

## Clinical Characteristics, Risk Factors, and Population Attributable Fraction for Campylobacteriosis in a Nicaraguan Birth Cohort

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**Abstract.** Campylobacteriosis is an important contributor to the global burden of acute gastroenteritis (AGE). In Nicaragua, the burden, risk factors, and species diversity for infant campylobacteriosis are unknown. Between June 2017 and December 2018, we enrolled 444 infants from León, Nicaragua, in a population-based birth cohort, conducting weekly household AGE surveillance. First, we described clinical characteristics of symptomatic *Campylobacter* infections, and then compared clinical characteristics between *Campylobacter jejuni/coli* and non-*jejuni/coli* infections. Next, we conducted a nested case-control analysis to examine campylobacteriosis risk factors. Finally, we estimated the population attributable fraction of campylobacteriosis among infants experiencing AGE. Of 296 AGE episodes in the first year of life, *Campylobacter* was detected in 59 (20%), 39 were *C. jejuni/coli*, and 20 were non-*jejuni/coli* species, including the first report of *Campylobacter vulpis* infection in humans. Acute gastroenteritis symptoms associated with *C. jejuni/coli* lasted longer than those attributed to other *Campylobacter* species. In a conditional logistic regression model, chickens in the home (odds ratio [OR]: 3.8, 95% CI: 1.4–9.8), a prior AGE episode (OR: 3.3; 95% CI: 1.4–7.8), and poverty (OR: 0.4; 95% CI: 0.2–0.9) were independently associated with campylobacteriosis. Comparing 90 infants experiencing AGE with 90 healthy controls, 22.4% (95% CI: 11.2–32.1) of AGE episodes in the first year of life could be attributed to *Campylobacter* infection. *Campylobacter* infections contribute substantially to infant AGE in León, Nicaragua, with non-*jejuni/coli* species frequently detected. Reducing contact with poultry in the home and interventions to prevent all-cause AGE may reduce campylobacteriosis in this setting.

### INTRODUCTION

*Campylobacter* causes an estimated 400–500 million cases of acute gastroenteritis (AGE) annually.<sup>1</sup> In low- and middle-income countries (LMICs), the burden of campylobacteriosis in children is particularly high.<sup>2</sup> The Malnutrition and Enteric Disease (MAL-ED) Study, a birth cohort study of enteric infections in eight LMIC sites, detected *Campylobacter* in 85% of children using enzyme immunoassay, with an overall attributable fraction of 12% during the first year of life.<sup>3,4</sup> In the Global Enteric Multicenter Study, *Campylobacter* had the fifth highest attributable fraction for diarrhea among 32 tested enteric pathogens.<sup>5</sup>

*Campylobacter* prevention is essential to improving child health worldwide, as campylobacteriosis has been associated with harmful long-term sequelae, including reductions in linear growth, immune-mediated diseases, and functional gastrointestinal disorders.<sup>6–8</sup> Because there is no licensed *Campylobacter* vaccine and etiologic testing for AGE is rarely performed, it is important to identify risk factors for campylobacteriosis to guide prevention efforts. Known risk factors for campylobacteriosis include consumption of poultry, eggs, and other animal products that become contaminated during processing.<sup>9</sup> *Campylobacter* transmission through environmental contamination is more common in LMICs than in high-income settings, where individuals are more likely to have

direct contact with the feces of infected animals in and around the home.<sup>9–11</sup>

Although campylobacteriosis has been primarily attributed to the species *Campylobacter jejuni* and *C. coli*,<sup>2</sup> recent studies have shown that symptomatic AGE caused by *Campylobacter* species that historically have not been associated with human infection can occur.<sup>2,4,12</sup> To date, most studies have focused on the detection of *C. jejuni/coli* rather than targeting *Campylobacter* species. The aims of this study were to describe the incidence and risk factors for symptomatic campylobacteriosis, and to describe the distribution and clinical characteristics of *Campylobacter* species that circulate in a Central American urban center. This case-control study is nested in a birth cohort of infants residing in León, Nicaragua, who are being followed from birth to 36 months of age to observe and characterize pathogen-specific AGE episodes.

### METHODS

**Participants.** The Sapovirus-associated gastroenteritis study (SAGE) is an ongoing population-based birth cohort of 444 infants recruited in León, Nicaragua, conducting weekly household surveillance for AGE episodes from birth to 36 months. Data are collected from caregivers on household characteristics, hygiene, dietary patterns, social interactions, and AGE risk factors. We adapted the basic needs index used by the National Institute of Development Information of Nicaragua to identify children living in poverty, based on household construction, economic status, and educational attainment of household members.<sup>13</sup> Informed consent was requested of a parent or legal guardian of each participant. The study was approved by the institutional review boards of the

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**Samples.** Between June 12, 2017 and December 10, 2018, 286 children in the SAGE cohort (64%) experienced 603 AGE episodes. Acute gastroenteritis was defined as at least three loose stools in a 24-hour period, change in the consistency of stools (bloody, very loose, or watery), or the presence of vomiting. Acute gastroenteritis episodes were differentiated from one another by a period of three symptom-free days. When AGE episodes were reported, field-workers collected caregiver-reported data on symptoms, treatment received, and AGE risk factors, and collected acute stool samples within one to 3 days of symptom onset. We analyzed episodes that occurred during the first year of life (296 episodes among 173 children) for *Campylobacter*. Routine stools were collected monthly for all children. Stool samples were collected in a soiled diaper or in a sterile plastic container and transported at 4°C to the laboratory at UNAN-León for processing and analysis. For analysis, an aliquot of 1:10 stool suspension in phosphate-buffered saline was prepared and stored at –20°C.

**Sample analysis.** *Quantitative PCR (qPCR) analysis.* *Campylobacter* species DNA was extracted using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany). Primers for *Campylobacter* 16S rRNA and *Campylobacter* adhesin to fibronectin (*cadF*) were used to detect *Campylobacter* spp. and *C. jejuni/coli*, respectively. Two separate qPCRs of 25- $\mu$ L for 16S rRNA and *cadF* were prepared using 1  $\mu$ L of DNA sample, 12.5  $\mu$ L of Bio-Rad iQ Multiplex Powermix (Bio-Rad Laboratories, Hercules, CA), 1  $\mu$ L of a primer-probe mix at final concentrations of 0.2  $\mu$ M for each primer and 0.1  $\mu$ M for each probe, and nuclease-free water making up the remaining volume. Genomic DNA from *C. jejuni* subsp. *jejuni* was used as a positive control. Negative controls included water only and reactions containing no DNA template. Quantitative PCR was performed with a LightCycler<sup>®</sup> 96 instrument (Roche Diagnostics, Mannheim, Germany). Positive and negative control samples were run in triplicate (see Supplemental Table 1 for primers, probes, and cycling conditions).

Samples were considered positive at cutoff threshold (Ct)  $\leq$  35, obtained from a sensitivity analysis of the relationship of 16S rRNA Ct versus *cadF* Ct values (data not shown). 16S rRNA-positive/*cadF*-positive were considered positive for *C. jejuni/coli*, whereas 16S rRNA-positive/*cadF*-negative were considered non-*jejuni/coli* *Campylobacter* species. Samples were also tested by reverse transcriptase–qPCR using multiplex panels for *Astrovirus*, *Norovirus*, *Sapovirus*, and *Rotavirus* using methods previously described.<sup>14</sup>

*Speciation of Campylobacter cases by Sanger sequencing.* To further determine *Campylobacter* species, DNA was amplified by PCR of 16S rRNA. PCR was performed using a Bio-Rad T100 (Bio-Rad Laboratories; Supplemental Table 1). Amplicons were Sanger sequenced in both directions (Eton Bioscience, Durham, NC; Genewiz, Morrisville, NC), and the resulting sequences were queried in the National Center for Biotechnology Information GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) using the Basic Local Alignment Search Tool (National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda, MD) and SILVA nucleotide database (<https://www.arb-silva.de/aligner/>) using the alignment, classification, and tree service (ACT) (Max Planck Institute for Marine Microbiology and Jacobs University, Bremen, Germany). Species were assigned according to the highest nucleotide homology.

**Statistical methods.** We first described the clinical presentation of symptomatic AGE episodes for which *Campylobacter* was detected, including the presence and duration of diarrhea and vomiting, maximum number of stools per day, fever, treatment received, healthcare utilization, and coinfection with other enteric pathogens. We also described the *Campylobacter* species that were isolated from AGE stools. We used the Mantel–Haenszel chi-squared test to compare the clinical presentation of AGE episodes between *C. jejuni/coli* and other *Campylobacter* species.

Next, we conducted two distinct case–control analyses, which required selection of two distinct case and control groups. First, to identify risk factors for symptomatic campylobacteriosis, we randomly selected two controls for each case of *Campylobacter*-associated AGE in the same age-group as the case (0–2 months, 3–5 months, 6–8 months, 9–11 months, 12–14 months, 15–17 months, and 18+ months), with no history of symptomatic campylobacteriosis. We used the Mantel–Haenszel chi-squared test to compare the prevalence of campylobacteriosis risk factors between cases and controls. Risk factors from the univariate analysis at  $P \leq 0.1$  were included in the multivariable analysis, excluding variables with five or fewer children. Finally, we used conditional logistic regression to estimate odds ratios (ORs) and 95% CIs for independent predictors of campylobacteriosis, controlling for the matching structure. We restricted this analysis to the first episode of symptomatic campylobacteriosis in each child.

In the second case–control analysis, we estimated the attribution of AGE cases to *Campylobacter*. We randomly selected one control per AGE case of any etiology who was in the same age-group as the case and had been free of AGE during the 28 days before the onset of the case. We screened the most recent stool collected during a routine monthly visit from each control, and then calculated the relative odds of having AGE among children who were positive for *Campylobacter* to those who were negative. Finally, we estimated the population attributable fraction (PAF) for *Campylobacter*, or the proportion of symptomatic AGE episodes that are attributable to *Campylobacter*, and 95% CI using the epiR package developed by Stevenson et al.<sup>15</sup> The following formula is used to estimate the PAF:  $P_e \times \left( \frac{OR-1}{OR} \right)$ , where  $P_e$  is the proportion of AGE cases that were positive for *Campylobacter* and  $\left( \frac{OR-1}{OR} \right)$  is the proportion of AGE cases attributable to *Campylobacter* infection among children positive for *Campylobacter*.<sup>16</sup> All analyses were completed in R version 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

***Campylobacter* burden and speciation.** *Campylobacter* spp. was identified in 59 (20%) stool of 296 episodes from 54 children with AGE; five children experienced two campylobacteriosis episodes. *Campylobacter jejuni/coli* was detected in 39 (66%) of 59 *Campylobacter*-positive stools. We identified non-*jejuni/coli* *Campylobacter* species as follows: *Campylobacter concisus* ( $n = 3$ ), *Campylobacter hyointestinalis* ( $n = 1$ ), *Campylobacter hominis* ( $n = 1$ ), and *Campylobacter vulpis* ( $n = 1$ ). *Campylobacter* species were undeterminable in the remaining 14 samples because of no resulting match or identity score  $< 40$  in the GenBank and SILVA databases, respectively.

TABLE 1

Clinical characteristics of campylobacteriosis in Nicaraguan children, 2017–2018 (*n* = 59)

Clinical characteristic	<i>n</i> (%) or median (IQR)
Presence of diarrhea	55 (93)
Median duration (days)	6 (4, 8)
Median maximum number of stools in a 24-hour period	6 (5, 8)
Presence of vomiting	14 (24)
Median duration (days)	2 (1, 4)
Fever	18 (30)
Blood in stool	6 (10)
Received care at primary care clinic	21 (36)
Received care in hospital	2 (3)
Received care at emergency department	9 (15)
Received zinc	17 (29)
Received intravenous fluid	1 (2)
Received other medications in the last 7 days	32 (54)
Reason for receiving other medications	
For treatment of gastroenteritis	27 (46)
For treatment of other illness	9 (15)
Coinfected with an enteric virus (five missing)	24 (44)
Rotavirus	13 (24)
Sapovirus	6 (11)
Norovirus	6 (11)
Astrovirus	3 (6)

**Clinical characteristics of symptomatic campylobacteriosis.**

Of the 59 *Campylobacter* spp. episodes, 55 (93%) experienced diarrhea, which lasted a median 6 days, and 14 (24%) were associated with vomiting, lasting a median 2 days (Table 1). Bloody stool occurred in six (10%) episodes. Twenty-one cases (36%) received care in a primary care clinic, nine (15%) in an emergency department, and two (3%) were hospitalized. Forty-four percent of cases were coinfecting with at least one enteric virus. Forty-six percent received medication to treat AGE symptoms, primarily antibiotics and probiotics (Table 1). Episodes associated with *C. jejuni/coli* AGE demonstrated longer duration of diarrhea and vomiting relative to non-*jejuni/coli* AGE (Table 2).

However, other *Campylobacter* cases had more stools in a 24-hour period (*P* = 0.07) and were more likely to seek medical care.

**Campylobacteriosis risk factors.** In the first case–control analysis, we selected 108 controls for the 54 children (*n* = 162). Child gender, race, birth weight, delivery mode, gestational age, and maternal education were similar between cases and controls. Controls were more likely than cases to reside in poor households (*P* = 0.02), and cases were more likely than controls to have a chicken in the home (*P* = 0.02), have experienced a prior AGE episode of any cause (*P* = 0.001), and have had contact with a person experiencing AGE (*P* = 0.02) (Table 3). In a conditional logistic regression model adjusting for predictors of *Campylobacter* AGE, a chicken in the home (OR: 3.8; 95% CI: 1.4–9.8) and a prior AGE episode (OR: 3.3; 95% CI: 1.4–7.8) were independent predictors of *Campylobacter* AGE (Table 4). Poverty was independently protective against *Campylobacter* AGE (OR: 0.4; 95% CI: 0.2–0.9).

**Population attributable fraction for *Campylobacter*.** To estimate the proportion of AGE cases that were attributable to *Campylobacter*, we randomly selected 90 infants who experienced AGE and 90 age-matched controls with no AGE in the 28 days before the onset of the case. Twenty-four (27%) of the AGE cases were positive for *Campylobacter* spp., compared with five (6%) of the controls. The odds of AGE among children infected with *Campylobacter* were 6.2 times the odds for children without *Campylobacter* (95% CI: 2.2–17.1). Among cases and controls, 22.4% (95% CI: 11.2–32.1) of AGE cases could be attributed to *Campylobacter* infection.

DISCUSSION

These data from Nicaragua contribute to the literature supporting the significance of rarer *Campylobacter* species, as data from Central America have been scarce.<sup>17–19</sup> In this nested case–control study, 34% of *Campylobacter* AGE cases were non-*jejuni/coli* species. Non-*C. jejuni/coli* AGE episodes demonstrated a slightly shorter duration of both

TABLE 2

Clinical characteristics of *C. jejuni/coli* compared with other *Campylobacter* species (*n* = 59)

Clinical characteristic, <i>n</i> (%) or median (IQR)	<i>C. jejuni/coli</i> ( <i>n</i> = 39)	Other species* ( <i>n</i> = 20)	<i>P</i> -value
Diarrhea	37 (95)	18 (90)	0.5
Median duration (days)	7 (4, 8)	4 (3, 7)	0.04
Median maximum number of stools in a 24-hour period	6 (5, 7)	7 (6, 8)	0.07
Vomiting	9 (23)	5 (25)	1.0
Median duration (days)	2 (1, 4)	1 (1, 1)	0.2
Fever	11 (28)	7 (35)	0.6
Blood in stool	6 (15)	0 (0)	0.09
Received care at primary care clinic	11 (28)	10 (50)	0.1
Received care in hospital	2 (5)	0 (0)	0.5
Received care at emergency department	5 (13)	4 (20)	0.5
Received zinc	10 (26)	7 (35)	0.5
Received intravenous fluid	1 (3)	0 (0)	1.0
Received other medications in the last 7 days	19 (49)	13 (65)	0.2
Reason for receiving other medications			
For treatment of gastroenteritis	15 (39)	12 (60)	0.1
For treatment of other illness	6 (15)	3 (15)	1.0
Coinfected with an enteric virus (five missing)	19 (54)	5 (26)	0.08
Rotavirus (five missing)	8 (23)	5 (26)	1.0
Sapovirus (five missing)	5 (14)	1 (5)	0.4
Norovirus (five missing)	5 (14)	1 (5)	0.4
Astrovirus (five missing)	3 (9)	0 (0)	0.5

*C. jejuni* = *Campylobacter jejuni*.

\* Other species include *Campylobacter concisus* (*n* = 3), *Campylobacter hyointestinalis* (*n* = 1), *Campylobacter hominis* (*n* = 1), *Campylobacter vulpis* (*n* = 1), and untypeable (*n* = 14).

TABLE 3  
 Characteristics of first campylobacteriosis cases as compared with age-matched controls\*

Characteristic, n (%) or mean (SD)	Cases (n = 54)	Controls (n = 108)	P-value
Mean age at the time of campylobacteriosis case (months)	5.8 (3)	5.4 (3)	0.4
Birth characteristics			
Gender (% female)	26 (48)	56 (52)	0.7
Race (% Latino/Mestizo)	54 (100)	107 (99)	0.5
Birth route (% vaginal delivery)	33 (61)	57 (53)	0.3
Gestational age at birth (completed weeks)	39 (1)	39 (1)	0.7
Mean birth weight (grams)	3,156 (463)	3,138 (415)	0.8
Mean age of mother (years) at child's birth	24 (5)	24.5 (5)	0.4
Socioeconomic indicators			
Maternal educational attainment (nine missing)	–	–	–
Completed any primary education	13 (25)	28 (28)	–
Completed any secondary education	28 (53)	40 (40)	–
Completed any university/vocational school	12 (23)	27 (27)	0.4
Illiterate	0 (0)	5 (5)	–
Mother employed at time of child's birth (six missing)			
Not formally employed			
Skilled	37 (70)	77 (75)	–
Unskilled	4 (8)	6 (6)	–
Student	5 (9)	4 (4)	0.8
Other	4 (8)	10 (10)	–
Poverty index (% poor or extremely poor) (two missing)	3 (6)	6 (6)	–
Crowding index (> 2.5 people/bedroom) (three missing)	25 (46)	70 (66)	0.02
Non-dirt floor	16 (30)	33 (31)	0.8
Wall composition (% brick/cement)	37 (69)	74 (69)	1.0
Electricity (% yes)	42 (78)	90 (83)	0.4
Household sanitation	54 (100)	108 (100)	N/A
Water source (% municipal in home)	45 (83)	90 (83)	1.0
Sanitation type (% indoor toilet)	38 (70)	73 (68)	0.7
Shares sanitation (toilet/latrine) with another home	3 (6)	4 (4)	0.7
Any water storage in the home	38 (70)	68 (63)	0.4
Always uses ≥ 1 effective means of water purification†	14 (26)	25 (23)	0.7
Water source interruption in the past week	5 (9)	14 (13)	0.5
Animals in the home, any			
Dog	31 (57)	59 (55)	0.7
Cat	11 (20)	23 (21)	0.9
Chickens	15 (28)	14 (13)	0.02
Pig	3 (6)	4 (4)	0.7
Cow	0 (0)	1 (1)	1.0
Goat	0 (0)	0 (0)	N/A
Rabbit	4 (7)	5 (5)	0.5
Bird	7 (13)	11 (10)	0.6
Turtle	0 (0)	0 (0)	N/A
Mice	3 (6)	10 (9)	0.4
Bat	0 (0)	0 (0)	N/A
Other	7 (13)	11 (10)	0.6
Personal hygiene			
Practices handwashing at appropriate moments‡ (two missing)	44 (83)	80 (75)	0.2
Use of alcohol hand sanitizer in home (% using at least sometimes)	14 (26)	25 (23)	0.7
Nutrition			
Ever breastfed	53 (98)	108 (100)	0.2
Currently breastfeeding	43 (80)	85 (79)	0.9
Mean age of introduction of any supplementary foods/drinks (weeks)	3.5 (3)	3.3 (1)	0.7
Mean weight-for-age Z-score	0.62 (1)	0.59 (1)	0.8
Mean length-for-age Z-score	0.28 (1)	0.45 (1)	0.4
Mean BMI-for-age Z-score	0.65 (1)	0.47 (1)	0.4
Ate uncooked fruit/vegetable in the past week	23 (43)	54 (50)	0.4
Ate seafood in the past week	2 (4)	4 (4)	1.0
Ate outside the home in the past week	8 (15)	15 (14)	0.9
Shared a bottle or cup with another person in the past week	11 (20)	15 (14)	0.3
Interpersonal contact			
Other child in home in diapers	7 (13)	22 (20)	0.2
Attended a social event in the past week	11 (20)	31 (29)	0.3
Attended day care in the past week	0 (0)	0 (0)	N/A
Met/played with a child outside the home in the past week (two missing)	12 (23)	35 (33)	0.2

(continued)

TABLE 3  
Continued

Characteristic, n (%) or mean (SD)	Cases (n = 54)	Controls (n = 108)	P-value
Used public transportation in the past week	20 (37)	35 (32)	0.6
Went swimming in the past week	2 (4)	1 (1)	0.3
Had contact with any person with diarrhea and/or vomiting, inside or outside the home, in the past week (one missing)	9 (17)	5 (5)	0.02
<b>Gastroenteritis risk factors</b>			
Completed rotavirus vaccination (two missing)	31 (59)	55 (51)	0.4
Prior episode of gastroenteritis (of any cause)	25 (46)	22 (20)	0.001

BMI = body mass index; SD = standard deviation.  
 \* Cases were matched 2:1 with controls within 3-month age-groups. Infants with a history of campylobacteriosis as indicated by reverse transcriptase-PCR were not eligible to be controls. Controls selected more than once were included twice, and controls who later became cases were included in both case and control columns.  
 † Water treatment options include using a water filter (including sand and mud/ceramic filters), boiling water, adding bleach or chlorine, solar disinfection, straining through a cloth, letting water settle, purchasing purified water.  
 ‡ Appropriate moments include after caring for a sick person, before eating, before preparing food, after using the bathroom, after changing diapers.

diarrhea and vomiting. As a whole, *Campylobacter* species contributed to 22.4% (95% CI: 11.2–32.1) of AGE episodes in this setting. The presence of a chicken in the home increased the odds of *Campylobacter* AGE by four times, whereas experiencing a prior AGE episode of any cause conferred a threefold increase in odds; poverty was protective against *Campylobacter* AGE.

That poverty was less prevalent in campylobacteriosis cases than controls runs counter to expectations. Although data from other LMICs are scarce, this finding differs from a population-based study in the United States, which found low socioeconomic status to be associated with *Campylobacter* detection in infants of low compared with high socioeconomic status.<sup>20</sup> Given the important role of chickens in *Campylobacter* transmission, our finding could be explained by the fact that wealthier households may have more opportunities for poultry consumption and thus a higher risk of exposure, which was not captured in our study. More data are needed to understand the relationship between poverty and AGE with respect to campylobacteriosis in LMICs.

The zoonotic potential of *Campylobacter* transmission is well documented.<sup>21,22</sup> An estimated 50–80% of all human campylobacteriosis cases are thought to be related to chicken consumption or exposure<sup>2</sup>; exposure to poultry and consumption of undercooked or incorrectly cooked chicken are commonly identified risk factors for campylobacteriosis in humans.<sup>23–25</sup> Whereas campylobacteriosis is a common foodborne illness, *Campylobacter* can also be transmitted between chickens and humans via environmental contamination with chicken feces. Study participants were asked about the presence of chicken in the home rather than chicken consumption, so we are unable to distinguish the relative contributions of foodborne and environmental exposure in this study. We also did not gather contextual background about household animal husbandry practices in the study setting, such as quantity of chickens or poultry housing practices.

Although León is a relatively urbanized setting and only 18% chicken ownership was described across study participants, national survey data from Nicaragua<sup>26</sup> estimate household chicken ownership to be about 55%, whereas estimates for neighboring Latin American countries<sup>27,28</sup> are as high as 70%. If these findings are generalizable, they would support the allocation of resources toward sustainable, community-level poultry manure management interventions, and calls to incorporate animal husbandry management into water, sanitation, and hygiene (WaSH) management.<sup>29,30</sup> Future environmental studies in LMICs should consider the consumption of poultry and eggs on *Campylobacter* infection. A variety of domesticated animals, specifically pigs, cattle, sheep, goats, dogs, and cats, have also been suspected as carriers of *Campylobacter*.<sup>31–34</sup> However, the presence of these animals was not associated with campylobacteriosis in our study.

Notably, WaSH characteristics did not predict *Campylobacter* AGE. Low-cost WaSH interventions such as improved pit latrines and implementation of handwashing stations are often highlighted as solutions to combat childhood diarrhea in LMIC settings.<sup>35</sup> We found that personal hygiene such as appropriate handwashing and use of alcohol-based sanitizer were insufficient to prevent campylobacteriosis. Water purification measures and sanitation also had no apparent preventive benefit. This is consistent with recent evidence from large randomized controlled trials, which found mixed effects of WaSH interventions on diarrhea, ranging from no effect in Kenya<sup>36</sup> and Zimbabwe<sup>37</sup> to a large relative risk reduction in Bangladesh.<sup>38</sup>

Because *C. jejuni* and *Campylobacter coli* are considered the most clinically relevant species of *Campylobacter*, other *Campylobacter* species are understudied and underestimated as causes of human AGE.<sup>2,12</sup> However, in our study, 34% of children with symptomatic *Campylobacter* AGE were infected with other *Campylobacter* species, with *C. concisus* being the most common of these among samples where sequencing

TABLE 4  
Crude and conditional predictors of *Campylobacter* spp.

Characteristic	Crude OR (95% CI)	Adjusted OR (95% CI)*
Poverty index (poor vs. not poor)	0.39 (0.18, 0.83)	0.38 (0.16, 0.91)
Chicken in the home	2.29 (1.06, 4.95)	3.75 (1.44, 9.79)
Prior episode of AGE (of any cause)	3.55 (1.66, 7.60)	3.33 (1.42, 7.78)
Had contact with any person, inside or outside the home, with AGE in the past week†	4.17 (1.27, 13.65)	–

AGE = acute gastroenteritis; OR = odds ratio.  
 \* Model adjusted for 3-month age-group, residual age difference between cases and controls after matching, poverty index, chicken in the home, and prior episode of gastroenteritis.  
 † Contact with a person experiencing AGE was excluded from the adjusted OR calculation due to cell sizes ≤ 5.

identified a species. Similarly, studies among children experiencing AGE in MAL-ED,<sup>4</sup> South Africa,<sup>17</sup> and Peru<sup>12</sup> have reported a prevalence of other *Campylobacter* species ranging from 28% to 76%. Furthermore, children in our study infected with other *Campylobacter* species experienced more stools per day and were more likely to seek treatment for their symptoms, suggesting that these species can cause severe disease. That bloody diarrhea was uncommon in these children is also consistent with other community-based studies. Among the other *Campylobacter* species, *C. concisus*, *Campylobacter ureolyticus*, *Campylobacter upsaliensis*, and *Campylobacter lari* are known as “emerging” *Campylobacter* species to underscore their growing recognition as human pathogens.<sup>2,38</sup> In fact, some studies have reported the prevalence of *C. concisus* and *C. upsaliensis* as comparable to *C. jejuni/coli*, among children experiencing AGE.<sup>31,33</sup> *Campylobacter hyointestinalis* and *C. hominis*, each of which was detected in one child in our study, are less common and have been rarely reported in prior studies of symptomatic AGE.<sup>38</sup>

A 2018 study first documented *C. vulpis* in canines in Italy.<sup>39</sup> Our study is the first published report of *C. vulpis* in humans. The episode lasted for 10 days with no vomiting, fever, bloody stool, or viral coinfections reported. This child had also experienced one AGE episode approximately 2 months before the *Campylobacter* episode, which was determined negative for *Campylobacter* spp. via qPCR. Chickens and dogs were present in the home at the time of infection, which may suggest that the child experienced contamination with the canine strain. More research is needed to understand whether *C. vulpis* can directly infect and cause AGE symptoms in humans.

Furthermore, we found that prior AGE episodes of any etiology increased the risk for *Campylobacter* AGE. In our study, over twice as many cases as controls (46% versus 20%) had experienced a prior episode of AGE before testing positive for *Campylobacter*. Prior enteric infections may modify or perturb the composition of the gut microbiome, which may alter susceptibility to *Campylobacter* and shape enteric immunity.<sup>40–42</sup> Interventions to increase the abundance of beneficial species in the gut microbiome to prevent enteric infections are under study.<sup>43–45</sup> At present, existing WaSH interventions to prevent all-cause AGE episodes should be strengthened.

There were several limitations in our study. First, of the samples positives for non-*jejuni/coli* *Campylobacter* species, many were unable to be speciated. This reflects the need for state-of-art technology to be used for sequencing to identify emerging rare or new *Campylobacter* species that increasingly contribute to the global campylobacteriosis burden. Also, because of the low prevalence of potentially important risk factors, such as contact with another person experiencing AGE in the past week, not all risk factors could be adjusted for in the conditional logistic model. Finally, as found in other settings in LMICs,<sup>46</sup> the prevalence of coinfections in this population was high (44%) and makes it challenging to attribute all of the observed clinical characteristics to *Campylobacter* infection. However, *Campylobacter* infections have detrimental impact on child health even when not causing AGE episodes.<sup>6</sup> To address this, we calculated a PAF to more clearly communicate the public health impact of *Campylobacter* and the proportion of AGE that could be eliminated with effective campylobacteriosis prevention.

Findings from this nested case-control study in Central America support recent findings that *Campylobacter* is a significant contributor to the burden of AGE among infants in LMICs.<sup>47</sup> Although non-*jejuni/coli* *Campylobacter* in this population causes AGE of mixed clinical severity, our findings suggest that other *Campylobacter* species as a whole are an important contributor to the burden of campylobacteriosis. Our study also highlights that prior AGE episodes and contact with household chickens are important risks for disease. Further research is needed to elucidate the best ways to prevent and mitigate AGE due to diverse *Campylobacter* species in LMIC settings.

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