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THE PREVALENCE AND RISK FACTORS OF *HELICOBACTER PYLORI* INFECTION AND *cagA* VIRULENCE GENE CARRIAGE IN ADULTS IN THE NAVAJO NATION

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Abstract – *Objective:* American Indian and Alaska Native people in the United States experience high rates of stomach cancer. *Helicobacter pylori* infection is a significant risk factor for stomach cancer, and *H. pylori* strains that carry the *cagA* gene are linked to greater gastrointestinal disease severity. Yet, little is known about *H. pylori* and *cagA* infections in American Indian and Alaska Native people, particularly at the tribal level. We assessed the prevalence and risk factors of *H. pylori* infection and *cagA* gene carriage in tribal members from the Navajo Nation.

Patients and Methods: We conducted a cross-sectional study with adults from the Navajo Nation. Stool samples collected from participants were analyzed with droplet digital PCR for *H. pylori* 16S ribosomal and *cagA* virulence genes. Self-administered health and food questionnaires were mailed to participants to collect information on sociodemographic, health, lifestyle, and environmental risk factors for *H. pylori* infection. Logistic regression assessed the association between risk factors and *H. pylori* infection and *cagA* gene carriage.

Results: Among 99 adults, the median age was 45 (age range: 18 to 79 years), and 73.7% were female. About 56.6% (95% CI: 46.2-66.5) of participants were infected with *H. pylori*. Of *H. pylori*-infected participants, 78.6% (95% CI: 65.6-88.4) were *cagA*-gene positive. No significant associations of relevant risk factors with *H. pylori* and *cagA*-gene positive infections were noted.

Conclusions: In a community-based study population, a substantial proportion of adult tribal members had *H. pylori* and *cagA*-gene positive infections. Given these high proportions, culturally appropriate prevention strategies and interventions addressing *H. pylori* infections present an avenue for additional research and stomach cancer prevention in the Navajo Nation.

Keywords: Indigenous, Stomach cancer, Cancer disparities, Risk factors, *Helicobacter pylori*, *cagA*.

INTRODUCTION

A substantial burden of stomach cancer incidence continues to be observed in American Indian and Alaska Native populations in the United States (US)[1,2]. The stomach cancer incidence rate was 1.98 times higher in American Indian and Alaska Native people than in White people (10.4 per 100,000 *vs.* 5.3 per 100,000)[2]. For the largest American Indian tribe in the US, the Navajo Nation is experiencing higher incidence and mortality rates for stomach cancer than

the surrounding White population (Incidence: 15.0 per 100,000 *vs.* 4.3 per 100,000; Mortality: 9.8 per 100,000 *vs.* 2.2 per 100,000)[3]. A contributing factor to this elevated burden of stomach cancer in the Navajo people may be related to a high burden of *Helicobacter pylori* (*H. pylori*) infection, an infectious pathogen that is an important risk factor for stomach cancer[4]. Therefore, identifying the prevalence and risk factors of *H. pylori* and its most important virulence factor, *cagA*, in American Indian people can inform prevention strategies and interventions to reduce the burden of stomach cancer.

H. pylori is a spiral-shaped bacterium that colonizes and infects the stomach lining and causes gastrointestinal diseases, such as gastric ulcers and stomach cancer[5,6]. *H. pylori* infections are predominately acquired during childhood, although transmission can occur at any time during a person's life through person-to-person contact, oral-to-oral or fecal-to-oral routes, consuming contaminated food and/or water[7]. Other known risk factors associated with *H. pylori* infection include older age, lower socioeconomic status, household crowding, and living with someone infected with *H. pylori*[7-14].

The virulence factors of the *H. pylori* strain further determine the development of severe gastrointestinal disease. *H. pylori* strains vary in their production of virulence factors (e.g., CagA, VacA, BabA), which form the causal link between *H. pylori* and stomach cancer[4]. In particular, *H. pylori* strains that contain the cytotoxin-associated antigen gene pathogenicity island (*cag* PAI) encode more than 20 proteins that produced a specialized secretion system that delivers the CagA protein effector and other bacterial metabolites into host cells[15,16]. CagA interrupts normal gastric epithelial cell activity, promotes inflammation, and increases gastrointestinal disease severity, including stomach cancer risk[17,18]. Further, the *cagA* gene has two allele variations: classified as East Asian or Western. The *cagA* gene with the East Asian allele encodes an EPIYA-D motif and is associated with a greater risk of stomach cancer than the *cagA* gene with the Western allele with an EPIYA-C motif[19].

Given the long-standing stomach cancer disparity among American Indian people in the US, gaining insight into the epidemiology of *H. pylori* among American Indian people can provide valuable information for prevention efforts. Therefore, we assessed the prevalence of *H. pylori* and *cagA*-positive infections in adults from the Navajo Nation. We also explored sociodemographic, health, lifestyle, and environmental risk factors associated with *H. pylori* and *cagA*-positive infections[6-9,20]. We hypothesized that adults from the Navajo Nation would have a disproportionately higher prevalence of *H. pylori* and *cagA*-positive infections than White people in the US.

PATIENTS AND METHODS

Study Methods and Participants

A cross-sectional community-based study was conducted from January to November 2021 in two regions of the Navajo Nation: the central and northeast regions. Participants learned about the Navajo ABID (Assessing the gut microbiota and Individual Diet) Study through online and offline recruitment platforms, such as a study website, social media pages (i.e., Facebook and Instagram), newspaper ads, flyers/postcards posted in the community, and in-person community events. Participants opted into the study by contacting the study team by phone or email or completing a participant intake form on the study website. Due to the COVID-19 pandemic, online recruitment platforms were used throughout the study, and offline recruitment platforms were used in the last four months of the study when COVID-19 vaccines were available, and the tribal community lifted physical distancing mandates.

Eligible participants had to identify as a Navajo tribal member, be at least 18 years old, reside in the study regions, not be pregnant, not have used oral or intravenous antibiotics in the past 3 months, not be using proton pump inhibitors, and not be undergoing any cancer treatment. Each eligible participant received a copy of a consent form in either English or Navajo. Those who participated gave verbal consent over the phone and were mailed a study packet containing questionnaires, a stool sample kit, instructions for completing the study packet, and prepaid envelopes to mail back the questionnaires and stool samples to the study group in Seattle, WA.

This study was approved by the Navajo Nation Human Research Review Board (NNR-20.384T) and the University of Washington Human Subjects Division (00011217). Verbal consent from each eligible participant was obtained and recorded by the study group.

Measurement of *Helicobacter pylori* and *cagA* Genotypes with Droplet Digital PCR (ddPCR)

The presence of *H. pylori* (positive or negative) and *cagA* genotypes (positive or negative and EPIYA-C or EPIY-D allele type) were determined from stool samples collected by the participants in their homes. Self-sampling stool collection instructions were provided to participants with the following modifications[21]. Participants placed stool samples (approximately 1 teaspoon) in a vial with 5 mL of 95% ethanol preservative (Fisher Scientific), rather than RNAlater, and stored at room temperature before mailing to the Salama Lab at the Fred Hutchinson Cancer Center (FHCC) in Seattle, WA. Pilot studies showed similar ddPCR assay performance between the two nucleic acid preservatives.

Bacterial DNA was extracted from stool samples using the QIAamp Stool DNA Mini Kit (Qiagen) and analyzed in duplicates using droplet digital Polymerase Chain Reaction (ddPCR) assays according to the manufacturer instructions for the QX200 ddPCR System (BioRad), which have been validated in adult stool in other studies in the Salama Lab[22]. Briefly, 0.5 ml of stool-ethanol slurry was extracted for each subject, yielding concentrations from 5-200 ng/μl stool DNA. We utilized 10 μl stool DNA for duplicate reactions for each sample and assay. *H. pylori* gene detection was not correlated with stool DNA concentration. Separate sample reactions contained probes and primers for *H. pylori* 16S and *cagA*, or for *cagA* EPIYA-C and EPIYA-D genotyping using primers and probes described previously[22]. Droplets were generated using the QX200 Droplet Generator (BioRad). Droplets were then analyzed for fluorescent amplitude using the QX200 Droplet Reader (BioRad). Data were analyzed using the QuantaSoft software version 1.6.6 (BioRad). For quality control, a positive control (stool DNA from a confirmed *H. pylori*-positive volunteer) and a negative control (molecular grade water) were included in each batch of samples analyzed. Additional controls included 0.2 pg/μl genomic DNA from strains EM17-1 (*cagA*+, EPIYA-C), EM41-2 (*cagA*+, EPIYA-D), EM16-4 (*cagA*-) as appropriate[23]. A sample with greater than 5 droplets observed above the recommended fluorescence intensity thresholds for *H. pylori*, *cagA*, and *cagA* EPIYA typing was categorized as positive. The fluorescence intensity threshold values were set to 4500 for *H. pylori* 16S assay, 2000 for the *cagA* assay, and 2000 for the EPIYA-C and EPIYA-D assays[22]. In addition, the thresholds for each assay were visually evaluated by inspecting the amplitude plot of the fluorescence intensity threshold between a positive control sample and the cluster of positive droplets and the cluster of negative droplets, as well as ensuring the threshold was greater than two standard deviations from the negative droplets.

Participant Data Collection

Data on study variables were collected from self-administered health and food questionnaires that were mailed to consented participants. Self-reported sociodemographic characteristics (age, sex, educational attainment); health conditions (i.e., diabetes, family history of stomach cancer, body mass index); medication use (daily aspirin use, monthly over the counter stomach medicine use, monthly vitamin use); lifestyle factors (smoking, alcohol use, physical activity); and environmental exposure (drinking water source) were adapted by prior items developed by Harris et al. and the Centers for Disease Control and Prevention, Behavioral Risk Factor Surveillance Survey[24,25].

The Food Frequency Questionnaire (FFQ), developed by the Nutrition Assessment Shared Resource (NASR) of Fred Hutchinson Cancer Center, Seattle, WA, assessed dietary intake. Participants were mailed an FFQ to report the frequency of consumption and portion size of 181-food items over the last year. Nutrient calculations were performed with the Nutrient Data System for Research software version v2020, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN. The annual intake of each food item was calculated using the frequency of consumption (never or less than once per month, 1 per month, 2-3 per month, 1 per week, 2 per week, 3-4 per week, 5-6 per week, 1 per day, 2+ per day) and portion size (small, me-

dium, large). Three diet variables derived from these data were included in the present analysis: daily sodium intake, daily vegetable intake (including green salad, tomatoes, carrots, peppers, broccoli, cauliflower, cabbages, green beans, peas, corn and hominy, squash, zucchini, yams, sweet potatoes, cooked greens potatoes, coleslaw, tomato juice and other vegetable juice), and daily fruit intake (including apples, applesauce, pears, bananas, peaches, plums, apricots, dried fruits, citrus fruits, berries, melons, orange juice, grapefruit juice, and other 100% fruit juice). Dietary cutoffs were established with the US Department of Agriculture and US Department of Health and Human Services Dietary Guidelines for Americans for daily intakes of vegetables (3.0 cups/day) and fruit (2.0 cups/day)[26]. Sodium daily intake was categorized into a tertile (low, medium, high) based on the sodium daily intake distribution in *H. pylori*-negative participants (≤2,028 mg/day, 2,209-3,765 mg/day, >3,765 mg/day).

Statistical Analyses

The sample size for this study was determined based on practical considerations (cost and feasibility) and formal statistical considerations. We assumed the *H. pylori* prevalence in Navajo adults to be 58%[27]. Therefore, in a sample of 150 participants, the *H. pylori* prevalence in this study was estimated with an absolute precision of 8% with 95% confidence.

For descriptive and univariate analyses of study variables, we calculated the frequency and percentages for categorical measures for the overall study population and by *H. pylori* and *cagA*-gene positive infection status. We then analyzed the distribution of study variables by *H. pylori* and *cagA*-gene positive infection status (positive vs. negative) using Pearson's Chi-squared () test. Study variables included birth year cohort (years; 1940-1965, 1966-1975, 1976-1985, 1985+), age quartiles (18-35, 36-45, 46-55, 56+), sex (male, female), education (≤high school, >high school), aspirin daily use (yes, no), monthly medication use (over the counter stomach medication, vitamins), family history of stomach cancer (yes, no), self-reported diabetes health condition (yes, no), body mass index (<25.0, 25.0-30.0, >30.0 kg/m^2), smoking (never, ever smoked, current smoker), alcohol use (never, drank in the past, current use), sodium daily intake (low, medium, high), vegetable daily intake (≤3.0 cups/day, >3.0 cups/day), fruit daily intake (≤2.0 cups/day, >2.0 cups/day), and type of drinking water consumed (filtered water or unfiltered tap water, bottled water).

Multivariate logistic regression analyses were used to calculate odds ratios (OR) and 95% CI to test associations of sociodemographic, family history, lifestyle, and environmental variables with *H. pylori* infection and *cagA*-gene positive infection. All models were adjusted for birth year cohort and sex, and the reference group was *H. pylori*-negative people. All analyses were performed using R Studio version 4.2.2 (R Core Team, Vienna, Austria). A value of $p<0.05$ was considered statistically significant.

RESULTS

From January to November 2021, 260 potential participants expressed interest in participating in the study (Figure 1). After attempting to contact all 260 individuals regarding the study, 115 were ineligible, and 145 were eligible. Of the 145 consented participants, 99 (68%) participants with complete questionnaires and *H. pylori* and *cagA* ddPCR results were included in these analyses. The remaining 41 individuals never completed the study procedures or decided not to participate, and five individuals were excluded due to calculated extreme energy intake values. Participants with extreme calorie intake were removed because these extreme values would have influenced the calculated nutrient variables analyzed in this study. It is likely that these 5 participants were extremely malnourished (<500 kcal/day), overnourished (>5,500 kcal/day), or did not understand the food frequency questionnaire.

Participant Characteristics

Of the 99 participants, the median age was 45 years old (age range: 18 to 79 years), 25.3% were in the 1940-1965 birth year, 21.2% in the 1966-1975 birth year, 29.3% in the 1976-1985

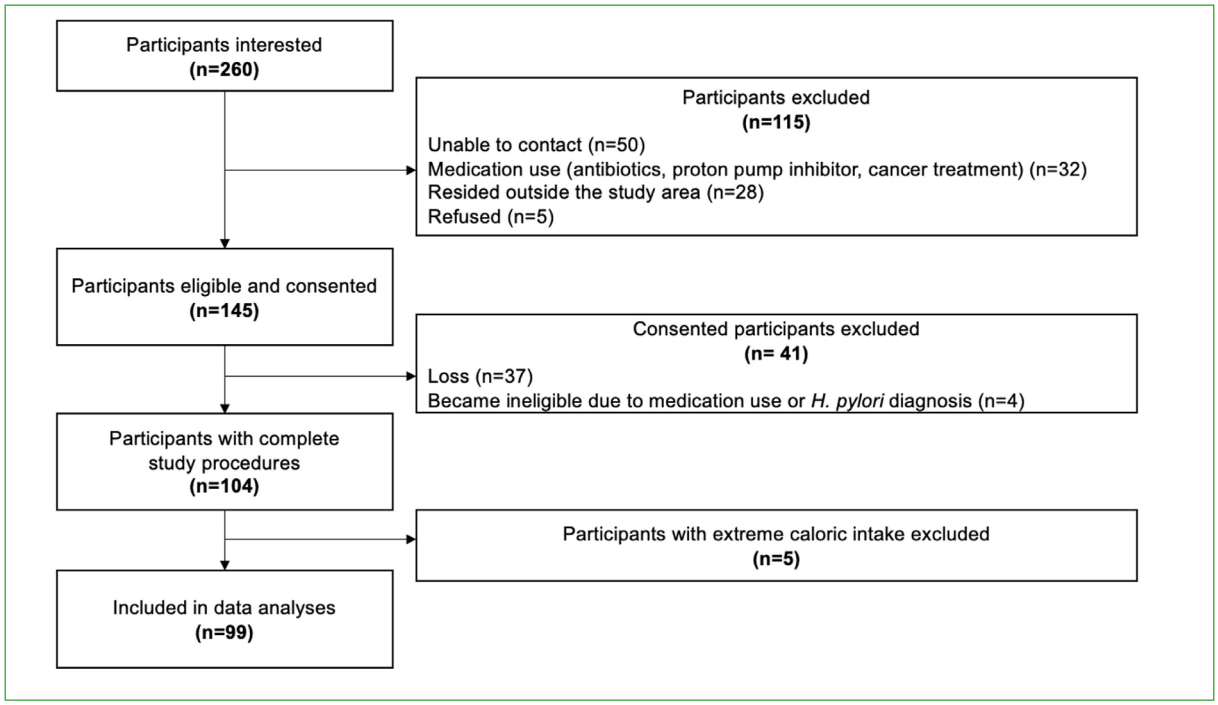

Figure 1. Flowchart of participant recruitment to the Navajo ABID study: January to November 2021.

birth year, 24.2% in the 1985+ birth year, 73.7% were female, 77.8% had greater than high school education, 16% had a family history of stomach cancer, 21.2% reported they had diabetes, 49.5% had a body mass index greater than or equal to 30 kg/m^2, 36.4% ever smoked, 29.3% consumed alcohol in the past month, 40.4% reported high sodium daily intake, and 53.5% consumed filtered or unfiltered tap water (Table 1).

Prevalence of *H. pylori* Infection

Based on analysis of ddPCR results of collected stool samples, active *H. pylori* infection was found in 56 participants, with an overall prevalence of 56.6% (95% CI: 46.2-66.5). Overall, the distribution of sociodemographic, health, lifestyle, and environmental factors was similar across *H. pylori* status (Table 1).

Prevalence of *cagA* Gene Carriage

The *cagA* genotyping assay detected the *cagA* gene in 78.6% (44/56, 95% CI: 65.6%-88.4%) of *H. pylori*-positive participants. Of these samples, 95.5% (42/44, 95% CI: 84.5%-99.4%) were the Western allele type (EPIYA-C motif). The distribution of sociodemographic, health, lifestyle, and environmental factors was similar between *H. pylori*-negative and *cagA*-positive participants (Table 2).

Association Between Risk Factors and *H. pylori* Infection and *cagA* Gene Carriage

Table 3 shows the crude and adjusted odds ratios (ORs) for *H. pylori* infection and *cagA* gene carriage associated with selected risk factors. Older birth year cohort, being male, lower education, family history of stomach cancer, ever smoking, current alcohol use, high daily sodium intake, and drinking bottled water were not statistically significantly associated with *H. pylori* infection or *cagA* gene carriage.

TABLE 1. PERCENT DISTRIBUTION OF OVERALL PARTICIPANT CHARACTERISTICS AND BY *HELICOBACTER PYLORI* (*H. PYLORI*) STATUS.

Characteristics	Overall (n=99)	*H. pylori*-negative (n=43)	*H. pylori*-positive (n=56)	*p*-value[a]
Age group, n (%)				
18-35	24 (24.2)	13 (30.2)	11 (19.6)	0.190
36-45	29 (29.3)	8 (18.6)	21 (37.5)	
46-55	21 (21.2)	11 (25.6)	10 (17.9)	
55+	25 (25.3)	11 (25.6)	14 (25.0)	
Birth year, n (%)				
1940-1965	25 (25.3)	11 (25.6)	14 (25.0)	0.190
1966-1975	21 (21.2)	11 (25.6)	10 (17.9)	
1976-1985	29 (29.3)	8 (18.6)	21 (37.5)	
1985+	24 (24.2)	13 (30.2)	11 (19.6)	
Sex, n (%)				
Female	73 (73.7)	32 (74.4)	41 (73.2)	1.000
Male	26 (26.3)	11 (25.6)	15 (26.8)	
Education, n (%)				
<High school	21 (21.2)	12 (27.9)	9 (16.1)	0.257
>High school	77 (77.8)	31 (72.1)	46 (82.1)	
Aspirin daily use, n (%)	18 (18.2)	9 (20.9)	9 (16.1)	0.752
OTC stomach medicine, monthly use, n (%)	27 (27.3)	8 (18.6)	19 (33.9)	0.142
Vitamins, monthly use, n (%)	60 (60.6)	22 (51.2)	38 (67.9)	0.140
Family health history of stomach cancer, n (%)	16 (16.2)	8 (18.6)	8 (14.3)	0.762
Diabetes, n (%)	21 (21.2)	10 (23.3)	11 (19.6)	0.851
BMI (kg/m^2), n (%)				
<25.0	16 (16.2)	5 (11.6)	11 (19.6)	0.449
25.0-30.0	31 (31.3)	13 (30.2)	18 (32.1)	
>30.0	49 (49.5)	24 (55.8)	25 (44.6)	
Physical activity, n (%)				0.147
Low (0-149 mins/week)	47 (47.5)	17 (39.5)	30 (53.6)	
Moderate (150-300 mins/week)	24 (24.2)	12 (27.9)	12 (21.4)	
High (>300 mins/week)	14 (14.1)	9 (20.9)	5 (8.9)	
Smoking, n (%)				
Never	61 (61.6)	29 (67.4)	32 (57.1)	0.376
Ever smoked	36 (36.4)	13 (30.2)	23 (41.1)	
Alcohol use, n (%)				
Never	17 (17.2)	9 (20.9)	8 (14.3)	0.132
Drank in the past	50 (50.5)	17 (39.5)	33 (58.9)	
Current use	29 (29.3)	16 (37.2)	13 (23.2)	
Sodium daily intake (mg/day), n (%)				
Low (<2,028)	21 (26.3)	14 (32.6)	12 (21.4)	0.307
Medium (2,209-3,765)	33 (33.3)	15 (34.9)	18 (32.1)	
High (>3,765)	40 (40.4)	14 (32.6)	26 (46.4)	
Vegetable daily intake (cups/day), n (%)				
<3.0	63 (63.6)	28 (65.1)	35 (62.5)	0.954
>3.0	36 (36.4)	15 (34.9)	21 (37.5)	
Fruit daily intake (cups/day), n (%)				
<2.0	58 (58.6)	27 (62.8)	31 (55.4)	0.590
>2.0	41 (41.1)	16 (37.2)	25 (44.6)	
Type of water consumed, n (%)				
Filtered or Unfiltered Tap water	53 (53.5)	24 (55.8)	29 (51.8)	0.998
Bottled water	44 (44.4)	19 (44.2)	25 (44.6)	

Abbreviations: SD=standard deviation; BMI=Body mass index, OTC=Over the Counter. [a]Pearson's Chi-squared test.

DISCUSSION

This is the first study to investigate the prevalence and correlates of *H. pylori* infection and *cagA* status from stool samples in the Navajo Nation. We observed an *H. pylori* prevalence of 56.6% (95% CI: 46.2%-66.5%) in Navajo adults and a *cagA* prevalence of 78.6% (95% CI: 65.6%-

TABLE 2. PERCENT DISTRIBUTION OF DEMOGRAPHIC CHARACTERISTICS BY *cagA* STATUS.

Characteristics	*H. pylori*-negative (n=43)	*H. pylori*-positive (n=44)	*p*-value[a]
Age group, n (%)			
18-35	13 (30.2)	8 (18.2)	0.340
36-45	8 (18.6)	15 (34.1)	
46-55	11 (25.6)	10 (22.7)	
55+	11 (25.6)	11 (25.0)	
Birth year, n (%)			
1940-1965	11 (25.6)	11 (25.0)	0.340
1966-1975	11 (25.6)	10 (22.7)	
1976-1985	8 (18.6)	15 (34.1)	
1985+	13 (30.2)	8 (18.2)	
Sex, n (%)			
Female	32 (74.4)	33 (75.0)	1.000
Male	11 (25.6)	11 (25.0)	
Education, n (%)			
<High school	12 (27.9)	7 (77.8)	0.299
>High school	31 (72.1)	36 (78.3)	
Aspirin daily use, n (%)	9 (20.9)	7 (15.9)	0.782
OTC stomach medicine, monthly use, n (%)	8 (18.6)	16 (36.4)	0.107
Vitamins, monthly use, n (%)	22 (51.2)	31 (70.5)	0.104
Family history of stomach cancer, n (%)	8 (18.6)	7 (15.9)	0.961
Diabetes, n (%)	10 (23.3)	10 (22.7)	1.000
BMI (kg/m^2), n (%)			
<25.0	5 (11.6)	9 (20.5)	0.514
25.0-30.0	13 (30.2)	13 (29.5)	
>30.0	24 (55.8)	21 (47.7)	
Physical activity, n (%)			
Low (0-149 mins/week)	17 (39.5)	22 (50.0)	0.260
Moderate (150-300 mins/week)	12 (27.9)	10 (22.7)	
High (>300 mins/week)	9 (20.9)	4 (9.1)	
Smoking, n (%)			
Never	29 (67.4)	25 (56.8)	0.413
Ever smoked	13 (30.2)	18 (40.9)	
Alcohol use, n (%)			
Never	9 (20.9)	5 (11.4)	0.074
Drank in the past	17 (39.5)	28 (63.6)	
Current use	16 (37.2)	10 (22.7)	
Sodium daily intake (mg/day), n (%),			
Low (<2,028)	14 (32.6)	11 (25.0)	0.654
Medium (2,209-3,765)	15 (34.9)	15 (34.1)	
High (>3,765)	14 (32.6)	18 (40.9)	
Vegetable daily intake (cups/day), n (%)			
<3.0	28 (65.1)	29 (65.9)	1.000
>3.0	15 (34.9)	15 (34.1)	
Fruit daily intake (cups/day), n (%)			
<2.0	27 (62.8)	25 (56.8)	0.727
>2.0	16 (37.2)	19 (43.2)	
Type of water consumed, n (%)			
Filtered or Unfiltered Tap water	24 (55.8)	24 (54.5)	1.000
Bottled water	19 (44.2)	19 (43.2)	

Abbreviations: BMI=Body mass index, OTC=Over the Counter. [a]Pearson's Chi-squared test.

88.4%) in *H. pylori*-infected Navajo adults. The *H. pylori* prevalence in our study was 2.7 times higher than the prevalence reported in non-Hispanic White adults from the 1999-2000 National Health and Nutrition Examination Survey (21% *H. pylori* seroprevalence)[28,29], and the *cagA* prevalence among *H. pylori*-positive adults was 1.3 times higher than the prevalence reported in *H. pylori*-positive White adults (non-Hispanic and Hispanic) from five prospective cohorts in the US (59% *cagA* seroprevalence)[29,30]. We further found no statistically significant associations between birth year cohort, sex, education, family history of stomach cancer, smoking, alcohol use, sodium daily intake, and drinking water source with *H. pylori* infection.

TABLE 3. ASSOCIATION BETWEEN RISK FACTORS AND *HELICOBACTER PYLORI* (*H. PYLORI*)-POSITIVE INFECTION AND *cagA*-POSITIVE GENOTYPE.

Characteristics	*H. pylori*-positive			*cagA*-positive		
	n (%)	Univariate OR (95% CI)	Adjusted[a] AOR (95% CI)	n (%)	Univariate OR (95% CI)	Adjusted[a] AOR (95% CI)
Birth year						
1940-1965	14/25 (56.0)	Ref.	Ref.	11/22 (50.0)	Ref.	Ref.
1966-1975	10/21 (47.6)	0.71 (0.22-2.29)	0.71 (0.22-2.30)	10/21 (47.6)	0.91 (0.27-3.03)	0.90 (0.27-3.02)
1976-1985	21/29 (72.4)	2.06 (0.67-6.60)	2.06 (0.67-6.61)	15/23 (65.2)	1.88 (0.57-6.40)	1.88 (0.57-6.42)
1985+	11/24 (45.8)	0.66 (0.21-2.04)	0.66 (0.21-2.04)	8/21 (38.1)	0.62 (0.18-2.06)	0.62 (0.18-2.06)
Sex						
Female	41/73 (56.2)	Ref.	Ref.	33/65 (50.8)	Ref.	Ref.
Male	15/26 (57.7)	1.06 (0.43-2.68)	0.98 (0.38-2.55)	11/22 (50.0)	0.96 (0.37-2.57)	0.93 (0.34-2.54)
Education						
<High school	9/21 (42.9)	Ref.	Ref.	7/19 (36.8)	Ref.	Ref.
>High school	46/77 (59.7)	1.98 (0.75-5.39)	1.76 (0.63-5.06)	36/67 (53.7)	1.99 (0.71-5.94)	1.83 (0.61-5.80)
Family history of stomach cancer						
No	48/83 (57.8)	Ref.	Ref.	37/72 (84.1)	Ref.	Ref.
Yes	8/16 (50.0)	0.73 (0.25-2.16)	0.74 (0.24-2.30)	7/15 (15.9)	0.87 (0.26-2.54)	0.80 (0.25-2.57)
Smoking						
Never	32/61 (52.5)	Ref.	Ref.	25/54 (46.3)	Ref.	Ref.
Ever smoked	23/36 (63.9)	1.60 (0.69-3.80)	1.52 (0.62-3.79)	18/31 (58.1)	1.61 (0.66-3.98)	1.54 (0.60-4.03)
Alcohol use						
Never	8/17 (47.1)	Ref.	Ref.	5/14 (35.7)	Ref.	Ref.
Drank in the past	33/50 (66.0)	2.18 (0.71-6.84)	2.04 (0.56-7.67)	28/45 (62.2)	2.96 (0.88-11.07)	3.06 (0.76-13.64)
Current use	13/29 (44.8)	0.91 (0.27-3.08)	0.58 (0.14-2.41)	10/26 (38.5)	1.13 (0.30-4.56)	0.87 (0.18-4.16)
Sodium daily intake (mg/day)						
Low (<2,028)	12/26 (46.2)	Ref.	Ref.	11/25 (44.0)	Ref.	Ref.
Medium (2,209-3,765)	18/33 (54.5)	1.40 (0.50-3.98)	1.27 (0.43-3.80)	15/30 (50.0)	1.27 (0.44-3.75)	1.25 (0.41-3.90)
High (>3,765)	26/40 (65.0)	2.17 (0.80-6.05)	2.12 (0.74-6.15)	18/32 (56.3)	1.64 (0.57-4.78)	1.70 (0.57-5.19)
Type of water consumed						
Filtered or Unfiltered Tap water	29/53 (54.7)	Ref.	Ref.	24/48 (50.0)	Ref.	Ref.
Bottled water	25/44 (56.8)	1.09 (0.49-2.45)	0.96 (0.41-2.25)	19/38 (50.0)	1.00 (0.43-2.35)	0.84 (0.34-2.07)

Abbreviations: OR=Odds ratio; AOR=Adjusted odds ratio; CI=Confidence Interval. [a]Adjusted for sex and birth year cohort.

Participants in our study experienced a high burden of *H. pylori* infections (56.6%). This finding is comparable with a study of *H. pylori* infections in a community-based random sampling of Navajo adults residing in the western region of the Navajo Nation [56.4% (Urea Breath Test (UBT)][27]. Because evidence has shown *H. pylori* infection increases the risk of peptic ulcers and stomach cancer and successful eradication of *H. pylori* infection is associated with reduced risk of these conditions, these findings may explain the stomach cancer disparity in the Navajo Nation and the need to address *H. pylori* with prevention and eradication strategies[6,31]. Also, the *H. pylori* prevalence among the birth year cohort was as follows: 1940-1965, 56.0% *H. pylori* (95% CI, 34.9-75.6); 1966-1975, 47.6% (95% CI, 25.7-70.2); 1976-1985, 72.4% (95% CI, 52.8-87.3); and 1985+, 45.8% (95% CI, 25.6-67.2) (Table 3). Statistical analyses show no significant difference or decrease over the generations, as seen in other studies[32,33]. A relative stable *H. pylori* prevalence across the birth cohort may indicate continued challenges with socioeconomic factors such as living in a crowded household and access to clean drinking water across the birth cohorts, as was observed during the COVID-19 pandemic[34,35]. Therefore, culturally appropriate *H. pylori* prevention strategies and education for all generations in the Navajo Nation are needed.

Among *H. pylori*-positive participants, we found a *cagA* prevalence of 78.6% (95% CI: 65.6-88.4), which was consistent with a reported 77.0% *cagA* prevalence from a study of biopsy samples of Navajo *H. pylori* patients with gastric disease[36]. Additional genes encoded in the *cag* PAI assemble into a specialized secretion system to deliver CagA protein and bacterial metabolites into host cells that promote inflammation and increase gastrointestinal disease severity by activating or disrupting normal cellular pathways[17,18]. Further, the *cagA* gene can possess a C-terminal region with a motif of five amino acid residues, which is known as the EPIYA motif, that play an important role in the relationship of CagA proteins with cell-to-cell interaction and tyrosine phosphorylation-dependent interactions[37]. There are four EPIYA segments, EPIYA-A, -B, -C, and -D defined by the surrounding amino acid sequence. The EPIYA-A, -B, and -C segments are characteristic of CagA of *H. pylori* in non-Asian countries, known as the *cagA* Western allele type, while the EPIYA-A, -B, and D segment is specific to CagA of *H. pylori* in Asian countries, known as the *cagA* East Asian allele type; some strains contain neither EPIYA-C or EPIYA-D motifs[37]. Both EPIYA-C and EPIYA-D motifs bind to SHP2 (SH2-containing protein tyrosine phosphatase), a known oncogene. While EPIYA-D motifs have higher affinity for SHP2 and the *cagA* East Asian allele type is associated with a greater risk of stomach cancer than *cagA* Western allele type, recent studies have shown that duplication of the EPIYA-C motif leads to substantial increases in SHP2 activation[38] and presence of two more EPIYA-C motifs is associated with increased cancer risk[39,40]. In our study, we found that 95.5% of *H. pylori-cagA*-positive participants showed presence of an EPIYA-C motif or *cagA* Western allele type. This finding is consistent with another study in Navajo *H. pylori*-infected patients, where the majority carried a *cagA* Western allele type[36]. However, the ddPCR method used in our study does not indicate the number of EPIYA-C motifs present. Future studies that isolate Navajo *H. pylori* strains and characterize the full sequence and cellular activities of their *cagA* alleles should be prioritized to further understand factors contributing to stomach cancer risk in this population. Because stomach cancer is associated with the *cagA* gene, there may be a large segment of the Navajo Nation at much higher risk for stomach cancer. To address stomach cancer in the Navajo Nation, local cancer prevention strategies may require the consideration of the greater burden of the *cagA* gene in the Navajo people.

Our study used stool-based ddPCR to detect *H. pylori* shed into the stool. Compared to serum-based analysis methods, which measure the antibody response to *H. pylori* and determine asymptomatic or previously exposed individuals, the ddPCR approach determines active *H. pylori* infection[41]. Therefore, the use of stool-based ddPCR for the detection of active *H. pylori* infection in our study was appropriate, and the disparity between the prevalence of stool-based *H. pylori* prevalence in our study (56.6%) and the seroprevalence in non-Hispanic Whites in the US (21.0%) is much greater because seroprevalence includes inactive and active infections. Moreover, a study by Talarico et al. reported that the stool-based ddPCR assay method had a sensitivity of 84% and 100%, and specificity of 100% and 71% compared to serology and stool antigen tests, respectively[22].

Strengths and Limitations

This study was subject to several limitations. First, our study is cross-sectional and not able to determine causality. It is uncertain when *H. pylori* infection was acquired, which could be during childhood or earlier compared to when the risk factors were assessed in the study. Thus, it cannot be determined if the risk factors preceded *H. pylori* infection. Second, our study population was a non-random sample of the Navajo adult population. Those motivated to participate in the study may have been influenced to participate because of a history of gastrointestinal conditions and/or symptoms they were experiencing at the time of recruitment or having a family history of stomach cancer. In addition, the demographic distribution of our study population does not fully reflect the demographic profile of the Navajo adult population. Based on the 2021 American Community Survey (ACS) 5-year Census estimates, our study did not adequately capture the male population (23.1% vs. 48.1%, respectively)[42]. Therefore, the findings may not generalize to the general Navajo adult population. In addition, our *H. pylori* prevalence is likely an underestimate because of the low participation rate of males because *H. pylori* is more common in males. Third, while we made efforts to recruit participants through offline approaches (i.e., flyers, word of mouth, and in-person community events), this study primarily used online approaches (i.e., website, social media) to recruit participants due to the COVID-19 pandemic, which may have unintentionally excluded a large segment of the Navajo population who do not have internet access[43]. Fourth, our study has limited power to detect statistical significance in several analyses, particularly among the adjusted models. Thus, some of our analyses may have imprecise estimates and should be interpreted with caution. Fifth, participants may not have accurately recalled dietary information such as specific foods they consumed, frequency, or portion sizes, thus limiting our ability to obtain accurate estimates, even though we accounted for energy intake to reduce potential measurement error. Sixth, the FFQ used in our study was not designed for the Navajo people and may not have fully captured nutrient intake. Therefore, developing a culturally appropriate FFQ with a comprehensive food list and food/beverage portion sizes with the tribe can improve the assessment of dietary intake in the Navajo people. Moreover, food sources were limited in these Navajo communities due to the COVID-19 pandemic, which impacted the study area throughout the data collection period, and thus the foods consumed may have differed from the usual foods participants ate. Seventh, because the ddPCR method used to analyze stool samples is not a clinically approved test, we notified all participants of their *H. pylori* results with recommendations for further testing; however, we did not confirm our results with any follow-up clinical tests participants received. Eighth, we did not assess additional antigens of *H. pylori* because of the problem of multiple testing, and *cagA* was the focus of this study because it has a strong association with stomach cancer. Lastly, the *H. pylori* and *cagA* prevalence comparisons between the study population in the Navajo Nation and White adults in the US are not direct statistical conclusions. These studies have distinct age- and sex-distribution and have been conducted at different times.

Our study has several strengths. This study is among a few studies that investigated *H. pylori* infection and *cagA* status in an Indigenous tribal population with disproportionate stomach cancer rates. Secondly, our study focuses on a community-based population and not a hospital-based population, which adds to the evidence of *H. pylori* infection in a "healthy" population. Thirdly, tribal leaders and community members of the two study regions supported the study, despite the logistic challenges and stress of the COVID-19 pandemic. Lastly, the testing of *H. pylori* infection was non-invasive, and the ddPCR methods used to detect *H. pylori* have a sensitivity and specificity greater than 84%[22].

CONCLUSIONS

In conclusion, our study showed a high prevalence of *H. pylori* and *cagA* infections in Navajo adults living in the Navajo Nation. Therefore, prevention strategies and interventions are warranted to reduce *H. pylori* infections in the Navajo Nation. These strategies should be tailored to the needs of Navajo people and individuals at higher risk for stomach cancer. Further larger studies are needed to elucidate the risk factors of *H. pylori* infection and stomach cancer in American Indian populations with high stomach cancer incidence and mortality.

Conflict of Interest

No conflict of interest to declare.

Acknowledgments

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Informed Consent

All participants gave informed consent for participation in this study.

Ethics Statement

This study was approved by the Navajo Nation Human Research Review Board (NNR-20.384T) and the University of Washington Human Subjects Division (00011217).

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Authors' Contributions

DP contributed to the conception and design of the study, was responsible for data collection and statistical analyses, and drafted the manuscript. AIP, NRS, JWL, and MCW contributed to the study's design and statistical analysis and provided revisions to the manuscript. All authors read and approved the final manuscript.

Data Availability

The Navajo Nation oversees the data, and requests for data can be made through the Navajo Nation Human Research Review Board (https://nnhrrb.navajo-nsn.gov/index.html).

Significance Statement

This original study provides an understanding of the *Helicobacter pylori* disease burden in adults from the Navajo Nation, a tribal nation with higher rates of stomach cancer than White populations in the Southwest. These tribal-specific results inform tribal cancer prevention strategies to reduce the *Helicobacter pylori* burden to ensure the health of the Navajo Nation.

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