Epidemiology of *Campylobacter* Infections among Children in Egypt

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Abstract. *Campylobacter* is a frequently isolated bacterial pathogen among children with diarrhea. Data are lacking on the distribution and spectrum of disease associated with *Campylobacter* species and *Campylobacter jejuni* capsular polysaccharide (CPS) types. This information is essential because current vaccine research seeks to target specific CPS types. An effective CPS-conjugate vaccine will need to cover CPS types that are both common and associated with severe disease. The US Naval Medical Research Unit-3 conducted several prospective cohort studies researching diarrheal disease in Egypt from 1995 to 2003. In total, 1,057 children were enrolled and followed to a maximum age of 36 months. We analyzed *Campylobacter*-positive stool samples that were collected while subjects were symptomatic, along with corresponding clinical data. Of 441 *Campylobacter* isolates, 322 represented primary infections (189 *C. jejuni*, 127 *Campylobacter coli*, six unspciated). There were 19 *C. jejuni* CPS types identified; eight accounted for 63.5% of primary *C. jejuni* infections. We also screened for the presence of the type-6 secretion system (T6SS), a putative virulence determinant. The T6SS was found in 18.0% of *C. coli* isolates and 57.6% of *C. jejuni* isolates ($P < 0.001$), and was not uniformly distributed among CPS types ($P < 0.001$). Strains with the T6SS were not associated with more severe disease. Clinical presentations across species and CPS types appeared similar. This study adds to the growing epidemiological data and also provides some analysis of the clinical spectrum associated with infection by specific *Campylobacter* species, *C. jejuni* capsule types, and possible virulence determinants.

INTRODUCTION

Campylobacter is a zoonotic disease and a frequent bacterial pathogen seen in children with diarrhea.^{1,2} Little is known regarding the mechanism(s) of *Campylobacter* virulence.^{3,4} *Campylobacter jejuni* expresses a capsular polysaccharide (CPS),⁵ but the role of CPS in *C. jejuni*-associated disease is undefined.⁴ The classification of *C. jejuni* historically has relied on a passive slide hemagglutination assay for Penner serotyping, for which CPS is the major serodeterminant. More recently, polymerase chain reaction (PCR) has been used to identify CPS type. Currently there are 47 recognized *C. jejuni* Penner serotypes, represented by 35 CPS complexes.⁶ The capsules are characterized by the presence of heptoses in various configurations (e.g., *altro*, *gulo*) and by nonstoichiometric modification of the sugars with O-methyl-phosphoramidate (MeOPN).⁷ *Campylobacter jejuni* is also one of several gram-negative bacteria known to harbor the type-6 secretion system (T6SS).^{8,9} The T6SS has been associated with both pathogen–pathogen and pathogen–host interactions among certain diarrheal agents, including *Vibrio cholerae*.⁹ Its role in *C. jejuni* is unclear, although it may be associated with invasive disease.⁸ The distribution of the T6SS among *C. jejuni* CPS types and among other *Campylobacter* species is unknown.

Although symptomatic infections impact children and adults in industrialized nations, in the developing world *Campylobacter* enteritis is almost exclusively a pediatric disease.² Seroprevalence of *C. jejuni*-specific IgG antibodies increases with age and older patients are much more likely to develop infections caused by uncommon CPS types compared with their younger counterparts.¹⁰ These findings suggest that prior infection may confer some degree of CPS-specific immunity. CPS has been an effective target for other vaccines against mucosal pathogens including

Streptococcus pneumoniae.¹¹ In addition, preliminary studies demonstrate that *C. jejuni* CPS-conjugate vaccines protect against diarrheal disease in nonhuman primates.⁷ With 35 known *C. jejuni* CPS types, an efficacious vaccine will need to target prevalent and disease-associated strains.

The distribution of *C. jejuni* CPS types along with their contribution to morbidity has not been defined worldwide. A recent meta-analysis evaluated 21,000 sporadic cases of *C. jejuni* infection, of which nearly 85% came from Europe; there were only 566 samples from Africa. HS4 was the most common *C. jejuni* CPS type overall (15%) and within Africa (7%). Three *C. jejuni* CPS types, (HS1/44, HS2, and HS4), accounted for 37% of the total isolates; however, there appeared to be a greater distribution of CPS types in Africa, where HS1/44, HS2, and HS4 combined represented just 20% of the isolates.¹²

In the current study, we sought to expand the epidemiological data regarding *Campylobacter*, while evaluating for associations between clinical presentation, *Campylobacter* species, *C. jejuni* CPS types, and the T6SS.

MATERIALS AND METHODS

In collaboration with the Egyptian Ministry of Health,¹³ the Naval Medical Research Unit Number 3 (NAMRU-3) and the National Institutes of Health (NIH) began active surveillance for pediatric diarrhea in Abu Homos, Egypt, in February 1995, as part of an observational pediatric diarrhea cohort study.¹⁴ Abu Homos is an agricultural community of approximately 350,000 persons in the Nile Delta in northern Egypt, located 35 kilometers (22 miles) southeast of Alexandria. Before the start of the initial cohort in February 1995, a team of investigators, field workers, and data management personnel mapped and numbered every house in the 16-village complex. A detailed house-by-house census was conducted, with periodic updates to ensure accurate data. With guardian consent, children younger than 24 months of age were enrolled in a prospective study to

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evaluate pediatric diarrhea in this region. Subsequently, newborns were eligible for enrollment.

From February 1995 through December 2006, four prospective studies of at least 3-year duration were undertaken. Each study was approved by the Institutional Review Board at NAMRU-3 and was overseen by infectious disease physicians and epidemiologists located at NAMRU-3 in collaboration with the Egyptian Ministry of Health. Surveillance methodology was consistent across all studies. Specifically, from enrollment to 36 months of age, each participant was visited twice weekly in the home. Trained field workers administered structured questionnaires soliciting data on the child's health. Diarrhea was defined as at least three loose or liquid stools or one bloody stool in a 24-hour period. For breast-fed infants, the mother also must have indicated an increase in the frequency or decrease in consistency of stools, in relation to normal stool patterns. A diarrheal episode began on the first diarrheal day following at least three nondiarrheal days, and ended on the last diarrheal day followed by at least three consecutive nondiarrheal days. For all but the last study, in which surveillance was halted after children were 24 months of age, surveillance was continued until the third birthday or until study completion.

Stool samples were inoculated into Cary-Blair transport medium and stored at the project field laboratory for up to 4 days at 4°C before being transported to the laboratory at NAMRU-3. At NAMRU-3, all samples were inoculated on a modified Skirrow's medium and grown for 48 hours in a microaerophilic environment at 42°C. Colonies that appeared grossly consistent with *Campylobacter* were confirmed by Gram stain, oxidase and catalase activity, and sensitivity to nalidixic acid. All laboratory-confirmed *Campylobacter* samples have remained frozen at -80°C.

Isolates were sent to the Naval Medical Research Center in Silver Spring, MD, where they were regrown on Mueller-Hinton agar. DNA was extracted from each sample for PCR analysis. The PCR consists of a *C. jejuni* CPS multiplex with 35 capsule primer sets spread over four mixes: alpha, beta, gamma, and delta.⁶ In addition, all strains were tested for a *C. jejuni* amplicon.¹⁵ When negative for *C. jejuni*, they were additionally tested for a *C. coli* amplicon.¹⁵ Because the *hcp* gene is a consistent marker of the complete T6SS cluster,^{8,9} samples were screened for the presence of the *hcp* gene using a multiplex PCR.¹⁶

Infections were categorized as primary (no prior *Campylobacter* infections isolated) and nonprimary (isolation of any heterologous capsule type following the primary *Campylobacter* infection or isolation of a homologous capsule type $\geq$ 90 days from the primary *Campylobacter* infection). Isolates that were found to be homologous, and obtained from the same subject within 90 days, were excluded from the analysis.

The percentage of infections caused by each *Campylobacter* species and CPS type was determined along with the corresponding proportions that were positive for the T6SS. The prevalence of the T6SS was estimated by species for all symptomatic infections and limited to primary symptomatic infections. The distribution of *Campylobacter* species and CPS type, along with the prevalence of each clinical characteristic, was analyzed by age category. The prevalence of clinical disease signs and symptoms was compared by species, CPS type, presence of T6SS, and presence of heptose, deoxyheptose, and MeOPN.

This study protocol was approved by the Naval Medical Research Center Institutional Review Board in compliance with all applicable regulations governing the protection of human subjects. A Mantel-Haenszel χ^2 test of homogeneity was used to compare proportions across various categories, whereas Student's *t* test was used for comparisons of continuous variables. Confidence intervals (CI) for point estimates of prevalence were calculated using Exact Binomial proportions at the 95% level. All analyses were conducted using Stata V13 (SataCorp, College Station, TX) and statistical significance assessed using a *P* value = 0.05.

RESULTS

A total of 1,057 children were enrolled and 6,562 stools collected. *Campylobacter* was isolated in 615 samples from symptomatic subjects; 54 samples were excluded from the analysis based on our criteria, and another 120 samples were no longer viable and so were not analyzed. As shown in Table 1, of the 441 *Campylobacter* isolates tested, 322 were from primary symptomatic *Campylobacter* infections, whereas 119 were from nonprimary symptomatic *Campylobacter* infections. Most isolates (*N* = 272, 61.7%; 95% CI: 57.0–66.2%) were speciated as *C. jejuni*, whereas 37.0% (*N* = 163; 95% CI: 32.4–41.7%) were *C. coli* and 1.4% (*N* = 6; 95% CI: 0.5–2.9%) were unable to be speciated. The majority of all *Campylobacter* isolates came from the primary symptomatic infection (*C. jejuni*: 69.4%, *C. coli*: 77.9%, *C. non-jejuni/non-coli*: 100%).

Among the primary *Campylobacter* infections, 103 (31.9%) were co-infected with *Shigella* (1.6%), rotavirus (13.0%), and/or enterotoxigenic *Escherichia coli* (19.8%). Of these 103, 57.3% (*N* = 59) were *C. jejuni* and 40.8% (*N* = 42) were *C. coli* infections. Subjects who were co-infected were 1.5 times more likely to have decreased activity (CI: 1.22–1.93) and were 1.5 times more likely to experience vomiting (CI: 1.02–2.29) than those who were not co-infected. There were no other significant differences in the clinical presentation between those with and without a co-infection.

A CPS type was identified in 88.2% (95% CI: 83.8–91.8%) of all *C. jejuni* isolates with the most prevalent types being HS2 (11.4%), HS3 (11.4%), HS15 (9.2%), HS4 (8.1%), HS5/31 (7.0%), HS10 (6.6%), HS6/7 (6.3%), and HS1/44 (5.5%). Although those eight capsule types accounted for 65.4% (95% CI: 59.5–71.1%) of the *C. jejuni* isolates, no other capsule type accounted for more than 5%. In addition, no capsule type was identified in 11.8% (*N* = 32; 95% CI: 8.2–16.2%) of the *C. jejuni* isolates. HS5/31 was identified more commonly among nonprimary symptomatic *C. jejuni* infections (12.0%) than in primary infections (4.8%) (Fisher's Exact *P* value: 0.04). There were no other differences in the proportion of *C. jejuni* capsule types in primary and nonprimary symptomatic *C. jejuni* infections.

Among the 424 isolates available for *hcp* analysis, the *hcp* gene was found in 29 (18.0%; 95% CI: 12.4–24.8%) *C. coli* isolates compared with 148 (57.6%; 95% CI: 51.3–63.7%) *C. jejuni* isolates and five (83.3%; 95% CI: 35.9–99.6%) unspciated isolates (*P* < 0.001). Among the identified *C. jejuni* capsules, *hcp* prevalence varied significantly from 0% to 100%. The most prevalent *hcp* positive capsule type was HS3 in which 26 of the 29 (89.7%; 95% CI: 72.7–97.8%) tested isolates had the *hcp* gene.

TABLE 1

Distribution of *Campylobacter* capsular polysaccharide (CPS) types causing diarrheal illness in a pediatric population living in Abu Homos, Egypt, from 1995 to 2006

<i>Campylobacter</i> species	<i>Campylobacter jejuni</i> capsule type	Primary infection	Nonprimary infection	All infections	<i>hcp</i> ⁺
All	N/A†	322	119	441	182/424 (42.9%)
Untypable	N/A†	6	0	6	5/6 (83.3%)
<i>Campylobacter coli</i>	N/A†	127	36	163	29/161 (18.0%)
<i>C. jejuni</i>	All	189	83	272	148/257 (57.6%)
<i>C. jejuni</i> capsule type	NT‡	21 (11.1%)	11 (13.3%)	32 (11.8%)	24/31 (77.4%)
	HS1/44	10 (5.3%)	5 (6.0%)	15 (5.5%)	8/13 (61.5%)
	HS2	19 (10.1%)	12 (14.5%)	31 (11.4%)	9/27 (33.3%)
	HS3	22 (11.6%)	9 (10.8%)	31 (11.4%)	26/29 (89.7%)
	HS4	18 (9.5%)	4 (4.8%)	22 (8.1%)	11/21 (52.3%)
	HS5/31	9 (4.8%)	10 (12.0%)	19 (7.0%)	7/17 (41.2%)
	HS6/7	12 (6.3%)	5 (6.0%)	17 (6.3%)	5/17 (29.4%)
	HS8/17	1 (0.5%)	0 (0.0%)	1 (0.4%)	1/1 (100.0%)
	HS9	3 (1.6%)	1 (1.2%)	4 (1.5%)	2/2 (100.0%)
	HS10	12 (6.3%)	6 (7.2%)	18 (6.6%)	12/18 (66.7%)
	HS12	1 (0.5%)	0 (0.0%)	1 (0.4%)	1/1 (100.0%)
	HS15	18 (9.5%)	7 (8.4%)	25 (9.2%)	11/25 (44.0%)
	HS18	1 (0.5%)	0 (0.0%)	1 (0.4%)	1/1 (100.0%)
	HS19	4 (2.1%)	0 (0.0%)	4 (1.5%)	1/4 (25.0%)
	HS22	8 (4.2%)	1 (1.2%)	9 (3.3%)	9/9 (100.0%)
	HS23/36	9 (4.8%)	2 (2.4%)	11 (4.0%)	9/11 (81.8%)
	HS41	5 (2.6%)	1 (1.2%)	6 (2.2%)	1/6 (16.7%)
	HS42	8 (4.2%)	4 (4.8%)	12 (4.4%)	2/11 (18.2%)
	HS52	0 (0.0%)	1 (1.2%)	1 (0.4%)	0/1 (0.0%)
	HS53	8 (4.2%)	4 (4.8%)	12 (4.4%)	8/12 (66.7%)

Bold values indicate the two-tailed *P* value for Chi-square test of homogeneity, comparing *C. coli* to *C. jejuni* and untypable to *C. jejuni*, are both <0.001.

* *hcp*, a genetic marker for the type 6 secretion system (denominator is different because not all isolates were available for *hcp* testing).

† N/A = not applicable (i.e., not *C. jejuni*).

‡ NT = nontypeable *C. jejuni* capsule.

The mean number of loose stools for each primary *C. jejuni*-attributed illness was 6.7. In addition, 23.9% experienced vomiting, 22.2% experienced dehydration, 35.2% had a documented fever, 4.5% experienced hematochezia, and just under 50% had parental report of lower than normal activity levels. There were no significant differences in the clinical profile of cases with *hcp*⁺ *C. jejuni* and those with *hcp*[−] *C. jejuni* infections. Primary *C. coli*-attributed illnesses had a similar clinical presentation to primary *C. jejuni*-attributed illnesses with no significant differences in the prevalence of signs and symptoms or in the frequency of stool output per day. *Campylobacter coli* isolates with the *hcp* gene were associated with a higher number of loose stools than *C. coli* isolates without the *hcp* gene (*P* = 0.001); however, no other significant differences were observed (Table 2).

Among primary *C. jejuni* symptomatic infections for which we identified a capsule type, low activity was reported more commonly from cases with MeOPN negative *C. jejuni* capsules (63.2%) compared with MeOPN positive (42.3%) (*P* = 0.02). A similar trend was seen with heptose, although the

difference was not statistically significant (*P* = 0.06). There appeared to be no difference in the clinical profile of primary *C. jejuni* infections by strains predicted of expressing CPS containing deoxyheptose (Table 3).

DISCUSSION

This study describes the distribution of *Campylobacter* species and *C. jejuni* CPS types within a birth cohort in rural Egypt. Of the isolated species of *Campylobacter*, 61.7% were due to *C. jejuni*, and *C. coli* accounted for 37.0%. Although the prevalence of *C. coli* is higher than reported in South Africa (3%),¹⁷ Nigeria (17%)¹⁸ and other developing countries,^{19–21} it is similar to prevalence reported by Georges-Courbot et al.²² in Bangui (39%). Only six isolates were not speciated as *C. jejuni* or *C. coli*.

Data on *C. jejuni* CPS-type distribution in pediatric populations within the developing world are limited. Pike et al.¹² recently reviewed the published literature on CPS distribution and found that HS1/44, HS2, and HS4 are the CPS types reported worldwide most commonly, isolated in 37% of

TABLE 2

Clinical features of primary *Campylobacter* infection by species and presence of the *hcp*⁺ gene

	<i>Campylobacter jejuni</i>				<i>Campylobacter coli</i>			
	All (N = 176)†	<i>hcp</i> ⁺ (N = 101)	<i>hcp</i> [−] (N = 75)	<i>P</i> value	All (N = 126)†	<i>hcp</i> ⁺ (N = 22)	<i>hcp</i> [−] (N = 104)	<i>P</i> value
Number stools/day, mean (std dev)	6.51 (2.78)	6.52 (2.57)	6.49 (3.05)	0.941	6.36 (3.07)	8.23 (3.60)	5.96 (2.81)	0.001
Vomiting, <i>n</i> (%)	42 (23.9%)	21 (20.8%)	21 (28.0%)	0.267	26 (20.6%)	6 (27.3%)	20 (19.2%)	0.397
Dehydration, <i>n</i> (%)	39 (22.2%)	27 (26.7%)	12 (16.0%)	0.090	34 (27.0%)	7 (31.8%)	27 (26.0%)	0.574
Fever, <i>n</i> (%)	62 (35.2%)	33 (32.7%)	29 (38.7%)	0.410	34 (27.0%)	9 (40.9%)	25 (24.0%)	0.105
Hematochezia, <i>n</i> (%)	8 (4.5%)	3 (3.0%)	5 (6.7%)	0.244	1 (0.8%)	0 (0.0%)	1 (1.0%)	0.644
Low activity, <i>n</i> (%)	85 (48.3%)	51 (50.5%)	34 (45.3%)	0.498	56 (44.4%)	11 (50.0%)	45 (43.3%)	0.564

* *hcp*, a genetic marker for the type 6 secretion system.

† Denominators for total *C. jejuni* and *C. coli* are 176 and 126, rather than 189 and 127, respectively, because not all isolates were available for *hcp* testing.

TABLE 3
Clinical features of primary *Campylobacter jejuni* infections by putative CPS* structures

	All CPS types (N = 168)	O-methyl-phosphoramidate (MeOPN)			Heptose			Deoxyheptose		
		Present (N = 130)	Absent (N = 38)	P value	Present (N = 130)	Absent (N = 38)	P value	Present (N = 111)	Absent (N = 57)	P value
Number stools/day, mean (std dev)	6.72 (2.92)	6.80 (2.94)	6.45 (2.85)	0.51	6.60 (2.95)	7.13 (2.82)	0.33	6.63 (2.99)	6.89 (2.79)	0.58
Vomiting, n (%)	41 (24.4%)	28 (21.5%)	13 (34.2%)	0.11	47 (36.2%)	12 (31.6%)	0.60	25 (22.5%)	16 (28.1%)	0.43
Dehydration, n (%)	38 (22.6%)	32 (24.6%)	6 (15.8%)	0.25	27 (20.8%)	11 (28.9%)	0.29	25 (22.5%)	13 (22.8%)	0.97
Fever, n (%)	59 (35.1%)	47 (36.2%)	12 (31.6%)	0.60	47 (36.2%)	12 (31.6%)	0.60	40 (36.0%)	19 (33.3%)	0.73
Hematochezia, n (%)	7 (4.2%)	5 (3.8%)	2 (5.3%)	0.70	7 (5.4%)	0 (0.0%)	0.14	6 (5.4%)	1 (1.8%)	0.26
Low activity, n (%)	79 (47.0%)	55 (42.3%)	24 (63.2%)	0.02	56 (43.1%)	23 (60.5%)	0.06	49 (44.1%)	30 (52.6%)	0.30

* CPS = capsular polysaccharide.

infections. These three CPS types were identified in 25% (95% CI: 20.0–31.6%) of our *C. jejuni* isolates. Combined, eight CPS types accounted for 65.4% (95% CI: 59.5–71.1%) of *C. jejuni*; higher than the 50.4% reported by Pike et al.¹² This may be due to the fixed pediatric population or the limited period of study compared with the global distribution over a longer time period and various populations included in the systematic review.¹²

Similar to a report by Lee et al.,²¹ we were unable to clinically differentiate *C. jejuni* and *C. coli* infections. In addition, our study was limited to the available number of infections identified during active surveillance. As such, although there were variations in the prevalence of specific signs and symptoms across capsule types, our power to detect significant differences was limited. Recent studies suggest that specific *C. jejuni* capsule structures contribute to virulence. MeOPN appears to enhance rates of colonization of an HS2 strain in piglets²³ and loss of a heptose branch from the same HS2 strain has been associated with decreased susceptibility to bile salts and decreased colonization in chickens.²⁴ MeOPN also has been shown to contribute to serum resistance in vitro for both HS2 and HS23/36.²⁵ It is unclear how these structures may impact clinical illness in the host; however, we observed no increase in the severity or frequency of clinical signs and symptoms in subjects infected with strains predicted to express capsules with MeOPN, heptose, or deoxyheptose.

Few reports have investigated the T6SS within *Campylobacter*. Corcionivoschi et al.²⁶ reported that 56.1% of *C. coli* and 28.8% of *C. jejuni* isolated from retail chicken meat were *hcp*-positive in the United Kingdom. Harrison et al.⁸ analyzed 181 *C. jejuni* strains from human, chicken, and environmental sources in the United Kingdom, Vietnam, Pakistan, and Thailand. By region, he observed significant variability in *hcp*-positivity, from 7.6% in the United Kingdom to 54.4% in Vietnam.⁸ Similar to the prevalence of *hcp*-positive strains in Vietnam, we found that 57.6% of our primary *C. jejuni* isolates were positive for the T6SS, which contrasts with the < 30% prevalence in *C. jejuni* isolates from retail chicken meat in the United Kingdom. Interestingly, Bleumink-Pluym et al.¹⁶ reported that the T6SS was only present in 10% of *C. jejuni* isolates from animal and human infections but that among the T6SS positive strains, 50% (4/8) had caused bacteremia. Our comparison of clinical signs and symptoms across isolates, however, with and without the T6SS, was largely unrevealing. This variability may be due to the small number of T6SS positive *C. jejuni* isolates tested by Bleumink-Pluym et al.¹⁶ or potential variability across these study populations.

Our study had several inherent limitations. First, this study was not a priori designed to address the research

questions addressed here and may have been insufficiently powered to address our stated hypotheses. Such studies, however, are rarely conceived prospectively for a specific enteropathogen. Future studies should use the estimates from this study in framing similar research questions. In addition, approximately 30% of the *Campylobacter* cases were co-infected with more than one enteropathogen confounding disease attribution. Our methods assumed the presence of the T6SS and molecules composing CPS structure by the presence of a specific gene, which may be an inaccurate assumption. We did not account for asymptomatic infections, unidentified co-pathogens, or nutritional status, all of which may have impacted our results.^{27,28}

We have described the epidemiology of *Campylobacter* among an Egyptian pediatric population. These data highlight the variability in *Campylobacter* species as well as *C. jejuni* CPS diversity in this population. In addition, we observed a high prevalence of the T6SS, particularly among *C. jejuni* isolates. We found no significant associations between clinical illness and *Campylobacter* species, *C. jejuni* CPS types, or suspected virulence factors. Further prospective birth cohort studies are needed to determine the spectrum of disease associated with specific factors and the role of infection in establishing immunity.

Received June 14, 2017. Accepted for publication July 31, 2017.

Published online December 18, 2017.

Acknowledgments: We would like to acknowledge Nashiva McDavid and Caroline Tribble for their assistance with laboratory procedures.

Financial support: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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