

High Seroprevalence of *Helicobacter pylori* and CagA/VacA Virulence Factors in Northern Central America

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Abstract

Background: Northern Central America is unique in the Western Hemisphere, with a high incidence of gastric cancer, low/middle-income country (LMIC) status, and a substantial emigration to the United States. The two primary *Helicobacter pylori* (*H. pylori*) virulence factors related to carcinogenesis are cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA). The prevalence of these factors may help delineate gastric cancer risk in the region. We aimed to characterize the *H. pylori* seroprevalence and virulence factors in two Central American Countries (Honduras and Guatemala).

Methods: Healthy volunteers from Western Honduras and Central-Western Guatemala were recruited and tested for antibodies against

13 *H. pylori* antigens using a novel multiplex serology assay. *H. pylori* seropositivity was defined as positivity for ≥ 4 antigens, and active infection was defined as positivity for a combination of 2/4 antigens: VacA, GroEl, HcpC, and HP1564, based upon the literature. Multivariate logistic regression models were used to estimate the odds ratios for the association between *H. pylori* and CagA positivity.

Results: A total of 1,143 healthy adults were tested using the *H. pylori* multiplex serology assay (444 in Guatemala and 699 in Honduras). Mean age was 54.2 ± 14.5 years, 46.2% were male, 60% were from rural settings, and 56% lived $> 1,000$ meters above sea level. *H. pylori* prevalence was 87%, and 83% with active infection. The CagA and VacA seropositivity rates were 82% and 75%, respectively. No significant differences were noted according to country, age group, sex, or rural/urban location. None of the socioeconomic variables were significantly associated with the presence of *H. pylori* or CagA.

Conclusions: A high prevalence of *H. pylori*, CagA, and VacA is observed in Honduras and Guatemala, with implications for Northern Central America and immigrants from the region. Innovative and resource-appropriate primary and secondary prevention programs are needed.

Keywords: Gastric cancer; *Helicobacter pylori*; CagA; VacA; Central America

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Introduction

The infection-associated cancers, gastric and cervical cancers, are the leading cancers in Northern Central America. Northern Central America is unique in the Western Hemisphere with a high incidence and mortality of gastric cancer, low/middle-income country (LMIC) status, and a high US immigrant population [1-3]. Central America has high incidence rates, including Guatemala and Honduras, with an estimated 12.2 and 8.3 cases per 100,000 individuals, respectively, although data are limited given the lack of population-based cancer registries (PBCRs) in the region [4, 5]. Chronic infection with *Helicobacter pylori* (*H. pylori*), the strongest risk factor for gastric adenocarcinoma, has an estimated prevalence of 70% in the two nations [6, 7].

H. pylori strains are genetically diverse, and variations in bacterial virulence factors correlate with the development of

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gastric adenocarcinoma. Cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) are the principal virulence factors associated with mucosal inflammation and carcinogenesis in humans [8-11]. In addition to strain-specific factors, host characteristics such as age, gender, germline mutations (e.g., cytokine polymorphisms), environmental influences (e.g., tobacco, diet, wood cookstove use), and coinfection (e.g., Epstein-Barr virus (EBV)) further delineate risk [12-14].

The *H. pylori* CagA protein is translocated into host cells by the type IV Cag secretion system after bacterial attachment. CagA interacts with phosphatidylserine and plays an important role in *H. pylori* infection [8, 10]. Inside the host cell, CagA is phosphorylated and induces morphological changes in gastric cells. Although all *H. pylori* strains induce gastritis, strains containing CagA augment the risk of severe chronic gastritis, multifocal atrophic gastritis, gastric intestinal metaplasia, and non-cardia gastric adenocarcinoma [8, 11, 15]. Approximately 85-100% of patients with duodenal ulcers have CagA+ strains, compared to 30-60% of infected patients who do not develop ulcers [16] and 63% of gastric cancer patients have CagA+ strains compared to 37.5% in controls [15]. The VacA toxin is a passive urea transporter that increases the permeability of the gastric epithelium to urea, thereby creating a favorable environment for *H. pylori* to live and reproduce. VacA toxin has also been shown to injure human gastric cells both *in vitro* and *in vivo*. CagA and VacA act synergistically; even though all *H. pylori* contain the gene coding for VacA, only those strains with the Cag pathogenicity island, including CagA, express the VacA toxin [11, 17]. Both CagA and VacA toxins are antigenic, can be measured in serum, and represent more specific biomarkers for gastric cancer prevention.

Few studies have described the prevalence of CagA and VacA virulence factors in the LMIC setting. Population studies describing the demographic and socioeconomic characteristics associated with virulent *H. pylori* strains are also scarce. This study evaluated the seropositivity of *H. pylori* in healthy individuals in Northern Central America and explored the demographic and socioeconomic characteristics of individuals harboring CagA/VacA-positive strains.

Materials and Methods

Study design and setting

This study used data from two population-based studies conducted in Guatemala (2016) and Honduras (2006 - 2012), two countries representative of the Central America Four ("CA-4") region (Guatemala, Honduras, El Salvador, Nicaragua). The CA-4 is the largest LMIC region in the Western Hemisphere, with over 44 million inhabitants, which is linked to more than 5 - 8 million US immigrants [18]. The majority of the CA-4 population has Hispanic Mestizo ancestry 90-95%. In the areas of the study, Guatemala has a predominant Mayan-indigenous population in the Western highlands, and Honduras has a significant Maya-Cho'rti' and Maya-Lenca population in the West and South regions [19]. The region has one of the highest gastric cancer incidence rates in the Western Hemisphere [20].

Healthy volunteers from Guatemala and Honduras were invited to participate. Research staff obtained informed consent from all the participants, administered the questionnaires, and performed a clinical evaluation. The questionnaire included questions on demographic variables, dietary habits, alcohol consumption, smoking, occupation, household characteristics, family history, and past medical history. Participants also underwent anthropometric measurements (height, weight, body mass index (BMI)), blood pressure assessment, and attainment of blood samples.

In Guatemala, participants were recruited in 2016 from five communities located in the Central and Western regions: Chichicastenango, Escuintla, Mixco, San Lucas Toliman, and San Pablo Jocopilas. In Honduras, participants were recruited from three departments in the Western region: Copan, Ocotepeque, and Lempira, between 2006 and 2012 [21]. Two locations in Guatemala (Escuintla and Mixco) were considered urban, and the remaining six locations were considered rural [22] (Fig. 1).

Healthy volunteers were approached at the household level. Community maps were used to select households. In Guatemala, up to two non-genetically related persons per household were invited to participate in the study. In Honduras, every fifth household was chosen in two dozen villages in West-Central Honduras; the first appearing adult was invited to participate. The minimum ages of participation were 40 and 18 in Guatemala and Honduras, respectively. Exclusionary criteria included individuals with gastric cancer, peptic ulcer disease, prior abdominal surgery, and significant co-morbidities. Households were classified as urban or rural based on neighborhood characteristics. Alcohol consumption was defined as drinking ≥ 7 drinks per week in women and ≥ 14 drinks per week in men. Tobacco use was defined as individuals that have smoked at least 100 cigarettes or cigars in their lifetime. Antibiotic exposure was defined as the use of any antibiotics for any reason in the last 6 months. Additional details of the sampling methodology have been provided in previous publications [21, 23].

Laboratory testing

H. pylori seropositivity was determined using multiplex serology performed at the German Cancer Research Center in Heidelberg, Germany. The *H. pylori* multiplex serology assay measures antibodies against 13 *H. pylori* antigens (GroEl, UreA, HP0231, HP0305, NapA, HpaA, CagA, VacA, HcpC, HP1564, Catalase, Cad, and HyuA) measured by glutathione-S-transferase (GST)-tag fusion protein, which was performed based on published methodology [24, 25]. *H. pylori* positivity was defined as positivity for ≥ 4 antigens, and active infection was defined as the presence of two out of the four antigens: VacA, GroEl, HcpC, and HP1564 [24, 26]. Individuals who were seropositive for *H. pylori* were subdivided into those who expressed CagA antigens (CagA+) and those without CagA antigens (CagA-).

Statistical analysis

Descriptive statistics were calculated for each country and overall. The BMI values had a skewed distribution and were

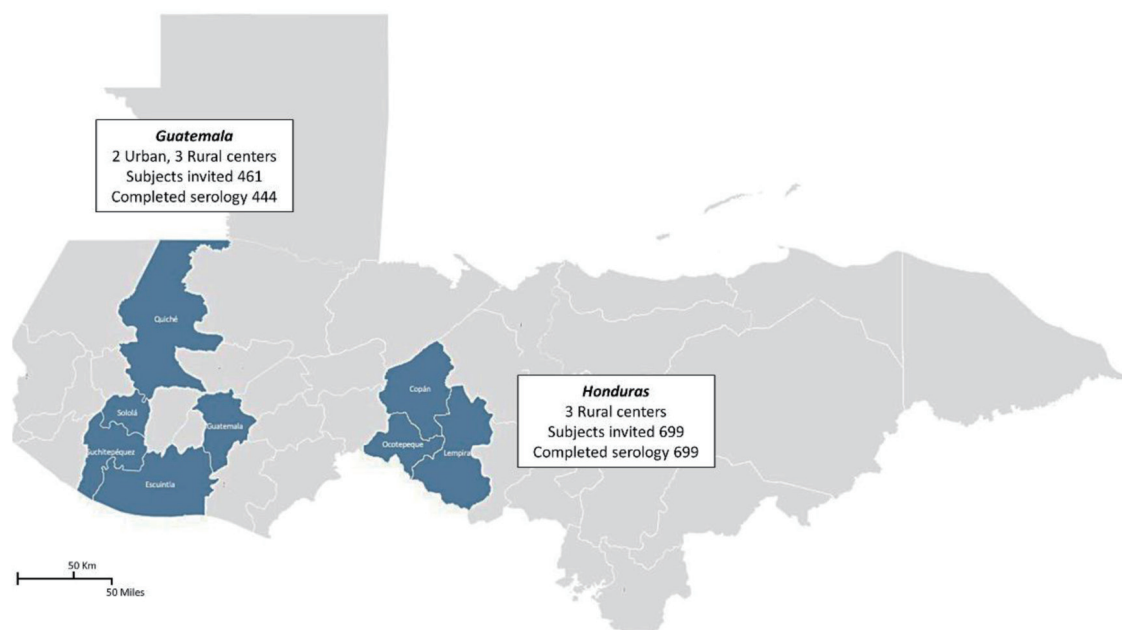


Figure 1. Sampling locations and participant recruitment across Northern Central America. This figure depicts the distribution and response rates of the subjects invited to participate in the study conducted in Northern Central America. In total, 1,143 participants were invited from various urban and rural centers within Guatemala and Honduras. The map highlights Guatemala with two urban centers and three rural centers, where 444 of the 461 subjects completed the serology tests. Similarly, in Honduras, all 699 invited subjects from three rural centers completed the tests. The shaded areas indicate the regions where the subjects were sampled, and the details provided within the insets specify the number of participants and completion rates of serological testing.

reported as median values with interquartile ranges (IQRs). Age was analyzed both as a continuous variable and as a categorical variable by decade. The primary analysis compared three groups based on multiplex serology: *H. pylori*-, *H. pylori*+ CagA-, and *H. pylori*+ CagA+. Analysis was performed with all participants who met the inclusion criteria, as well as for patients ≥ 40 years old (with exclusion of Honduras participants aged 18 - 39). The Pearson Chi-squared test and two-sample *t*-test were used to calculate differences in distributions between groups for categorical and continuous variables, respectively.

A secondary exploratory analysis was performed comparing three groups (*H. pylori*-, *H. pylori*+ CagA-, and all individuals CagA-) with individuals *H. pylori*+ CagA+, to identify any demographic and socioeconomic variables associated with CagA positivity. Univariate logistic regression was used to screen for any socioeconomic and clinical characteristics associated with CagA positivity, with a P-value < 0.1 . Using only the variables considered to be associated with CagA positivity in the univariate analysis, multivariate models were computed to obtain adjusted odds ratios (ORs) for CagA positivity (the covariates included were age, sex, BMI, altitude, smoking, access to a refrigerator, and access to an electric or gas stove).

STATA software (STATA Corp, College Station, TX, USA) was used for statistical analysis. All tests for significance were two-sided. This study was conducted and reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for observational research. All the participants provided informed consent. This

study was conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol and questionnaires were approved by the respective IRBs: Guatemala (#053-2015 Institute of Nutrition of Central America and Panama in Guatemala City, and #6877 Johns Hopkins Bloomberg School of Public Health in Baltimore, MD), Honduras (#IRB00011680 - IRB Western Hospital IRB FWA 00027925; UAB IRB 300003672), and analysis of de-identified information from both countries (exempt status 45 CFR 46.101(b)(4) Presbyterian Healthcare Services in Albuquerque, NM).

Results

Overall, 1,143 adults were enrolled in the studies and tested using the *H. pylori* multiplex assay (Guatemala, $n = 444$; Honduras, $n = 699$). The mean age was 54.2 ± 14.5 years and 46.2% were male. Nearly two-thirds of the participants (60%) lived in rural settings and 56% lived $> 1,000$ meters above mean sea level (mamsl). The most common occupations were domestic work (40%) and agriculture (19%). Approximately 53% ($n = 608$) of the individuals had a refrigerator, and 33.9% ($n = 387$) had an electric or gas stove. The percentage of underweight patients (BMI < 18.5) was 3.2%, while 23.5% were obese (BMI > 30). Twenty-seven percent ($n = 310$) were tobacco smokers and 43% ($n = 488$) consumed alcohol. The participants' characteristics were similar in both countries, with only minor

Table 1. Socioeconomic, Clinical, and Related Characteristics of Study Participants

	Guatemala	Honduras	Total
N	444	699	1,143
Age, mean (SD)	55.4 (10.7)	53.4 (16.5)	54.2 (14.5)
Age categories			
18 - 45	90 (20.3%) ^a	219 (31.3%)	309 (27.0%)
45 - 54	138 (31.1%)	159 (22.7%)	297 (26.0%)
55 - 64	122 (27.5%)	147 (21.0%)	269 (23.5%)
> 65	94 (21.2%)	174 (24.9%)	268 (23.4%)
Sex			
Female	255 (57.4%)	360 (51.5%)	615 (53.8%)
Male	189 (42.6%)	339 (48.5%)	528 (46.2%)
Rural setting	272 (61.3%)	411 (58.8%)	683 (59.8%)
Altitude (mamsl), mean (SD)	1,250.8 (644.6)	983.4 (209.2)	1,087.9 (453.7)
Altitude > 1,000 mamsl	272 (61.3%)	369 (53.3%)	641 (56.4%)
Occupation			
Professional	9 (2.0%)	29 (4.1%)	38 (3.3%)
Technician	2 (0.5%)	37 (5.3%)	39 (3.4%)
Artisan or vendor	182 (41.0%)	57 (8.2%)	239 (20.9%)
Construction	21 (4.7%)	23 (3.3%)	44 (3.8%)
Agriculture	54 (12.2%)	167 (23.9%)	221 (19.3%)
Housewife	144 (32.4%)	313 (44.8%)	457 (40.0%)
Other or not available	32 (7.2%)	73 (10.4%)	105 (9.2%)
BMI, median (IQR)	27.4 (23.9 - 30.7)	24.8 (21.9 - 28.7)	25.7 (22.7 - 29.7)
BMI categories			
Underweight (BMI < 18.5)	5 (1.1%)	31 (4.5%)	36 (3.2%)
Normal (18.5 - 24.9)	148 (33.6%)	323 (47.3%)	471 (41.9%)
Overweight (25 - 29.9)	155 (35.2%)	197 (28.8%)	352 (31.3%)
Obese (> 30.0)	132 (30.0%)	132 (19.3%)	264 (23.5%)
Current tobacco smoker ^b	196 (44.1%)	114 (16.3%)	310 (27.1%)
Recent use of antibiotics ^c	55 (12.4%)	23 (3.3%)	78 (6.8%)
Refrigerator	200 (45.0%)	408 (58.4%)	608 (53.2%)
Alcohol consumption ^d	321 (72.3%)	167 (23.9%)	488 (42.7%)
Electric or gas stove	265 (59.7%)	122 (17.5%)	387 (33.9%)
<i>H. pylori</i> serostatus (positive)	387 (87.2%)	606 (86.7%)	993 (86.9%)
<i>H. pylori</i> active infection	378 (85.1%)	571 (81.7%)	949 (83.0%)
CagA status (positive)	402 (90.7%)	539 (77.1%)	941 (82.4%)
VacA status (positive)	368 (83.1%)	493 (70.5%)	861 (75.4%)
CagA and VacA positive	397 (89.4%)	553 (79.1%)	950 (83.1%)

^aIn Guatemala, all these individuals were 40 - 45 years old. ^bTobacco use was defined as an adult who has smoked at least 100 cigarettes in his or her lifetime. ^cAntibiotic exposure was defined as any antibiotics for any reason in the last 6 months. ^dAlcohol consumption was defined as at least 12 drinks in any one year in lifetime. BMI: body mass index; CagA: cytotoxin-associated gene A; IQR: interquartile range; mamsl: meters above mean sea level; SD: standard deviation; VacA: vacuolating cytotoxin A.

differences. Recent antibiotic use, access to a refrigerator, alcohol consumption, and tobacco smoking were more frequent in Guatemala (3.7, 3.4, 3.0, and 2.7 times higher, respectively) compared to Honduras (Table 1).

H. pylori positive serostatus was 86.9% (n = 993), and active *H. pylori* infection was observed in 83.0% (n = 949) of participants. CagA and VacA seropositivities were 82.4% (n = 941) and 75.4% (n = 861), respectively. In these prevalence

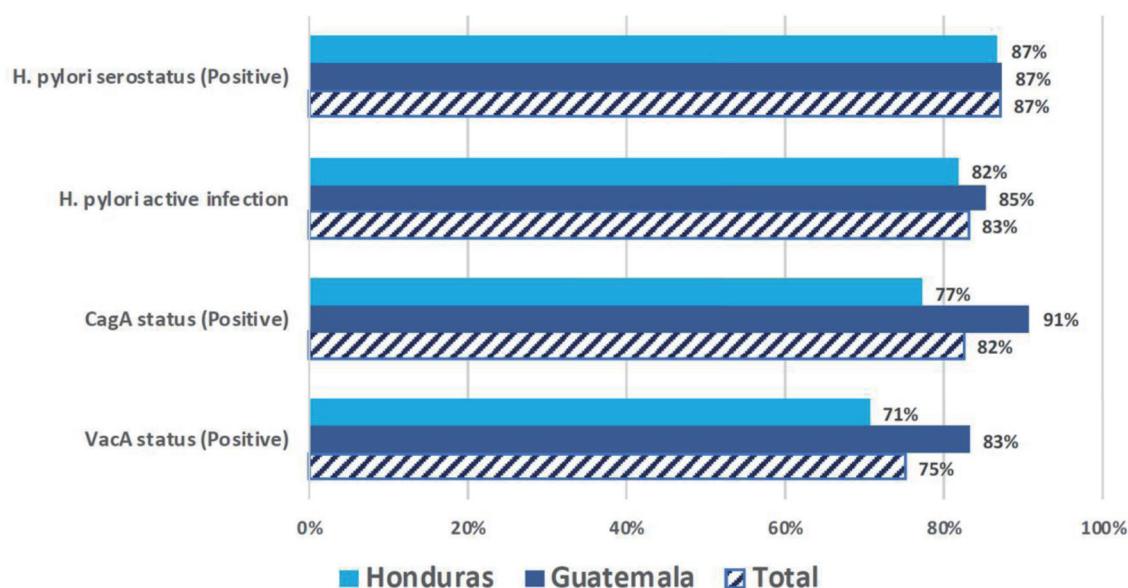


Figure 2. Prevalence of *H. pylori*, CagA, and VacA seropositivity by country (N = 1,143). This figure presents the prevalence rates of *H. pylori* serostatus and associated virulence factors among the populations sampled in Honduras and Guatemala. The bar chart compares the positive rates of *H. pylori* serostatus, active infection, and the presence of CagA and VacA virulence factors between the two countries as well as the total combined prevalence.

estimates, there were no significant differences between countries (Fig. 2).

The majority of participants who were *H. pylori* seropositive harbored a CagA+ strain (86.7%). No significant differences in *H. pylori* prevalence or CagA positivity were observed among the seven age groups (Fig. 3). Socioeconomic

characteristics were similar across participants of the three serological groups (*H. pylori*-, *H. pylori*+ CagA-, and *H. pylori*+ CagA+), with several exceptions (Table 2). Smoking was more common among *H. pylori* CagA+ individuals. Participants with CagA+ *H. pylori* were less likely to have a refrigerator compared to *H. pylori* CagA- and seronegative participants.

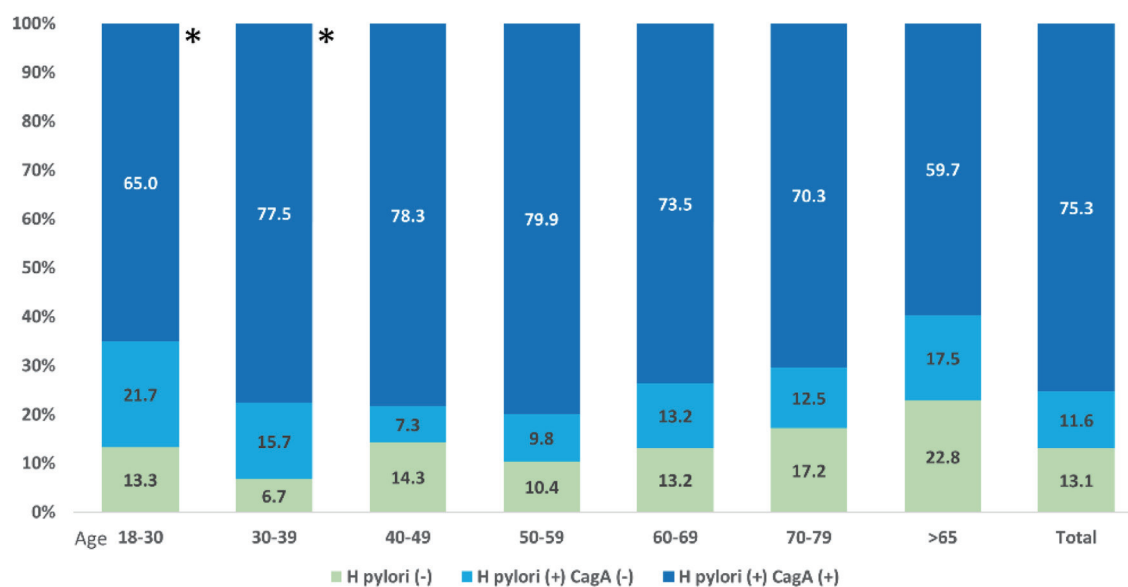


Figure 3. *H. pylori* and CagA seropositivity by age groups (N = 1,143). This figure illustrates the distribution of *H. pylori* and CagA seropositivity across different age groups among the 1,143 individuals sampled. The stacked bar chart shows the percentages of participants who were *H. pylori*-positive with and without CagA positivity (shown in darker and lighter shades of blue, respectively) and those who were *H. pylori*-negative (shown in green). Age groups were segmented as 18 - 30, 30 - 39, 40 - 49, 50 - 59, 60 - 69, and over 65 years (*Honduras participants only).

Table 2. Socioeconomic and Clinical Characteristics Associated With CagA Positivity (N = 1,143)

	<i>H. pylori</i> (-)	<i>H. pylori</i> (+) CagA (-)	<i>H. pylori</i> (+) CagA (+)	P-value
N	150	132	861	
Age, mean (SD)	56.6 (15.7)	54.4 (16.8)	53.7 (13.8)	0.08
Age categories				0.13
18 - 45	36 (24.0%)	39 (29.5%)	234 (27.2%)	
45 - 54	34 (22.7%)	28 (21.2%)	235 (27.3%)	
55 - 64	32 (21.3%)	31 (23.5%)	206 (23.9%)	
> 65	48 (32.0%)	34 (25.8%)	186 (21.6%)	
Sex				0.61
Female	78 (52.0%)	76 (57.6%)	461 (53.5%)	
Male	72 (48.0%)	56 (42.4%)	400 (46.5%)	
Rural setting	91 (60.7%)	77 (58.3%)	515 (59.8%)	0.92
Altitude (mamsl), mean (SD)	1,079.3 (425.0)	1,031.2 (338.5)	1,097.9 (473.1)	0.29
Altitude > 1,000 mamsl	81 (54.4%)	82 (63.1%)	478 (55.8%)	0.25
BMI, median (IQR)	25.5 (22.1 - 28.9)	25.4 (22.3 - 30.3)	25.7 (22.8 - 29.7)	0.29
BMI categories				0.054
Underweight (BMI < 18.5)	10 (6.8%)	5 (3.8%)	21 (2.5%)	
Normal (18.5 - 24.9)	57 (38.8%)	56 (43.1%)	358 (42.3%)	
Overweight (25 - 29.9)	54 (36.7%)	36 (27.7%)	262 (31.0%)	
Obese (> 30.0)	26 (17.7%)	33 (25.4%)	205 (24.2%)	
Current tobacco smoker	37 (24.7%)	25 (18.9%)	248 (28.8%)	0.046*
Recent use of antibiotics	15 (10.0%)	5 (3.8%)	58 (6.7%)	0.12
Refrigerator	87 (58.0%)	82 (62.1%)	439 (51.0%)	0.026*
Alcohol consumption	66 (44.0%)	46 (34.8%)	376 (43.7%)	0.15
Electric or gas stove	63 (42.0%)	34 (25.8%)	290 (33.7%)	0.016*
<i>H. pylori</i> active infection	27 (18.0%)	109 (82.6%)	813 (94.4%)	< 0.001*
VacA status (positive)	36 (24.2%)	53 (40.2%)	772 (89.7%)	< 0.001*

*P < 0.05. BMI: body mass index; CagA: cytotoxin-associated gene A; mamsl: meters above the mean sea level; SD: standard deviation; VacA: vacuolating cytotoxin A.

Individuals with *H. pylori* CagA+ were less likely to have an electric or gas stove than *H. pylori* seronegative participants. Analyses excluding Honduran participants 18 - 39 years old (N = 994, Supplementary Material 1, gr.elmerpub.com) showed only minor differences. Individuals with *H. pylori*+ CagA+ strains were significantly younger than the other two groups (mean age was 59.5, 60.6, and 57.0 years, respectively, in *H. pylori*- individuals, *H. pylori*+ CagA-, and *H. pylori*+ CagA+; P = 0.02). Antibiotic exposure was significantly lower in *H. pylori*+ CagA+ individuals (59.6% for *H. pylori*+, 63.8% for *H. pylori*+ CagA-, and 50.9% for *H. pylori*+ CagA+; P = 0.014).

In the unadjusted analyses, advanced age, low BMI, access to household appliances (refrigerator and gas/electric stoves) were inversely associated with CagA positivity (Table 3). Tobacco use was directly associated with CagA positivity. In multivariate analyses, both low BMI (< 18.5) and lack of a household refrigerator were significantly and independently associated with increased odds of *H. pylori* CagA+ status. Compared to individuals who were *H. pylori*-negative, underweight

participants had a lower likelihood of being CagA+ (adjusted OR (aOR) 0.37, 95% confidence interval (CI): 0.16 - 0.84, P = 0.018), as did those with both *H. pylori* and CagA negativity (aOR 0.47, 95% CI: 0.23 - 0.96, P = 0.039). Similarly, participants without a refrigerator had higher odds of CagA positivity when compared to *H. pylori*-negative individuals (aOR 0.56, 95% CI: 0.38 - 0.83, P = 0.004) and those negative for both *H. pylori* and CagA (aOR 0.67, 95% CI: 0.50 - 0.89, P = 0.006). Access to an electric or gas stove was inversely associated with CagA positivity (compared to *H. pylori*- individuals, aOR 0.65 (0.45 - 0.95)). Smoking was positively associated with CagA positivity (compared to *H. pylori*+ CagA- individuals, aOR 1.44 (1.04 - 1.99)).

Discussion

We observed an enduring high prevalence of *H. pylori* infection and CagA positive strains in the LMICs of Northern Cen-

Table 3. Exploratory Univariate and Multivariate Analyses of Socioeconomic and Clinical Characteristics Associated With CagA Positivity (N = 1,143)

	<i>H. pylori</i> (-) vs. CagA (+)			CagA (-) vs. CagA (+)			<i>H. pylori</i> (-) and CagA (-) vs. CagA (+)		
	Unadjusted OR	Adjusted OR	P-value	Unadjusted OR	Adjusted OR	P-value	Unadjusted OR	Adjusted OR	P-value
	OR (95% CI)	OR (95% CI)		OR (95% CI)	OR (95% CI)		OR (95% CI)	OR (95% CI)	
Age									
45 - 54	1.06 (0.64 - 1.76)	1.12 (0.67 - 1.87)	0.65	1.39 (0.93 - 2.34)	1.16 (0.67 - 1.98)	0.21	1.21 (0.82 - 1.78)	1.19 (0.81 - 1.76)	0.37
55 - 64	0.99 (0.59 - 1.65)	1.09 (0.64 - 1.84)	0.75	1.1 (0.66 - 1.83)	0.89 (0.51 - 1.53)	0.69	1.04 (0.71 - 1.53)	1.05 (0.71 - 1.55)	0.80
> 65	0.59 (0.37 - 0.96)	0.65 (0.39 - 1.05)	0.081	0.91 (0.55 - 1.50)	0.79 (0.46 - 1.37)	0.72	0.72 (0.50 - 1.05)	0.74 (0.51 - 1.08)	0.13
Sex (male)	0.94 (0.66 - 1.33)	-	-	1.17 (0.81 - 1.70)	0.96 (0.61 - 1.50)	0.39	1.04 (0.79 - 1.36)	-	-
BMI									
Underweight	0.33 (0.14 - 0.74)	0.37 (0.16 - 0.84)*	0.018*	0.65 (0.23 - 1.81)	0.42	0.42	0.44 (0.22 - 0.88)	0.47 (0.23 - 0.96)*	0.039*
Overweight	0.77 (0.51 - 1.15)	0.80 (0.52 - 1.21)	0.29	1.13 (0.72 - 1.78)	0.57	-	0.91 (0.66 - 1.26)	0.94 (0.68 - 1.31)	0.75
Obese	1.25 (0.76 - 2.05)	1.29 (0.78 - 2.17)	0.32	0.97 (0.61 - 1.54)	0.90	0.90	1.09 (0.76 - 1.56)	1.11 (0.77 - 1.61)	0.56
Altitude (> 1,000 mamsl)	1.06 (0.75 - 1.50)	-	-	0.73 (0.50 - 1.08)	0.11	0.75 (0.51 - 1.10)	0.89 (0.68 - 1.17)	0.44	-
Current smoker	1.24 (0.83 - 1.84)	-	-	1.73 (1.09 - 2.74)	0.019	1.52 (0.91 - 2.54)	1.43 (1.05 - 1.97)	0.026	0.028*
Recent use of antibiotics	0.65 (0.36 - 1.18)	-	-	1.83 (0.72 - 4.66)	0.20	-	0.94 (0.55 - 1.60)	0.84	-
Refrigerator	0.75 (0.53 - 1.07)	-	-	0.63 (0.43 - 0.92)	0.018	0.56 (0.38 - 0.83)*	0.69 (0.52 - 0.91)	0.009	0.006*
Alcohol consumption	0.99 (0.69 - 1.39)	-	-	1.44 (0.98 - 2.12)	0.06	-	1.17 (0.89 - 1.54)	0.24	-
Electric or gas stove	0.70 (0.49 - 0.99)	0.65 (0.45 - 0.95)*	0.027*	1.46 (0.96 - 2.21)	0.07	-	0.96 (0.72 - 1.28)	0.83	-
Urban Setting	1.03 (0.72 - 1.47)	-	-	0.94 (0.64 - 1.36)	0.74	-	0.99 (0.75 - 1.30)	0.94	-

Initial univariate analyses were performed with $P < 0.1$. Variables with a significant association were included in the multivariate analysis. *Variables with $P < 0.05$ in multivariate analysis. BMI: body mass index; CagA: cytotoxin-associated gene A; CI: confidence interval; mamsl: meters above mean sea level; OR: odds ratio; VacA: vacuolating cytotoxin A.

tral America. *H. pylori* prevalence was 87% and 83%, overall and with active infection, respectively. The CagA and VacA seropositivity rates were 82% and 75%, respectively. Our results suggest a higher *H. pylori* prevalence than in previous studies in Guatemala (66.8%, N = 6,733) [6] and Honduras (82.6%, N = 259) [7], as well as a higher CagA prevalence compared with a prior report from Guatemala (53%) [27].

Despite the clear association between CagA and VacA strains and human disease, only a small proportion of individuals colonized by CagA+ *H. pylori* develop gastric cancer, which underscores the importance of host and environmental factors in gastric carcinogenesis. We found no statistically significant differences in *H. pylori* infection according to sex, age, BMI, or rural vs. urban residency. In multivariate analyses, refrigerator ownership and underweight status were inversely associated with *H. pylori* CagA+ infection. Compared to *H. pylori*-negative individuals, the aORs were 0.56 (refrigerator) and 0.37 (underweight); compared to individuals negative for both *H. pylori* and CagA, the aORs were 0.67 and 0.47, respectively. No other socioeconomic or clinical characteristics were statistically significant. The current literature suggests that *H. pylori* is acquired via fecal-oral or oral-oral transmission during childhood [5, 28]. We had hypothesized that improved living conditions may lower *H. pylori* seropositivity with a smaller proportion of CagA+ strains in younger participants; however, no significant trends were observed among the age groups.

Guatemala and Honduras are primarily populated by Mayan-Mestizo populations, with modest ethnic differences [19]. The population in western regions in Honduras includes populations of Maya-Cho'rti and Maya-Lenca groups. This ethnic composition is similar to that of the Guatemalan Western highlands, and yet the plethora of Mayan indigenous groups in the Highlands are well recognized. The diets in the rural areas are primarily based on maize and beans, and similar environmental exposures have been reported in rural areas (e.g., woodstove cooking) [29, 30]. We did not identify significant differences in *H. pylori* prevalence, active infection, CagA positivity, or VacA positivity between the two countries. Our findings suggest that the prevalence of *H. pylori* high-risk strains are common to the CA-4 region. We report a higher prevalence of *H. pylori*+ CagA+ strains among smokers and individuals without access to refrigerators.

In endemic *H. pylori* areas with a high incidence of gastric cancer, the confirmation of the high prevalence of high-risk strains may inform prevention strategies. Mass screening and eradication of *H. pylori* should be considered in populations at higher risk of gastric cancer per the Taipei Consensus [31]. Novel strategies would be needed given the lack of resources in the health sector in Northern Central America. Previous geospatial analyses in western Honduras have demonstrated the utility of geospatial analysis to identify hot spots of gastric cancer and high-risk strains [32].

To our knowledge, this is the first large community study to examine the prevalence of *H. pylori* and risk strains in Northern Central America. The age ranges for inclusion in Honduras and Guatemala were modestly different (> 18 and > 40 years old, respectively), but exclusion of the younger in-

dividuals in Honduras did not have a significant effect on the overall results. The novel and validated *H. pylori* multiplex assay was used. This multiplex serology assay has adequate sensitivity (85.7%, 95% CI: 68.5-94.3%) and negative predictive value (97.3%, 95% CI: 93.2-98.9%) when compared to the fecal antigen test [25], and has been validated against enzyme-linked immunosorbent assay, western blot, and urea breath tests [24, 26]. Serologic tests may be less sensitive both with decreased *H. pylori* burden (e.g., intestinal metaplasia, cancer) or with decreased immune response (e.g., age, advanced cancer), which were not significant factors in our study.

Despite adequate test performance, this assay has limitations inherent to antigen testing. Antigen expression can change during the natural history of *H. pylori* infection. As humans develop atrophic gastritis and intestinal metaplasia, the *H. pylori* bacterial load decreases, lowering CagA exposure. However, CagA can still be detected in patients with advanced disease, as observed in patients with gastric adenocarcinoma after tumor resection.

Conclusion

H. pylori infection with high-risk strains remains endemic in the Northern Central America, and supports the reality of the high incidence of gastric cancer in the area. This has implications for cancer control in this LMIC region, wherein the infection-associated cancers, gastric and cervical, continue to define a large proportion of the cancer burden. There are additional implications for Mesoamerica and immigrants to the USA from this region. Novel approaches are imperative in light of the resource limitations in the Ministries of Health.

Supplementary Material

Suppl 1. Socioeconomic and clinical characteristics associated with CagA positivity, individuals 40 years or older (N = 994).

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Conflict of Interest

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflict of interest.

Author Contributions

CSA, DRM, and JEC involved in conceptualization. DAN, DHK, EEMS, ARA, MRZ, and RLD involved in data acquisition. CSA, KAM, TW, and DRM involved in methodology. DAN, CSA, DRM, and JEC involved in data analysis/interpretation. DAN, CSM, DRM, and JEC wrote the original draft. CSA, ARA, MRZ, KAM, RLD, and TW critically reviewed and edited the manuscript.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Abbreviations

BMI: body mass index; CagA: cytotoxin-associated gene A; *H. pylori*: *Helicobacter pylori*; IQR: interquartile range; LMICs: low/middle-income countries; mams: meters above mean sea level; SD: standard deviation; VacA: vacuolating cytotoxin A

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