is insufficiently sensitive for use in epidemiologic studies of *Campylobacter* infection in these settings. EIA appears to be a reasonable alternative to PCR; however, the specificity for detection of *Campylobacter jejuni/C. coli* infection is poor, in part due to detection of non-*jejuni/coli Campylobacter* species. The clinical rel-

evance of infections with these species in these settings remains unclear and warrants further study. Nucleic acid-based diagnostics offer increased sensitivity, can determine both the presence and burden of infection, and can distinguish between *Campylobacter* infections at the species level. We therefore promote PCR, if feasible, as the preferred diagnostic modality for detection of *Campylobacter* infection for epidemiologic studies in the developing world. This will allow the fullest ascertainment of the relevance of *Campylobacter* infections in these settings.

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Drake H. Tilley is an employee of the U.S. government. Title 17 U.S.C. §101 defines U.S. government work as work prepared by a military service member or employee of the U.S. government as part of that person's official duties. This work was prepared as part of his official duties.

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