Assessing Helicobacter pylori infections among adults from the Navajo Nation

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A dissertation
submitted in partial fulfillment of the
requirement for the degree of

Doctor of Philosophy

University of Washington

2022

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Program Authorized to Offer Degree:
Epidemiology

University of Washington

Abstract

Assessing the gut microbiota and Individual Diet in the Navajo Nation (ABID study)

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Background:

Although the burden of gastric cancer is low in the United States (US) overall, a substantial burden of gastric cancer continues to be observed in American Indians and Alaska Natives (AI/AN), particularly in the Southwest and Alaska regions of the US. In particular, gastric cancer incidence is 3.5 times higher in the Navajo Nation, a tribe of 157,000 tribal members, than in the general Arizona and New Mexico population. Contributing factors underlying this disparity are not well understood, although it is plausible that *Helicobacter pylori* (*H. pylori*), an infectious pathogen that colonizes the stomach mucosa and is a major risk factor for gastric cancer, could be a significant contributor to the elevated burden of gastric cancers in the Navajo Nation.

Objectives:

This dissertation presents the findings from the Navajo ABID (Assessing the microBiota and Individual Diet) study, the first study to assess risk factors for gastric cancer in two regions of the Navajo Nation. We assessed the prevalence of and risk factors for *H. pylori* infection and *H.*

pylori cagA virulence gene carriage, as well as the association between diet and *H. pylori* infection in Navajo adults residing on the Navajo Nation.

Methods:

We launched a cross-sectional study in the central and northeast regions of the Navajo Nation in 2021. Demographic, health, behavioral, environmental, and diet data were collected from health and food questionnaires. *H. pylori* infection and *cagA* virulence gene status were detected from stool samples. We calculated the prevalence of *H. pylori* infection and odds ratios for associations between infection status and potential risk factors (including demographics, medical history of gastrointestinal conditions, family history of gastrointestinal conditions, aspirin use, body mass index, smoking, alcohol use, drinking water source, and own livestock). We used principal component analysis to identify dietary patterns and assessed the associations between dietary patterns and *H. pylori* infection using logistic regression.

Results:

We recruited and obtained data on 104 eligible adults for the Navajo ABID study. We found that 57.7% (95% CI: 47.6-67.3) of participants were infected with *H. pylori* and, among *H. pylori*-infected participants, 76.7% (95% CI: 64.0-86.6) were infected with a *cagA*-positive *H. pylori* strain. Having a history of *H. pylori* infection was inversely associated with *H. pylori* infection; no significant associations were observed with other known risk factors. We identified three dietary patterns and found that a diet pattern of Soups and Mixed dishes was positively associated with *H. pylori* infection in Navajo participants after adjusting for confounders; we found no significant associations between the Western or the Fruits and Vegetable pattern and *H.*

pylori infection. No signification associations were found between selected nutrients (i.e., sodium, alcohol, vitamin C, vitamin A, vitamin E, and folate) and *H. pylori* infection, although positive associations with sodium and folate, and inverse associations with vitamin A, vitamin C, and alcohol were suggestive.

Conclusions:

The prevalence of *H. pylori* infection was two times higher in Navajo adults in the Navajo ABID study compared to the US population, while the prevalence of the *cagA* gene (77%) in *H. pylori*-infected participants was disproportionately higher than the US population *cagA* gene prevalence in whites (19%). With the exception of the observed Soups and Mixed dishes dietary pattern, few dietary factors were associated with *H. pylori* infection. Our findings provide a greater understanding of the burden of *H. pylori* and *cagA*-positive infections needed to inform public health prevention efforts in the Navajo Nation. These results may also allow for the design of health education material that focuses on *H. pylori* prevention and promotes general recommendations on healthy eating.

Acknowledgments

First and foremost, I want to express my deepest gratitude to the Navajo participants of the Navajo ABID study. It would not have been possible to complete this research without your participation and interest in improving the health of the Navajo people. My interaction with you was always uplifting, especially during the COVID-19 pandemic and being away from Dine Bi'keyah. Many of you reminded me about the importance of establishing K'e while doing research, in that establishing our relationship with one another from the beginning of our conversation sets us in a good way in talking about cancer and health. As a Dine' person, I believe this has been one of the most significant cultural lessons I have learned from the project. I will carry this practice with me going forward in my research career.

I am deeply grateful for the endless support from the most loving and giving partner any person can ask for, Joyce Becenti. You have been my greatest support through the numerous highs and lows of graduate school. Your optimistic spirit strengthened me each step of the way and allowed me to overcome unimaginable obstacles. For that, I am forever grateful to you for taking this journey with me, and I love you with all my heart. To our beautiful Kendra, Yuki, and Cedar, thank you for being the best kiddo, kitty, and corgi, especially during the most stressful moments of the dissertation!

This journey would be impossible without my mom Grace Tsosie and my sisters Daniela Roseen and Dionne Daly. You, above and beyond all people in life, continue to be my unwavering foundation when things were unbalanced in life. The love I have for you is immeasurable, and I thank you for impressing on me from my earliest memory that education could make a difference in a place I have been rooted.

Thank you to all my family from the Water Flows Together, Water's Edge, and Black Streak of Wood clans, especially my niece Annika Roseen, my brother-in-laws Jon Roseen and Tom Daly, and wonderful in-laws Abel and Marilyn Becenti, Rebecca, Jeff, Allee, and Rhlyee Manuelito, and LuJuana Becenti, and friends, especially my best friend Rae Warner. Without your encouraging words, talks, laughs, food, and prayers throughout these six years, I would not have seen the light at the end of the tunnel. I look forward to spending more time together now that this dissertation is complete. And I hope grandpa Calvin Coolidge, grandma Pearl Coolidge, uncle Jimmy Coolidge, and my father Daniel Pete would have been proud of this work.

I have benefited greatly from the mentorship, advice, and trust of my advisor, Amanda Phipps. Her passion for using epidemiology to reduce cancer disparities in her research is an inspiration and I hope to carry her superhero qualities and passion in my work. Countless conversations with my committee members Nina Salama, Johanna Lampe, and Michael Wu have also shaped my dissertation and they have given me invaluable skills and knowledge in study designs, *H. pylori*, laboratory methods, and statistical methods to use in my research endeavors.

This work also rests heavily on the shoulders of other individuals. My understanding of cancer disparities and research began with the Navajo Cancer Workgroup and conversations with colleagues like Deborah Klaus, Carmelita Sorrelman, Melissa Jim, Del Yazzie, Carol Goldtooth, Sheldwin Yazzie, Marc Emerson, Chuck Wiggins, Susie John, Priscilla Sanderson, Kevin English, Tom Vaughan, Stephanie Melkonian, and Dian Million. Thank you for all our enlightening conversations about cancer and health in the Navajo Nation and among American Indian people in general.

Throughout the last four years, Adrian Dominguez and Abigail Echo-Hawk have allowed me to give back to the Native community by using my epi skills to work on a range of projects as

a part-time Epidemiologist, as well as mingle and have a little piece of a Native community in Seattle at the Urban Indian Health Institute. My deepest thanks to you both for showing me how to use data to tell stories and to always serve our Native relatives with respect and care.

This dissertation could not have been successful without the support from community members and tribal leaders from the Northern Navajo and Fort Defiance Agencies, as well as health providers Chris Percy and Michael Tutt. My thanks to Frank Morgan and Martha Garrison for ensuring this project honored the Navajo language. They translated study documents, provided translation during recruitment, and offered cultural insight into the project. Also, huge gratitude to Note Louis for designing an amazing study website for people to learn about the study and participate, to Pearl Liu for assisting me with understanding the PCA analysis and wrangling through the R code, to the Salama Lab in particular to Ali Meyer and Eli Le for receiving and analyzing specimens, to Carolyn Ehret for sharing your expertise on diet data and making collecting diet data easier than expected, and to Rebecca Becenti, Marilyn Becenti, and Karla Chavez for weathering the cold weather to post flyers and set up a booth at local flea markets to have people learn about the study and sign up.

A special shoutout to my cohort friends – Yu Ni, Yan Chen, Hongjie Chen, Xinwei Hau, Gui Liu, Valentine Wanga, Arthur Sillah, and Natasha Ludwig-Barron. Over the years, you made getting through the program fun and I will remember the bonds we formed with food, conversation, and laughter. I look forward to staying in touch!

Finally, my research and education received generous financial support from the Tribal Researchers' Cancer Control Fellowship Program (funded by the National Institute of General Medical Sciences and the National Cancer Institute of the National Institutes of Health, S06GM123543), Navajo Nation Scholarship, American Indian Graduate Center, Native

American Cancer Initiative, University of Washington Multicultural Alumni Partnership Scholarship, University of Washington Cancer Epidemiology and Biostatistics Pre-Doctoral Nation Cancer Institute T32 Fellowship, and F99/K00 Pre-Doc to Post-Doc Transition Fellowship (the National Cancer Institute of the National Institutes of Health, F99CA253685). Many thanks to Thomas Becker, Ashley Thomas, Linda Burhansstipanov, and Jennifer Bills for their investment in my dissertation and for always connecting me with the resources needed for this research.

~Ahe'hee

TABLE OF CONTENTS

List of Tables

List of Figures

Introduction

Gastric cancer, a highly fatal cancer (approximately 32% survival at 5 years), is the fifth most common cancer worldwide, accounting for 780,000 deaths annually.[1,2] In the United States (US), a substantial burden of gastric cancer continues to be observed in American Indians and Alaska Natives (AI/AN).[3–5] One study of cancer surveillance data from 2010-2015 revealed that, although the burden of gastric cancer is low in the US as a whole, there is a "*disproportionate burden of gastric cancer in the AI/AN population, particularly in the Southwest and Alaska*."[5] The Navajo Nation is one of the largest tribes in the Southwest, and has been particularly impacted by a high burden of gastric cancer: recent cancer surveillance data have shown that the gastric cancer incidence and mortality rates are 3.5 times and 4.4 times higher, respectively, in the Navajo Nation than in the general Arizona and New Mexico population (age-adjusted incidence: 15.0 per 100,000 persons vs. 4.3 per 100,000 persons, respectively; age-adjusted mortality: 9.8 per 100,000 persons vs. 2.2 per 100,000 persons).[6] Contributing factors underlying this disparity are not well understood. However, previous studies have indicated that Alaska Natives, who also experience elevated rates of gastric cancer (age-adjusted incidence: 28.2 per 100,00 persons), are exposed to *Helicobacter pylori* (*H. pylori*) at a prevalence of 75% - much higher than in other population groups within the US.[7–9] *H. pylori* is an infectious pathogen that colonizes the stomach mucosa and is a significant risk factor for gastric cancer.[10,11] Although not yet established, *H. pylori* may also plausibly be a significant contributor to the elevated burden of gastric cancers in the Navajo Nation.

H. pylori is a gram-negative, acid tolerant bacterium and is present in the stomachs of at least half the world's population.[12] The World Health Organization has characterized *H. pylori* as a class I carcinogen that plays a vital role in the etiology of gastric cancer.[10] The prevalence of *H.*

pylori in Indigenous populations is disproportionately high compared to their non-Indigenous counterparts. Studies have shown the prevalence in the Alaska Natives, Inuit, and First Nations of Canada to range from 60% to 90%; however, there have not been published assessments of *H. pylori* infections in the Navajo Nation, where the risk for *H. pylori* exposure may be similarly high.[13–15]

Certain *H. pylori* strains vary in their production of virulence factors, which have been credited with the causal link between *H. pylori* and gastric cancer.[12,16,17] In particular, cytotoxin-associated gene A (*cagA*) and its pathogenicity island (*cag* PAI) function together to produce a specialized secretion system that delivers CagA protein and other bacterial metabolites from the bacterial cytosol into host cells. Once injected into host cells, CagA interrupts normal gastric epithelial cell activity to promote inflammation and gastric cancer.[18–22] The *cagA* gene is split into two allele types: East Asian and Western. The *cagA* gene with the East Asian allele drives greater increased risk for gastric cancer.[23] Of relevance, studies of Alaska Natives found disproportionate burdens of *H. pylori* infection (75%-90%), of which an unexpectedly high proportion were *cagA*-positive *H. pylori* strains (85%) and Western *cagA* allele type (90%-95%).[24–26] In the Navajo Nation, we do not know if the prevalence of *cagA*-positive *H. pylori,* and *cagA*-positive *H. pylori* with the East Asian allele type, is a concern.

Epidemiological studies of non-AI/AN populations have shown that diets with high-salt, high-processed meats, and low fruits and vegetables are associated with *H. pylori* infection and gastric cancer risk.[27–30] The effects of these diets involve changes to the protective lining of the stomach and enhanced expression of the pathogenic *cagA* gene.[30,31] Given that diet is an important modifiable factor and offers promise in cancer prevention, studies on the association of

diet and *H. pylori* infection specific to American Indian populations, such as the Navajo Nation tribe, are needed.

To address these knowledge gaps, we aimed to determine the prevalence and correlates of *H. pylori* infection and *H. pylori cagA* genotype in tribal members from the Navajo Nation, with an emphasis on dietary factors.

The following dissertation consists of two research chapters and concluding statements. In **Chapter 1** we describe a cross-sectional study (Navajo ABID study) conducted to collect primary epidemiological data on *H. pylori* and *cagA* gene status, including known risk factors for *H. pylori*, from Navajo adults. In using these data, we first determined the prevalence of *H. pylori* infection and *H. pylori cagA* genotype and made comparisons with the prevalence published in the literature of other US populations. Secondly, we determined the association between known risk factors, such as demographics, health conditions, behavioral, and environmental factors, and *H. pylori* infection.

In **Chapter 2** we used diet data from the Navajo ABID study to identify dietary patterns of study participants and evaluated the association between dietary patterns and *H. pylori* infection using principal component analysis and logistic regression. Additionally, we assessed the protective and adverse relationships between selected dietary nutrients such as sodium, alcohol, vitamin C, vitamin A, vitamin E, and folate, and *H. pylori* infection.

Overall, this research has vast implications for cancer prevention efforts, particularly for American Indian tribes such as the Navajo Nation. This dissertation addressed key gaps in the literature and provides public health workers, clinicians, and researchers with the information needed to develop future in-depth studies and interventions to address gastric cancer risk in the Navajo Nation.

Chapter 1. Prevalence and risk factors of *Helicobacter pylori* and its *cagA* virulence genotype in Navajo adults: Navajo ABID study.

Abstract

Background

American Indians in the Southwest US, including the Navajo tribe, are experiencing a disproportionate burden of gastric cancer incidence. *Helicobacter pylori* (*H. pylori*), a bacterium known to infect the stomach of over half of the world's population, is a primary risk factor for gastric cancer and *H. pylori* strains that carry the *cagA* gene are linked to greater gastrointestinal disease severity. However, the prevalence of *H. pylori* and its *cagA* virulence genotype in the Navajo Nation is uncertain. In this study, we assessed the prevalence and risk factors of *H. pylori* and *cagA* virulence genotype in Navajo adults living in the Navajo Nation.

Materials and Methods

Navajo adults were recruited in two areas of the Navajo Nation from January to November 2021. Stool samples were analyzed with droplet digital PCR assays for *H. pylori* 16S gene and *cagA* virulence genotyping. Questionnaires were used to collect information on risk factors for *H. pylori* infection. We calculated odds ratios and 95% confidence intervals (CI) for the association between risk factors and *H. pylori* infection, including *cagA* virulence gene carriage, using logistic regression models.

Results

Of the 104 participants recruited, 57.7% (95% CI: 47.6-67.3) were infected with *H. pylori*; among *H. pylori*-infected participants, 76.7% (95% CI: 64.0-86.6) were *cagA*-gene positive and of the *cagA*-gene positive participants, 100% were of the Western *cagA* allele subtype. Having a prior history of *H. pylori* infection was inversely associated with detected *H. pylori* infection (OR=0.13, 95% CI, 0.01-0.81). No significant associations were observed with other known risk factors.

Conclusions

The prevalence of *H. pylori* infection (58%) is two-fold higher in Navajo adults in this study compared to the US population (2% seroprevalence). The prevalence of the *cagA* gene (77%) in *H. pylori*-infected participants was disproportionately higher than the US population *cagA* gene prevalence (19% seroprevalence); and of the *cagA*-positive participants, all (100%) were of the Western *cagA* allele subtype. Our findings provide a greater understanding of the burden of *H. pylori* and *cagA*-positive infections and inform cancer prevention efforts in the Navajo Nation.

Introduction

Helicobacter pylori (*H. pylori*) infection is one of the most common infections in humans, with a seroprevalence of about 27% to 35% in the United States and 50% globally.[26,32,33] *H. pylori* is a spiral-shaped gram-negative bacterium that colonizes and infects the stomach lining and causes gastrointestinal diseases such as gastritis, gastric ulcers, and gastric cancer.[10,11] *H. pylori* infections are predominately acquired during childhood, although common transmission occurring through person-to-person contact, oral-to-oral or fecal-to-oral routes, consuming contaminated food and/or water, and contact with infected animals can occur at any point during a person's life.[34] Other known risk factors related to *H. pylori* infection include older age, lower socioeconomic status, household crowding, living with someone infected with *H. pylori*, and dietary factors.[34–36] While a majority of people with *H. pylori* infection will experience no symptoms (80-90%), 3% develop gastric ulcers, and <1% develop gastric cancer during their lifetime, which may seriously affect a person's quality of life.[33,37]

The development of severe *H. pylori* disease among those infected is further determined by the virulence of the *H. pylori* strain, as well as host genetics (e.g., L-1β, IL-10, TNF-α) and environmental factors (e.g., diet and smoking).[38] Certain *H. pylori* strains vary in their production of virulence factors (e.g., CagA, VacA, BabA), which have been credited with the causal link between *H. pylori* and gastric cancer.[17] In particular, the cytotoxin-associated antigen (*cagA*) gene and its pathogenicity island (cag PAI) function together to produce a specialized secretion system that delivers CagA proteins and other bacterial metabolites from the bacterial cytosol into host cells.[25,39] Once injected into host cells, CagA interrupts normal gastric epithelial cell activity, promotes inflammation, and increases gastrointestinal disease severity, including

gastric cancer risk.[21,22] 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 gastric cancer than the cagA gene with the Western allele with an EPIYA-C motif.[23]

According to the World Health Organization, *H. pylori* is classified as a Group 1 carcinogenic agent.[40] About 89% of gastric cancers are attributable to *H. pylori* infection, and eradication of infection has been known to reduce the burden of gastric cancer.[33,40] Investigations of *H. pylori* infection in American Indians are limited, especially when considering the substantial burden of gastric cancer that continues to be observed in American Indians.[4,5] Previous studies of *H. pylori* infection have focused on Alaska Natives and found a disproportionate burden of *H. pylori* infection (75%-90%) in Alaska Natives compared to other populations in the United States (27% seroprevalence),[8–11] of which an unexpectedly high proportion were *cagA*-positive *H. pylori* strains (~85%).[24–26] The Navajo Nation is one of the largest tribes in the United States; yet there are limited epidemiological investigations of *H. pylori* infections in the Navajo people, where the incidence and mortality rates of gastric cancer are 3.5 and 4.5 times higher, respectively, than in the general Arizona and New Mexico population (incidence: 15.0 per 100,000 persons vs. 4.3 per 100,000 persons; mortality: 9.8 per 100,000 persons vs. 2.2 per 100,000 persons).[6]

To advance our understanding of *H. pylori* infections in the Navajo people, we assessed the prevalence of active *H. pylori* infections and the *cagA* virulence gene in Navajo adults from two regions of the Navajo Nation. We also evaluated demographic factors known to be associated with *H. pylori* status overall (e.g. age, gender, education, household crowding), as well as health

conditions (e.g., aspirin use, family history of gastrointestinal disease, body mass index), environmental (e.g., drinking water source), and lifestyle (e.g., physical activity, smoking, alcohol use, diet) factors associated with *H. pylori* infection.[11,34–36,41]

Methods

Participants and Data collection

We conducted a cross-sectional study from January to November 2021 in two geographic regions of the Navajo Nation: the central and northeast regions. Participants were informed 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 (i.e., Facebook and Instagram), newspaper ads, flyers/postcards, and community events (i.e., local flea markets). The information provided on these platforms summarized information about the study, eligibility requirements, and how to participate. Eligible participants had to identify as Navajo, be 18 years old or older, reside in the two study areas on the Navajo Nation, not be pregnant, not have used oral or intravenous antibiotics in the past 3 months, not be using proton pump inhibitors, not have been treated for *H. pylori* infection in the past 3 months, 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 sample 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 participant was obtained and recorded by the study group.

Data Collection

Measurement of Helicobacter pylori and cagA genotypes

The presence of *H. pylori* (positive or negative) and *cagA* genotypes (positive or negative and EPIYA-C or EIPY-D allele type) were determined from stool samples collected by the participants. Stool samples were collected at home and placed in a sample vial with 5-mL of 95% ethanol preservative and stored at room temperature before mailing to the Salama Lab at the Fred Hutchinson Cancer Center (FHCC, Seattle, WA).

In the lab, bacterial DNA was extracted from stool samples using the QIAamp Stool DNA Mini Kit (Qiagen) and then analyzed in duplicates using droplet digital PCR (polymerase chain reaction) assays and methods in accordance with the manufacturer's instructions, which have been validated in adult stool in the Salama Lab at FHCC.[42] For quality control, a positive control (stool DNA from a confirmed *H. pylori*-positive volunteer) and negative control (molecular grade water) were included in each batch of samples analyzed. A sample with greater than 5 droplets above recommended thresholds for *H. pylori*, *cagA*, and *cagA* EPIYA typing was considered to be positive. The 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.[42] Additionally, the thresholds for each assay were visually evaluated by inspecting the amplitude plot of the 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.

Measurement of H. pylori covariates

Data on potential risk factors for *H. pylori* and *cagA* presence were collected from a self-administered health questionnaire. The 26-item health questionnaire accessed characteristics associated with *H. pylori* infection, such as demographic factors, drinking water consumption, health conditions, medication use, family health history, and health behaviors.

Statistical analyses

The sample size for this study was determined based on practical considerations (i.e., cost and feasibility), and formal statistical considerations. According to published data, we assumed the *H. pylori* prevalence in Navajo adults to be 58%.[43] 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.

We calculated the prevalence and 95% confidence interval (CI) of *H. pylori* and *cagA* presence and compared them with previously reported prevalence estimates for other US populations. Notable differences in the prevalence were determined when the prevalence of other US populations exceeded the boundaries of the 95% CI of *H. pylori* and *cagA* positivity determined in the study.

For our descriptive and univariate analyses of relevant factors (demographic, health conditions, environmental, and lifestyle factors), we calculated the frequency, means (standard deviations) for continuous measures, and percentages for categorical measures for the overall study population and by *H. pylori* infection status. We then tested the distribution of these factors by *H. pylori* infection status (positive versus negative) using a two-sample t-test for continuous measures and either a χ^2 or Fisher exact test for categorical measures. The relevant factors in the study included age (years; 18-44, 45-54, 55+), gender (male, female), education (<high school or high school, >high school), the number of people living in the household (<3, 3+), history of *H. pylori* (yes, no), previous history of gastritis or ulcers (yes, no), family history of stomach cancer (yes, no), family history of peptic ulcers (yes, no), aspirin daily use (yes, no), BMI (kg/m^2; <25.0, 25.0-29.9, 30 or higher), smoking (never, ever smoked, current smoker), alcohol use in the past month (never drank, past use, current use), type of drinking water consumed (filtered water, bottled water, windmill water), and owned livestock (yes, no).

To assess other potential risk factors, we examined the univariate associations between running water in the home (yes, no), diabetes (yes, no), heartburn (yes, no), monthly prescription medication use (yes, no), monthly over the counter stomach medication use (yes, no), monthly vitamin use (yes, no), monthly Navajo herbal medicine use (yes, no), and physical activity (no physical activity, low (<150 mins/week), moderate (150-300 mins/week), high activity (>300 mins/week)) and *H. pylori* status.

Multivariate logistic regression analyses were used to test the association between relevant factors and *H. pylori* infection and *cagA* presence, and to calculate odds ratios (OR) and 95% CI.

We applied two models to control for potential confounding characteristics. Model 1 adjusted for age and sex. Model 2 adjusted additionally for education, number of people living in the household, history of *H. pylori*, history of gastritis or ulcers, family history of stomach cancer, family history of peptic ulcers, daily aspirin use, BMI, smoking, alcohol use, type of water consumed, and owned livestock. All analyses were performed using R Studio version 4.0.3 (R Core Team, Vienna, Austria). A value of $p<0.05$ was considered statistically significant. We used complete case analysis for our regression analyses, as data for several relevant factors, particularly education, type of water consumed, number of people living in the household, daily aspirin use, BMI, smoking, and alcohol use, were missing or classified as refused/unknown for <5% of study participants.

Results

From January to November 2021, we had 260 potential participants express interest in participating in the study (Figure 1.1). After attempting to contact all 260 individuals regarding the study, 115 were ineligible and 145 were eligible. Of the 145 consented participants, 104 (71%) participants with complete questionnaires and *H. pylori* and *cagA* ddPCR results were included in these analyses. The remaining 41 individuals never completed study procedures or decided not to participate.

Participant characteristics

The mean age of the study population was 46.8 years (range: 18–79 years), 76.9% were female, 47.1% had some college education, 49.5% had a BMI greater than or equal to 30 kg/m^2, 10.6%

were current smokers, 29.8% consumed alcohol in the past month, 91.3% had running water in the home, and 52.9% consumed filtered or unfiltered tap water (Table 1.1).

Gastrointestinal Conditions

When asked about having experienced gastrointestinal (GI) conditions, most participants reported no previous GI conditions (78.8%). The majority reported having experienced heartburn (11.5%), followed by having a previous *H. pylori* infection (9.6%), and previous history of gastritis or ulcers (7.7%). The majority of participants did not report a family history of gastric diseases (80.8%). In addition, 15.7% reported a family history of stomach cancer, and 7.7% reported a family history of peptic ulcers. Additionally, about a third of participants (26.9%) had taken monthly over-the-counter gastrointestinal medication such as an antacid, H2-blocker, or proton pump inhibitor.

Prevalence of *H. pylori* infection

Based on analysis of ddPCR results of collected stool samples, active *H. pylori* infection was found in 60 participants, with an overall prevalence of 57.7% (95% CI: 47.6-67.3). Male participants were slightly more prone to *H. pylori* infection (62.5%, 15/24) compared to female participants (56.3%, 45/80), however, this difference was not statistically significant.

Overall, the distribution of demographic, health, environmental, and lifestyle factors was similar across *H. pylori* status, with the exception that a significantly lower proportion of *H. pylori*-positive participants reported having a prior history of *H. pylori* (3.3%, p=0.017). Participants

did not differ significantly in the distribution of other relevant factors by *H. pylori* infection status (Table 1.1).

Prevalence of *cagA* virulence gene in *H. pylori*-positive participants

As shown in Table 1.2, the *cagA* genotyping assay detected *cagA* virulence in 76.7% (46/60, 95% CI: 64.0-86.6) of *H. pylori*-positive participants. The type of *cagA* expressed was solely the Western allele type (46/46, 100%). Although not statistically different, *cagA*-positive participants were more likely to be female and greater than 45 years old (Table 1.2). Due to the small sample size, particularly of *cagA*-negative participants, the association between other covariates and *cagA* virulence gene carriage was not assessed.

Association between *H. pylori* infection and demographic, health, environmental, and lifestyle factors

Table 1.3 shows the crude and adjusted ORs for *H. pylori* infection associated with demographic, health, environmental, and lifestyle factors. Both the crude and adjusted ORs revealed that having a history of *H. pylori* infection was inversely associated with *H. pylori* infection compared to participants with no history of *H. pylori* infection (crude OR=0.16, 95% CI: 0.02-0.66; AOR_2=0.13, 95% CI: 0.01-0.81). Other factors such as age, gender, education, number of people living in the household, history of gastritis or ulcers, family history of stomach cancer and peptic ulcers, aspirin daily use, BMI, alcohol and smoking use, type of drinking water consumed, and owning livestock were not statistically significantly associated with *H. pylori* infection in both the crude and adjusted models.

Discussion

H. pylori is considered a causative agent for gastritis and peptic ulcer disease and has been identified as a main risk factor for the development of gastric cancer. Navajo people in the Navajo Nation are at an increased risk for gastric cancer. This is the first study to investigate the prevalence of *H. pylori* infection and *cagA* status from stool samples in the central and northeast regions of the Navajo Nation, and shows that the prevalence of *H. pylori* infections is much higher in asymptomatic Navajo adults than in other US populations. We observed an *H. pylori* prevalence of 57.7% (95% CI: 47.6-67.3) in Navajo adults and a *cagA* prevalence of 76.7% (95% CI: 64.0-86.6) in *H. pylori*-infected Navajo adults. In comparison, the *H. pylori* prevalence in our study was 2.1 times higher than the prevalence reported in other US populations (27-35% *H. pylori* seroprevalence)[26,32,33], and the *cagA* prevalence was 4.0 times higher than the prevalence reported in whites in the US (19% *cagA* seroprevalence).[44] We further found that having a prior history of *H. pylori* infection was inversely associated with *H. pylori* infection; and no significant association was observed between other factors such as age, gender, education, number of people living in the household, history of gastritis or ulcers, family history of stomach cancer and peptic ulcers, aspirin daily use, BMI, alcohol and smoking use, type of drinking water consumed, and owning livestock and *H. pylori* infection.

Previous studies have found a comparable high prevalence of *H. pylori* infection in asymptomatic Native American people as our study (57.7%): the prevalence in 2018 was 56.4% (Urea Breath Test (UBT)) among Navajo adults in western Navajo Nation and 68-69% (anti-*H. pylori* IgG and UBTs) in Alaskan people living in rural communities, most of whom were Alaska Native people.[43,45] Moreover, the prevalence of *H. pylori* infection determined in our study is

much higher than the prevalence in the general US population (27% to 35% seroprevalence).[8–11] Since the incidence of gastric cancer in the Navajo Nation is much higher than in the general population of Arizona and New Mexico, a high prevalence of *H. pylori* infection may explain this disparity. However, because only 1-3% of people with *H. pylori* infection develop gastric cancer, it is uncertain if *H. pylori* infection is the main risk factor for gastric cancer in the Navajo people, as there are other factors, such as host genetic factors related to inflammatory responses not measured in this study that can contribute to the development of gastric cancer in the context of *H. pylori* infection.[11,46,47] Further research is needed to understand how *H. pylori* infection attributes to gastric cancer in the Navajo people.

The only risk factor significantly inversely associated with *H. pylori* infection was having a history of *H. pylori* infection. While *H. pylori* infection may be protective against other diseases like asthma[48] and inflammatory bowel disease[49], it is uncertain whether prior *H. pylori* infection is protective of recurrent *H. pylori* infection.[50] It has been hypothesized that prior *H. pylori* infection may promote an immune response that is protective against future reinfection; however, an experimental study of healthy patients found no immune protection of prior *H. pylori* infection with recurrent *H. pylori* infection.[51] Moreover, because an individual is exposed to many other infections that influence immune protection, it is difficult to distinguish if a prior *H. pylori* infection itself could be a protective factor, or if it is a marker for some other unidentified factor. Further investigation of this relationship is warranted. Similarly, participants with a history of gastritis/ulcers showed a decreased risk of *H. pylori*, which was consistent with a large cross-sectional study in China that found an inverse association between a history of peptic ulcer and *H. pylori* infection.[52] It is possible people with a prior diagnosis of

gastritis/ulcers may have changed their diet and/or lifestyle in response to that diagnosis, which could impact the risk of *H. pylori* infection; it is also possible that people with a prior diagnosis of gastritis/ulcers may exhibit greater health care seeking behaviors to prevent *H. pylori* exposure, thus contributing to reduced risk of *H. pylori* infection.

Other demographic, health, environmental, and lifestyle factors assessed in the study were not significantly associated with *H. pylori* infection; however, we found suggestive associations that are notable. Increased age is generally accepted as a risk factor for *H. pylori* infection and many population-based studies report an increasing prevalence of *H. pylori* infection with increasing age.[53–55] In contrast, our findings are consistent with other population-based studies that find a decreased prevalence of *H. pylori* with increasing age, including a study of 101 asymptomatic Navajo adults in the western region of the Navajo Nation.[43,52,56] Explanations of a lower risk of *H. pylori* in older age can be due to older individuals having a low *H. pylori* load, which cannot be detected by ddPCR, and/or *H. pylori* could have been present earlier in life but was eliminated due to an unfavorable gastric environment due to age.[56]

Our results showed no significant association between gender and *H. pylori* infection, which was found in other studies including a meta-analysis study of 183 studies from 73 countries.[32,52–55,57] However, we observe a suggestive positive association of *H. pylori* in males, which was confirmed in other studies.[43,58] While the reasons for the possible gender differences are still unknown, a higher risk of *H. pylori* infection in males could be linked to males being more active and having lower handwashing practice than females.[59]

Regarding education, higher educational attainment is generally believed to be associated with a lower risk of *H. pylori* infection because individuals may have a good job, income, and living environment. However, we observed no association between education and *H. pylori* infection, which was seen in another cross-sectional study.[55] Although our finding did not reach statistical significance, the odds of *H. pylori* was higher in participants with some college education compared to participants without college education. This finding has been found in other community-based studies, particularly in a study of Navajo adults residing in the Navajo Nation.[43,56] It may be that individuals with high education and income have more opportunities to travel to work or across the Navajo Nation to interact with people and their environment, thus increasing their chance of *H. pylori* exposure.

Living in an overcrowded household is considered a strong risk factor for *H. pylori* infection[53] because of the close space between people which can foster the transmission of *H. pylori*. However, we observed no significant association between higher household size (≥3 people) and *H. pylori*, which has been observed in other community-based cross-sectional studies.[52,55,57] In fact, we see a decreased risk of *H. pylori* in higher household sizes. It is possible that people in larger households have lower odds and prevalence for *H. pylori* infection because of other household characteristics such as better living conditions with separate rooms for family members and less confined living areas. Further studies looking closely at household characteristics and *H. pylori* infection are needed.

Aspirin is a type of nonsteroidal anti-inflammatory drug (NSAID) and can increase the likelihood of developing peptic ulcers in the presence of *H. pylori* infection.[60] Because long-term

use of NSAIDs can modify the gastric environment, *H. pylori* infection in NSAIDs users may be more common. In our study, we found no association between daily aspirin use and *H. pylori* infection, which was confirmed in another cross-sectional study of hospital patients.[59] A possible explanation for our null result may be due to our measure of aspirin use. In addition to asking participants about their current daily aspirin use, information about their duration of aspirin use could have elucidated more about the relationship between aspirin use and *H. pylori* infection.

Our finding on BMI showed that high BMI was not associated with a higher odds of *H. pylori* infection, which has been found in other studies.[52,54,55,57,61] Yet, other studies have shown positive[62,63] and inverse [64] associations. While we found no association, we can observe a suggestive negative association between BMI >30 kg/m^2 and *H. pylori* infection. Previous studies have mentioned that persistent *H. pylori* infection from childhood and uncontrolled gastric inflammation can lead to dysregulation of appetite and caloric intake.[64,65] Thus, individuals exposed to *H. pylori* earlier in life may be prone to decreased appetite and food intake due to defective signaling of hormones (i.e., leptin and ghrelin) in the stomach.[65] Additional epidemiological studies on the potential inverse effect of high BMI with *H. pylori* infection in Navajo adults are needed.

A strong suggestive positive association between current everyday smoking and *H. pylori* infection was found in our study, which was similar to other population-based studies.[52,53,61] An explanation for this finding may relate to the nicotine effects on gastric mucosal blood flow and mucus secretion, facilitating *H. pylori* colonization.[66]

In regards to alcohol use, no association between current alcohol consumption and *H. pylori* was observed in our study, which aligns with findings from other population-based studies.[52,56,57,61] Despite these null results, a suggestive inverse association between current alcohol use and *H. pylori* infection can be noticed. A previous study showed similar protective effects of alcohol at moderate consumption levels.[67] A negative association may be observed because alcohol may reduce the risk for *H. pylori* infection through antimicrobial activities and increased gastric acid secretion which can compromise the pH conditions for new or existing *H. pylori* infections.[68] Yet in another study, they observed a positive association between heavy alcohol consumption and *H. pylori*.[69] In addition, we can observe a suggestive higher odds of *H. pylori* infection among participants who drank in the past; however, information on the amount of alcohol consumed in the past was incomplete due to participants not answering the survey question (41% missing). Perhaps participants who drank heavily in the past have compromised their stomach and gastrointestinal system making them vulnerable to *H. pylori* infection. Additional study of the role of past and current alcohol consumption, including duration of use, alcohol type, and amount consumed, and *H. pylori* infection is needed.

Environmental exposure to contaminated drinking water may pose a risk for *H. pylori* infection, and various sources of drinking water can yield varied results. A previous study by Harris et al. found a significantly increased risk of *H. pylori* infection among Navajo adults using unregulated tap water in their home (water from a natural spring, community spigot, windmill, and/or private well).[43] Moreover, a study of population-based survey data from the United States' National Health and Nutrition Examination Survey found a suggestive positive association between well water and *H. pylori* infection.[53] However, in our study, we observe a suggestive increased risk

for *H. pylori* in participants consuming bottled water. To our knowledge, there are limited studies on the association between bottled water and *H. pylori* infection. One study in Iran found about 2% of bottled mineral water samples (8/450) were contaminated with *H. pylori*, and certain brands harbored greater proportions of *H. pylori*.[70] In the Navajo Nation, where drinking water sources are limited and of low water quality, the use of bottled water can be quite common. More research on the role of various drinking water sources and *H. pylori* infection in the Navajo Nation is warranted.

It is common for Navajo people to raise sheep and livestock for subsistence and income. Studies have shown livestock animals can harbor *H. pylori* and may possibly play a role in the zoonotic transmission of *H. pylori*.[71,72] In our study, we found no association between owning livestock such as sheep, goats, cows, cattle, horses, and llamas and *H. pylori* infection. However, studies with larger samples size can confirm or shed more insight into the relationship between owning livestock and *H. pylori* infection.

H. pylori strains with a cytotoxin-associated antigen (*cagA*) gene in its genome are known to biologically disrupt normal gastric cellular activity and increase gastric cancer risk.[22,73] The *cagA* gene is located at one end of the cytotoxin-associated antigen pathogenicity island (*cag* PAI), a 37-kilobase genomic DNA segment containing about 29 genes. Among *H. pylori* strains carrying the *cagA* gene (i.e., *cagA*-positive strains), *H. pylori* attaches to the surface of gastric epithelial cells and several of the *cag* PAI genes work together to produce proteins used to build a specialized type IV secretion system, which delivers CagA proteins and other bacterial metabolites from the bacterial cytosol into host cells.[25,39] CagA proteins interrupt normal gastric

epithelial cell activity after tyrosine phosphorylation (via EPIYA motif) by causing a growth factor-like cellular response and cytokine production to promote inflammation and increase gastrointestinal disease severity, including gastric cancer risk.[21,22] Further, the *cagA* gene has 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.[74] There are four types of EPIYA segments, EPIYA-A, -B, -C, and -D. The EPIYA-A,-B, and -C segments are characteristic of CagA of *H. pylori* in non-Asian countries, known as the Western CagA type, while the EPIYA-, -B, and D segment is specific to CagA of *H. pylori* in Asian countries, known as the East Asian CagA type.[74] The East Asian CagA type has a greater binding affinity to host cell protein kinases (SHP2) and induce morphological changes in the epithelial cells compared to the Western type.[74] Thus, it is thought the *cagA* gene with the East Asian CagA type is associated with a greater risk of gastric cancer than the *cagA* gene with the Western CagA type.[23] In our study, we observed the prevalence of *cagA* and its allele type in asymptomatic *H. pylori-infected* participants. We found the *cagA* gene prevalence of 76.7% (95% CI: 64.0-86.6) in *H. pylori*-positive participants to be consistent with a reported 75.0% in Navajo *H. pylori* patients with gastric disease[75] and other non-Native American people in Alaska (67.0%); however, our cagA prevalence was much higher than the prevalence of white people in the US (19% seroprevalence).[45,76] Our data showed all of the *H. pylori*-cagA positive participants were infected with the less pathogenic Western genotype (EPIYA-C). This finding is consistent with another study in Navajo *H. pylori*-infected patients where the majority carried a Western type *cagA* gene.[75]

We also found that the overall study population reported no previous history of gastrointestinal (GI) conditions (79%) such as heartburn, ulcers, gastritis, or previous *H. pylori* infection. Interestingly, *H. pylori*-positive participants were less likely to report a previous history of GI conditions compared to *H. pylori*-negative participants. A possible explanation for this finding is that *H. pylori*-negative participants may have experienced ulcers or gastritis because of a prior *H. pylori* infection, which they reported at a higher proportion (18.2%). Furthermore, because the GI questions in the questionnaire focused on GI conditions and less on symptoms of *H. pylori* we cannot determine if participants were experiencing GI symptoms such as abdominal pain, nausea, and vomiting because of an active *H. pylori* infection, which has been observed in other studies of people harboring *H. pylori*.[59,77–80]

In our study, we used a stool-based analysis approach to detect *H. pylori* infection. Stool-based tests such as stool antigen test, PCR, and ddPCR, detect *H. pylori* shed into the stool and can determine active *H. pylori* infection compared to serum-based analysis methods.[81] Serum-based tests, such as the enzyme-linked immunosorbent assay (ELISA) test, Western blotting, and agglutination test, measure the antibody response to *H. pylori* and are useful for determining asymptomatic or previously exposed individuals and cannot distinguish between current and previous infection as antibodies are present after infection is cleared.[81] Therefore, the use of stool-based analysis for the detection of active *H. pylori* infection in our study was appropriate. Moreover, results of the sensitivity of stool-based tests and serum-based tests for *H. pylori* detection vary, likely due to differences in tests performed, sample integrity (storage conditions), or methods not standardized across laboratories. According to previous studies, Khalifehgholi et al. found a higher sensitivity of serum-based ELISA test (91.3%) when compared to stool

antigen tests (73.9%)[82], Talarico et al. reported a similar sensitivity result between the stool-based ddPCR method (84%) and serum ELISA tests (85%)[42], and Kazemi et al. observed much lower sensitivity in serum vitek immunodiagnostic assay tests (50%) compared to stool antigen tests (96%).[83]

Several important limitations of the study should be considered. 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 at an earlier time point compared to when the risk factors were assessed in the study, particularly the socioeconomic, health, environmental, and lifestyle factors. Thus, it cannot be determined if the risk factors preceded *H. pylori* infection and/or were related to duration of infection. Second, our study population was a convenience sample of the Navajo adult population and those motivated to participate in the study may be influenced to participate because of a prior history of GI conditions and/or symptoms they were experiencing at the time of recruitment; such selection bias may bias the results to participants not representative of the Navajo adult population. 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.[84,85] Fourth, the demographic distribution of our study population does not fully reflect the demographic profile of the Navajo adult population. Based on 2010 Census, our study did not adequately capture the younger age groups (18-29 years, 11% versus 22%) and male population (23.1% versus 51.9%).[86] Fifth, our study has limited power to detect statistical significance in our analyses. This limits our ability to

generalize to the Navajo adult population and the relatively small cases across risk factors in some of our analyses may have imprecise estimates and thus should be interpreted with caution. Lastly, because the ddPCR test 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 monitor or confirm our results with any follow-up clinical tests participants received.

Despite these limitations, 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 gastric cancer rates and has increased our knowledge about risk factors related to gastric cancer. Secondly, our study was supported by the tribal leaders and community members of the two study regions, despite the logistic challenges and stress of the COVID-19 pandemic. Thirdly, the testing of *H. pylori* infection was non-invasive and ddPCR methods were used to detect *H. pylori* in stool specimens with a sensitivity and specificity greater than 84%.[42] Furthermore, our *H. pylori* testing required a small quantity of stool, was highly sensitive because PCR is carried out in each droplet for detection, and highly specific because assays and multiple fluorescent probes can be used to determine different alleles of the target gene (e.g., detect 16S and cagA genes in one run) also known as multiplexing.[42,87,88]

In summary, our study showed the prevalence of *H. pylori* infection to be high among Navajo adults living in the two study regions of the Navajo Nation compared to the US *H. pylori* prevalence, and that a history of *H. pylori* is inversely associated with active *H. pylori* infection. While the clinical implication of *H. pylori* virulence factors is a matter of debate, the prevalence

of *cagA* in our study is comparable to the *cagA* prevalence of other populations in the US. Further studies are needed to elucidate the risk factors of *H. pylori*, including its virulence genes, and understand the inverse association of previous *H. pylori* infection with active *H. pylori* infection found in this study. Overall, these results point to a need of developing prevention strategies to reduce *H. pylori* infection in the study regions of the Navajo Nation in order to prevent gastric cancer.

Figure 1.1. Participant recruitment to the Navajo ABID study from January to October 2021.

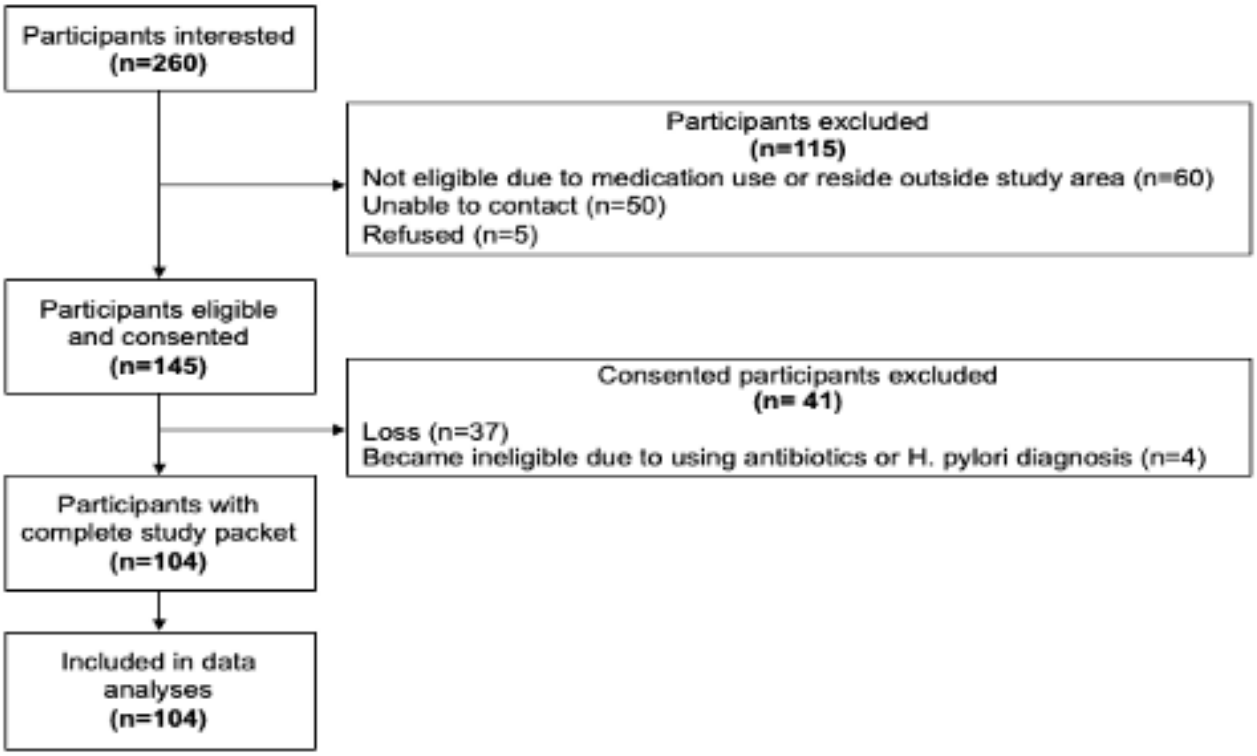


Table 1.1 Percent distribution of selected characteristics overall and by *Helicobacter pylori* (*H. pylori*) status.

Characteristics	Overall (N=104)	*H. pylori*-negative (n=44)	*H. pylori*-positive (n=60)	P-value[a]
Age, mean (SD)	46.8 (14.4)	46.5 (15.0)	47.0 (14.0)	0.857
Age group (%)				
18-44	50 (48.1)	20 (45.5)	30 (50.0)	0.820
45-54	23 (22.1)	11 (25.0)	12 (20.0)	
55+	31 (29.8)	13 (29.5)	18 (30.0)	
Gender (%)				
Female	80 (76.9)	35 (79.5)	45 (75.0)	1.000
Male	24 (23.1)	9 (20.5)	15 (25.0)	
Education (%)				
Less than High school	2 (1.9)	1 (2.3)	1 (1.7)	0.317
High school	21 (20.2)	12 (27.3)	9 (15.0)	
Some college	49 (47.1)	17 (38.6)	32 (53.3)	
College degree	31 (29.8)	14 (31.8)	17 (28.3)	
Missing	1 (1.0)	0 (0)	1 (1.7)	
People living in house, mean (SD)	3.4 (1.8)	3.5 (1.8)	3.4 (1.9)	0.731
Running water in home (%)	95 (91.3)	41 (93.2)	54 (90.0)	0.730
Type of water consumed (%)				
Filtered or Unfiltered Tap water	55 (52.9)	24 (54.4)	31 (51.7)	1.000
Bottled water	47 (45.2)	20 (45.5)	27 (45.0)	
Windmill water	1 (1.0)	0 (0)	1 (1.7)	
Don’t know / Unsure	1 (1.0)	0 (0)	1 (1.7)	
Owned livestock (%)	21 (28.0)	9 (30.0)	12 (26.7)	1.000
Health Conditions (%)				
Diabetes	23 (22.1)	10 (22.7)	13 (21.7)	1.000
Heartburn	12 (11.5)	6 (13.6)	6 (10.0)	0.793
History of *H. pylori*	10 (9.6)	8 (18.2)	2 (3.3)	0.017[b]
History of gastritis or ulcers	8 (7.7)	5 (11.4)	3 (5.0)	0.278
Aspirin daily use (%)	20 (19.6)	9 (20.5)	11 (19.0)	1.000

Medication use, once a month (%)				
Prescription medicine	43 (41.3)	19 (43.2)	24 (40.0)	0.901
OTC stomach medicine	28 (26.9)	8 (18.2)	20 (33.3)	0.117
Vitamins	62 (59.6)	22 (50.0)	40 (66.7)	0.131
Navajo herbal medicine	15 (14.4)	3 (6.8)	12 (20.0)	0.108
Family health history (%)				
Stomach cancer	16 (15.7)	8 (18.2)	8 (13.3)	0.688
Peptic ulcers	8 (7.7)	4 (9.1)	4 (6.7)	0.719
BMI (kg/m^2) (%)				
Below 24.9	17 (16.3)	5 (11.4)	12 (20.0)	0.273
25.0 – 29.9	34 (32.7)	13 (29.5)	21 (35.0)	
30 or higher	50 (48.1)	25 (56.8)	25 (41.7)	
Missing	3 (2.9)	1 (2.2)	2 (3.3)	
Physical activity (%)				
No physical activity	11 (10.6)	5 (11.4)	6 (10.0)	0.157
Low (<150 mins / week)	41 (39.4)	13 (29.5)	28 (46.7)	
Moderate (150-300 mins / week)	24 (23.1)	12 (27.3)	12 (20.0)	
High activity (>300mins / week)	14 (13.5)	9 (20.5)	5 (8.3)	
Missing	14 (13.5)	5 (11.4)	9 (15.0)	
Smoking (%)				
Never smoked	64 (61.5)	29 (65.9)	35 (58.3)	0.757
Ever smoked	27 (26.0)	10 (22.7)	17 (28.3)	
Current smoker	11 (10.6)	4 (9.1)	7 (11.7)	
Missing	2 (1.9)	1 (2.3)	1 (1.7)	
Alcohol use in the past month (%)				
Never drank	18 (17.3)	9 (20.5)	9 (15.0)	0.134
Past	51 (49.0)	17 (38.6)	34 (56.7)	
Current	31 (29.8)	17 (38.6)	14 (23.3)	
Refused	4 (3.8)	1 (2.3)	3 (5.0)	

[a]Chi-square test, Fisher-exact test, or t-test; [b]statistical significance $p<0.05$. Abbreviation: SD=standard deviation; BMI=Body Mass Index

Table 1.2 Percent distribution of demographic characteristics by *cagA* status among *Helicobacter pylori*-positive participants.

Characteristics	*cagA*-negative (n=14)	*cagA*-positive (n=46)	P-value[a]
Age, mean (SD)	48.1 (17.8)	46.7 (12.9)	0.449
Age group (%)			
18-44	8 (57.1)	22 (47.8)	1.000
45+	6 (42.9)	24 (52.2)	
Gender (%)			
Female	10 (71.4)	35 (76.1)	0.425
Male	4 (28.6)	11 (23.9)	

[a]Chi-square test or t-test; [b]statistical significance $p<0.05$

Table 1.3 Association between risk factors and *Helicobacter pylori* infection.

Characteristics	*H. pylori*-positive n (%)	Univariate OR (95% CI)	Adjusted Model 1[a] AOR_1 (95% CI)	Adjusted Model 2[b] AOR_2 (95% CI)
Overall	60/104 (57.7)			
Age, years				
18-44	30/50 (60.0)	1.00	1.00	1.00
45-54	12/23 (52.2)	0.73 (0.27-1.98)	0.75 (0.27-2.08)	0.66 (0.18-2.37)
55+	18/31 (58.1)	0.92 (0.37-2.32)	0.94 (0.38-2.39)	0.54 (0.14-1.98)
Gender				
Female	45/80 (56.3)	1.00	1.00	1.00
Male	15/24 (62.5)	1.30 (0.51-3.41)	1.25 (0.49-3.32)	2.02 (0.55-8.17)
Education				
< HS or HS graduate	10/23 (43.5)	1.00	1.00	1.00
> HS	49/80 (61.3)	2.05 (0.81-5.37)	2.16 (0.83-5.76)	3.15 (0.94-11.37)
People living in house				
<3 people	22/34 (64.7)	1.00	1.00	1.00
3+ people	37/69 (53.6)	0.63 (0.26-1.46)	0.60 (0.23-1.50)	0.56 (0.16-1.78)
History of *H. pylori*				
No	58/94 (61.7)	1.00	1.00	1.00
Yes	2/10 (20.0)	0.16 (0.02-0.66)[c]	0.15 (0.02-0.68)[c]	0.13 (0.01-0.81)[c]
History of gastritis/ulcers				
No	57/96 (59.4)	1.00	1.00	1.00
Yes	3/8 (37.5)	0.41 (0.08-1.77)	0.41 (0.08-1.79)	0.49 (0.05-4.28)
Family history of stomach cancer				
No	52/88 (59.1)	1.00	1.00	1.00
Yes	8/16 (50.0)	0.69 (0.23-2.04)	0.69 (0.23-2.09)	0.79 (0.16-3.78)
Family history of peptic ulcers				
No	56/96 (58.3)	1.00	1.00	1.00
Yes	4/8 (50.0)	0.71 (0.16-3.18)	0.68 (0.15-3.08)	0.65 (0.07-7.37)
Aspirin daily use				
No	47/82 (57.3)	1.00	1.00	1.00
Yes	11/20 (55.0)	0.91 (0.34-2.48)	0.88 (0.31-2.57)	1.08 (0.26-4.47)

BMI (kg/m^2)				
Below 24.9	12/17 (70.6)	1.00	1.00	1.00
25.0 – 29.9	21/34 (61.8)	0.67 (0.18-2.28)	0.74 (0.19-2.64)	0.61 (0.11-2.97)
30 or higher	25/50 (50.0)	0.42 (0.12-1.30)	0.44 (0.12-1.40)	0.55 (0.11-2.33)
Smoking				
Never smoked	35/64 (54.7)	1.00	1.00	1.00
Ever smoked	17/27 (63.0)	1.41 (0.57-3.64)	1.47 (0.58-3.88)	1.61 (0.49-5.63)
Current smoker	7/11 (63.6)	1.45 (0.40-5.99)	1.49 (0.40-6.32)	2.01 (0.32-13.45)
Alcohol use in past month				
Never drank	9/18 (50.0)	1.00	1.00	1.00
Past	34/51 (66.7)	2.00 (0.67-6.06)	1.97 (0.58-6.77)	1.78 (0.37-8.95)
Current	14/31 (45.2)	0.82 (0.25-2.66)	0.81 (0.23-2.84)	0.45 (0.08-2.33)
Type of water consumed				
Filtered/Unfiltered Tap water	31/55 (56.4)	1.00	1.00	1.00
Bottled water	27/47 (57.4)	1.05 (0.48-2.30)	1.06 (0.47-2.37)	1.39 (0.47-4.24)
Own livestock				
No	45/79 (56.9)	1.00	1.00	1.00
Yes	14/25 (56.0)	0.91 (0.37-2.30)	0.89 (0.35-2.30)	1.04 (0.29-3.82)

[a]Model 1 adjusted for sex and age; [b]Model 2 adjusted for all other variables in the table; [c]statistical significance $p<0.05$. Abbreviation: OR=Odds ratio; AOR=adjusted odds ratio; CI=Confidence Interval; HS=High school; BMI=Body Mass Index

Chapter 2. Association of Diet and *Helicobacter pylori* infection in Navajo adults: the Navajo ABID study.

Abstract

Background

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that colonizes the stomach and causes chronic gastritis, peptic ulcers, and gastric cancer. Diets high in sodium and processed meats, and low in fruits and vegetables have been associated with *H. pylori* infection and may influence the course of *H. pylori* infection and associated disease. In this study, we examined dietary patterns associated with *H. pylori* infection in Navajo adults, a population that experiences a disproportionate burden of stomach cancer.

Methods

The Navajo ABID study is a cross-sectional community-based study conducted in two regions of the Navajo Nation. Diet information was collected from 104 adults using a detailed food frequency questionnaire and a tribal foods questionnaire. Stool samples were collected for assessment of *H. pylori* infection status. Principal component analysis was used to identify dietary patterns. The association of dietary patterns and specific nutrients with *H. pylori* infection was assessed by logistic regression models.

Results

Three dietary patterns were identified: Western, Soups and Mixed dishes, and Fruits and Vegetables. We found that a Soups and Mixed dishes diet pattern was positively associated with *H. pylori* infection after adjusting for confounders. No significant associations with *H. pylori*

infection were observed for the Western or the Fruits and Vegetable diet patterns or evaluated nutrients.

Conclusions

Nutritional recommendations for *H. pylori* infection prevention in the Navajo Nation should consider whole dietary patterns instead of individual foods. We found important differences between the Soups and Mixed dishes dietary pattern, which was associated with a higher risk of *H. pylori* infection, and Western and Fruits and Vegetables dietary patterns, which had no relationship with *H. pylori* infection risk.

Introduction

Helicobacter pylori (*H. pylori*) is a bacterial pathogen that is prevalent in half of the world's population.[89] *H. pylori* is known to colonize the stomach and cause gastrointestinal conditions such as gastritis, peptic ulcers, and gastric cancer, and is classified as a Group 1 carcinogen by the World Health Organization and International Agency for Research on Cancer.[10,11,13] Known risk factors associated with the acquisition and aggressiveness of *H. pylori* infection include the allele or presence of virulence factor genes (e.g. *cagA*, *vacA*), and certain individual characteristics (gender, socioeconomic status), lifestyle (diet, smoking), and environmental factors (drinking water source, overcrowded household, living with *H. pylori*-infected family member). Dietary factors have been identified as particularly important to the risk of *H. pylori* acquisition and aggressiveness.[27,28,30,90] However, the role of diet and dietary factors are not given ample attention, especially since diet offers promise in cancer prevention and may benefit populations with high *H. pylori* prevalence.

Diet is an important contributor to the gastric environment and may influence the course of *H. pylori* infection. Several epidemiological studies have shown that diets high in sodium and processed meats, and low in fruits and vegetables are associated with *H. pylori* infection,[27,28,30,89,90] although at least one study has shown no association.[91] Mechanistically, diets high in sodium consumption have been linked to increased pathogenicity of *H. pylori* through changes in the stomach's protective mucous lining, aiding in the colonization and expression of virulence genes of *H. pylori*, and initiating inflammatory responses of the gastric epithelium that increase epithelial cell proliferation.[30,31] In contrast, certain nutrients, such as

beta-carotene, vitamin C, or foods such as garlic, peppers, and indigenous plants containing phytochemicals with antibacterial properties may prevent or reduce *H. pylori* infection.[92,93]

H. pylori prevalence is twice as high among American Indian adults in the Navajo Nation (57% to 65%) compared to the US population (27%, seroprevalence).[43,94] The high *H. pylori* prevalence in the Navajo people may also contribute to their high gastric cancer incidence and mortality rates.[95] Unfortunately, there are limited published studies on diet and *H. pylori* infection in American Indian people, particularly at the tribal level. The extent of dietary investigation and *H. pylori* infection has been limited to an assessment of a single effect of a nutrient or food item such as the type of water consumed and consumption of alcohol.[9,43,96] In particular, a study of 101 Navajo adults found that individuals who consumed unregulated water (i.e. not disinfected and monitored for safety) had a higher odds of *H. pylori* infection than individuals who consumed regulated water (i.e. disinfected and monitored).[43]

Given the high prevalence of *H. pylori* infection in the Navajo Nation, a study investigating the role of both dietary patterns and nutrient factors with *H. pylori* infection in the Navajo people was conducted. Our primary focus was to use an *a posteriori* approach to derive diet patterns to examine the association between diet and *H. pylori* infection. Dietary patterns allow for the aggregation of individuals with similar diets and foods and have the advantage over looking at a single nutrient or food.[97] Hence, we designed this cross-sectional study to identify dietary patterns in a sample of Navajo adults from the Navajo ABID study, and examined the association between dietary patterns and *H. pylori* infection using food questionnaires that accounted for tribal foods.

Methods

Study Participants

Details of Navajo ABID participants and study design have been described previously (Chapter 1). Briefly, eligibility of participants was limited to individuals who identified as Navajo, were aged 18 years old or older, resided in the two study areas (Northern Navajo or Fort Defiance Agencies) on the Navajo Nation, were not pregnant, had not used oral or intravenous antibiotics in the past 3 months, had not used proton pump inhibitors in the past 3 months, not treated for *H. pylori* infection in the past 3 months, and not undergoing any cancer treatment. The study was approved by the Navajo Nation Human Research Review Board and the University of Washington Human Subjects Division, and verbal consent for all participants was obtained and recorded.

Dietary assessment

Two food questionnaires assessed dietary intake. Participants were asked about their usual diet in the past year using a food frequency questionnaire and a Navajo Food Questionnaire.

Food Frequency Questionnaire (FFQ)

The FFQ, developed by the Nutrition Assessment Shared Resource (NASR) of Fred Hutchinson Cancer Center, Seattle, Washington, assessed dietary intake by asking participants to report the frequency of consumption and portion size of 181-food items over the last year. Each item was defined by a series of foods or beverages that were categorized into 10 major food groups: CEREALS, BREADS, SNACKS; MEAT-FISH-EGGS; SPAGHETTI, MIXED DISHES, SOUPS; DAIRY PRODUCTS; VEGETABLES AND GRAINS; SAUCES AND

CONDIMENTS; FRUITS; SWEETS; BEVERAGES AND ALCOHOL. 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, medium, large).

Nutrients that have been previously associated with *H. pylori* infection were chosen for these analyses. These included sodium, alcohol, vitamin C, vitamin A, vitamin E, and folate.[93,98] We also explored other nutrient variables, such as energy (calories), carbohydrates, fiber, total cholesterol, total fat, and protein.[98]

Navajo Food Questionnaire

To assess the frequency of consumption of tribal foods over the last year, we developed a culturally appropriate Navajo Food Questionnaire by referring to tribal food lists from previous studies[99,100] and following the same consumption categorization as the FFQ. We selected 13 tribal foods based on foods consumed among American Indian people and on the cultural characteristics of the Navajo people.[100–102] The 13 food items selected were frybread, Navajo taco, Navajo roast mutton, Navajo burger, pueblo bread, kneel down bread, blue corn mush, mutton and/or beef stew with dumplings and/or macaroni, mutton and/or beef stew with corn or any vegetables, blood sausages, achee', Navajo cake, and chilchin. These tribal foods were grouped into 5 food groups: BREADS, SOUPS OR STEWS, CEREAL, MEATS, AND DESSERTS AND SNACKS. Two Navajo researchers reviewed the questionnaire to determine

the appropriateness of selected foods and the length of the questionnaire. We did not add or remove any food items after reviews; however, we added an additional open-ended question for participants to report foods not listed. The values used for the frequency of consumption were the following: 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. Annual servings for each tribal food were calculated using the frequency of consumption reported and a set medium portion size (1.0 serving ratio).

Identification of dietary patterns

Of the combined 181- and 13-food items, we first removed food items with low consumption (-≤10%) and then categorized food items into 20 food groups based on culinary value and nutritional content (Table 2.1).[92,93,98,99,103–105] Subsequently, we applied principal component analysis (PCA) with varimax rotation to all 20 food groups in two steps using R studio (version 4.0.3, psych package). First, we applied PCA to generate a set of initial rotated principal components. Second, after evaluation of the eigenvalues and Scree plot of the initial components, PCA was applied to a subset of components above an eigenvalue of >1.0. Among the subset of principal components, only the components that greatly explained the variance of diet were selected in our analysis. Food groups with a factor score greater than an absolute value of 0.30 were considered main contributors to the dietary component and represented the character of each dietary component.[103,104] The labels of dietary components were named based on the food groups listed in Table 2.1 with a factor score ≥ |0.30| and informed by other studies.[103,104] For each diet pattern, the factor scores were grouped into tertiles based on the distribution of factor scores among *H. pylori* negative participants.

Measurement of *Helicobacter pylori*

The presence of *H. pylori* (positive or negative) was determined from stool samples collected by the participant. Stool samples were collected at home and placed in a sample vial with 5-mL of 95% ethanol preservative and stored at room temperature before mailing to the Salama Lab at the Fred Hutchinson Cancer Center (Seattle, WA).

At the lab, samples were analyzed using droplet digital PCR (polymerase chain reaction) assays following standard protocols which have been validated in adult stool in the Salama Lab.[42] A sample with greater than 5 positive *H. pylori* 16S-DNA droplets above a recommended threshold of 4500 was considered to be positive for *H. pylori*.[42]

Measurement of covariates

Data on covariates were collected from self-administered questionnaires. The 26-item health questionnaire accessed characteristics previously associated with *H. pylori* infection in other populations, such as demographics, drinking water consumption, health conditions, medication use, family health history, and health behaviors. Relevant covariates were classified as presented in Table 2.2.

Statistical analyses

We were most interested in examining the association between dietary patterns and *H. pylori* infection, particularly dietary patterns with high sodium foods (processed meats, refined grains, salty snacks, soups, mixed dishes, and sauces) and/or low consumption of fruits and vegetables.

We further analyzed the association between the following selected nutrient factors and *H. pylori* infection: intake of sodium, alcohol, vitamin C, vitamin A, vitamin E, and folate.

Three logistic regression models were used to examine the relationship between dietary pattern tertile scores and *H. pylori* infection. Odds ratios (OR) and 95% CI were calculated using participants in the lowest tertile (T1) as the referent group. Model 1 was used to calculate the crude OR. Model 2 adjusted for age, sex, and total energy intake (log-transformed kcal/day). Model 3 adjusted additionally for education, daily aspirin use, BMI, smoking, and variables that were significantly associated with *H. pylori* infection from univariate analyses. Due to confounding and effects of measurement error by energy intake, we adjusted Models 2 and 3 for total energy intake.[106] Energy intake was not normally distributed and was therefore log-transformed in the model. To test for significant increasing or decreasing trends across the tertile categories, we created a continuous variable from the median factor score values of each tertile and modeled this continuous variable in a logistic regression model. In addition, we used χ^2 and Fisher's exact tests to examine differences in the percent distribution of participants' characteristics between the lowest and highest tertiles of each diet pattern.

Dietary factors (i.e., nutrients and food groups) were analyzed both continuously and categorically as tertiles. As continuous measures, dietary factors were log-transformed and examined by a two-sample t-test for association with *H. pylori* infection. Dietary variables were categorized into tertiles based on the distribution in *H. pylori*-negative participants and modeled in three logistic regression models, as described above, comparing the highest tertile (T3) nutrient intake with *H. pylori* status using the lowest tertile (T1) as the referent group.

We further calculated the frequency, means (standard deviations) for continuous measures, and percentages for categorical measures for the overall study population and by *H. pylori* infection status for our covariates. We then tested the distribution of covariates by *H. pylori* infection status (positive versus negative) using a two-sample t-test for continuous measures and either a χ^2 or Fisher's exact test for categorical measures, depending on case count. The relevant covariates in the study, parameterized as in Table 2.2, included age, gender, education, the number of people living in the household, history of *H. pylori*, history of gastritis or ulcers, family history of stomach cancer, family history of peptic ulcers, aspirin daily use, BMI, smoking, alcohol use in the past month, type of drinking water consumed, and owned livestock. Other variables were explored, such as having running water in the home, health conditions (diabetes, heartburn), medication use (prescription, over the counter, vitamins, Navajo herbal medicine), physical activity, eating a special diet due to a health condition, food questionnaire assessment, and Navajo cultural connection.

All analyses were performed using R Studio version 4.0.3 (R Core Team, Vienna, Austria). A value of $p<0.05$ was considered statistically significant. We used complete case analysis for our regression analyses, as data for several relevant covariates, particularly total energy intake, daily aspirin use, BMI, and smoking were missing for <5% of study participants.

Results

Study population

A total of 104 participants completed questionnaires and provided stool samples. From these, we excluded participants with incomplete dietary data (n=6) or extreme energy intakes (<500 (n=4) or >5,500 (n=1) kcal/day for females and <500 or >8,000 for males).[107,108] The final analyses were based on 93 participants.

In the study population, the *H. pylori* prevalence was 57.0% (95% CI, 46.3-67.2). The characteristics of participants overall and by *H. pylori* status are presented in Table 2.2. Overall, the majority of participants were between 18-44 years old, female, had some college education, lived in a household with 3 people or more, consumed tap water, had a BMI equal to or greater than 30, were not on a special diet due to a health condition, and had a high connection (practiced tribal traditions, participated in tribal ceremonies, lived off the land, and/or ate traditional foods/ herbs "All the time or "Most of the time"). to the Navajo culture. Participants with *H. pylori* infection were significantly less likely to have a prior history of *H. pylori* (p=0.036) and more likely to use monthly Navajo herbal medicine (p=0.021) compared to participants without *H. pylori* infection.

Dietary patterns

After varimax rotation, the principal component analysis revealed six components with eigenvalue >1.0 (Figure 2.1). Three dietary components were subsequently selected because they explained the largest variance, and the main factor loadings of each pattern are shown in Table 2.3. The first three rotated diet components explained 45.3% of the total variation. Individually,

the first component explained 26.5% of variation followed by 10.0% for the second, and 8.8% for the third. The three patterns were identified as follows: component 1 was labeled as the "Western" pattern characterized by the intake of processed meats, refined grains, sweets and pastries, salty snacks, meat, dairy, potatoes, and vegetables; component 2 was the "Soups & Mixed dishes" pattern which included the intake of soups and mixed dishes such as spaghetti, pasta, asian noodles, burritos, and enchiladas; component 3 was identified as the "Fruits & Vegetables" pattern characterized by the intake of fruits and vegetables.

Dietary patterns and participant characteristics

Participants in the highest tertile (T3) of the Western pattern were more likely to have a BMI $\geq$30 and to have indicated the FFQ used in the study completely represented the foods they ate compared to participants in the lowest tertile (T1) (Table 2.4). For the Soups and Mixed Dishes pattern, participants in the highest tertile were more likely to be males and to have a family history of peptic ulcers compared to participants in the lowest tertile. Moreover, participants of the highest tertile of the Fruits and Vegetable pattern were more likely to never have smoked compared to participants of the lowest tertile.

Association between dietary patterns and *H. pylori* infection

The associations between dietary patterns and *H. pylori* infection are shown in Table 2.5. The second tertile (T2) of the Western diet pattern in the adjusted model 3 was inversely associated with the prevalence of *H. pylori* infection (OR=0.26, 95% CI, 0.07-0.92); however, the association was not statistically significant in the highest tertile (T3) of the same model (OR=0.37, 95% CI, 0.09-1.37). The Soups and Mixed dishes pattern was positively associated

with *H. pylori* infection status in the fully adjusted model 3 for tertiles 2 (OR=4.32, 95% CI, 1.05-20.99) and 3 (OR=5.21, 95% CI, 1.10-29.00) compared to tertile 1; however, the trend for increasing odds of *H. pylori* across the tertiles was not significant (p=0.074). There was no significant association observed between the Fruits and Vegetable pattern and *H. pylori* infection status in the unadjusted and adjusted models.

Association between nutrient intake and *H. pylori* infection

Comparisons of the distribution of mean daily nutrient intake of selected dietary factors are shown in Table 2.6. There were no significant differences in the mean distribution of daily nutrient intake by *H. pylori* infection status. Yet, compared to *H. pylori*-negative participants, *H. pylori*-positive participants had a lower mean daily intake of vitamin A and higher mean daily intake of sodium, alcohol, vitamin C, vitamin E, and total folate. The mean daily intake of energy and macronutrients such as carbohydrate, fiber, total cholesterol, total fat, and protein was also not statistically different between *H. pylori*-negative and *H. pylori*-positive participants; however, the mean intakes of energy, carbohydrate, fiber, total fat, and protein were slightly higher in *H. pylori*-positive participants.

In Table 2.7 we examined the association between relevant dietary nutrient intake (sodium, alcohol, vitamin C, vitamin A, vitamin E, and folate) and *H. pylori* infection. In the fully adjusted models, comparing highest tertile to lowest tertile, no individual nutrient was significantly associated with *H. pylori* infection. However, although based on small numbers, the results suggest that high sodium, vitamin E, and folate intake were associated with a higher odds of *H. pylori* infection after adjusting for confounders (OR_{sodium}= 5.88, 95% CI, 0.38-107.2;

$OR_{vitaminE}$ = 1.44, 95% CI, 0.18-11.92; OR_{folate}= 3.57, 95% CI, 0.37-37.97). Being in the highest tertile of alcohol, vitamin C, and vitamin A intake was suggestively associated a decreased odds of *H. pylori* infection compared to the lowest tertile ($OR_{alcohol}$ = 0.42, 95% CI, 0.11-1.50; $OR_{vitaminC}$ = 0.36, 95% CI, 0.08-1.49; $OR_{vitaminA}$ = 0.46, 95% CI, 0.46-4.14).

Discussion

In this study, we identified three dietary patterns and found that a Soups and Mixed dishes diet pattern was positively associated with *H. pylori* infection in Navajo participants. After adjusting for confounders, no significant associations were observed between either the Western or the Fruits and Vegetable diet pattern and *H. pylori* infection. Although findings with respect to nutrient intake were not statistically significant, we found suggestions that a greater intake of sodium and folate was positively associated with *H. pylori* infection, while also suggesting a stronger inverse association for *H. pylori* with a greater intake of vitamin A, vitamin C, and alcohol.

The positive association we found between the Soups and Mixed dishes pattern and *H. pylori* infection may be attributed to the consumption of foods with high sodium and carbohydrate content, as this pattern includes meat/chili/Navajo stews, ramen, spaghetti, pasta, Asian noodles, burritos, and enchiladas. To our knowledge, there are no studies, especially among American Indian people, identifying a Soups and Mixed dishes diet pattern associated with *H. pylori* infection. Nonetheless, consistent with our findings, Tsugane et al. found a positive association of daily consumption of miso soup, a high salt content food, and *H. pylori* infection risk in Japanese men.[109] Similarly, a large cross-sectional study in China identified a positive

association between a “high salt” diet pattern, that included noodles and instant noodles, such as ramen, and *H. pylori* infection in 3,014 older adults (45-59 years old).[103] On the other hand, another large cross-sectional study in China found a negative association between a “high protein/cholesterol” diet pattern that included instant noodles and *H. pylori* in 10,407 adults; however, instant noodles were not the main contributors to that diet pattern.[104] An explanation for our positive association may be related to the high sodium content of soups and noodle dishes in our pattern[110–112], which can contribute to the pathogenicity of *H. pylori* by direct gastric mucosal damage and increased colonization of the bacteria.[28,90] Another suggested explanation is the link between carbohydrates and *H. pylori* risk, as the foods in the Soups and Mixed dishes pattern can be considered refined carbohydrates (e.g., noodles, pasta).[98,104,113] Although the mechanism is not clear, it is hypothesized that high consumption of carbohydrates can play a significant role in *H. pylori* infection through abnormal glucose metabolism.[113] Finally, Navajo stew, pasta, burritos, and enchiladas may be consumed in the restaurant or purchased from local food stands, food trucks, or mobile vendors[114], which can be processed and prepared with added sodium and carboyhydrates.[112] It is also possible that environmental transmission of *H. pylori* may occur through consuming contaminated water and food or interacting with vendors in these food settings.[115] More research may be needed on the role of environmental transmission of *H. pylori* at local food vendors in the Navajo Nation.

We found no association between our Western diet pattern and *H. pylori* infection, as also found in another cross-sectional study of about 3,000 Chinese adults.[103] Shu et al. found a Western dietary pattern characterized by high consumption of red and/or processed meat and energy-dense foods[103] was not associated with *H. pylori* infection.[103] In contrast, our results differed

from the results in another large cross-sectional study based in China of 10,407 adults (Xia et al.).[104] They identified two diet patterns similar to our Western diet pattern and found that a high carbohydrate/sweet pattern was positively associated with *H. pylori* and a high protein/cholesterol pattern was inversely associated with *H. pylori*.[104] Because the foods in our Western diet pattern aligned with foods in both the high carbohydrate/sweet and high protein/cholesterol patterns of the Xia et al. study, and the results were opposing, this may be evidence for our non-significant finding. It may be that individual foods within our Western diet pattern attenuate the effect of the association between our Western diet pattern and *H. pylori*. On the one hand, high consumption of processed meat, salty snacks, and refined grains contain high sodium content and studies have shown high sodium intake promotes the colonization and viability of *H. pylori* through deterioration and damage to the gastric mucosa.[116] Similarly, an elevated risk of *H. pylori* infection was reported with high consumption of carbohydrates, bread, and refined grains in several studies,[104,113]owing to altered glucose metabolism because of type II diabetes and changes in the gastric mucosa that promote *H. pylori* colonization. On the other hand, animal foods such as red meat are rich in selenium, vitamin A, and vitamin D, which can decrease the risk for *H. pylori* infection. Specifically, selenium is an essential nutrient that protects membranes from oxidative damage[117], and vitamins A and D are known to play a critical role in immune response and aid in suppressing *H. pylori* inflammation by reducing inflammatory signaling.[118] Also of note, the large sample size in the Xia et al. study may have allowed them to observe an effect not seen in our study of 93 Navajo adults. Furthermore, the lack of association between this Western pattern and *H. pylori* could also be due to reverse causality. Participants who may be experiencing gastrointestinal symptoms may have limited their intake of food containing sugar and meats.

Further, we found no significant association between a Fruits and Vegetable diet pattern, which is characterized by fruits and vegetables only, and *H. pylori*. This supports previous studies involving a large sample of Asian adults that showed no association between a "balanced" and "health-conscious" diet pattern and *H. pylori*.[103,104] The healthy dietary constituents from fruits and vegetables have shown protective effects against *H. pylori*. The fruits and vegetables in this diet pattern should contain flavonoids that protect the gastric lining[119], and other phytochemicals, such as isothiocyanates. The isothiocyanate sulforaphane, has been shown to have antibacterial activity and reduce *H. pylori* growth.[93,120] However, in our study we saw no association.

After adjusting for confounders, we found no associations with selected dietary nutrients previously suggested to be associated with *H. pylori*. A likely explanation for no significant differences could be due to the small sample size which can be seen in the estimated wide confidence intervals. Nonetheless, we did observe suggestive directions of the odds ratio for *H. pylori* across the nutrient tertiles. In particular, we observed a suggestive higher odds for *H. pylori* infection associated with a higher intake of sodium, which is consistent with other epidemiological studies.[28,90,121] As mentioned previously, a high sodium intake is responsible for the deterioration and damage to the gastric mucosa and *H. pylori's* successful colonization in the stomach.[31]

Previous results of the association between alcohol intake and *H. pylori* are mixed. Our finding of a suggestive inverse association between high alcohol intake and *H. pylori* aligns with previous large epidemiological studies, including a meta-analysis study.[67,122,123] In particular, based on national survey data in Germany (~1,800 adults), Brenner et al. found a dose-response

between alcohol consumption and *H. pylori* infection risk, demonstrating decreasing odds ratio for *H. pylori* infection in adults with increasing consumption of alcohol (0-10, 10-20, 20+ grams of alcohol/day).[67] An inverse association may be observed because alcohol may reduce the risk for *H. pylori* infection through antimicrobial activities and increased gastric acid secretion which provides less desirable pH conditions for new or existing *H. pylori* infections.[69] Yet in other studies, results showed a positive association between alcohol consumption and *H. pylori* in Korea and India possibly due to the focus on heavy and binge alcohol drinkers in one study (Ma et al., 2015) and dyspeptic patients (Kanakala et al., 2017) in the other.[68,69]

The association between vitamin C and *H. pylori* is mixed. Vitamin C is a micronutrient that comes from fresh vegetables and fruits, particularly citrus fruits. The suggestive inverse associations of high vitamin C intake with *H. pylori* in our study are in line with other findings. Based on two randomized trials, vitamin C supplements improved the eradication and suppression of *H. pylori* in *H. pylori*-infected patients.[124,125] In addition, in vitro studies showed that gastric cell lines with *H. pylori* strains treated with vitamin C had altered cell growth and interrupted cell cycle events.[126–128] In contrast, null associations were found from a case-control study of dietary intake of vitamin C in 1,245 Korean adults (OR= 0.72 (0.32 – 1.65))[129], a randomized study of oral vitamin C supplements in 38 patients,[130] and meta-analysis of six randomized studies on vitamin C and E supplement efficacy.[131] Similar to alcohol, vitamin C appears to show an inverse association with *H. pylori* but the evidence is mixed.

Vitamin A comes from animal products, vitamin supplements, fortified foods, and many fruits and vegetables. To our knowledge, there are no epidemiological studies of the direct relationship

between dietary vitamin A intake and *H. pylori* infection to compare our results. Nonetheless, in vitro studies found vitamin A-treated gastric epithelial AGS cells inhibited *H. pylori*-induced expression of tumor necrosis factor receptors (TRAF1 and TRAF2) and β-catenin-regulated genes (c-myc and cyclin E), thus reducing *H. pylori* hyper cell proliferation.[126,132] Furthermore, vitamin A is known to inhibit *H. pylori*-induced inflammatory responses and suppress oxidative stress.[133] This supports our suggestive inverse finding between vitamin A intake and *H. pylori* infection.

Vitamin E is found in plant-based oils, nuts, seeds, fruits and vegetables. The association between vitamin E with *H. pylori* is not definitive, despite our observation of a suggestive positive association between vitamin E and *H. pylori*. To our knowledge, there are limited epidemiological studies that have directly examined the independent effect of high dietary vitamin E intake and *H. pylori*. In a meta-analysis of six randomized controlled trials of *H. pylori* patients, the data are not conclusive on the effectiveness of vitamin C and E with *H. pylori* infection due to small sample size and issues with double blinding and allocation methods.[131] However, compared to an in vitro study, vitamin E may have an antibacterial effect and that combined with vitamin C during antibiotic treatment may suppress *H. pylori* colonization in Mongolian gerbils.[134]

Lastly, folate is naturally found in dark green leafy vegetables, beans, nuts, seafood, eggs, fruits; and is added to foods like breads, cereal, pasta, and other grains. We observed that a high intake of folate suggests a positive association with *H. pylori*. Because there are limited studies to our knowledge that directly study the direct association between dietary folate intake and *H. pylori*, it

is difficult to compare our findings with current knowledge.[135] Among patients with dyspepsia, a study looking at serum folate and B_{12} levels and *H. pylori* found individuals with *H. pylori* have deficient folate and B_{12} serum levels likely due to *H. pylori* infection, which may impact a person's risk for other health conductions such as atherosclerosis.[136]

Many of the demographic, health, environmental, and lifestyle factors assessed in this study are considered risk factors for *H. pylori* infection; however, most were not differently distributed by *H. pylori* status in our study, except for having a history of *H. pylori* infection and using monthly Navajo herbal medicine (see Chapter 1). Compared to *H. pylori*-negative participants, having a history of *H. pylori* infection was significantly less prevalent in participants who were *H. pylori*-positive while monthly use of Navajo herbal medicine was significantly more prevalent. The relationship of these factors with diet and *H. pylori* is interesting because these factors may influence dietary habits and may be linked to unidentified dietary constituents, particularly in Navajo herbal medicines, not characterized in this study. Certain medicinal plants used in Native cultures have been correlated with antibacterial activity[137] and medicinal plants from cultures worldwide have shown protective effects against *H. pylori*.[138,139] Additional research on the role of Navajo herbal medicine is needed in the Navajo Nation, especially when over half of study participants are highly connected to their culture and 15% use monthly Navajo herbal medicines.

While these results provide a greater understanding of the association between diet and *H. pylori*, it is imperative to provide context about the food system in the Navajo Nation as this could also be a strong risk factor for *H. pylori,* and health in general.[140,141] Foremost, Indigenous food systems have been displaced since the start of colonization, industrialization, and economic,

political, and environmental changes that have disrupted the food environments of Indigenous people, including the Navajo Nation.[141] Today, structural barriers exist as large distances to grocery stores, income disparities, and lack of access to transportation, present a challenging food environment in the Navajo Nation.[142] There are 15 grocery stores and 85 smaller convenience stores that sell food across the vast 17 million acres that constitute the Navajo reservation.[143,144] Therefore, low access to affordable and healthy food subsequently can result in low reporting of fruits and vegetables and high intake of fats and calorie-dense foods in the Navajo people. As such, the Navajo people may have moved away from a traditional way of life, including a traditional diet, due to the influence of Western culture and structural barriers.[145] Moreover, the existing and continued environmental pollution due to mining and resource extraction has further compromised the essential water sources in the Navajo Nation.[146,147] It is therefore important to shed light on the historical injustices and policies that have shaped the food choices and diet of the Navajo people.

Notwithstanding, there are some limitations of the study that should be considered when interpreting these results. First, a convenience sampling method was used to select participants, thereby limiting the generalizability of results to the Navajo Nation. Moreover, 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. This may have unintentionally excluded a large segment of the Navajo population who do not have internet access.[84,85] Second, our study has limited power to detect statistical significance in our analyses. This limits our ability to generalize to the Navajo adult population and the relatively small sample of cases

across dietary patterns in some of our analyses may have resulted in imprecise estimates and thus should be interpreted with caution. Third, the a posteriori approach we used uses a data-driven approach to derive the diet patterns that are specific to the Navajo study population and results are less likely to be comparable to different study populations, especially over time, due to changes in population, society, and economic profiles, as well as changes to food intake assessments. Fourth, dietary assessment was based on an FFQ and tribal food questionnaire, and 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 and included tribal foods. Fifth, 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. Sixth, because our study is cross-sectional, the associations between our dietary patterns and nutrients with the odds of *H. pylori* infection must be interpreted with caution, given the possibility of reverse causation. Seventh, 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. Lastly, PCA is an exploratory method, and we subjectively decided on the number and types of dietary patterns created, and how these patterns were analyzed.[148]

Our study also presents some notable strengths. Firstly, this study is the only study addressing the association between dietary patterns and *H. pylori* infection in American Indian populations

at the tribal level. Secondly, our sample of participants comes from the community population, which is more representative than the numerous hospital-based studies in the literature. Thirdly, in our multivariate logistic models, we adjusted for a variety of potential confounders for reliability. Fourthly, this study takes into consideration dietary behaviors of a specific place (i.e., geography, socioeconomic status, culture, and policies) and time in the Navajo Nation, as well as captures foods (i.e., Navajo food questionnaire) local to the food system. Lastly, the PCA method helped summarize dietary behaviors across many variables into a smaller grouping of variables, which was useful for identifying major dietary patterns. The identified dietary patterns in our study may drive further research questions about specific foods or nutrients.

In conclusion, we found the Soups and Mixed dishes dietary pattern was associated with *H. pylori* infection, whereas the Western and Fruits and Vegetable dietary patterns were not associated with *H. pylori* infection among Navajo adults. We also found that none of the selected nutrients were significantly associated with *H. pylori* infection despite suggested directions of association. Further studies are needed to understand the relationship between diet and *H. pylori* infection in American Indian populations with high *H. pylori* prevalence, recognizing the heterogeneity of potential risk factors across diverse population groups. Also, more *in vitro* and animal studies are needed to elucidate the mechanisms and effects of how dietary nutrients increase or decrease *H. pylori* infection risk. Our findings add to the *H. pylori* body of literature and derive from the first study in Navajo Nation examining the relationship between dietary patterns and *H. pylori* infection. These results can inform nutritional counselors, public health professionals, and clinicians about population dietary and lifestyle habits. It may also allow for

the design of health education material that promotes general recommendations on healthy eating patterns and encourage the availability of healthy foods and choices.

Table 2.1. Food groups used in the principal component analysis.

Criteria for group	Food group	Food items
Nutrient profile (sodium)	Processed meats	Bacon, sausage, hot dogs, bratwurst, lunch meat, spam, fried fish, fried chicken
Nutrient profile (sodium/carbohydrates)	Refined grains	Cereals, breads, pancakes, white rice, frybread
Nutrient profile (carbohydrates)	Sweets & pastries	Jams, cereal bars, cookies, candy, donuts, Navajo cake
Nutrient profile (carbohydrates)	Sweets	Ice cream, puddings
Nutrient profile (sodium)	Salty snacks	Chips, popcorn, crackers
Nutrient profile (protein)	Meat	Eggs, red meat, white meat, fish, and seafood
Culinary use	Organ meat	Liver, organ meats, achee, blood sausage
Nutrient profile (dairy)	Dairy	Cream, milk, yogurt, sour cream
Nutrient profile (fats)	Fats	Butter, margarine, lard, meat drippings, oils
Nutrient profile (sodium)	Soups	Meat stews, chili, ramen, Navajo stews (dumplings or vegetable)
Nutrient profile (sodium)	Mixed dishes	Spaghetti, pasta, Asian noodles, burritos, enchiladas
Nutrient profile (carbohydrates)	Potatoes	Fried, boiled, baked, mashed, salads
Nutrient profile (phytochemicals)	Vegetables	Green lettuce/spinach/collards, carrots, tomatoes, peppers/chili, broccoli, cabbage, peas, beans, squash, sweet potatoes, onions, garlic, avocado, kneel down bread
Nutrient profile (phytochemicals)	Fruits	Apples, bananas, peaches, apricots, dried fruits, oranges, berries, melons, grapes, cherries, pineapple, fruit cocktail
Nutrient profile (caffeine)	Coffee	Coffee, latte, tea
Nutrient profile (vitamins)	Juices	Fruit juices, vegetable juices
Nutrient profile (carbohydrates)	Soft drinks	Diet and regular soft drinks
Nutrient profile (alcohol)	Alcohol	Beer, wine, liquor
Nutrient profile (sodium, fats)	Sauces	Cheese or cream sauces, gravies, salad dressing, salsa, mayonnaise
Nutrient profile (fiber)	Whole grain/High fiber	Whole-grain crackers, brown rice, whole-grain bread, high fiber cereal

Table 2.2 Percent distribution of selected characteristics by *Helicobacter pylori* (*H. pylori*) status.

Characteristics	Overall (N=93)	*H. pylori*-negative (n=40)	*H. pylori*-positive (n=53)	P-value[a]
Age, mean (SD)	46.0 (14.1)	47.3 (15.2)	45.0 (13.4)	0.453
Age group (%)				
18-44	47 (50.5)	17 (42.5)	30 (56.6)	0.386
45-54	21 (22.6)	10 (25.0)	11 (20.8)	
55+	25 (26.9)	13 (32.5)	12 (22.6)	
Gender (%)				
Female	68 (73.1)	30 (75.0)	38 (71.7)	0.905
Male	25 (26.9)	10 (25.0)	15 (28.3)	
Education (%)				
Less than High school	2 (2.2)	1 (2.5)	1 (1.9)	0.491
High school	19 (20.4)	11 (27.5)	8 (15.1)	
Some college	43 (46.2)	16 (40.0)	27 (50.9)	
College degree	29 (31.2)	12 (30.0)	17 (32.1)	
People living in house, mean (SD)	3.54 (1.8)	3.53 (1.7)	3.6 (1.9)	0.954
People living in house (%)				
<3	28 (30.1)	11 (27.5)	17 (32.1)	0.804
≥3	65 (69.9)	29 (72.5)	36 (67.9)	
Running water in home (%)	84 (90.3)	37 (92.5)	47 (88.7)	0.727
Type of water consumed (%)				
Filtered or Unfiltered Tap water	51 (54.8)	23 (57.5)	28 (52.8)	0.906
Bottled water	40 (43.0)	17 (42.5)	23 (43.4)	
Windmill water	1 (1.1)	0 (0)	1 (1.9)	
Don't know / Unsure	1 (1.1)	0 (0)	1 (1.9)	
Owned livestock (%)	23 (24.7)	10 (25.0)	13 (24.5)	1.000
Health Conditions (%)				
Diabetes	20 (21.5)	10 (25.0)	10 (18.9)	0.647
Heartburn	11 (11.8)	5 (12.5)	6 (11.3)	1.000
History of *H. pylori*	9 (9.7)	7 (17.5)	2 (3.8)	0.036[b]
History of gastritis or ulcers	8 (8.6)	5 (12.5)	3 (5.7)	0.283
Aspirin daily use (%)	18 (19.4)	9 (22.5)	9 (17.0)	0.601

Medication use, once a month (%)				
Prescription medicine	41 (44.1)	19 (47.5)	22 (41.5)	0.715
OTC stomach medicine	26 (28.0)	7 (17.5)	19 (35.8)	0.064
Vitamins	58 (62.4)	21 (52.5)	37 (69.8)	0.136
Navajo herbal medicine	14 (15.1)	2 (5.0)	12 (22.6)	0.021[b]
Family health history (%)				
Stomach cancer	16 (17.2)	8 (20.0)	8 (15.1)	0.587
Peptic ulcers	8 (8.6)	4 (10.0)	4 (7.5)	0.212
BMI (kg/m^2) (%)				
Below 24.9	16 (17.2)	5 (12.5)	11 (20.8)	0.458
25.0 – 29.9	29 (31.2)	12 (30.0)	17 (32.1)	
30 or higher	45 (48.4)	22 (55.0)	23 (43.4)	
Missing	3 (3.2)	1 (2.5)	2 (3.8)	
Physical activity (%)				
No physical activity	8 (8.6)	4 (10.0)	4 (7.5)	0.145
Low (<150 mins / week)	36 (38.7)	11 (27.5)	25 (47.2)	
Moderate (150-300 mins / week)	23 (24.7)	11 (27.5)	12 (22.6)	
High activity (>300mins / week)	14 (15.1)	9 (22.5)	5 (9.4)	
Missing	12 (12.9)	5 (12.5)	7 (13.2)	
Smoking (%)				
Never smoked	57 (61.3)	28 (70.0)	29 (54.7)	0.312
Ever smoked	25 (26.9)	8 (20.0)	17 (32.1)	
Current smoker	9 (9.7)	3 (7.5)	6 (11.3)	
Missing	2 (2.2)	1 (2.5)	1 (1.9)	
Alcohol use in the past month (%)				
Never drank	15 (16.1)	9 (22.5)	6 (11.3)	0.090
Past	48 (15.6)	16 (40.0)	32 (60.4)	
Current	28 (30.1)	15 (37.5)	13 (24.5)	
Refused	2 (2.2)	0 (0)	2 (3.8)	
Eat a special diet due to a health condition (%)				
Yes	13 (14.0)	6 (15.0)	7 (13.2)	0.616
No	78 (83.9)	34 (85.0)	44 (83.0)	
Prefer not to answer	2 (2.2)	0 (0)	2 (3.8)	

FFQ represented participant diet[c] (%)				
Yes, completely	24 (25.8)	13 (32.5)	11 (20.8)	0.353
Yes, mostly	49 (52.7)	21 (52.5)	28 (52.8)	
Yes, somewhat	17 (18.3)	5 (12.5)	12 (22.6)	
No, not at all	1 (1.1)	0 (0)	1 (1.9)	
Missing	2 (2.2)	1 (2.5)	1 (1.9)	
Navajo acculturation scale (%)				
Low cultural connection	45 (45.2)	22 (55.0)	20 (37.7)	0.228
High cultural connection	48 (51.6)	18 (45.0)	30 (56.6)	
Missing	3 (3.2)	0 (0)	3 (5.7)	

[a]Chi-square test or t-test; [b]statistical significance $p<0.05$; [c]Participants were asked if the Fred Hutch FFQ used in the study represented the foods they normally ate; Abbreviation: SD=standard deviation; BMI=Body Mass Index

Table 2.3 Rotated factor-loading matrix for the 3 dietary patterns*.

Food groups	Western	Soups/Mixed Dishes	Fruits/Vegetables
Processed meats	0.866	-	-
Refined grains	0.696	-	-
Sweets & pastries	0.668	-	-
Sweets	0.776	-	-
Salty snacks	0.745	-	-
Meat	0.847	-	-
Organ meat	-	-	-
Dairy	0.376	-	-
Fats	-	-	-
Soups	-	0.941	-
Mixed dishes	-	0.924	-
Potatoes	0.580	-	-
Vegetables	0.341	-	0.791
Fruits	-	-	0.872
Coffee	-	-	-
Juices	-	-	-
Soft drinks	-	-	-
Alcohol	-	-	-
Sauces	0.603	-	-
Whole grain/High fiber	0.740	-	-
Variance explained (%)	26.5	10.0	8.8

*For simplicity, absolute values <0.3 were excluded.

Table 2.4 Characteristics of study participants across tertiles[a] (T) categories of factor loading values for each diet pattern.

	Western			Soups/Mixed Dishes			Fruits/Vegetables		
Characteristics	**T1 (n= 41)**	**T3 (n= 28)**	**P-value[b]**	**T1 (n= 20)**	**T3 (n=40)**	**P-value[b]**	**T1 (n= 30)**	**T3 (n=33)**	**P-value[b]**
Age group (%)									
18-44	20 (48.8)	15 (53.6)	0.740	8 (40.0)	25 (62.5)	0.265	12 (40.0)	17 (51.5)	0.472
45-54	8 (19.5)	8 (28.6)		4 (20.0)	7 (17.5)		8 (26.7)	6 (18.2)	
55+	13 (31.7)	5 (17.9)		8 (40.0)	8 (20.0)		10 (33.3)	10 (30.3)	
Gender (%)									
Female	32 (78.0)	17 (60.7)	0.208	19 (95.0)	25 (62.5)	0.028[c]	23 (76.7)	24 (72.7)	0.842
Male	9 (22.0)	11 (39.3)		1 (5.0)	15 (37.5)		7 (23.3)	9 (27.3)	
Education (%)									
Less than High school	1 (2.4)	0 (0)	0.819	1 (5.0)	0 (0)	0.113	0 (0)	1 (3.0)	0.335
High school	7 (17.1)	6 (21.4)		4 (20.0)	5 (12.5)		7 (23.3)	5 (15.2)	
Some college	18 (43.9)	13 (46.4)		11 (55.0)	17 (42.5)		15 (50.0)	12 (36.4)	
College degree	15 (36.6)	9 (32.1)		4 (20.0)	18 (45.0)		8 (26.7)	15 (45.5)	
Type of water consumed (%)									
Filtered or Unfiltered Tap water	26 (63.4)	14 (50.0)	0.233	9 (45.0)	22 (55.0)	0.406	16 (53.3)	19 (57.6)	0.874
Bottled water	14 (34.1)	14 (50.0)		11 (55.0)	18 (45.0)		14 (46.7)	14 (42.4)	
Windmill water	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)	
Don't know / Unsure	1 (2.4)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)	
Health Conditions (%)									
Diabetes	7 (17.1)	9 (32.1)	0.307	5 (25.0)	6 (15.0)	0.416	5 (16.7)	8 (24.2)	0.814
Heartburn	2 (4.9)	5 (17.9)	0.143	3 (15.0)	5 (12.5)	0.779	3 (10.0)	3 (9.1)	0.719
History of *H. pylori*	4 (9.8)	4 (14.3)	0.546	3 (15.0)	5 (12.5)	0.260	3 (10.0)	5 (15.2)	0.314
History of gastritis or ulcers	1 (2.4)	5 (17.9)	0.082	3 (15.0)	4 (10.0)	0.249	3 (10.0)	4 (12.1)	0.526
Aspirin daily use (%)	11 (26.8)	4 (14.3)	0.297	4 (20.0)	6 (15.0)	0.627	7 (23.3)	6 (18.2)	0.849

Medication use, once a month (%)									
Prescription medicine	14 (34.1)	16 (57.1)	0.168	10 (50.0)	16 (40.0)	0.742	12 (40.0)	15 (45.5)	0.899
OTC stomach medicine	7 (17.1)	10 (35.7)	0.104	5 (25.0)	11 (27.5)	0.914	9 (30.0)	9 (27.3)	1.000
Vitamins	28 (68.3)	17 (60.7)	0.513	9 (45.0)	28 (70.0)	0.167	15 (50.0)	23 (69.7)	0.229
Navajo herbal medicine	7 (17.1)	3 (10.7)	0.811	1 (5.0)	9 (22.5)	0.214	4 (13.3)	6 (18.2)	0.875
Family health history (%)									
Stomach cancer	6 (14.6)	8 (28.6)	0.154	0 (0)	7 (17.5)	0.158	4 (13.3)	6 (18.2)	0.836
Peptic ulcers	4 (9.8)	4 (14.3)	0.196	2 (10.0)	8 (20.0)	0.035[c]	1 (3.3)	5 (15.2)	0.268
BMI (kg/m^2) (%)									
Below 24.9	10 (24.4)	0 (0)	0.013[c]	1 (5.0)	8 (20.0)	0.437	4 (13.3)	6 (18.2)	0.659
25.0 – 29.9	13 (31.7)	7 (25.0)		7 (35.0)	10 (25.0)		8 (26.7)	13 (39.4)	
30 or higher	17 (41.5)	19 (67.9)		12 (60.0)	19 (47.5)		17 (56.7)	13 (39.4)	
Missing	1 (2.4)	2 (7.1)		0 (0)	3 (7.5)		1 (3.3)	1 (3.0)	
Physical activity (%)									
No physical activity	3 (7.3)	5 (17.9)	0.169	1 (5.0)	4 (10.0)	0.739	4 (13.3)	1 (3.0)	0.821
Low	12 (29.3)	12 (42.9)		8 (40.0)	17 (42.5)		10 (33.3)	15 (45.5)	
Moderate	12 (29.3)	7 (25.0)		3 (15.0)	11 (27.5)		7 (23.3)	8 (24.2)	
High	7 (17.1)	2 (7.1)		5 (25.0)	4 (10.0)		4 (13.3)	6 (18.2)	
Missing	7 (17.1)	2 (7.1)		3 (15.0)	4 (10.0)		5 (16.7)	3 (9.1)	
Smoking (%)									
Never smoked	22 (53.7)	19 (67.9)	0.886	10 (50.0)	28 (70.0)	0.155	16 (53.3)	27 (81.8)	0.0001[c]
Ever smoked	12 (29.3)	7 (25.0)		8 (40.0)	9 (22.5)		13 (43.3)	4 (12.1)	
Current smoker	5 (12.2)	2 (7.1)		0 (0)	23 (7.5)		0 (0)	2 (6.1)	
Missing	2 (4.9)	0 (0)		2 (10.0)	0 (0)		1 (3.3)	0 (0)	
Alcohol use in the past month (%)									
Never drank	8 (19.5)	2 (7.1)	0.170	5 (25.0)	8 (20.0)	0.110	5 (16.7)	8 (24.2)	0.303
Past	19 (46.3)	20 (71.4)		8 (40.0)	23 (57.5)		16 (53.3)	17 (51.5)	
Current	13 (31.7)	6 (21.4)		6 (30.0)	8 (20.0)		9 (30.0)	7 (21.2)	
Refused	1 (2.4)	0 (0)		1 (5.0)	1 (2.5)		0 (0)	1 (3.0)	
Eating a special diet due to health condition(%)									
Yes	7 (17.1)	3 (10.7)	0.419	4 (20.0)	5 (12.5)	0.920	2 (6.7)	5 (15.2)	0.497
No	34 (82.9)	23 (82.1)		16 (80.0)	34 (85.0)		27 (90.0)	27 (81.8)	
Prefer not to answer	0 (0)	2 (7.1)		0 (0)	1 (2.5)		1 (3.3)	1 (3.0)	

Fred Hutch FFQ represented diet (%)									
Yes, completely	7 (17.1)	8 (28.6)	0.022[c]	4 (20.0)	9 (22.5)	0.678	9 (30.0)	4 (12.1)	0.205
Yes, mostly	22 (53.7)	14 (50.0)		13 (65.0)	20 (50.0)		15 (50.0)	18 (54.5)	
Yes, somewhat	12 (29.3)	5 (17.9)		2 (10.0)	9 (22.5)		6 (20.0)	8 (24.2)	
No, not at all	0 (0)	1 (3.6)		0 (0)	1 (2.5)		0 (0)	1 (3.0)	
Missing	0 (0)	0 (0)		1 (5.0)	1 (2.5)		0 (0)	2 (6.1)	
Navajo acculturation scale (%)									
Low cultural connection	14 (34.1)	15 (53.6)	0.280	12 (60.0)	14 (35.0)	0.283	15 (50.0)	12 (36.4)	0.601
High cultural connection	24 (58.5)	13 (46.4)		8 (40.0)	23 (57.5)		14 (46.7)	19 (57.6)	
Missing	3 (7.3)	0 (0)		0 (0)	3 (7.5)		1 (3.3)	2 (6.1)	

[a]tertile categories based on the distribution of *H. pylori*-negative participants; [b]Chi-square test or Fisher's exact test; [c]statistical significance $p<0.05$; Abbreviation: BMI=Body Mass Index, OTC=over the counter, FFQ=Food Frequency Questionnaire, Western pattern: T1 (-1.585, -0.193) and T3 (0.371, 3.758), Soups/Mixed dishes pattern: T1 (-0.880, -0.386) and T3 (-0.151, 1.615), Fruits/Vegetables pattern: T1 (-0.975, -0.499) and T3 (-0.360, 4.190); Notes: Low physical activity (<150 mins / week), Moderate physical activity (150-300 mins / week), High physical activity (>300mins / week).

Table 2.5 Multivariate adjusted ORs (95% CI) for *Helicobacter pylori* (*H. pylori*) infection across tertiles[a] (T) categories of dietary pattern loading scores.

	Tertiles of factor scores (n=93)			
Dietary patterns	**T1**	**T2**	**T3**	**p for trend**
Western	(-1.585, -0.193)	(-0.192, 0.370)	(0.371, 3.758)	
No. of *H. pylori* infection	28	11	14	
Model 1	1.00	0.39 (0.14-1.10)	0.46 (0.17-1.24)	0.122
Model 2	1.00	0.35 (0.11-1.04)	0.39 (0.13-1.17)	0.097
Model 3	1.00	0.26 (0.07-0.92)[b]	0.37 (0.09-1.37)	0.130
Soups/Mixed dishes	(-0.880, -0.386)	(-0.385, -0.152)	(-0.151, 1.615)	
No. of *H. pylori* infection	6	20	27	
Model 1	1.00	3.59 (1.14-12.45)[b]	4.85 (1.57-16.52)[b]	0.017[b]
Model 2	1.00	5.62 (1.54-24.57)[b]	6.70 (1.75-30.48)[b]	0.019[b]
Model 3	1.00	4.32 (1.05-20.99)[b]	5.21 (1.10-29.00)[b]	0.074
Fruits/Vegetables	(-0.975, -0.499)	(-0.498, -0.361)	(-0.360, 4.190)	
No. of *H. pylori* infection	17	16	20	
Model 1	1.00	0.87 (0.64-2.75)	1.18 (0.43-3.24)	0.613
Model 2	1.00	0.72 (0.02-2.13)	0.95 (0.03-2.87)	0.857
Model 3	1.00	0.47 (0.13-1.63)	1.02 (0.27-3.80)	0.744

[a]tertile categories based on the distribution of *H. pylori-negative* participants; [b]statistical significance $p<0.05$; Model 1 is unadjusted; Model 2 is adjusted for age, gender, calories (log-transformed); Model 3 is adjusted for age, gender, calories (log-transformed), education, daily aspirin use, BMI (categorical), smoking, history of *H. pylori*, monthly use of Navajo herbal medicine.

Table 2.6 Mean distribution of daily dietary nutrient intake of Navajo ABID study participants by *Helicobacter pylori* (*H. pylori*) infection status.

Nutrient	Overall (N=93) Mean (SD)	*H. pylori*-negative (n=40) Mean (SD)	*H. pylori*-positive (n=53) Mean (SD)	P-value[a]
Energy (kcal)	2205 (1385)	2058 (1520)	2328 (1265)	0.189
Carbohydrate (g)	267 (167)	244 (172)	285 (161)	0.162
Fiber (g)	22 (14)	21 (15)	24 (13)	0.082
Total Cholesterol (mg)	354 (234)	360 (273)	350 (198)	0.616
Total Fat (g)	88 (60)	84 (66)	93 (55)	0.231
Protein (g)	91 (62)	87 (72)	93 (53)	0.281
Sodium (mg)	3890 (2776)	3663 (3108)	4078 (2484)	0.497
Alcohol (g)	3 (11)	2 (6)	3 (14)	0.412
Vitamin C (mg)	160 (255)	130 (82)	186 (337)	0.442
Vitamin A (mcg)	1363 (1042)	1520 (1395)	1232 (599)	0.725
Vitamin E (IU)	15 (11)	15 (12)	16 (9)	0.205
Total Folate (mcg)	400 (248)	381 (278)	417 (221)	0.179

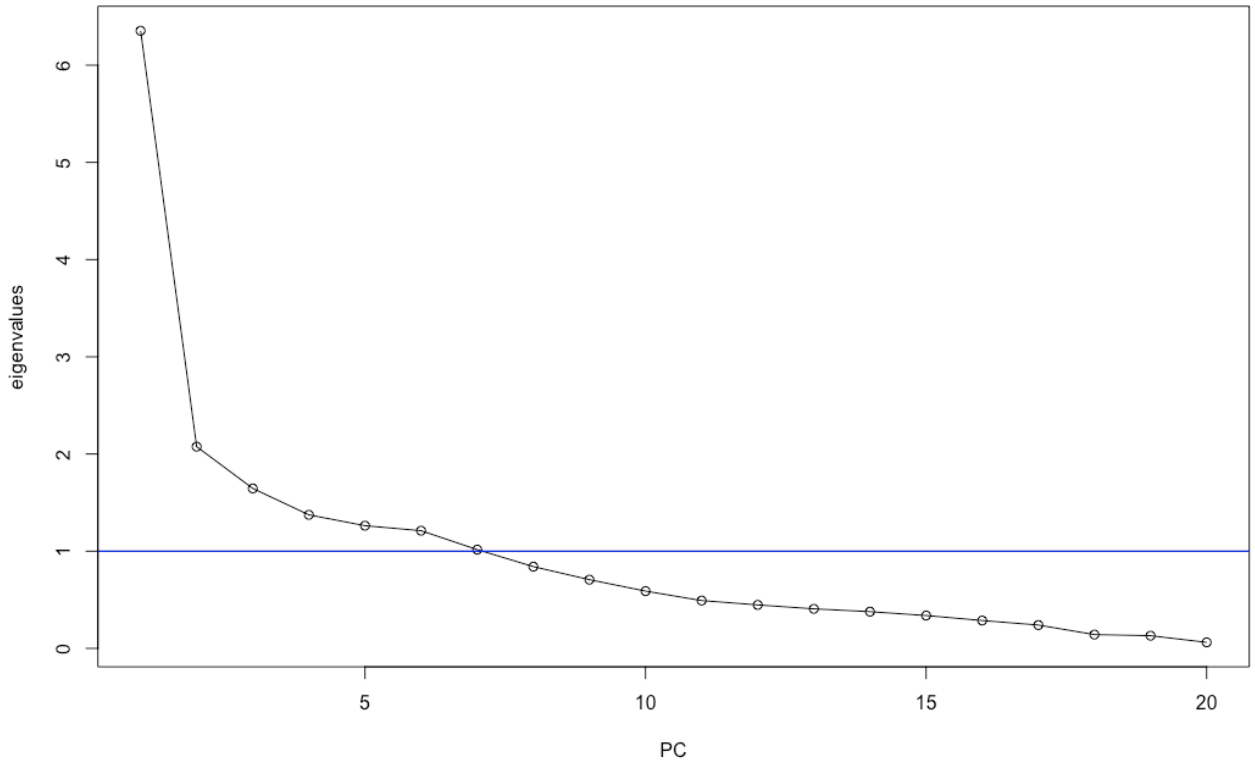
[a]t-test statistic (nutrients were log-transformed); Abbreviations: SD=standard deviation.

Table 2.7. Adjusted ORs for *Helicobacter pylori* (*H. pylori*) infection by tertiles[a] of daily nutrient intake.

Nutrient	T1	T2	T3	p for trend
Sodium				
Intake value (g)	≤2001	2002-3590	>3590	
No. of *H. pylori*-positive	8	16	24	
Total	21	29	38	
Model 1	1.00	2.00 (0.64-6.49)	2.79 (0.94-8.68)	0.547
Model 2	1.00	2.49 (0.63-10.58)	4.21 (0.53-37.40)	0.269
Model 3	1.00	3.65 (0.65-23.20)	5.88 (0.38-107.2)	0.387
Alcohol				
Intake value (g)	≤0.013	0.014-0.435	>0.435	
No. of *H. pylori*-positive	16	21	11	
Total	30	34	24	
Model 1	1.00	1.41 (0.52-3.87)	0.74 (0.25-2.17)	0.166
Model 2	1.00	1.14 (0.36-3.59)	0.67 (0.22-2.00)	0.346
Model 3	1.00	1.31 (0.35-5.02)	0.42 (0.11-1.50)	0.097
Vitamin C				
Intake value (g)	≤82.4	82.5-137.6	>137.6	
No. of *H. pylori*-positive	17	12	19	
Total	30	25	33	
Model 1	1.00	0.71 (0.24-2.05)	1.04 (0.38-2.83)	0.497
Model 2	1.00	0.55 (0.17-1.70)	0.56 (0.15-1.93)	0.453
Model 3	1.00	0.51 (0.13-1.85)	0.36 (0.08-1.49)	0.195
Vitamin A				
Intake value (g)	≤633	634-1627	>1627	
No. of *H. pylori*-positive	5	36	7	
Total	19	48	21	
Model 1	1.00	8.40 (2.64-30.80)[b]	1.40 (0.36-5.76)	0.132
Model 2	1.00	6.16 (1.62-26.79)[b]	0.65 (0.10-4.15)	0.044[b]
Model 3	1.00	8.03 (1.76-44.95)[b]	0.46 (0.04-4.14)	0.067
Vitamin E				
Intake value (g)	≤8.6	8.7-13.0	>13.0	
No. of *H. pylori*-positive	10	12	26	
Total	23	25	40	
Model 1	1.00	1.20 (0.38-3.80)	2.41 (0.85-7.07)	0.538
Model 2	1.00	1.20 (0.37-3.87)	2.33 (0.82-6.87)	0.208
Model 3	1.00	1.13 (0.23-5.46)	1.44 (0.18-11.92)	0.717
Folate				
Intake value (g)	≤228.9	229.0-368.2	>368.2	
No. of *H. pylori*-positive	7	18	23	
Total	21	30	37	
Model 1	1.00	3.00 (0.96-10.06)	3.29 (1.10-10.60)[b]	0.621
Model 2	1.00	3.51 (0.89-15.26)	4.72 (0.72-34.87)	0.269
Model 3	1.00	4.46 (0.87-26.40)	3.57 (0.37-37.97)	0.749

[a]tertile categories based on the distribution of *H. pylori-negative* participants; [b]statistical significance $p<0.05$; Model 1 is unadjusted; Model 2 is adjusted for age, gender; Model 3 is adjusted for age, gender, calories (log-transformed), education, daily aspirin use, BMI (categorical), smoking, history of *H. pylori*, monthly use of Navajo herbal medicine.

Figure 2.1 Scree plot of eigenvalues.



Conclusion

In this dissertation, we aimed to determine the prevalence and correlates of *Helicobacter pylori* (*H. pylori*) infections and *H. pylori cagA* virulence gene carriage, as well as the association between diet and *H. pylori* infection in Navajo adults residing in the Navajo Nation. To achieve this aim, we implemented a cross-sectional study to collect primary data and conduct in-depth analyses on the prevalence and association of known risk factors for *H. pylori* infection and *H. pylori cagA* virulence gene carriage.

We first launched a cross-sectional study (Navajo ABID study) using online and offline platforms online and offline recruitment platforms, such as a study website, social media (i.e., Facebook and Instagram), newspaper ads, flyers/postcards, and community events (i.e., local flea markets) to recruit and collect data on 104 Navajo adults residing in the central and northeast regions of the Navajo Nation.

In the first chapter, we determined the prevalence of *H. pylori* and *cagA* virulence gene carriage and established risk factors for *H. pylori* infection. We observed a prevalence of *H. pylori* infection (58%) that was two-fold higher in Navajo adults compared to the US population (23% seroprevalence). Also, the prevalence of the *cagA* gene (77%) in *H. pylori*-infected participants was disproportionately higher than the US population *cagA* gene prevalence (19% seroprevalence); and of the *cagA*-positive participants, all were of the Western *cagA* allele subtype. After adjusting for confounders, having a history of *H. pylori* infection was significantly inversely associated with *H. pylori* infection. No significant associations were observed with other known risk factors despite some noteworthy suggestive associations. Not only do these

study results shed light on the disease burden of *H. pylori* infection, but our study allowed for fourteen individual and environmental risk factors of *H. pylori* infection to be studied, which provided insights that were not previously studied in the Navajo Nation, particularly health history, family health history, aspirin use, body mass index, smoking, and owning livestock. If confirmed with additional large tribally based research, we can obtain a greater understanding of the association between individual and environmental risk factors and *H. pylori* infection in American Indian people, which can help target prevention strategies for *H. pylori* risk.

In the second chapter, we identified three dietary patterns using principal component analysis and reported the logistic regression analysis of diet patterns and odds of *H. pylori* infection in 93 participants. This is the first study in the Navajo Nation to collect and use a large amount of diet data, including tribal food data, to derive diet patterns and detect the relationships between diet patterns and *H. pylori* infection. In our analyses, we found that a Soups and Mixed dishes diet pattern was positively associated with *H. pylori* infection after adjusting for confounders, which has never been reported before in the Navajo Nation or in American Indian people. No significant associations with *H. pylori* infection were observed for the Western or the Fruits and Vegetable diet patterns or six dietary nutrients (sodium, alcohol, vitamin C, vitamin A, vitamin E, and folate). However, we did observe some suggestive associations. Overall, our study allowed for the control of a wide range of individual, environmental, and cultural confounders for our logistic regression models, which allowed for insights that were not previously studied particularly health history and Navajo herbal medicine use. By conducting large epidemiological studies that use both a posteriori and a priori data approaches, we can enhance our understanding

of the complex role of diet (including tribal herbs and foods) and *H. pylori* infection risk in American Indian communities.

Gastric cancer is a common cause of morbidity and mortality in the Navajo people and *H. pylori* infection is one likely contributing factor to this disease. We hope our findings add to the Navajo Nation's remarkable efforts to address the gastric cancer disease burden and sustain the health of the Navajo people.

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