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Effect of *Helicobacter pylori* Infection on Symptoms of Gastroenteritis Due to Enteropathogenic *Escherichia coli* in Adults

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Abstract

Background—*Helicobacter pylori* can cause hypochlorhydria in some hosts and predispose to diarrheal infections. *Aims* We tested the hypothesis that chronic *H. pylori* infection increases the risk of diarrheal illness due to an acid-sensitive organism: enteropathogenic *Escherichia coli* (EPEC).

Methods—After testing healthy adult volunteers for *H. pylori*, 19 infected and 26 uninfected subjects had gastric pH probes placed and were given 5–10 × 10^9 EPEC organisms; six had previously received a proton pump inhibitor. We measured diarrhea and created a composite gastroenteritis severity score based on symptoms in the 48 h following exposure. Outcomes were compared using logistic regression and analysis of covariance.

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Results—More *H. pylori*-infected (36.8%) than *H. pylori*-uninfected subjects (7.7%) were hypochlorhydric ($P = 0.02$). Six (31.6%) *H. pylori*-infected and five *H. pylori*-uninfected subjects (19.2%) developed diarrhea ($P = 0.34$). Hypochlorhydria was a strong risk factor for diarrhea [odds ratio (OR) 6.25, confidence interval (CI): 1.29-30.35]. After adjusting for hypochlorhydria and EPEC dose, *H. pylori* was not associated with diarrhea (OR 0.89, CI: 0.17–4.58). Among those with symptoms, *H. pylori*-infected subjects had lower gastroenteritis severity score than did *H. pylori*-uninfected subjects (2.6, CI: 1.9–3.4 versus 1.5, CI: 1.1–1.9, $P = 0.01$), particularly if they were also hypochlorhydric (3.8, CI: 2.3–5.3 versus 1.9, CI: 1.3–2.5, $P = 0.02$).

Conclusions—In adults, *H. pylori* infection was associated with hypochlorhydria but had no detectable effect on occurrence of diarrhea. Among symptomatic subjects, *H. pylori* infection decreased severity of gastroenteritis.

Keywords

Helicobacter pylori; Hypochlorhydria; Gastroenteritis; Enteropathogenic *Escherichia coli*; Diarrhea; Interaction

Introduction

Helicobacter pylori is one of the world's most common infections, with person-to-person transmission the predominant mode of acquisition. In a previous study, we showed that *H. pylori* is recoverable from vomitus and stools during induced emesis and catharsis but is undetectable in normal stools [1]. We hypothesized that *H. pylori* facilitates its own transmission by increasing the risk of gastroenteritis due to other enteric pathogens. One method by which *H. pylori* may increase the risk of gastroenteritis is through hypochlorhydria. In chronically infected adults, *H. pylori* can lead to atrophic gastritis and reduced acid secretion capacity, making the host more susceptible to acid-sensitive pathogens [2, 3].

Because *H. pylori* is an enteric infection, its prevalence may be linked to exposure to other enteric pathogens. Results of observational studies on the association between *H. pylori* and gastroenteritis, however, have been conflicting. Some have shown increased incidence of diarrhea in children with *H. pylori* infection, with one study attributing 11% of diarrhea cases to *H. pylori* [4–6]. Other studies found no association [7, 8], and still others found a protective effect of *H. pylori* against gastroenteritis [9–11]. Dissecting out confounding from true physiological associations can be difficult in observational studies.

To better elucidate the association between *H. pylori* and gastroenteritis, we performed a direct challenge experiment with a well-characterized gastrointestinal pathogen, enteropathogenic *Escherichia coli* (EPEC). EPEC is a leading cause of infantile gastroenteritis in the world and has a long history of safe use in human experiments [12–19]. It is also acid sensitive: in our laboratory less than 0.001% of inoculated EPEC organisms survived at pH 2.5. Our goal was to test the hypothesis that chronic infection with *H. pylori* increases the risk of diarrheal illness after direct challenge with EPEC.

Methods

Patient Selection

Healthy volunteers aged 35–59 years were recruited through local advertising and invited to undergo a screening medical evaluation. Exclusion criteria included chronic use of antacids or opiates, pregnancy, prior history of gastritis, treatment for *H. pylori*, peptic ulcer disease, irritable or inflammatory bowel disease, gastroesophageal illness requiring treatment, or comorbidities requiring physician treatment. Subjects had normal hematology and chemistry serum panels and negative EPEC serology. *H. pylori*-infected subjects were selected if they were positive in both *H. pylori* serology and C13 urea breath test, while *H. pylori*-uninfected subjects were negative in both. Subjects with discordant *H. pylori* test results were excluded from the study. Only subjects with no gastrointestinal symptoms were included in the study, and those who were *H. pylori* positive were assumed to have chronic infection [20]. Subjects provided informed consent. The study complied with all requirements of the Stanford Institutional Review Board. The study was registered in the clinical trials registry (clinicaltrials.gov, number NCT00550368), and a Stanford Data Safety Monitoring Board reviewed results for safety. Using alpha of 0.10, power of 80%, and a baseline rate of diarrhea of 15%, we determined that 60 subjects, evenly split between *H. pylori*-infected and *H. pylori*-uninfected, would be required to find a 30% difference in percentages of subjects who developed diarrhea between the study groups. The data safety and monitoring board protocol specified interim power analysis for the protection of human subjects. The study was stopped after enrollment of 45 subjects when it was determined that increasing the sample size to the prespecified target would not yield a statistically significant difference in the primary outcome.

Experiment Protocol

A random subsample of three *H. pylori*-infected and three *H. pylori*-uninfected subjects were asked to begin 40 mg pantoprazole once daily starting 72 h prior to admission to confirm the effects of hypochlorhydria on gastroenteritis. Subjects provided a baseline stool sample and fasted for 3 h prior to admission to the Stanford General Clinical Research Center (GCRC). Nasogastric pH probes (Medtronic Digitrapper MkIII) were calibrated before each recording according to the manufacturer's directions using both pH 7 and pH 1 solutions. Subjects then had the pH probes inserted, and placement was confirmed in the distal stomach by sustained pH reading of less than 2.5 or by chest radiography if gastric pH was not acidic.

Subjects were then asked to ingest 5×10^8 EPEC O111: NM organisms suspended in phosphate-buffered saline. This dose induced mild diarrhea in 50% of subjects in studies conducted at Stanford [18]. After the first 21 subjects were enrolled, due to lower occurrence of diarrhea than expected (14.3%), the dose was increased to 1×10^9 organisms. After EPEC ingestion, subjects were kept fasting for three more hours for pH monitoring before probe removal.

Each stool produced during the first 48 h was recorded, its weight and volume measured, and a sample taken for culture. Vitals were monitored every 8 h, and subjects were asked to

rate and score their symptoms of malaise, headache, nausea, and abdominal pain from 0 (none) to 3 (severe). Subjects were discharged home with a prescription for 500 mg ciprofloxacin twice daily for 5 days. After completion of antibiotics subjects had one return visit to monitor their well-being and to provide a final stool sample for confirmation of EPEC clearance. Four weeks after discharge, subjects returned for follow-up EPEC serology.

Laboratory Methods

EPEC was obtained fromthe laboratory of Dr. Gary Schoolnik (Stanford University, CA). Based upon the work by Donnenberg [17], we developed an in-house enzyme-linked immunosorbent assay (ELISA) for EPEC O111:NM lipopolysaccharide. Serial dilutions of ten controls from a prior study were used to determine cutoff optical densities (OD). For this study, ODs outside of ±3 standard deviation (SD) from the mean were considered positive. For the postchallenge serology, an increase of ≥0.1 OD from the prechallenge titer was considered as seroconversion. *H. pylori* serology assays were performed as described previously [1].

All subjects also had C13 urea breath test (Meretek, Nashville, TN) according to the manufacturer's directions. Each stool sample was cultured for EPEC by suspending in pre-enrichment buffer in serial dilutions, and incubating at 42°C for 24 h. Immunomagnetic separation was used to isolate EPEC from other flora using Dynabeads (Dynal, Invitrogen, Carlsbad, CA) according to the manufacturer's directions. To verify bacterial identity from suspected colonies, a polymerase chain reaction detecting an EPEC-specific gene (*eaeA*) was used. Stools were also cultured for *H. pylori* as previously described [1].

Study Definitions

Subjects were defined as hypochlorhydric when fasting gastric pH was >4 more than 25% of the time during the 3 h of monitoring. This cutoff was selected based on the plotted distribution of gastric acidity in our study population and was the point that showed clear segregation between the groups, after excluding the six subjects who had been randomized to take a proton pump inhibitor (PPI). This definition of hypochlorhydria was consistent with other published reports [21, 22]. Based on this definition, the incidence of hypochlorhydria in our *H. pylori*-infected subjects was similar to that reported in the literature [21–24].

Using criteria previously established in EPEC studies, diarrhea was defined as having one loose stool that weighed at least 300 g, or two or more loose stools each weighing at least 200 g, in the 48 h following inoculation with EPEC [18, 25]. Loose stools were any that did not maintain their shape in the container. A daily loose stool score, defined a priori, was calculated from the sum weight of loose stools as follows: 0 (none), 1 (1–150 g), 2 (151–300 g), 3 (301–450 g), 4 (451–600 g), 5 (greater than 601 g). Total daily symptom scores for malaise, headache, nausea, and abdominal pain were tabulated from the subjects' rating of their symptoms on a scale of 0–3 as described in the experiment protocol. Symptom scores and loose stool scores for each day were then added to create a 48-h composite gastroenteritis severity score.

Data Analysis

The primary outcome of our study was difference in risk of diarrhea between *H. pylori*-infected and *H. pylori*-uninfected subjects. Prespecified secondary outcomes were differences in composite gastroenteritis severity score and recovery of *H. pylori* and EPEC in subjects' stool cultures. Differences in proportions between study groups were analyzed using the chi-square test and Fisher's exact test. Differences in means and distributions were compared using the *t*-test or the Wilcoxon rank-sum test, respectively, depending on the normality of the data. Multivariate analyses were conducted using logistic regression. Severity of gastroenteritis was analyzed using ordinal logistic regression, categorizing subjects into three prespecified groups based on the composite severity score: group 1 (score = 0), group 2 (score = 1–4), and group 3 (score >4), and after testing the assumption of proportional odds. Severity of gastroenteritis was also analyzed using analysis of covariance (ANCOVA), treating the composite severity score as a continuous variable after logarithmic transformation to achieve normality. All multivariate models included the terms *H. pylori* infection, hypochlorhydria, race/ethnicity (Hispanic versus other), and EPEC dose. Effect heterogeneity was assessed by including interaction terms for *H. pylori* and hypochlorhydria in the multivariate models. Analyses were corrected for multiple comparisons using the Tukey method. Time to stool shedding of EPEC organisms was calculated using the generalized Wilcoxon test for nonparametric data to account for censoring and early appearance of events. All analyses were conducted using SAS version 9.1 (North Cary, NC).

Results

Patient Characteristics

A total of 45 subjects completed the study, 19 and 26 being *H. pylori* positive and negative, respectively. Racial/ethnic distribution differed between the two groups: 63.2% of *H. pylori*-positive subjects were of Hispanic ethnicity compared with 19.2% of *H. pylori*-negative subjects (Table 1). Mean age of all study subjects was 48.6 ± 7.0 years, and mean body mass index was 27.7 ± 4.3 kg/m^2. There were no differences in either age or body mass index between *H. pylori*-positive and *H. pylori*-negative subjects. All subjects also had negative EPEC serology.

Of the nine subjects (20% of all subjects) who were hypochlorhydric during the 3 h fasting, more were *H. pylori* infected than uninfected (36.8% versus 7.7%, $P = 0.02$), even after excluding recipients of proton pump inhibition. During the 3 h monitoring, on average, *H. pylori*-positive subjects maintained an acidic gastric environment (pH < 4) 88.6% of the time compared with *H. pylori*-negative subjects, who maintained gastric acidity 99.8% of the time ($P = 0.004$). More *H. pylori*-positive than *H. pylori*-negative subjects received the higher dose of EPEC (73.7% versus 38.5%, $P = 0.02$). PPI recipients were evenly distributed between the *H. pylori*-infected versus *H. pylori*-uninfected groups (15.8% versus 11.5%, respectively, $P = 0.68$).

Diarrhea

Diarrhea was the primary outcome of our study. Eleven subjects (24.4%) developed diarrhea according to the study definition (Table 2). Nineteen (42.2%) developed at least one loose

stool in the 48 h following EPEC ingestion. On bivariate analyses, *H. pylori* infection, EPEC dose, being of Hispanic ethnicity, and receiving PPI were not risk factors for diarrhea (Table 3). Hypochlorhydria, in contrast, was a strong risk factor and increased the odds of diarrhea more than sixfold ($P = 0.02$). On multivariate analysis, *H. pylori* infection did not increase the odds of diarrhea, whereas hypochlorhydria increased the odds of diarrhea fivefold ($P = 0.08$). We detected no effect modification between *H. pylori* infection and hypochlorhydria on occurrence of diarrhea.

Severity of Gastroenteritis

Ingestion of EPEC at either dose was generally well tolerated in our study (Table 2). Thirty-two (71%) study subjects reported any symptom during the 48 h of follow-up. The most common symptoms were generalized malaise ($N = 17$, 37.8%) and headache ($N = 15$, 33.3%). Abdominal pain ($N = 7$, 15.6%) and nausea ($N = 10$, 22.2%) were less frequent. Incidence of gastroenteritis and mean/median composite severity scores were not different between *H. pylori*-infected and *H. pylori*-uninfected subjects. The higher dose of EPEC elicited a wider range of severity scores than the lower dose, but mean and median scores were similar ($P = 0.45$). No associations were found between severity of gastroenteritis and *H. pylori* infection, EPEC dose, hypochlorhydria, Hispanic ethnicity, or use of PPI on bivariate analyses that included all 45 study subjects. For the multivariate analysis, we grouped the subjects into three categories based on their composite severity scores: 13 subjects had absolutely no symptoms and severity score of 0, 19 subjects had severity score between 1 and 4, and 13 subjects developed severity score >4. On ordinal logistic regression of the three levels of gastroenteritis scores, there was also no effect on severity of illness due to *H. pylori* infection (OR 0.50, 95% CI 0.14–1.76), EPEC dose (OR 1.09, 95% CI 0.85–1.40), hypochlorhydria (OR 2.84, 95% CI 0.61–13.20) or Hispanic ethnicity (OR 1.61, 95% CI 0.43–6.06). However, among the subgroup of 32 subjects who did experience some degree of gastroenteritis (Table 4), after adjusting for EPEC dose, Hispanic ethnicity, and hypochlorhydria, *H. pylori*-infected subjects had lower gastroenteritis severity score than did *H. pylori*-uninfected subjects ($P = 0.007$). Hypochlorhydria was a strong independent risk factor for higher gastroenteritis severity scores during the 48-h period ($P < 0.001$) and appeared to modify the effect of *H. pylori* infection: *H. pylori*-infected subjects had lower composite symptom score if they were also hypochlorhydric ($P = 0.07$ for interaction).

Organism Shedding in Stool

Regardless of stool consistency, *H. pylori* was not isolated in any of the stool samples collected. EPEC was recovered in the stool of 12/19 (63.2%) *H. pylori*-infected versus 21/26 (80.8%) *H. pylori*-uninfected subjects ($P = 0.19$). Those with no EPEC shedding did not differ in baseline characteristics from the rest of the participants.

EPEC was shed as early as 4 h after ingestion. Median time to EPEC shedding was longer for *H. pylori*-infected than for *H. pylori*-uninfected subjects: 42.4 h compared with 23.2 h, respectively, although this difference did not reach statistical significance ($P = 0.13$). EPEC shedding was not associated with stool consistency (51.0% solid stool versus 56.9% loose stool, $P = 0.47$), but participants with loose stools shed EPEC faster than those with no loose stools, with median time of 18 versus 32.2 h, respectively ($P = 0.01$). At the end of antibiotic

treatment, all subjects had negative EPEC stool cultures; five subjects developed detectable EPEC IgG antibodies 1 month after challenge.

Discussion

Our study is the first direct-challenge experiment to examine the independent effects of *H. pylori* infection and gastric acidity on susceptibility to an acid-sensitive enteric pathogen. We found that *H. pylori* did not alter the risk of diarrhea (our primary outcome). Hypochlorhydria, in contrast, was a strong predictor of diarrhea on bivariate analysis, albeit with borderline significance after adjusting for *H. pylori*, EPEC dose, and race. Although *H. pylori* was more likely to be associated with hypochlorhydria, the effect size was not large enough to make *H. pylori* infection a significant risk factor for diarrhea. We did not recover any *H. pylori* in loose stools, even in subjects with rapid transit times based on EPEC recovery, also arguing against our initial hypothesis that *H. pylori* induces gastroenteritis as a method to facilitate its own transmission. The recovery of EPEC organisms in stool samples showed a trend towards delayed EPEC shedding and fewer positive stools in *H. pylori*-infected subjects. This could suggest that *H. pylori* infection plays a protective role against EPEC infection. Neither comparison reached statistical significance, however, and whether *H. pylori* infection alters adhesion or pathogenesis of EPEC will need to be clarified in mechanistic studies.

With respect to severity of gastroenteritis, we were interested in evaluating severity of illness by taking into consideration other symptoms that commonly occur in gastroenteritis in addition to loose stool. For example, a subject who developed nausea and abdominal pain in addition to diarrhea would be considered to be more ill than one with just diarrhea. Donnenberg et al. previously reported symptom scores that correlate with severity of illness [17]. In their study, they evaluated subjective symptoms separately from loose stool, but also found some subjects with more symptoms but fewer loose stools than others. For the composite severity score, we aggregated both reported symptoms and measured loose stool to provide a better reflection of the degree of illness due to EPEC ingestion. Overall, neither *H. pylori* nor hypochlorhydria was associated with higher gastroenteritis symptom score. However, among those who were symptomatic, hypochlorhydria led to higher severity score, as expected. In addition, we found that *H. pylori* infection was associated with lower severity of gastroenteritis among those who developed gastroenteritis. The composite severity score provided greater power to discriminate differences between the subjects when used as a numeric covariate.

Plausible biological explanations exist for why *H. pylori* infection diminishes severity of gastroenteritis. *H. pylori* infection causes local inflammation of the gastric mucosa and upregulates B-cell and Th-1-type T-cell adaptive immune responses, both of which may protect against enteric pathogens [26–28]. In addition, studies have uncovered antibacterial properties of peptides derived from *H. pylori* ribosomal proteins [29, 30]. The protection provided by *H. pylori* infection in our study appeared to primarily affect hypochlorhydric individuals. This suggests that the protective effect is small and perhaps detectable only in those at increased risk for gastroenteritis, such as individuals with diminished gastric acidity. The small number of hypochlorhydric subjects in our study, however, precluded us from

further exploring the effect heterogeneity of *H. pylori* infection or the effect of proton pump inhibitor use.

Our study was limited by the lower than expected occurrence of symptoms and diarrhea following ingestion of EPEC. In prior ingestion studies, EPEC, an acid-sensitive pathogen, was administered in an alkaline solution of sodium bicarbonate to counteract the protection afforded by gastric acid [17–19]. This method was not possible in our study because we wished to separate the effect of *H. pylori* infection from the effect of gastric acidity on susceptibility to EPEC infection. At the higher dose of EPEC, we continued to observe diarrhea in only a minority of subjects, but a subset experienced significant discomfort. Higher doses in prior studies also caused severe illness [18]. These safety concerns prevented us from further increasing the dose of EPEC. Although power to detect our primary outcome may have been limited, the findings regarding our secondary outcome were at a significance level that is unlikely to be due to chance. Our findings run contrary to and question the prevailing dogma that *H. pylori* increases the risk of gastroenteritis, at least among adults.

Interpretation of our results needs to take into account that both diarrheal diseases and *H. pylori* infection are more commonly found in groups of lower socioeconomic status [31–33]. *H. pylori*-infected individuals may be more likely to have been exposed to EPEC in the past. Existing but undetected EPEC immunity would reduce the likelihood of gastroenteritis in *H. pylori*-infected subjects and confound the study results. Although there are at least 12 serogroups of EPEC that cause diarrheal diseases in the world [34], prior EPEC infection provides no protection against reinfection with a heterologous strain. With the same strain, however, disease is milder and subjects have higher baseline IgG titers, suggesting that pre-existing EPEC immunity confers protection [25]. EPEC O111:NM is the most common cause of diarrhea in some parts of the world such as Brazil and Iran [34, 35]. The epidemiology of this strain in other countries is less well characterized, but it is possible that some of our *H. pylori*-infected subjects of Hispanic ethnicity were previously exposed. At baseline, all of our subjects had no EPEC O111:NM in their stools and negative serology. Both *H. pylori*-positive and *H. pylori*-negative groups also had similar baseline EPEC IgG titers, which would argue against increased exposure to the study strain in the *H. pylori*-infected subjects. Ultimately, however, because our assay may not identify all prior exposure to EPEC O111:NM, we cannot completely exclude the possibility that pre-existing immunity in the *H. pylori*-infected subjects protected that group preferentially against diarrhea.

It is unlikely that differential reporting of symptoms caused the observed differences in severity of gastroenteritis between the study groups. Although subjects were informed of their *H. pylori* infection status, they did not know the study hypothesis. Furthermore, we originally hypothesized that *H. pylori*-infected subjects would be more likely to develop symptoms of gastroenteritis, which was contrary to our findings. The possibility of differential reporting of symptoms due to the subjects' racial backgrounds was also addressed. As is typical in the USA, *H. pylori*-positive subjects in our study were more likely to be Hispanic than were *H. pylori*-negative subjects. However, on bivariate and multivariate logistic regression we found that race had no effect on gastroenteritis.

Ultimately, however, it is possible that some other unknown factor may have led to differential reporting of symptoms.

Given its home at the gateway to the intestinal tract, *H. pylori* could theoretically influence the incidence of diarrheal disease. Whether it actually does so, however, remains unclear. *H. pylori* is a pathogen that causes variable amounts of inflammation and hypochlorhydria depending on age at infection acquisition and host and bacterial genetics. In the end, we believe this variability limited our ability to identify differences in response to EPEC. Our study, however, suggests that any impact of *H. pylori* on risk of diarrhea is not large and certainly not as potent as the presence of gastric hypochlorhydria. Although *H. pylori* infection was associated with hypochlorhydria, which increased the risk of diarrhea, it had no additional effect on the occurrence of diarrhea. Moreover, if any trend was observed, it refuted our original hypothesis, i.e., *H. pylori* infection was not associated with risk of diarrhea independent of hypochlorhydria, but in those who developed gastroenteritis after EPEC challenge, *H. pylori* was associated with less severe disease.

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Table 1
Participant characteristics and intervention received

	H. pylori negative (*N* = 26)	*H. pylori* positive (*N* = 19)	*P*-value[a]
Age [mean (SD)], years	48.1 (7.3)	49.4 (6.7)	0.56
Male [*N* (%)]	15 (57.7)	15 (78.9)	0.14
Race [*N* (%)]			<0.001
White	16 (61.5)	1 (5.3)	
Hispanic	5 (19.2)	12 (63.2)	
Black	2 (7.7)	3 (15.8)	
Asian	2 (7.7)	2 (10.5)	
Multiracial	1 (3.8)	1 (5.3)	
Mean body mass index, kg/m^2	27.0 (4.5)	28.7 (3.8)	0.21
Gastric pH [median (range)]	1.5 (1.1–6.8)	1.6 (1.2–7.5)	0.08
Hypochlorhydric[b] [*N* (%)]	2 (7.7)	7 (36.8)	0.02
PPI recipient [*N* (%)]	3 (11.5)	3 (15.8)	0.68
With EPEC dose [*N* (%)]			0.02
5×10^8 organisms	16 (61.5)	5 (26.3)	
1×10^9 organisms	10 (38.5)	14 (73.7)	

PPI proton pump inhibitor, *EPEC* enteropathogenic *Escherichia coli*

[a] *t*-Test for difference of means; chi-square test for differences of proportions

[b] pH >4 more than 25% of the time

Table 2

Diarrhea and severity of gastroenteritis following EPEC ingestion for all 45 study subjects

	***H. pylori* negative [*N* = 26]**	***H. pylori* positive [*N* = 19]**	***P*-value[a]**
Developed diarrhea [*N* (%)]	5 (19.2)	6 (31.6)	0.34
Developed any gastroenteritis symptoms [*N* (%)]	18 (69.2)	14 (73.7)	0.74
Reported any symptoms[b]	15 (57.6)	9 (47.3)	0.49
Developed any loose stool	10 (38.5)	9 (47.4)	0.55
Median (range) composite gastroenteritis severity score[c]	3.0 (0–43.5)	3.0 (0–15.0)	0.95[d]

[a] Chi-square test or Fisher's exact test except as noted

[b] Malaise, headache, nausea, or abdominal pain

[c] Sum of independent scores for malaise, headache, nausea, vomiting, and loose stool during 48 h

[d] Wilcoxon rank-sum test

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Table 3

Odds ratios for diarrhea following EPEC ingestion for all 45 study subjects

Diarrhea	Unadjusted			Adjusted[a]		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
H. pylori infection	1.94	(0.49–7.66)	0.34	1.22	(0.19–7.69)	0.83
EPEC dose[b]	1.25	(0.93–1.68)	0.13	1.23	(0.87–1.73)	0.24
Hypochlorhydria	6.25	(1.29–30.35)	0.02	4.89	(0.85–28.19)	0.08
Hispanic versus other	1.08	(0.27–4.44)	0.91	0.50	(0.08–3.27)	0.47
PPI	3.88	(0.65–22.96)	0.14			

EPEC enteropathogenic *Escherichia coli*, *PPI* proton pump inhibitor

[a] Multivariate logistic regression model including terms *H. pylori* infection, hypochlorhydria, race/ethnicity, and EPEC dose

[b] Inoculation with 1×10^9 versus 5×10^8 EPEC organisms

Table 4

Composite gastroenteritis severity scores (sum of independent scores for malaise, headache, nausea, vomiting, and loose stools) for only symptomatic subjects

	***H. pylori* negative [*N* = 18]**	***H. pylori* positive [*N* = 14]**	***P*-value**
Severity score [median (range)]	4.3 (1–43.5)	4.0 (1–15.0)	0.77[a]
Normalized severity score[b]			
Unadjusted [mean (95% CI)]	1.6 (1.1–2.0)	1.5 (1.0–1.9)	0.66[c]
Adjusted[d] [mean (95% CI)]	2.8 (1.9–3.4)	1.5 (1.1–1.9)	0.007
For normal gastric acidity subjects	1.6 (1.1–1.8)	1.1 (0.6–1.7)	0.17
For hypochlorhydric subjects	4.1 (2.3–5.3)	1.9 (1.3–2.5)	0.01

	Normal gastric acidity [*N* = 25]	**Hypochlorhydria [*N* = 7]**	***P*-value**
Unadjusted [mean (95% CI)]	1.3 (1.0–1.7)	2.2 (1.3–3.0)	0.02[c]
Adjusted[e] [mean (95% CI)]	1.3 (1.0–1.6)	2.9 (2.0–3.7)	<0.001

[a] Wilcoxon rank-sum test

[b] Log-transformed severity score

[c] *t*-Test for difference of means

[d] Adjusting for hypochlorhydria, race/ethnicity, and EPEC dose in analysis of covariance

[e] Adjusting for *H. pylori* infection, race/ethnicity, and EPEC dose in analysis of covariance