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Environmental Exposures, *Helicobacter pylori* Infection and Gastritis in Canadian Arctic Communities

by

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Abstract

The role of environmental exposures in the acquisition of *H.pylori* and related disease is not yet understood. This analysis examined two hypotheses regarding how environmental exposures may affect digestive health in northern Canada. First, environmental sources of biological contamination may facilitate transmission of *H.pylori*. Second, exposure to environmental sources of chemical contamination may influence the development of severe gastritis.

Data from three northern Canadian communities were used to examine relationships between environmental exposures and digestive health. Using logistic regression, odds ratios and 95% confidence intervals were estimated for the effect of investigated exposures on prevalence of *H.pylori* and severe gastritis.

Findings showed a positive association between exposure to mice and prevalent *H.pylori* infection. Other zoonotic and waterborne exposures did not appear associated with this infection in the study populations. This analysis also provided evidence of a possible link between untreated water consumption and prevalence of severe gastritis.

Table of Contents

List of Tables

Environmental Exposures and Prevalent *H. pylori* Infection

Untreated Water Consumption and Severe Gastritis Prevalence

List of Figures

List of Abbreviations

H. pylori	***Helicobacter pylori***
HP	*Helicobacter pylori*
CAN*Help*	Canadian North *Helicobacter pylori*
NT	Northwest Territories
YT	Yukon Territory
ISR	Inuvialuit Settlement Region
SAT	Stool Antigen Test
UBT	Urea Breath Test
95%CI	95% Confidence Interval
OR	Odds Ratio
HAV	Hepatitis A Virus
GHLOs	Gastric *Helicobacter*-like Organisms
DNA	Deoxyribonucleic Acid
VNC	Viable but Not Culturable
PCR	Polymerase Chain Reaction
rRNA	Ribosomal Ribonucleic Acid
E. coli	*Escherichia coli*
C. jejuni	*Campylobacter jejuni*
CWI	Clean Water Index
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
PAF	Platelet Activating Factor
NACTRC	Northern Alberta Clinical Trials and Research Centre
LR	Likelihood-Ratio
CO_2	Carbon dioxide

Chapter 1: Introduction

Helicobacter pylori (*H. pylori*) are bacteria that infect the lining of the stomach and/or duodenum. Persistent infection with *H. pylori* leads to chronic gastritis, a spectrum of chronic inflammation that has been linked to the development of peptic ulcers and gastric cancer. Believed to have once infected the majority of humans worldwide, a decline in prevalence has been observed in major urban centers across Canada, as it has elsewhere in the developed world. In contrast, evidence has highlighted a disproportionately high prevalence in northern Aboriginal populations [1–8]. This has instigated growing concern in these populations, as the observed frequency and severity of digestive conditions related to *H. pylori* infection are also higher than those observed across southern Canada. The issue is further complicated by the difficult nature of treatment and low rates of treatment success, particularly in communities where the infection is more common. While public health providers and communities in northern Canada have identified *H. pylori* infection and associated diseases as a major health concern, research specific to northern communities is relatively limited.

In response to questions raised by community leaders and health care providers, the Canadian North *Helicobacter pylori* (CAN*Help)* Working Group was established in 2006. The CAN*Help* Working Group is a collaborative effort, which brings together Arctic communities, Northwest Territory and Yukon Health Agencies and Alberta Health Services with investigators from a variety of departments at the University of Alberta. This research program has three main objectives: 1. Obtain representative data from diverse settings in northern Canada for informing regional public health strategies for reducing risks from *H. pylori;* 2. Conduct a policy analysis to identify cost-effective *H. pylori*

management strategies that are ethically, economically and culturally appropriate for northern communities; 3. Develop knowledge exchange strategies that help community members understand *H. pylori* health risks as well as available solutions and unsolved challenges for reducing these risks.

The collaborators in the Northwest Territories identified the Hamlet of Aklavik as the target community for the beginning of this research, due to the high level of concern regarding *H. pylori* infection and gastric cancer outcomes expressed by community members. In response to these concerns, the Aklavik *H. pylori* project was launched in 2007. Since that time, additional communities in the Northwest Territories and Yukon Territory expressed interest in working with the CAN*Help* research program to address concerns about *H. pylori* in their respective communities. The Old Crow *H. pylori* Project was established in 2010 in Old Crow, Yukon Territory and the Inuvialuit Settlement Region (ISR) *H. pylori* Project, the expansion of the Aklavik project to all ISR communities, was launched in 2011 in Tuktoyaktuk, Northwest Territories.

The science surrounding the transmission of *H. pylori* remains unclear. Evidence suggests that the most likely mode of transmission is through close personal contact with an infected individual, occurring more readily during bouts of acute gastroenteritis with vomiting and/or diarrhea [9]. The human stomach is the only known source of the infection and evidence from extra-gastric sources has been inconclusive [10], leaving the question of how the environment impacts transmission unanswered to date. Each individual's environment is all things external to them, including their physical, cultural, social and biological surroundings [11]. The physical environment can be further divided into the built and the natural environment, the latter being comprised of air, water and soil. For the purposes of this research, the term environment was used to refer to the natural environment. Aspects of the environment that were investigated in relation to *H. pylori* infection and related digestive conditions included water (fresh water and sewage systems) and exposure to animals. Soil was not

examined in relation to *H. pylori* infection, as evidence pertaining to the ability of the organism to survive in soil has not been documented in the scientific literature.

A common belief across Aboriginal cultures is the interconnectedness of human health and environmental health [12]. Disruptions in human health are often linked to disruptions in the surrounding environment [12]. With this in mind it is reasonable that individuals from northern communities frequently speculate that *H. pylori* infection has an environmental source. The fact that environmental aspects of the acquisition of *H. pylori* and related disease have not been resolved by science to date provided a compelling reason to investigate *H. pylori* as an environmental health concern through research conducted in partnership with northern Aboriginal communities. The present analysis investigated the relationship between environmental exposures and the digestive health of individuals from the Canadian Arctic. The present analysis had three main objectives:

1. Conduct a comprehensive review of the literature to investigate the degree of consensus in the scientific community regarding environmental exposures and their association with *H. pylori* infection and related digestive conditions.

2. Using CAN*Help* project data collected in Aklavik and Tuktoyaktuk NT, and Old Crow, YT, investigate the hypothesis that environmental sources of biological contamination (untreated fresh water, wastewater, dogs, mice/mice dropping or animals innards) are sources of *H. pylori* transmission by estimating the association between exposure to the specified environmental sources of biological contamination and the prevalence of *H. pylori* infection in residents of the target communities.

3. Using CAN*Help* project data, investigate the hypothesis that chemical irritants in untreated drinking water from local polluted rivers and other groundwater sources increase the frequency of severe *H. pylori*-associated gastritis by estimating the association between the consumption of untreated water and the severity of gastritis among *H. pylori*-positive participants with histopathologically evaluated gastric biopsies in the Aklavik and Old Crow *H. pylori* projects.

Chapter 2: Literature Review

Background and Significance

H. pylori is a helical, flagellar gram-negative bacterium that inhabits the lining of the stomach and/or duodenum[13]. Chronic *H. pylori* infection is involved in the pathogenesis of chronic atrophic gastritis, peptic ulcer disease and gastric cancer [13,14], digestive diseases responsible for a large disease burden worldwide. This was particularly so in eras that preceded modern sanitation[15,16], and remains so in settings where sanitation does not meet modern standards *H. pylori* was identified in 1982 by Barry Marshall and Robin Warren, following isolation of the organism from biopsies of human gastric mucosa. For this, they were awarded the Nobel Prize in 2005, a testament to the global public health importance of this discovery [17]. While developed areas are experiencing a decline in the prevalence of this infection and associated disease, the impact of this bacterium is still prominent in less developed regions [18-20]. In the absence of infrastructural and socioeconomic development, associated with better management of infectious disease, economically disadvantaged communities are at a higher risk of *H. pylori* infection. Barriers to successful treatment *H. pylori* infection are also common in communities at higher risk of acquiring the infection[18-20]. This disparity is also reflected in the occurrence of *H. pylori*-related diseases; for example stomach cancer, now rare in affluent communities, is a major cause of death in economically disadvantaged areas [21].

Prevalence in Canada and Northern Aboriginal Populations

Prevalence in the Canadian population

Evidence from major urban centers across Canada shows the prevalence of *H. pylori* infection is relatively low. Some studies have reported increasing

prevalence with age, younger age being associated with a lower prevalence. This was demonstrated in a 1997 study of 734 healthy individuals from Manitoba, 469 individuals 20 to 34 years of age and 265 between 35 and 65 years, with 35% and 46% prevalence of *H. pylori* infection, respectively [2]. In a prevalence study published in 2003 of 1013 patients with uninvestigated dyspepsia aged 18 to 86 years from 49 physician clinics in 6 Canadian provinces, 30% were infected with *H. pylori* [22]. In another prevalence study published in 2005 of 309 patients aged 18 to 83 years with uninvestigated heartburn-dominant dyspepsia from 46 physician clinics across Canada, 31% were infected with *H. pylori* [23]. A random sample of 316 individuals aged 18 to 72 years from the provincial medical insurance registry in Nova Scotia from 2005 had a prevalence of 38% [24]. Lower prevalence in pediatric populations has been shown in a study from 2005 of 246 pediatric endoscopy patients aged 5 to 18 years from four academic centers, with a prevalence of 5% [1]. The literature suggests that chronic *H. pylori* infection is most readily acquired during childhood in association with household crowding [14]. Therefore these data from across the country showing higher prevalence in older age groups and low prevalence in pediatric populations indicate a trend towards decreasing rates of transmission characterized by greater spread of *H. pylori* in earlier eras, with a prominent reduction in transmission in major urban centers in recent years [1,2].

Prevalence in Northern Aboriginal Populations

The literature has shown that Aboriginal communities in the circumpolar region are disproportionately affected by *H. pylori* infection. While the few studies from across Canada indicate the estimated prevalence for adults is around 30-40%, studies of Aboriginal populations in Canada, Alaska, Greenland and Russia estimate *H. pylori* prevalence in the range of 51-95% [3-8]. In a study of a 306 adults from a Wasagamack Cree community in Northern Manitoba, 95% were found to be *H. pylori*-positive through serology [5]. A study investigating *H.*

pylori infection using the stool antigen test in 163 children aged 0 to 12 years from the same community reported 56% were positive [6]. Finally, an investigation of *H. pylori* infection in Inuit communities from Chesterfield Inlet, Repulse Bay and Nunavut found that of 256 individuals of all ages, 51% were positive [25]. These data indicate that although the prevalence of *H. pylori* infection varies within and between Aboriginal populations in the circumpolar north, it is consistently higher than in their southern counterparts.

Detection Methods

Biopsy-Based Methods

The original methods for detecting this infection were based on the collection of biopsies through endoscopy [26]. Included in this category of tests are culture, histology and urease tests [26]. These tests are thought to have excellent diagnostic accuracy and are often used as the gold standard when assessing the diagnostic accuracy of less invasive methods [26–29]. These methods are thought to be 100% specific, however sensitivity is less than 100% due to the heterogeneous distribution of the bacteria in the stomach and the localized sampling of biopsy methods [26,29]. Further disadvantages are the associated costs and highly invasive nature of endoscopy procedures [26].

Serology

Serology is a commonly used detection method, which employs a nonquantitative enzyme-linked immunosorbent assay to identify *H. pylori* antibodies in serum samples [26]. This test is often employed in epidemiologic studies due to the ability to screen large numbers of people quickly and inexpensively [26]. There are several disadvantages to this method, however, including the need for several reagents to account for differing strains and subsequent antibodies in a given population of infected individuals [26]. Further, the cutoff used to define a positive versus negative test is somewhat arbitrary

and difficult to define [26]. Finally, antibodies can remain in the sera of a previously infected individual for several months following treatment and as such serology is not an appropriate method for determining whether eradication therapy was successful [26].

Stool Antigen Test

The stool antigen test (SAT) is a non-invasive detection method that functions by detecting the presence of *H. pylori* antigens in fecal matter [27]. The SAT demonstrates good diagnostic accuracy in both adult and pediatric populations. When the rapid urease test (RAT), histology and culture are used as the gold standard, sensitivity ranges from 73-100% and specificity from 78-100% in adult populations [28]. In pediatric populations with the same gold standards, sensitivity ranges from 76-100% and specificity from 61-100% [28]. Due to the non-invasive nature of this test, it has the advantage of being suitable for a test-and-treat approach in a clinical setting, when more invasive methods are not required. Samples can also be stored frozen for prolonged periods of time and retain accuracy [28]. Further advantages as a non-invasive method for use in pediatric populations include being easy for a child to complete (does not require ability to control breathing) and it does not require a needle stick [28]. Disadvantages of the SAT include low sensitivity in post-eradication tests, likely due to lower density of *H. pylori* in feces, which could lead to lower *H. pylori* stool optical density [28]. Finally genetic variability of *H. pylori* from different geographical regions means variation in epitopes, a common problem to all immunological tests [28]. Therefore, geographically local validation of the test is necessary to ensure accuracy [28].

Urea Breath Test

The urea breath test (UBT) is a commonly used non-invasive and nonquantitative method for detecting the presence of *H. pylori* organisms [26,29]. There are two forms of UBT, using different carbon isotopes 14C and 13C [29]. *H.*

pylori organisms secrete the enzyme urease, which breaks urea down into carbon dioxide and ammonia [30]. These tests function by detecting the bacterial urease activity using either mass spectrometry or nondispersive isotope-selective infrared spectroscopy to measure the ratio of 13C/14C to 12C in breath samples collected before and after the consumption of 13C-labeled urea [29,31]. The standard test protocol involves the collection of a baseline breath sample, during which the participant is required to take a deep breath, release a puff of that breath and then exhale the remainder of that breath through a mouthpiece connected by a tube to fill the first side of an aluminum double-sample bag. The participant is then asked to consume an age-dependent dose of labeled urea, dissolved in citric acid and water. A second breath sample is collected 30 minutes following administration of the labeled urea, in the same manner described above to fill the second side of the sample bag [32]. The 13C-UBT is considered advantageous as it employs the stable isotope, whereas 14C is radioactive [29].

The 13C-UBT has demonstrated good sensitivity and specificity in both adult and pediatric populations [29]. When the RAT, histology and culture are used as the gold standard, the 13C-UBT has demonstrated a sensitivity ranging from 95-100% and specificity ranging from 89-100% in adult populations [29]. These estimates of sensitivity and specificity are consistent with those observed in pediatric populations when the same gold standards are used [29]. The estimate sensitivity in pediatric populations ranges from 91-100% and specificity ranges from 79-100% [29].

Beyond diagnostic accuracy, there are several advantages to the 13C-UBT; most notably the innocuous nature of the test makes it safe for unlimited repetition of the test and testing in children, pregnant women and elderly patients [29]. As a non-invasive method, the 13C-UBT is suitable for a test and treat approach when more invasive tests are not necessary [29]. The 13C-UBT also draws from the entire stomach, providing an advantage over biopsy-based methods, which are subject to the heterogeneous distribution of the bacteria [26,29]. Finally,

the breath test detects active infections, giving it a higher diagnostic accuracy than serology and allowing quicker detection of eradication following the administration of treatment [29].

Transmission of *H. pylori*

For this review, a search of medical literature databases revealed 535 publications pertaining to transmission of *H. pylori.* Of those, 135 were selected for inclusion in this review. Selected publications were written in English and presented evidence of transmission through specific pathways including: fecal-oral, oral-oral and gastro-oral, water- or food-borne, iatrogenic, blood-borne, perinatal, sexual contact, airborne, vector-borne or zoonotic. Investigations of transmission from environmental sources of biological contamination were also selected for inclusion. Specifically, publications presenting estimates of the effect of exposure to potentially contaminated water, sewage or animals (dogs, cats, mice and animal innards) on the frequency of *H. pylori* infection were included. The predominantly employed study design in the included analyses of specified transmission pathways was cross-sectional. A limitation of evidence generated by cross-sectional studies in the investigation of transmission pathways is that estimates reflect the relationships between exposures and prevalent *H. pylori* infection, rather than incidence. Among other limitations, prevalence-based study designs cannot distinguish variables that influence infection acquisition from those that influence duration.

Infectious disease can be defined as an illness occurring in response to the entry and development and/or propagation of an infectious agent or the toxic products thereof in a human or animal host [11]. Infectious agents or their toxic products are transmissible through a variety of mechanisms, originating from their source (an infected person or animal, or contaminated food, water or other reservoir) and transferred either directly or indirectly to a susceptible host [11]. Direct transmission involves the immediate transfer of the infectious agent from a host to a susceptible individual through direct contact such as touching,

biting, kissing, sexual intercourse or direct projection [11]. Indirect transmission involves passage through an intermediate, such as an animal host, insect vector or fomite[11]. A vehicle of infection transmission is a broad term used to describe the mode of transferring a pathogenic organism from its source to a new host [11]. By definition, a vehicle of transmission can refer to the person from whom the agent is passed, contaminated food or water, or an arthropod [11]. A vector describes a living carrier that transmits a pathogenic organism from its source to a susceptible host or the host's immediate surroundings [11]. An animal is considered a host in the event they are infected with a given pathogen, which may be transferred to a susceptible human [11]. A fomite is a specific type of vehicle, defined as an inanimate object or material that transfers a pathogenic organism from its source to new host [11].

Some pathogens can be transmitted through multiple routes, depending on their epidemiologic features [33]. Transmission of *H. pylori* has only been documented in three circumstances: patients undergoing endoscopic procedures, accidental infection through gastric pH electrodes and finally, through voluntary oral ingestion of the bacteria [14]. The final example has influenced the thinking around how an individual may acquire the infection, with the predominantly hypothesized routes involving oral ingestion of the bacteria.

Gastrointestinal Fluids

Gastrointestinal infections can be acquired through ingestion of fluids from an infected person's digestive tract, which includes fluid from the oral cavity, stomach and intestines [34]. There are several mechanisms that can facilitate spread via digestive fluids, including direct contact, through a fomite or through a vector [33]. Transmission may occur through either direct or indirect contact with one or more of the three main digestive fluids, including saliva, regurgitated stomach contents and feces. Fomites may also play a role, allowing transmission to occur through ingestion of contaminated water or food, or contact with a contaminated inanimate object or material [11].

The only known source of *H. pylori* is the human stomach. Therefore, transmission is thought to occur most readily during contact with and subsequent ingestion of fluids from an infected person's digestive tract. In the literature, these routes are referred to specifically as oral-oral, gastro-oral and fecal-oral [35]. The relative frequency of transmission through these particular routes is unclear. Further, the role, if any, of suitable intermediates, including fomites and vectors is not yet understood.

Fecal-Oral Route

In order to investigate the frequency of transmission through the fecal-oral route, information on proxy exposures are used. Common proxies include hand washing practices following washroom use or diaper changes [36], type of toilet (flush or pit) [36-40], type or presence of sewage system [38,41,42], occupational contact with feces [43] and co-infection with other pathogens for which the fecal-oral route is the primary mechanism of transmission [44].

Hand washing practices have been used as a proxy for exposure to potentially contaminated fecal matter [10,36]. Exposure is typically ascertained through self-report and defined as not washing hands after using the toilet or prior to eating [36]. The presence or absence of a washtub or sink in the bathroom has also been compared in relation to prevalent *H. pylori* infection [36]. The results produced by studies examining the association between hand washing practices and *H. pylori* infection have been inconsistent. In their cross-sectional analysis of 3, 347 children from the Liepzig *Helicobacter pylori* study in 1997/1998, Herbarth *et al*. (2001) [36] reported elevated prevalence in individuals without a wash tub in their washroom (91%), and in those who don't wash their hands after using the toilet (90%) and also in those who don't wash their hands before eating (90%). A birth cohort of 472 children from Juarez, Mexico and Texas, U.S. was followed from 1998 for changes in *H. pylori* status (based on the 13C-UBT) every 6 months from birth till 24 months of age [10]; hand-washing practices following diaper

changing was assessed through caretaker interviews at each follow-up exam. The estimated effect of hand washing habits of mothers following diaper changes was minimal [10].

Exposure of individuals to fecal matter has also been classified according to whether they predominantly use pit latrines or flush toilets [36–39,45]. Data from prospective investigation of this effect has indicated the association between toilet type and prevalent *H. pylori* infection is minimal or null [10,39]. A cohort of 397 Egyptian individuals aged 6 to 35 years were followed from June 1997 for one calendar year [39]. The proportion of participants with positive serostatus was 15% and did not vary across categories of sewage disposal type [39]. The estimated odds ratio for having a toilet versus not was 1.0 (95%CI: 0.40, 1.4) [39].

Co-infection with *H. pylori* and pathogens that are primarily transmitted through the fecal-oral route, such as hepatitis A virus (HAV), is considered evidence of a shared transmission pathway [44]. Positive associations have been observed between *H. pylori* and HAV status in studies around the world, with odds ratios ranging from 1.9 to 5.7 [44]. However, the observed effect size became consistently smaller across studies when important confounding variables, including age and indicators of socioeconomic status, were taken into account [44].

Oral-Oral Route

The likelihood of transmission through the oral-oral route has been investigated through attempts to isolate and culture the bacteria from dental plaque [46]. The results of these attempts have been inconclusive [47]. Commonly used proxies for exposure to potentially contaminated fluids from the oral cavity include frequency of sharing utensils [48] or dental care occupations [49]. Some research has explored the potential for dental plaque to act as a reservoir for *H. pylori* [50]. Exposure was defined using a dental plaque score (high versus low as graded by a dental professional) or number of dental visits per annum, with fewer visits presumed to indicate more plaque [50].

In a study of 328 Chinese individuals aged 25 years or older who had immigrated to Melbourne, Australia, the prevalence of *H. pylori* infection in persons who reported sharing chopsticks was 65%, compared to 42% in those who did not [48]. Data provided in the report was insufficient for approximation of the precision of these estimates. The effect of occupational exposure to dental plaque and saliva has been assessed by comparing dentists and dental assistants with other health professionals [49] or comparing dental hygienists and assistants to students [51]. Overall, an elevated frequency of *H. pylori* infection in dentists and hygienists has not been consistently observed [49,51]. However, in their analysis of 239 dental professionals, Malaty *et al.* (1992) [51] estimated the odds ratio for dental assistants compared to dentists was 2.5 (95%CI: 1.3, 5.0). However, this estimate was not adjusted for other variables that may influence the likelihood of acquiring *H. pylori*.

The relative odds of *H. pylori* infection in individuals with a high plaque score, indicating a large amount of plaque on their teeth, compared to persons with a low plaque score was assessed in 217 individuals from Victoria, Australia [50]. Compared to individuals with a low plaque score, those with a high plaque score had 1.7 (95%CI: 1.1, 2.7) times the odds of having *H. pylori* infection, after adjusting for socioeconomic status, age and sex [50]. From the same study, individuals who went to the dentist less than once annually had 4.4 (95%CI: 0.8, 23) times the odds of having *H. pylori* infection compared to persons who visited the dentist more than once per annum, after adjusting for amount of dental plaque, socio-economic status, age and sex [50].

Gastro-Oral Route

Evidence for this mode of transmission has been generated by investigation of individuals suffering from gastroenteritis [52]. In a prospective study initiated in 1998, volunteers with and without *H. pylori* infection aged 55 years or older provided samples of saliva, feces (from normal stools and induced

diarrhea) and vomit (from induced vomiting) [9]. In addition, the authors took samples from the air surrounding an infected individual during emesis [9]. *H. pylori* organisms were detected in each type of gastrointestinal fluid by PCR and the viability of the bacteria was tested by culture [9]. The highest quantities of *H. pylori* bacteria were recovered from vomitus samples, isolated from 100% of the samples provided by *H. pylori*–positive individuals [9]. *H. pylori* organisms were isolated from 38% of the air samples taken while an *H. pylori*–positive individual vomited [9]. Saliva samples were taken both pre and post emesis; the proportion of samples yielding *H. pylori* was 19% and 56% respectively [9]. The bacteria were not recovered from any of the normal stools sampled, although it was found in 22% of the medically induced stools [9]. These results provide evidence that the gastro-oral route may be an important mechanism in the transmission of *H. pylori* to a susceptible host.

Cohabiting Hispanic patients seeking care from a community clinic for gastrointestinal episodes including vomiting and/or diarrhea were followed from January 2000 till January 2004 [52]. The 1,303 participants were examined every 3 months, which included ascertainment of gastrointestinal symptoms and duration of gastroenteritis (occurring within the last 21 days) and collecting blood and stool samples in order to screen for *H. pylori* infection [52]. This study identified cohabitation with an *H. pylori*–positive individual with gastroenteritis as a strong risk factor for acquisition of *H. pylori* infection [52]. The odds ratio for exposure to an *H. pylori*-positive family member with gastroenteritis compared to not having an infected family member with gastroenteritis was 4.8 (95%CI: 1.4, 17), adjusting for age, sleeping density and completion of follow-up for each member of the same family) [52]. When the definition of exposure was restricted to *H. pylori*–positive individuals who were experiencing gastroenteritis with vomiting and/or diarrhea, their family members had 6.3 (95%CI: 1.6, 24) times the odds of acquiring *H. pylori* compared to people without *H. pylori*-positive family members, after adjusting for the same factors [52]. The effect of

cohabitation with an infected individual on acquiring *H. pylori* infection was consistently stronger when the index case had gastroenteritis with vomiting than with diarrhea alone. This suggests that *H. pylori* organisms transmitted most readily when a susceptible host is exposed to an infected individual who is experiencing gastroenteritis with vomiting.

Water- or Food-borne

Some gastrointestinal infections are water- or foodborne, transmitted by the ingestion of food or water that has become a reservoir of free-living bacteria, viruses, protozoa, parasites or the toxic products thereof [11]. In a food or water reservoir, the propagation or cyclic development of the pathogenic organism occurs [53], thus differentiating it from the role of food or water as a fomite. For example, meat can become contaminated through improper storage, which allows for the rapid propagation of bacteria [53]. As a fomite, water or food simply transfer an infectious substance, such as digestive fluids, to the new host [29]. Currently, the only known reservoir for *H. pylori* is the human stomach, and the potential for water or food to act as a frequent source of the bacteria is unresolved to date [35].

H. pylori organisms have consistently demonstrated fragility when exposed to aqueous environments [54–56]. Exposure to water induces the same fundamental reaction as exposure to medications that are effective at eliminating *H. pylori*, characterized by conversion to a viable but non-culturable state [54–56]. The questionable ability of *H. pylori* to survive following exposure to water has left unanswered the question of whether water is another source of the bacteria. Culture and PCR methods have been used to investigate both the presence of *H. pylori* in various food stuffs as well as the survival capabilities of the bacteria in these environments [57–66]. Milk is the most commonly investigated type of food [57-63], with some analysis of various types of meat [62,64,65] and raw vegetables [35,64,66,67].

Similar to evidence that supports the hypothesis of transmission of *H. pylori* through water, most evidence for transmission through milk is indirect [68]. While some authors have reported the ability to isolate *H. pylori* from samples of raw milk [57-60], detection of *H. pylori* in milk is not commonly reported in the scientific literature and isolation of *H. pylori* from milk is thought to be rare [68]. The rationale for examining whether *H. pylori* is present in milk stems from the hypothesis that the bacteria infect the stomach of cows, sheep or goats and are eliminated in viable forms through fecal matter, which contaminates milk during the milking process [69]. Studies of milk samples spiked in the laboratory have indicated that *H. pylori* are able to survive in milk for short time periods [61-63]. In all such studies, culture-based methods were used to determine whether the bacteria were alive following exposure to milk. In studies where the temperature of the milk was not altered, authors have reported survival up to 6 to 10 days after exposure to milk [61-63]. The longest survival time reported was 10 days, in fresh milk (type not specified) with no preservatives [63]. Increased temperatures reduced the reported survival times, with the longest duration at body temperature being 3 days (in cow's milk at 37°C) [62].

In a comparison of the prevalence of the *H. pylori*-specific glmM gene in samples of raw goat milk (n=160), raw sheep milk (n=130) and raw cow milk (n=110), the proportion positive was 26% (95%CI: 20%, 34%), 43% (95%CI: 34%, 52%) and 55% (95%CI: 46%, 65%), respectively (CIs estimated using reported data) [57]. These proportions are similar to those estimated by similar studies of *H. pylori* gene frequencies when only one gene is targeted [58-60]. When a second gene is targeted in the same samples, however, the proportion positive drops drastically [59,60]. For example, when 63 samples of raw sheep's milk were targeted for the 16 rRNA gene alone, the proportion positive was 60% [59]. However, when the *H. pylori*-specific vacA gene was targeted in addition to 16 rRNA gene, only 8% were positive [59]. The discrepancy between these estimates of prevalence is likely due to the fact that the vacA gene is not present in all strains of *H. pylori.*

Further, the 16 rRNA gene is present in many different organisms, although it contains a hypervariable region that has species-specific sequences. Given the inability to reliably identify genes specific to the bacteria and present in all strains, or a comparison of strains isolated from milk and those infecting humans, the role of milk in the transmission of *H. pylori* is undetermined [68].

Evidence of the survival of *H. pylori* in food products aside from milk is scarce [68]. *H. pylori* organisms introduced to raw vegetables such as lettuce [64,66] and carrots [66] in a laboratory setting, have demonstrated survival times of 72 hours [66] up to 2 days [64]. Epidemiologic data on the relationship between consumption of raw vegetables and prevalent *H. pylori* infection has been focused on raw vegetables as a proxy for exposure to untreated water. Few studies have examined this relationship and results are inconclusive. In 648 children aged 2 to 9 years from Colombia the odds ratio for consuming raw lettuce a few times per month compared to a few times per year or less was 1.1 (95%CI: 0.8, 2.0), adjusting for age, sex, occupation of the head of the household, exposure to rabbits, milk, fruit and vegetable consumption, height-for-age percentile, amebiasis, crowding, birth order, sharing drinking cups, mother's hand washing practices, latrine location, swimming in the river stream or pool and contact with sheep [35]. From the same analysis, consumption of a few servings of raw lettuce per week compared to per year or less yielded an odds ratio of 1.8 (95%CI: 0.9, 3.6) [35]. These data do not provide strong support for or against the role of raw vegetables in acquisition of *H. pylori* infection.

Evidence of the ability of *H. pylori* to survive following exposure to various food items, including tofu [64], yogurt [62,64], raw or cooked chicken [62,64], ground beef [65] and cheese [62], has been reported from experimental studies. Duration of survival in yogurt and tofu was either negligible or non-existent [62,64]. Jiang and Doyle (2002) [65] reported survival up to 3 days for *H. pylori* recovered from autoclaved ground beef and up to 7 days in bacteria recovered from irradiated ground beef. When recovered from both raw [64] and cooked [62] chicken,

H. pylori retained culturability up to 2 days. While these data indicate the plausibility of *H. pylori* contaminating foodstuffs, no data on the frequency of contamination in the natural environment exists. Authors examining 104 children from Lima, Peru compared *H. pylori* prevalence in children who reported consuming food from street vendors with the rationale that the unhygienic conditions under which this type of food is prepared are conducive to the spread of enteric disease [70]. The authors reported the prevalence of *H. pylori* infection was 38% (95%CI: 26%, 52%) in children who never ate food from street vendors, compared to 65% (95%CI: 47%, 80%) in those who ate from street vendors once a month and 75% (95%CI: 19%, 99%) in those who ate from street vendors weekly [70] (CIs calculated using data from the report). Given the wide overlapping confidence intervals and lack of confounder control, these data do not provide strong support for or against transmission of *H. pylori* through food either as a source of biological contamination or as a source of the bacteria.

Iatrogenic

Iatrogenic transmission involves the acquisition of an infection from a medical procedure [11]. As previously mentioned, one of the only documented examples of transmission occurred in a patient undergoing endoscopy [14]. Endoscopy would facilitate gastro-oral transmission, with gastric fluid being transferred through an endoscope from an infected person to a susceptible host. Some studies have demonstrated the presence of *H. pylori* in washout fluid from endoscope cleaning, and one report from Brazil identified endoscopy following successful treatment for *H. pylori* as a risk factor for reinfection [202-204]. However, current guidelines for disinfection of gastroscopes, which include the use of chemical disinfectants for a prolonged period of time, reduce the risk of this type of transmission [14,77].

While health care practitioners are not routinely exposed to procedures that are the pathways for iatrogenic transmission, they are exposed to some

sources of the pathogen transmitted in patient care settings. Therefore, occupation as a gastroenterologist or endoscopy nurse is the proxy most often used to assess the frequency of *H. pylori* transmission through medical procedures [71-76]. Reports of the relationship between occupational exposure to gastroscopes and prevalent *H. pylori* infection have been inconsistent. While some authors report an elevated frequency of infection in endoscopy staff [71-73], others have demonstrated a lower prevalence of *H. pylori* in these same groups[74,76]. In a study of 1460 randomly selected physicians and 235 nurses the odds ratios for the effect of regular performance of endoscopies compared to controls were 1.6 (95%CI: 1.3, 2.0) and 0.96 (95%CI: 0.73, 1.3) respectively [73]. However these estimates were not adjusted for potentially important confounders, for example age or indicators of socioeconomic status.

Blood-borne

Pathogens may be transferred through the blood, plasma, serum or organs if the organisms are present in the circulatory system at the time of exposure [33]. This can occur through open sores on an infected individual coming into direct contact with an open sore on a susceptible host [11]. Alternately, transmission can occur indirectly through medical procedures such as transfusions, organ transplants or injection drug use [11]. It is considered unlikely that *H. pylori* organisms are transmissible through blood, as the primary reservoir in the human body is the stomach, and the bacteria do not enter the circulatory system.

Perinatal

Perinatal transmission involves the vertical transfer of an infectious agent from a mother to her child and can occur in utero, intrapartum, or postpartum through breastfeeding [33]. A study in Belgium attempted to determine whether infants might acquire *H. pylori* from their mothers by testing the children of *H.*

pylori-positive women for antibodies associated with *H. pylori* every three months from 3 to 15 months of age [78]. Most of the infants were positive for anti-*H. pylori* IgG before 6 months of age and all became seronegative by 12 months. The investigators administered the 13C-urea breath test (UBT) to 67 infants with seropositive mothers at the age of 12 to 15 months. All but one of the infants had a negative breath test [78]. This study demonstrated the ability of *H. pylori*-specific antibody to cross the placental barrier but the infection itself did not appear to be transmitted from mother to child at birth or during infancy, except perhaps in rare cases [78].

Limited data on the effect of breastfeeding on prevalent *H. pylori* infection has been reported [79,80]. In both studies, breastfeeding was associated with decreased odds of the child acquiring the infection [79,80]. In their analysis of 1221 German children who attended grade 1 in 1997/1998, Rothenbacher *et al.* (2002) [80] reported the odds ratio for having never been breastfed compared to having been breastfed for 6 months or longer was 2.57 (95%CI: 1.19, 5.55) adjusting for infection status of the mother, ethnicity, age, sex, place of birth, birth weight, education level of parents, previous antibiotic use, housing density, and whether or not the parents smoke inside the household [80]. Similarly, in a study of 356 American children aged 2 to 16 years, those who were not breastfed had 2.7 (95%CI: 1.4, 4.2) times the odds of having *H. pylori* infection compared to those who were, adjusting for age, number of children and adults in the household, number of bedrooms, mother's education and type of daycare center attended [79].

Sexual Contact

Sexual intercourse is a broad term that encompasses a variety of sexual behaviours that facilitate the transmission of infectious agents from person to person, through a number of mechanisms [33]. There are over 25 different organisms that are transmissible through sexual contact, which can include vaginal, rectal and oral intercourse [33]. The mucosal membranes on both the

male and female genitalia make them susceptible to infections transmissible through contact with the skin of an infected host [33]. Although the mucosal membranes are the most common port of entry, infections can be spread to the skin surrounding the genitalia as well [33]. Sexual contact may also facilitate the transmission of pathogens through the blood or venereal fluids [33]. The number of sexual partners an individual has had is strongly associated with increased risk of acquiring a sexually transmitted infection, although specific patterns of behaviours put some individuals at a higher risk than others [33]. For instance, having multiple concurrent partners is considered higher risk than multiple successive partners [33]. Engaging in high-risk behaviours such as drug use or commercial sex work, or having a partner who does, is also associated with increased transmission [33]. Finally, adolescence is considered a high-risk period, due to the frequent turnover of sexual partners in this age group [33].

Since *H. pylori* is thought most readily transmitted through ingestion of infected gastrointestinal fluids, sexual behaviours such as kissing (contact with saliva or regurgitated stomach contents enabling oral-oral or gastro-oral transmission subsequently) and anilingus (enabling fecal-oral transmission) could conceivably facilitate the spread of the bacteria. While the transmission of some enteric pathogens through sexual contact (specifically anilingus) has been demonstrated, there is no evidence to suggest that *H. pylori* organisms are transmitted sexually [14,81]. A study of heterosexual and homosexual males attending an outpatient clinic assessed *H. pylori* serostatus in relation to common risk factors for contracting an infection through sexual contact, including lifetime number of sexual partners and history of sexually transmitted infections [81]. The authors reported no association between being *H. pylori*-positive and sexual behaviours characteristic of increased spread of sexually transmitted infections[81].

Airborne

Some pathogens can become aerosolized, allowing them to be disseminated through the air [11]. Microbial aerosols can be defined as particles that are comprised either partially or completely of microorganisms, that become suspended in the air in large droplets, droplet nuclei, or dust [11]. Droplet nuclei are residues of the evaporated fluids projected from an infected host that become widely dispersed and can remain suspended in the air for a long time before entering a susceptible host [11]. They are characterized by their small size (between 1 and 5 μ), which allows them to penetrate deep into the lung and be retained by the alveoli [11]. Transmission through dust particles behaves in a similar way, arising from fungus spores or contaminated fomites and becoming airborne, entering a new host through inhalation [11]. Due to their size, large droplets do not remain airborne, but instead transmit pathogens through the direct projection of infectious fluids onto the mucous membranes of a susceptible host, through coughing, sneezing or spitting [11]. Large droplets may also remain on the hands of an infected individual or be projected onto fomites. Transmission could occur if a susceptible individual came into contact with the large droplets either directly (touching the infected person's hand), or indirectly (touching a contaminated fomite) and transferring them to a suitable port of entry.

A portion of the mucosal fluid sprayed by a sneeze or cough will become droplet nuclei, although the ability of the organism to remain suspended and survive in aerosolized form is dependent on several factors [82–84]. It is thought that in order for bacteria to become aerosolized from saliva, the concentration of the bacteria in the saliva must be extremely high [82]. Further, survival as a microbial aerosol is predicated on specific environmental conditions [84]. High relative humidity, low ambient temperature and low solar irradiation are considered optimal conditions for improved survival and increased dispersal of bacteria in droplet nuclei form [83,84]. Survival is also dependent on the

characteristics of the specific organism, for instance, resistance to drying out [84]. While the ability of some gram-negative bacteria to survive in this form has been demonstrated, there has been no evidence to suggest *H. pylori* organisms are able to do so [85]. Further, as an enteric pathogen, the optimal port of entry for *H. pylori* is the oral cavity, whereas droplet nuclei typically enter through the respiratory tract [11,35].

Some evidence suggests *H. pylori* organisms are able to live in the oral cavity and are transmissible through saliva and thus have the potential for droplet transmission following coughing, spitting [26] or vomiting [9]. Large droplet projection is a plausible mechanism through which *H. pylori* may be transmitted from the oral cavity of an infected individual to a new host. This could occur through direct projection onto the mouth, or indirectly through contaminated fomites. Whether or not *H. pylori* are transmissible through fomites contaminated with large droplets depends on the ability of the bacteria to survive outside of the human digestive tract. The fragility of *H. pylori* in adverse environments has been consistently demonstrated in laboratory settings, indicating survival in droplets may be limited [14].

Vector-borne

Mechanical vector transmission involves the carriage of an infectious agent from its source to a susceptible host, their food or immediate surroundings on the feet or proboscis, or following passage through the gastrointestinal system of an arthropod [11]. Biological vector transmission is characterized by the propagation and/or cyclic development of the pathogenic organism within an arthropod [11]. Transmission occurs if the infected arthropod bites a susceptible host, transferring the pathogen either through the bite or by regurgitating or depositing infected waste in the bite or another area of trauma, with entry being facilitated by rubbing or scratching [11].

The potential role for mechanical vector transmission of *H. pylori* has been suggested, with flies acting as the intermediate in pathways involving

infectious digestive fluids, most prominently the fecal-oral route [26]. The presence of *H. pylori* both on the body hairs and in the gastrointestinal system of houseflies has been demonstrated [86]. A clear understanding of the role of houseflies in the transmission of *H. pylori* is hampered by observations of high concentrations of contaminated houseflies in populations with both high and low frequencies of *H. pylori* infection [26]. Further, inconsistent reports of the ability to isolate the bacteria from houseflies that were exposed to contaminated feces in a laboratory setting indicate the vector potential of houseflies is limited [26,87].

Zoonotic

Animals may also act as vectors in the transmission of disease, becoming infected with a pathogen and transferring the infection to a susceptible human host [33]. The isolation of *H. pylori* from non-human primates suggests the possibility of animal reservoirs and consequently zoonotic transmission pathways [88]. Demonstration of *H. pylori* organisms in the stomachs of cats, dogs, mice and sheep has lead to investigations of their potential to be a source of the bacteria [14,26]. Further, there has been some research examining increased frequencies of *H. pylori* infection in employees of an abattoir [89-91].

There is little evidence for naturally acquired *H. pylori* in animals; however the presence of other gastric Helicobacter-like organisms (GHLOs) has been demonstrated in a variety of animals [92]. The prevalence of these organisms has been shown to be quite high in some species, for example ranging from 67 to 100% in healthy pet dogs [93-98]. Although the common noninvasive diagnostic technique in humans (UBT) cannot discriminate between *H. pylori* and other GHLOs that produce urease, it is estimated that no more than 1% of humans are infected with other Helicobacter species [35,88]. The low prevalence of other GHLOs in humans indicates that they are not readily transmitted between animals and their owners [94]. In other epidemiologic studies examining the role of pets, lack of elevated frequencies of infection associated with increased contact with domestic pets (including dogs and cats) is reported [99].

Environmental Exposures and Transmission of *H. pylori*

Water

It has been suggested that water may play a role in the transmission of *H. pylori* in two ways. First, water may act as a vehicle through which the organisms are transferred from one individual to another, given contamination of the water with infective digestive fluids. Second, water may be another source of the bacteria. The potential for water to act in either capacity in the transmission of *H. pylori* continues to be a topic of debate in the epidemiologic literature. Much of the dispute is predicated on the morphological change the bacteria undergo when exposed to aquatic environments or adverse conditions. This change is characterized by the conversion from a spiral-shaped organism to coccoid and u-shaped forms, which are believed to be associated with a loss of culturability [54–56]. This occurs in response to physical or chemical stress placed on the organism; exposure to such stress causes changes in morphology, metabolic activity and growth behaviour [100,101]. The modified form is viewed as a viable but not culturable stage, characterized by the maintenance of basal metabolism but the inability to multiply [100]. When assessed under laboratory conditions, *H. pylori* in coccoid form have demonstrated the ability to sustain a high enough metabolism to preserve important cellular structures, including cell membrane, flagella and DNA [100]. However, the extent to which these structures are preserved and the associated ability to colonize a host remains a point of contention in the scientific community [18-20,23,25].

Laboratory experiments to determine the length of time required for *H. pylori* to convert to the coccoid form have indicated that conversion of all organisms in a given sample may take multiple days or weeks [102,103]. For example, Adams *et al.* (2003) [103] spiked several samples of filtered water from a stream with *H. pylori* and observed the conversion to the coccoid form through

epifluorescence microscopy. The authors reported only 75% of the bacteria had converted to the coccoid form following 10 days of observation. Similarly, Queralt and Araujo (2007) [102] examined several samples of bottled mineral water spiked with the bacteria, through scanning electron microscopy and concluded that conversion of all organisms took place after 14 days. Shorter time to complete conversion was reported by Nayak and Rose (2007) [104] when the temperature to which the bacteria were exposed while in the water was manipulated. The authors reported complete conversion to the coccoid form 24 hours after exposure to water at 15°C and after 72 hours at 4°C [104].

Detection in Water

The currently available detection methods are the fundamental limitation in our ability to say whether *H. pylori* organisms in these altered forms can be transmitted through water. Polymerase chain reaction (PCR) is the most commonly used technique, followed by autoradiography or microscopic examination of stained samples [105]. PCR employs chemically synthesized strands of nucleic acids (known as primers) to detect the presence of an organism's DNA in a given sample [106]. Primers are engineered to match either end of a target sequence believed to be specific to the organism, isolating the DNA and then amplifying it [106]. The advantages of this technique include the ability to identify *H. pylori* in either spiral or coccoid form, as well as to target specific genes of interest, for example, those that have implications for antibiotic resistance and virulence factors. While PCR has successfully identified *H. pylori* DNA in samples of water, the presence of the DNA does not indicate whether or not the bacteria are alive or dead [107].

Attempts to identify *H. pylori* DNA in water samples using PCR-based methods have been made for a variety of water types. Natural sources such as groundwater [108], surface water [108], river water [109–111], spring water [109], and seawater [111,112] have yielded prevalences of *H. pylori* DNA ranging from 0 to 68%

of tested samples [108–112]. A prevalence of 0% was found only in flowing natural water sources, including rivers [111], spring water [109] seawater [111]. When examining water from four unidentified rivers in Japan, Fujimura (2004) [110] reported negative results in samples taken close to the river source and positive results only in samples taken at middle and long range distances from the source, with *H. pylori* DNA identified in 45-50% of samples taken from those areas. These points along the river were selected based on the premise that contaminants are carried by the water downstream from the source, and therefore individuals consuming water further downstream would be more exposed to contaminants than those living closer to the source [110]. The highest reported prevalence of *H. pylori* DNA was found in surface water in Mexico [108]. Artificial sources such as municipally supplied drinking water [111,113-115] artificial recharge systems [116] and well water [111] have yielded prevalences of *H. pylori* DNA ranging from 0 to 50% of tested samples [108,111,113-116]. The highest reported prevalence of *H. pylori* DNA was in drinking water from Peru, estimated at 50% of samples [115]. However, when more than one gene was targeted to demonstrate the presence of *H. pylori*, the proportion of samples yielding a positive result reduced to 23% [115]. In contrast, several authors have reported finding no *H. pylori* DNA in various artificial sources [111,113,116].

The most commonly targeted gene for detection of *H. pylori* by PCR is 16 rRNA [108,111,113–117], followed by cagA and ureA [108–111,114,117]. While a larger proportion of studies targeted multiple genes [108,110–113,115,117], using a single ID to identify *H. pylori* DNA has also been reported [109,114]. Authors that report the prevalence of multiple genes in the same sample often show variation in prevalence estimates based on the targeted gene. For example, Horiuchi *et al.* (2001) [111] tested 6 samples of well water for the presence of both the 16S rRNA and ureA genes, with the estimated prevalence of DNA being 33% and 0%, respectively. Further, authors that report the prevalence based on a single ID followed by the prevalence based on multiple genes often report a reduction in

the proportion of samples from which *H. pylori* DNA is recovered [110,115]. These findings suggest targeting single genes may result in an inflated estimate of the proportion of samples containing *H. pylori*. Further, variability in prevalence estimates from the same sample depending on the targeted gene suggests that in addition to the previously mentioned limitations of PCR-based methods, the validity of these tests for detecting *H. pylori* may be poor.

Survival in Water

In an attempt to overcome the inability of PCR-based methods to determine whether *H. pylori* organisms remain alive in water, investigators have combined these procedures with immunomagnetic separation, epifluorescence microscopy, autoradiography and culture [118]. The findings of these approaches have indicated that *H. pylori* organisms may be able to survive in water for limited durations. Investigations of the length of time during which the bacteria are able to be cultured following exposure to water have had variable results. The shortest duration reported was 5 minutes from a sample of spiked chlorinated water [119], with the majority of authors demonstrating culturability up to 2 to 10 days [91,102,103,120]; the longest duration reported was 20 days in a sample of distilled water [121].

It has been suggested that the viability of the organism may be determined by examining the extent to which the cellular membrane remains intact [100,101]. To this end, procedures like epifluorescence microscopy and autoradiography have been employed in conjunction with culture and PCR to generate a well-rounded understanding of the effect of exposure to water on *H. pylori* [101,102,119]. In their examination of bottled mineral water spiked with *H. pylori* organisms, Queralt and Araujo (2007) [102] utilized culture, PCR and epifluorescence microscopy. The authors reported the ability to culture *H. pylori* up to 5 days following immersion in water, cellular viability up to 14 days and the ability to detect the ureA gene up to 3 months [102].

Attempts to understand the determinants of the organism's survival in water have aimed at identifying specific conditions under which the bacteria may be able to overcome adverse conditions. For example, it has been suggested that the presence of other microorganisms, such as zooplankton and free-living amoeba might influence both the ability of *H. pylori* to survive as well as the duration of survival [63,105,122,123]. This was demonstrated in a study of samples from the Adriatic Sea, analyzed by filtration (with a 200 mm filter), culture, and PCR, in which the ability to isolate *H. pylori* only in samples containing large zooplanktonic organisms was consistent across detection methods [123]. Studies have also reported increased survival times associated with lower temperatures [54,65,124,125], pH levels between 5.8 and 7.0 [125] and milk or saline solutions [26,105,126].

Biofilms

Another potential survival tactic exhibited by *H. pylori* in aquatic environments is the ability to readily incorporate into water insoluble biofilms, which can be defined as matrix-enclosed bacterial populations [121,127,128] within which cells adhere to one another on a surface [128]. In the process of incorporating in such an aggregate of microorganisms, *H. pylori* create a novel antibacterial peptide, thought to contribute to increased survival and assist in the maintenance of a niche within which the bacteria can multiply [121,129–131]. This is especially advantageous since other observations suggest that multiplication of *H. pylori* in water in the absence of a biofilm is rare if it occurs at all [60]. Further, observations suggest that symbiotic relationships with other microorganisms in the biofilm allow *H. pylori* organisms to survive for longer durations [132]. For example, some species of Acanthamoeba, a genus of amoeba commonly found in drinking water, have demonstrated the ability to protect *H. pylori* organisms when found in co-cultures, sustaining the bacteria for up to 8 weeks [118,132,133]. This adaptive mechanism is not unique to *H. pylori* and the general ability of waterborne bacteria to form biofilms is widely accepted, as organisms in aquatic

environments are more often found in these aggregates than in their natural state[134–136].

The capacity of *H. pylori* to incorporate into biofilms has been observed in laboratory settings [126,130] and includes adherence to plumbing materials such as copper and stainless steel [137]. Interestingly, Azevedo (2006) [137] found copper material was particularly suitable for sustaining *H. pylori* organisms in their spiral form. Outside of a laboratory setting, the presence of *H. pylori* containing biofilms in water-holding vessels such as pots or pipes from municipal systems has also been demonstrated [126,131,137–140]. For example, Watson *et al.* (2004) [138] identified *H. pylori* in showerhead biofilms in domestic homes. Observations of *H. pylori* containing biofilms in domestic settings indicate the plausibility of biofilms playing a role in the transmission of the bacteria. However, in order to fully understand the impact biofilms have on transmission, more evidence generated through isolating and culturing environmentally adapted forms is necessary [132].

Disinfection

While the ability of *H. pylori* to survive in aqueous environments in an infectious form remains in question, some literature has assessed the susceptibility of the bacteria to standard disinfection techniques. The literature pertaining to the effectiveness of standard water disinfection protocols against *H. pylori* is limited [132]. Some laboratory experiments have demonstrated that chemical additives, such as chlorine, are associated with the death of microorganisms like *H. pylori* [105,118]. However, other investigators have found that *H. pylori* organisms are more resistant to low levels of chlorine than other enteric pathogens like *E. coli* and *C. jejuni* [107,141]. These findings have lead to the belief that if the bacteria are able to persist in water, their presence may not be eliminated by inadequate levels of disinfectants in municipal water systems [107,141]. Further, standard disinfection methods may not prevent *H. pylori* from

entering and persisting in drinking water systems, particularly when incorporated in biofilms [132].

A number of studies have successfully identified *H. pylori* in water systems both pre and post chlorination [142]. When standard disinfection practices were mimicked in a laboratory setting, Moreno (2007) [119] observed the bacteria in the coccoid form, which retained culturability up to 5 minutes after exposure to chlorinated water. In the same study, the viability of the cells was examined through fluorescence *in situ* hybridization, which showed that the cells remained viable up to 3 hours [119]. Finally, two PCR methods positively identified the vacA gene in the sample up to 24 hours after exposure to chlorination [119].

While evidence suggests *H. pylori* are able to persist in chlorinated water, the length of survival time is markedly shorter than reported for *H. pylori* organisms in unchlorinated water [91,102,103,120]. Further, while the effect of disinfection techniques may be weaker against *H. pylori* than against other microorganisms in aqueous environments, the survival of *H. pylori* in such environments may be dependent on the presence of other microorganisms that are more susceptible to chlorination, as mentioned previously. Finally, the length of time between disinfection of the water and delivery to domestic settings is likely to surpass the amount of time *H. pylori* is able to survive in chlorinated water, and therefore the risk posed by resistance to disinfection is likely low.

Epidemiologic Evidence

Most of the epidemiologic evidence has been generated from cross-sectional studies [35–38,41,42,70,110,143–154] with few prospective studies [49,155]. The most frequently assessed water sources include natural sources such as streams or rivers [35,37,110,155], groundwater wells [35,37,42,143-145,150,151,155] and municipally supplied or piped water [35-39,41,42,143-145,147,148,151-153]. Typical exposure ascertainment is based on self-reported consumption of water that is not treated, such as water from rivers or groundwater wells that has not been

boiled, filtered or treated with chemicals, as well as consumption of raw unwashed vegetables or swimming/bathing in rivers or lakes. Another method of exposure assessment is the development of a clean water index (CWI), for which a score is generated for each person based on a set of pre-determined characteristics [156,157]. For example, frequency of boiling water, storing and reusing water, and frequency of bathing have been used to assess the cleanliness of water an individual is exposed to. Finally, in some studies that analyze river water use and consumption in relation to *H.pylori* prevalence, the point along the river where an individual collects their water indicates their exposure status. For example, in a study of 224 children who lived along a river in Japan, exposure was defined as living further downstream from the source, compared to living closer to the source [110].

In studies that use locations along the river or CWIs to assess level of exposure, dose-response trends have been reported for increasing exposure and *H. pylori* prevalence [110,156,157]. In the Japanese study, the prevalence of *H. pylori* infection among people living at lower, mid and upper stream of four unidentified rivers was 23.8% (95% CI: 15.8%, 33.2%), 9.8% (95% CI: 3.8%, 19.7%) and 0% respectively (CIs estimated using data from the report) [110]. In a study of 288 individuals from Kazakhstan that used a CWI to classify exposure to unclean water, the prevalence for low (representing the least clean water), medium or high score was 95%, 79% and 56% and associated odds ratios were 14 (95%CI: 4.8, 40), 2.9 (95%CI: 1.5, 5.8) and 1.0 (reference), respectively [156]. This was also observed in a study from South India, where the prevalence in individuals with a low score on the CWI (associated with the least clean water) was 88.2% (95% CI: 84.3%, 91.4%), compared to 80% (95% CI: 70.8%, 87.3%) for a middle score and 33.3% (95% CI: 20.9%, 45.3%) for a high score (CIs estimated using data from the report) [157]. However, these estimates may be biased by uncontrolled confounding given failure to control for factors that influence an individual's risk of acquiring the infection such as indicators of socioeconomic status.

There have been several reports of increased prevalence of *H. pylori* infection in populations with limited access to potable water systems [37,38,41,70,147,148,150,152,154,155]. For example, in a study of 261 Argentinians aged 2 months to 18 years, individuals who did not have piped water in their home (n=63) had a prevalence of *H. pylori* infection of 22.2% (95% CI: 12.7%, 34.4%) compared to 14.1% (95% CI: 9.5%, 19.8%) in those who had tap water (n=191) (CIs estimated using data from the report) [148]. However, this difference is not statistically precise and confounding was not considered in this study, a commonly pinpointed problem in the identified literature.

Most of the studies that examine the association between exposure to untreated water and *H. pylori* infection provide evidence of a positive association [36–38,41,42,144–150,155], although in a large proportion of these studies, the difference between individuals who consumed untreated water and those who did not was not greater than would be expected with random variation [36,38,42,145,146,148,155], and the possibility of publication bias that favors positive associations should be noted. The most notable associations between consumption of untreated water and prevalent *H. pylori* infection were reported by Klein *et al.* (1991) [144] and Rolle-Kampczyk *et al.* (2004) [149]. In their study of 407 Peruvian children (266 from families with low socio-economic status (SES) and 141 from families with high SES), Klein *et al.* (1991) [144] reported an odds ratio of 12.8 (CI not reported) comparing children who consumed water from an external tap to those with access to an internal tap. However, this estimate was only adjusted for the age, height and weight of the children [144], and not for striking socioeconomic differences in the neighborhoods where children with different water delivery systems resided. In their study of 91 individuals of all ages from Germany, Rolle-Kampczyk *et al.* (2004) [149] determined which wells contained water contaminated with *H. pylori* and compared frequency of *H. pylori* infection in individuals exposed to contaminated wells with those who obtained water from wells that were not contaminated. The authors reported that individuals who

consumed water from wells known to be contaminated with *H. pylori* had 10.4 times the odds of having *H. pylori* infection than individuals who consumed water with no evidence of *H. pylori* contamination [149]. However, this estimate did not account for any other variables that influence risk of acquiring the infection and may also be associated with the cleanliness of specific wells in the region.

The majority of authors (5 of 7) who reported a positive association beyond what would be expected from random variation estimated the odds ratio for the effect of consuming untreated water on prevalence odds of *H. pylori* infection between 1.2 and 3.0 [36,37,41,148,150]. Only 3 of the 16 studies identified by this search did not report a positive effect [35,39,143]. Limited prospective data indicates the effect of untreated water consumption on *H. pylori* infection frequency may be small, if it exists at all [39,155]. Lindkvist *et al.* (1999) [155] followed a cohort of 235 Ethiopian children aged 2 to 4 years for two and a half years, measuring *H. pylori* serostatus and assessing exposure to untreated water every 12 months. The relative risk for consuming water from the well compared to piped water was 1.4 (95%CI: 0.94, 2.1) [155]. However, the authors did not adjust for variables that are likely to influence the magnitude of the association. Further, there was a 33% loss to follow up, with a higher proportion of children with gastrointestinal symptoms remaining in the study, likely motivated by visits with a gastroenterologist as part of participation [155]. Naficy *et al.* (2000) [39] followed a cohort of 397 Egyptian children under the age of 36 months for half a year, beginning in June of 1997. The relative risk for consuming untreated water compared to municipally supplied water was 1.0 (95%CI: 0.15, 4.7) after adjusting for age [39]. While some advances have been made in understanding the role of water in the transmission of *H. pylori*, more data are needed to elucidate the relationship between exposure to contaminated water and frequency of *H. pylori* infection. Further, it could be that exposure classification based on whether or not water had been treated may not be a good proxy for exposure to water that is contaminated with *H. pylori.*

Contaminated Water (Sewage)

The presence of a sewage system servicing the home and its type have been used in the epidemiologic literature to ascertain exposure to water that is contaminated with potentially infectious fecal matter [36–39,41,42,45,146]. Several authors have reported increased odds of infection in individuals with poor sewage systems or non-flush toilets [37,38,41,42,45,146]. In a cross-sectional analysis of 263 subjects recruited from a blood donor clinic in Brazil from 1997-1999, the odds ratio for not having had a sewage system in their house during childhood compared to having one was 1.27 (95%CI: 1.08, 1.48), adjusting for rainwater invading the dwelling during childhood, poultry consumption, type of water ingested in adulthood, fruit consumption, milk and vegetable consumption [41]. In a similar study of 456 children under 6 years of age, whose mothers participated in the Pasitos Cohort Study, the odds ratio per 1 unit increase in number of indoor bathrooms in the home was 0.92 (95%CI: 0.35, 2.4), adjusting for location, age, maternal seroprevalence, maternal education and household crowding [42]. While estimates of the association between toilet type or sewage system and prevalent *H. pylori* infection generated from cross-sectional analyses indicate an association, estimates derived from two identified prospective studies, however, suggest the association is minimal or null [10,39].

Zoonotic Exposures

While science has been unable to determine whether a source of *H. pylori* outside of the human stomach exists, the potential for an animal source has been investigated [158]. Preliminary research of the CAN*Help* Working Group in Canadian Arctic communities indicates that exposure to dogs, cats, mice and animal innards are the most common animal exposures among participants in this research. The ownership of dogs is fairly ubiquitous in northern

communities, whereas only a small number of individuals reported owning cats. Contact with the innards of animals is also common, since hunting and cleaning game is an integral part of life in the Canadian Arctic. There have been some reports of contact with sheep in these communities, but the sample of individuals with this exposure is too small for precise estimates of its effect.
All of the estimates of the association between exposure to animals and prevalent *H. pylori* infection have been generated from cross-sectional studies [35,36,45,60,70,143,159–162]. The predominant techniques for classifying *H. pylori* infection status used in these studies were the UBT [35,36,160,162] and serology [45,46,60,143,161]. Additional methods included the stool antigen test [159] and histology[70]. In most of the literature on this association, an individual is considered exposed to animal sources of the bacteria if they report owning animals as pets [36,45,46,60,70,143,159–162]. Alternatively, the number of animals an individual is in regular contact with, occupational contact with animals and whether or not individuals share their living space with animals has been used to define exposure [46,60,160,162]. Animals that are most often investigated in relation to *H. pylori* transmission include cats, dogs, rabbits, birds, guinea pigs and sheep [35,36,45,46,70,160,162]. Other animals that have been examined include fish, hamsters, horses, donkeys, cows, chickens, geese, goats and pigs [36,46].

Some authors have reported a slightly elevated prevalence of *H. pylori* infection in individuals who own pets or are exposed to multiple animals in their home or through their occupation [45,46,60,162]. For example, in a study of 131 Polish individuals, in 42 shepherds the prevalence of *H. pylori* was 100% (95%CI: 91%, 100%) compared to 65% (95%CI: 52%, 77%) in 61 individuals with no contact with sheep (CIs estimated using reported data) [162]. However, the predominant finding is a decreased prevalence of *H. pylori* infection in individuals who are exposed to animals compared to those who are not [36,46,143,159–161]. For example, in a study of 2578 individuals from the United States, of the 1058 persons that reported having pets in their household, 19% (95%CI: 16%, 22%) were positive

for *H. pylori*, compared to 32% (95%CI: 5%, 38%) in 1520 persons who were not exposed to animals [161].

A large proportion of studies suggest there is no association between pet ownership and prevalent *H. pylori* infection [45,46,70,160]. A protective effect of owning animals has also been reported [46,70,143]. For example, in a study of 245 youth aged 3 to 20 years recruited from a surgical outpatient clinic in Arkansas, U.S from 1988 to 1989, the odds ratio for animal ownership compared to not owning pets was 0.52 (95%CI: 0.29, 0.97), adjusting for age, race, gender, annual income, community type (urban, suburban or rural) and water source (municipal or well) [143]. One study generated evidence of a positive association between frequent contact with animals and prevalent *H. pylori* infection [150]. In their study of 383 randomly selected adults from Ourense, Spain, Garcia *et al*. (2006) [150] found individuals who reported frequent exposure to animals had 1.7 (95%CI: 1.0, 2.7) times the odds of having *H. pylori* infection compared to individuals who reported infrequent contact with animals. However, this estimate was not adjusted for additional variables that influence an individual's risk of acquiring the infection.

Specific species that have been associated with increased odds of having *H. pylori* infection include sheep [35], cats [160], rabbits [160] and dogs [46]. However, the difference between individuals who were exposed to these animals and those who were not was not greater than would be expected from random variation. Some evidence of a protective effect of animals such as guinea pigs [160] and birds (including chickens, ducks and geese) [46,160] has been reported, however the difference between individuals exposed to these animals and those who were not was not greater than would be expected with random variation. It should be noted that in many settings, contact with animals including pets and livestock implies some degree of wealth; independent of contact with animals, wealth generally conveys an inverse association with *H. pylori* prevalence, and thus

represents an important source of potential confounding of associations between animal contact and the occurrence of *H. pylori* infection.

Exposure to Dogs

Experimental inoculation with *H. pylori* has been carried out in order to observe the pathogenicity of the organism in a canine host [95,163]. A pack of 4- to 6-month-old gnotobiotic beagles raised in a laboratory setting were successfully infected with the bacterium [95]. The beagles presented with gastritis approximately 4 weeks following inoculation [95]. This study also demonstrated the transmissibility of *H. pylori* from infected to non-infected gnotobiotic beagles [95]. Colonization of the stomachs of conventional puppies with *H. pylori* in a laboratory setting following intentional inoculation has also been reported [163]. The infected puppies presented with vomiting and loose stools 1 week after inoculation, followed by the development of chronic follicular gastritis [163].

There is limited epidemiologic data examining this association. The predominantly employed study design is cross-sectional [36,46,70,160], with one prospective analysis of dog ownership [164]. Two studies estimated an increased prevalence of *H. pylori* infection in dogs owners compared to individuals who do not own dogs [36,47]. In one cross-sectional study of exposure to domestic pets as an adult in China, an odds ratio of 1.2 (95%CI: 0.3-4.5) was reported for keeping a dog in the home compared to not keeping a dog in the home [47]. In the same study, retrospective assessment of exposure to dogs at the age of 10 years yielded an odds ratio of 1.5 (CI not reported) for the comparison of being exposed to dogs at this earlier age with not being so exposed [47]. These estimates were adjusted for current age and occupational contact with animals [47]. Conversely, several authors have reported a slight protective effect of dog ownership on the odds of *H. pylori* infection [47,70,164]. In a prospective study, farm workers from five English government districts were followed for two years and *H. pylori* status was measured annually through serological tests [164]. The odds

ratio for contact with dogs at the ages of 0 to 5 years compared to no such contact was 0.85 (95%CI: 0.75, 0.97), adjusting for age and sex [164]. However, the diagnostic accuracy of serological methods is poor in this age group. Further, important confounding variables such as indicators of socioeconomic status were not considered. In a cross-sectional study of children attending pediatric gastroenterology clinics in 1994 and 1995 in Peru, the odds ratio for exposure to domestic dogs compared to no such exposure was 0.89 (measures of statistical precision were not reported), although this estimate was not adjusted for factors likely to influence the odds of acquiring *H. pylori* [70]. While experimental inoculation of puppies with the bacteria indicate the potential for *H. pylori* to colonize canine stomachs and the consequent possibility of transmission to humans, more epidemiologic evidence is needed to elucidate the importance and frequency of this route of transmission.

Exposure to Cats

H. pylori has been identified in the stomach of cats, indicating their potential to transmit the bacteria to humans [93,99,165,166]. The ability of *H.pylori* to infect a feline host has been observed in cats obtained from commercial vendors of research animals, reported to have naturally acquired infections [166,167], and others raised in a laboratory setting that were successfully inoculated with the bacterium [93,165,168,169]. However, other attempts to isolate *H.pylori* from stray cats have not been successful [170]. Pathogenesis of *H. pylori* infection in cats appears to follow a similar pattern to human infection, with the presence of severe gastritis [165,168].

Epidemiologic data regarding the zoonotic risk presented by cats has been inconsistent [35,160,165,170,171]. An increased prevalence of *H. pylori* infection in individuals who own cats has been noted in report [36]. In a cross-sectional analysis of preschool children from Germany in 1996, the odds ratio for owning a cat compared to not was 1.9 (95%CI: 0.7, 5.1), although this estimate was not

adjusted for potential confounders [160]. However, a protective effect of cat ownership has also been reported [164]. In a prospective study of farm workers from five districts in England, the odds ratio for the effect of current contact with cats on the incidence odds of *H. pylori* infection over a 2-year period was 1.55 (95%CI: 0.96, 2.5), adjusting for age and sex [164]. The evidence produced by epidemiologic investigation of the association between cat ownership and *H. pylori* infection does not provide strong support for or against the possibility of cats playing a role in transmission to humans.

Exposure to Mice

Mice are consistently used as animal models for *H. pylori* infection in order to gain a better understanding of the pathogenicity and to develop potential vaccines [172–175]. The ubiquitous use of mice in this context and the repeated ability to infect mice with both mouse-adapted and human strains of *H. pylori* indicate their potential to act as a source [95,172–174]. Epidemiologic data on this association was not identified by this search; thus it is unclear whether or not there is an increase in the risk of infection associated with contact with mice.

Animal Innards

Three studies have reported an increased prevalence of *H. pylori* infection in abattoir workers [89,90,158]. In a study of 98 abattoir workers from Italy, comparing individuals with direct contact with animals to clerical workers, significantly lower prevalence of infection was observed in individuals without direct contact with animal remains [89]. This is consistent with the results of another study that compared abattoir workers with matched controls who were not abattoir employees, and observed a higher prevalence of *H. pylori* infection in abattoir employees [90]. Additionally, an increased prevalence of infection was found in individuals who worked with meat compared to random blood

donors[91]. However, important potential confounders such as age, education and socio-economic status were not adequately addressed in these studies. More data is needed to examine the association between regular contact with animal innards and increased risk of infection.

Gastritis

The chronic inflammation of the gastric mucosa that defines gastritis involves the degeneration of the surface epithelium, characterized by exaggeration of the normal cell loss and regeneration processes, also termed cellular exfoliation [176,177]. Common causes of gastritis are *H. pylori* infection and chemical irritation, most often resulting from bile reflux or regular use of non-steroidal anti-inflammatory drugs (NSAIDs) [176,177]. More broadly, the presence of irritants and a suboptimal supply of nutrients can induce gastritis [176]. Irritation of the gastric mucosa triggers an acute inflammatory response characterized by an influx of polymorphonuclear cells [176]. Persistent exfoliation and subsequent cell injury is associated with the development of erosions in mucosal tissue [177]. Complete erosions, defined as small erosions occurring in several areas of the gastric mucosa, are seen most frequently in patients with *H. pylori* infection [177]. In contrast, incomplete erosions, defined as localized defects of the mucosal lining that do not induce a reaction from the surrounding area, are often the product of acute damage caused by chemical degradation or ischaemia [177]. Following damage to the epithelial cells, higher concentrations of mononuclear leukocytes remain in the gastric mucosa [98]. This phenomenon is not observed in normal gastric mucosa and is considered characteristic of chronic inflammation[98].

***H. pylori*- Associated Gastritis**

The relationship between *H. pylori* infection and the development of chronic gastritis has been well-established [176,177]. The products of *H. pylori* bacteria have a direct toxic effect on the epithelial cells of the stomach lining [176,177]. There are several attributes of the bacteria that influence the development of gastritis, specifically including vacuolating toxins, urease and ammonia, acetaldehyde, phospholipases, and platelet activating factors [177].

1. **Vacuolating Toxins.** The *H. pylori* vacA gene codes for the vacA protein, which induces the formation of a vacuolating toxin (87-kd vacuolating toxin), which inhibits enzymes in the plasma membrane of gastric mucosal cells [177,178].
2. **Urease and Ammonia.** *H. pylori* organisms secrete the enzyme urease, which breaks urea down into ammonia and carbon dioxide and allows *H. pylori* to survive in the acidic environment of the stomach. The consequence of this is a higher concentration of ammonia in the stomach, which is associated with mitochondrial inhibition in gastric mucosal cells [177]. Further, ammonia reacts with neutrophils to create mono-N-chloramine, which is considered highly toxic [177].
3. **Acetaldehyde.** *H. pylori* organisms also produce the enzyme alcohol dehydrogenase, which breaks down ethanol substrates, producing acetaldehyde, a highly reactive substance [177].
4. **Phospholipases.** Phospholipases secreted by *H. pylori* have the potential to damage the surface epithelium by liberating acids that increase the permeability of the mucus membrane, and promote mucus production and discharge and other inflammatory effects [177]. Further, the mucus membrane in a stomach infected with *H. pylori* is less hydrophobic than a normal mucus membrane, and this has the potential to compromise its function as a barrier that protects the gastric epithelium [178].

5. **Platelet Activating Factor (PAF).** *H. pylori* organisms cause gastric cells to release PAF, which can lead to thrombosis and focal occlusion of circulation which damages the surface epithelium through ischaemia[177,179].

Chemical Degradation and Gastritis

In addition to *H. pylori*-associated inflammation, chemical degradation can be responsible for damaged gastric epithelial cells. This is most commonly a result of bile reflux and regular NSAID use [176,177].

1. **Bile Reflux.** The content of the reflux includes both alkaline and acidic substances, bile salts and lysolecithin [177,180,181]. The presence of these substances induces exfoliation of the surface epithelium, with increased concentrations of the acidic milieu being directly related to an increase in exfoliation and mucous production [177].
2. **NSAIDs.** Regular exposure to NSAIDs is associated with gastritis as they reduce the synthesis of prostaglandins, which are important for the maintenance of blood flow through the mucosal lining [177].

Prevalence of Severe Gastritis

Initial data collected by the *CANH*elp Working Group from Canadian Arctic communities has demonstrated higher frequencies of moderate to severe gastritis than would be expected in *H. pylori*-positive populations in western developed countries. The prevalence of severe gastritis in Old Crow in 2012 and Aklavik in 2008 was 65% and 43%, respectively. In contrast, the prevalence of severe gastritis observed in the *H. pylori*-positive patient population with gastric biopsies examined by pathologists at the University of Alberta Hospital in 2011 was 4.6% [201]. This discrepancy between Arctic populations and the Edmonton patient population indicate that the frequency of severe gastritis observed in the northern communities is not solely attributable to *H. pylori* infection.

Environmental Factors

The environmental impact of a warming global climate includes changes to the organic carbon cycle and degradation of Arctic permafrost; processes thought to be associated with increased levels of mercury in Arctic ecosystems[182]. Concordantly, contamination of Arctic animals and water sources with mercury has been documented extensively [183]. Residents of Arctic Aboriginal communities who continue to follow a traditional subsistence lifestyle are regularly exposed to these sources of mercury contamination. Exposure to inorganic mercury is of particular importance when investigating gastrointestinal (GI) outcomes. When ingested, approximately 7-15% of inorganic mercury is absorbed through the GI tract, leaving large amounts bound to the GI mucosa[184]. While the severity of GI outcomes following ingestion is dose dependent, chronic ingestion of low levels of mercury has been shown to induce gastritis [184]. Therefore, it is plausible to hypothesize that consumption of food and water that contains heavy metal contaminants such as inorganic mercury may be partially responsible for the severity of gastritis observed in Arctic communities.

Chapter 3: Methods

Definitions

The following definitions, from Porta's 2008 Dictionary of Epidemiology, 5th edition,[11] were used:

1. **Infectious Agent.** A factor that can cause illness through transmission of the factor or its products from an infected host to a susceptible host; an infectious agent is usually a type of microorganism that leads to a specific illness defined by the presence of this organism.
2. **Pathogen.** An organism capable of triggering a pathogenic process in a human or animal host.
3. **Infection**. The entry and subsequent development or propagation of an infectious agent in a human or animal host.
4. **Source or Reservoir of Infection.** The source or reservoir of an infection is environment in which the infectious agent normally lives and multiplies. This can include any person, animal, arthropod, water or soil, from which the organism originates.
5. **Vehicle.** A vehicle is a broad term used to describe the agent of transmission of an infectious agent from its source to a new host. By definition, a vehicle of transmission can refer to the person from whom the infectious agent is passed, contaminated food, water or other objects, or an arthropod. For the purposes of this research, the term vehicle will be used to refer specifically to an intermediary that facilitates transfer from a source to a susceptible host.
6. **Fomite.** A fomite is a specific type of vehicle, defined as an inanimate object or material that acts as an intermediary between the source and new host. Objects such as toys, soiled clothing, bedding, handkerchiefs,

cooking or eating utensils, medical equipment, water, food or milk, can act as fomites.

7. **Vector.** A vector is a living carrier that transfers an infectious agent from its source to a susceptible host, or to the host's food or immediate surroundings.
8. **Vector-borne.** A vector-borne infection refers specifically to arthropod vectors, and can involve either mechanical or biological transmission [11].

Literature Review

A comprehensive literature review was conducted, to investigate the degree of consensus in the literature regarding environmental exposures and digestive health. Two subjects were investigated, the first being the capacity for environmental factors to influence the transmission of *H. pylori*, either as sources of the bacteria or vehicles in the transmission from person to person. Second, literature examining the presence of environmental contaminants in the geographic regions of interest and their potential influence on digestive health was reviewed. The search strategy was developed with the guidance of a health sciences librarian. Relevant databases were reviewed, including: PubMed, CINHAL, Cochrane Library and Medline. For the first literature review, search terms included *Helicobacter pylori* OR *H* pylori* OR *Helicobacter* infections, pathology, therapy and transmission. The operation AND was used to link to the following terms: prevalence, incidence, acquisition, environment, water, sewage, zoonosis and biofilms. For the second topic, search terms included "gastritis", linked by the AND operator to the terms: chemical, pollution, mercury, lead and persistent organic pollutants. For both subjects, the review was limited to papers published in English.

Study Design and Research Program

Study Design

These analyses used data collected in a cross-sectional study of *H. pylori* infection in northern Canadian communities. Exposures were ascertained by structured interviews at approximately the same time infection status was ascertained by screening with the 13C-urea breath test. Gastritis severity was ascertained by histopathological examination of gastric biopsies collected during upper gastrointestinal endoscopy, which occurred after screening tests classified *H. pylori* status.

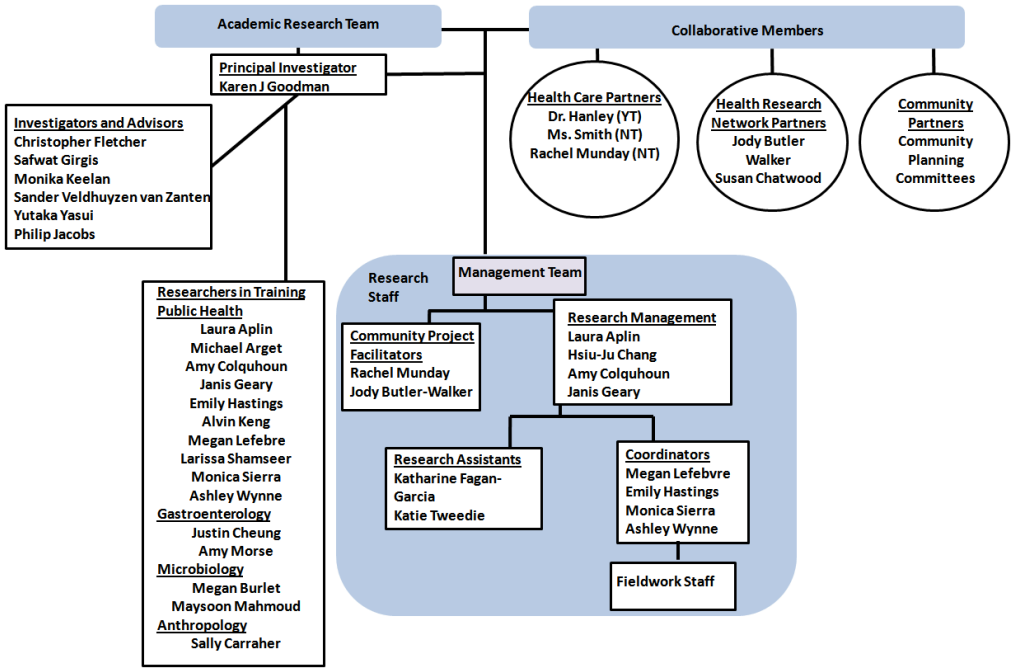
Community Projects

In order to conduct a comprehensive investigation of *H. pylori* infection in northern Aboriginal populations, projects were established in each community. Each community project was designed with six main components: questionnaire-based interviews to collect data on health and socio-environmental exposures, screening for *H. pylori* infection via 13C-UBT, endoscopy, treatment, knowledge exchange and policy development.

Research Program

The CAN*Help* Working Group was established in 2006 to address community concerns about *H. pylori* infection. This research program is a collaborative effort, linking northern Canadian communities, their health care providers and regional health authorities with investigators from a variety of disciplines at the University of Alberta (figure 1).

Figure 1: Organizational structure of the CAN*Help* Working Group



Ethics

Study Approvals and Licenses

Ethics approval was obtained from the University of Alberta's Human Ethics Research Board – Biomedical Panel. Additional approval was obtained from the Yukon Science Institute and the Aurora Research Institute, NWT. The principal investigator, Dr. Karen Goodman, obtained territorial research licenses. Project physicians obtained territorial medical practice licenses. Administrative and operational approval for the conduct of endoscopies, and collection and analysis of gastric biopsies was obtained from The Northern Alberta Clinical Trials and Research Centre (NACTRC) Research Administration on Behalf of Alberta Health Services.

Privacy and Anonymity

Each participant was assigned a 6-digit project identification number, to ensure privacy during data collection and analysis. The first number indicated which of the three communities the participant was from. The project identification numbers began with '1', '2' and '3' for residents of Aklavik, Old Crow and Tuktoyaktuk, respectively. The following three digits of the project identification number represented the household that the individual lived in. A random number list was generated and each house number in the community was assigned a random 3-digit number. The remaining digits in the project identification number represented the birth order of the individual in relation to other individuals residing in the same house. For example the project identification number for the oldest individual in the home ended with '01', with successive household members receiving numbers in ascending order.

Personal identifiers beyond the assigned project identification number were not transferred from questionnaires to electronic data files. All original copies of paper documents were scanned and the electronic copies were saved in a project folder with restricted access. Paper copies were either locked in a cabinet within a permanent project office in the community, or transported back to Edmonton where they were stored in a locked cabinet at the University of Alberta.

Informed Consent

In accordance with ethical and legal standards, interviewers outlined the research process to each participant, including the risks and expected benefits of taking part in a research study. Information was provided in the form of a study information sheet, which has received approval from the University of Alberta Health Research Ethics Board and was conveyed to the participant exactly as written. Once the participant reviewed the sheet, had the opportunity to ask questions and indicated they understood the information they had been given, they completed a consent form. For individuals under the age of 17, parental

consent was obtained. A second information sheet designed to convey the same information to children was given to participating youth who were old enough to understand the material and an assent form was signed in addition to the parental consent form. A second copy of each consent and assent form was made and given to the participant for their records. In the event that the interviewer felt the participant did not fully understand the material despite having expressed otherwise, it was the responsibility of the interviewer to review the material again to ensure comprehension. If the participant was unable to understand the material and a suitable assistant for the interview was not available, consent was not signed and the individual did not register as a study participant.

Possible Risks and Expected Benefits of Participation

Potential risks were outlined to each participant prior to their enrolment in the study. While the privacy of each participant was ensured, they were cautioned that they might feel uncomfortable providing some of the information requested. Because of this, they were reminded of their option to refuse to answer any given question and to remove themselves from the project at any time, without having to give a reason and without affecting their future medical care. Prior to endoscopy, participants were advised the procedure might induce some discomfort including nausea, gagging, uncontrolled swallowing and a mild sore throat or nose bleed, depending on the method chosen by the gastroenterologists. Participants were informed that some slight bleeding might occur at the site where biopsies were taken, although blood loss is typically minimal. The rare possibilities of serious complications were explained, including heavy blood loss requiring a transfusion; a hole in the esophagus, stomach or small intestine requiring surgery; or fluid or stomach contents entering the lungs. Participants were reminded that the doctors performing the endoscopies were experienced specialists who would act carefully to reduce the aforementioned

risks and that in the unlikely occurrence of an adverse event during the endoscopy, emergency measures would be taken as necessary.

Expected benefits of the project overall and for each participant were explained. Broad benefits of community-based research on *H. pylori* described to each individual included the provision of evidence to northern health officials to inform policies and clinical management of the infection. Individual benefits included determining whether the participant required more tests or treatment, which was arranged by project staff when necessary. If the participant consented to endoscopy, benefits of involvement included appointments with one or more project gastroenterologist who would examine each individual, explain the diagnosis at the time of the scope and the results of histopathological examination of biopsies taken. Participants were reminded that they would be able to consult the project gastroenterologists about any gastric problems they were experiencing, in addition to their test results.

Establishment of Community Projects

Study Populations

This research used data collected in 3 community projects conducted by the CAN*Help* Working Group. The Aklavik *H. pylori* Project was launched in 2007. According to the 2006 census, the population of Aklavik, NT was approximately 590 with roughly 92% of residents identifying with either Gwitch'in (First Nations) or Inuvialuit (Inuit) cultures [185]. Discussions with the community of Old Crow, YT began in 2008, with the official launch of the Old Crow *H. pylori* project in 2010. According to the 2006 census, the population of Old Crow was approximately 250, with roughly 86% identifying as Vuntut Gwitch'in [185]. In 2010, there were requests for expansion to the rest of the Inuvialuit Settlement Region (ISR), which comprises 6 NT communities, including Tuktoyaktuk, Sachs Harbour, Paulatuk, Ulukhaktok, Aklavik and Inuvik. Continued work in the ISR was initiated in Tuktoyaktuk, with a pilot project that was launched in 2011. According to the

2006 census, Tuktoyaktuk was a community of approximately 870 individuals, with about 85% identifying with Inuvialuit, Métis or First Nations cultures [185].

Many residents of these communities continue to follow a traditional lifestyle of hunting, trapping and fishing, while adopting modern technologies such as computers and snowmobiles. Both Aklavik and Tuktoyaktuk are accessible by water or air in the summer and ice road in the winter. Old Crow is accessible only by air [186–188].

Community Planning Committees

At the start of each community project, a community planning committee was established. These committees were comprised of representatives from the community and project staff from the University of Alberta. The goal of these committees was to ensure the projects being carried out in the respective communities were culturally appropriate and addressed community concerns. Regular teleconferences ensured the committee was able to discuss important aspects of the project, including: the name of the project, coordinating the appropriate times for project staff from the university to come to the community, review of information to be distributed in the community for clarity, review of the questionnaires to ensure cultural appropriateness, assisting with hiring an individual from the community and plans for the dissemination of results to participants. Community approval and meetings with planning committees began in February of 2007 for the Aklavik *H. pylori* Project, August 2008 for the Old Crow *H. pylori* Project and January 2010 for the ISR *H. pylori* Project in Tuktoyaktuk.

Recruitment

This research followed a participatory model, inviting all interested individuals to register during defined enrolment periods. Recruitment occurred in Aklavik from November of 2007 till February of 2008, in Old Crow from November of 2010 till February of 2011 and in Tuktoyaktuk from February-March

of 2011 and March-May of 2012. Recruitment activities aimed to disseminate information about the project and how to enrol. The planning committee guided the activities by recommending appropriate forums. These activities included community gatherings, flyers, announcements in newsletters or on the radio, information tables in high traffic locations and door-to-door outreach. Posters and flyers were placed on public bulletin boards around the community and are either put up at the time of project-staff's arrival in town, or emailed to community contacts to be put up ahead of time. Flyers contained basic information about the project, including the contact information of project-staff, the dates that project-staff would be in the community and where to go for more information. Short radio announcements that contained the same basic project information as the flyers were drafted and were also either delivered to the station by project-staff for sent ahead of time to community contacts. The announcements were read prior to BINGO, which is played over the radio and involving the majority of the town. This ensured the announcement would reach a wide audience.

When in the community, project-staff held community gatherings, typically in the form of a community feast. The planning committee advised project-staff of the individual or group of individuals in town to be contacted about catering. Community recreation centers were contacted and rented out for the evening. Pamphlets with information regarding the project, contact information and 'fast facts' about *H. pylori* infection, associated disease and courses of treatment were placed around tables for community members to read. Project-staff gave either a PowerPoint presentation or showed the documentary created through the Aklavik *H. pylori* Project, which describes the research process and what individuals can expect should they choose to participate.

Door-to-door outreach involved project-staff, ideally one person from the university and one hired from the community, approaching houses to engage in

a one on one dialogue. Households were chosen for door-to-door outreach on the basis of not having been contacted to date. Project-staff provided information, answered questions and made appointments for interested individuals. At the conclusion of the visit, project-staff recorded with whom they spoke and how the information was received. For community members that indicated they are not interested in participating, project-staff asked for and recorded the reason. Individuals who did not wish to participate were not contacted again, but invited to contact project-staff if they changed their mind. Finally, when individuals made their appointments, they were encouraged to have family members participate.

Appointments were documented on a calendar, which was kept by the phone in the project office. The name and phone number of each individual was recorded. Participants were contacted prior to their appointment to remind them of the time and instruct them how to prepare for the upcoming test. Individuals with morning appointments were contacted the night before and those with afternoon appointments are contacted in the morning before they are scheduled to come in. Individuals who required assistance during their interview were noted and the appropriate arrangements were made. For example an interpreter or translator was hired by the project to facilitate interviews with participants who were not comfortable with the English language.

Data Collection

Community Surveys

Data on health history, demographic characteristics and exposure to relevant socio-environmental factors were collected through structured interviews conducted by trained interviewers. The instruments included participant and household questionnaires, which ascertained socio-environmental exposures pertaining to individuals and their households,

respectively, and a clinical questionnaire, which ascertained individual health factors pertaining to individuals. The household questionnaire was administered to one member of each household and the participant and clinical questionnaires were administered to each participant.

Data on socio-environmental exposures at both household and individual levels were collected using the respective interviewer-administered questionnaire. Characteristics of interest included family size and structure, educational attainment, occupation, housing quality, residential crowding, water source, type of sewage disposal facility, hygienic practices, contact with animals, food preparation practices, and diet.

Using the clinical questionnaire to obtain data on health history, participants were interviewed about previous diagnoses of *H. pylori* infection and related diseases (gastritis, peptic ulcer disease, gastroesophageal reflux disease, stomach or esophageal cancer), previous treatment for *H. pylori* and the outcome of such treatment, frequency of relevant symptoms (upper abdominal discomfort), and other reasons to be tested for *H. pylori* (family history of stomach cancer, long-term use of NSAIDs or aspirin) or to be evaluated by upper gastrointestinal endoscopy (persistent esophageal reflux, diagnosis of anemia, dark stools, loss of appetite, recent weight loss). In order to enhance completeness of information and to help reduce recall bias, information relevant to the history of digestive disease was also extracted from participants' medical records. A chart review tool was used to collect information pertaining to relevant family history of disease, history of seeking care for stomach complaints, previous diagnosis or treatment of *H. pylori* infection, and related diagnoses and prescriptions. The questionnaire and charte review information was collected by several research assistants, all trained in proper interviewing and data recording procedures.

Structured Interviews

The main goal of the interview was to collect accurate and complete information from each participant. In order to achieve this, the interviewer followed specific protocol to facilitate the respondents' provision of accurate information by aiding in their complete understanding of what information is being requested through the use of unbiased probing techniques. Further, the interviewer was responsible for ensuring the respondent's comfort in providing complete answers to the questions being asked. Finally, interviewers assured complete and exact documentation of each response.

Training of interviewers focused on ensuring they had a thorough understanding of the goals of the research program and proper interviewing techniques, including the qualities of a good interviewer and correct usage of the interview instruments. Qualities of a good interviewer include an investigative approach, characterized by approaching each interview in a manner that encourages accurate responses and does not introduce bias. In order to achieve this, interviewers maintained neutrality and avoided suggesting that there were correct or expected answers or that the interviewer disapproved of or was surprised by a given response. A good rapport with the participant was developed through conveying interest in the wellbeing of the participant, respect and a non-judgemental approach. Project-staff were responsible for reviewing the schedule of appointments each morning, in order to ensure they were able to greet each participant by name, an important first step in establishing a rapport. Ensuring the interviewer dressed in accordance with local customs facilitated the participant's comfort in the interview and avoided introducing a potential source of distraction. Familiarity with the interview instruments was ensured through review of the project manual of procedures and meticulous review of the questions. Methods for explaining what information the question is aiming to solicit without altering the meaning of the question were practiced. Project-staff that were responsible for training new interviewers went through

the questionnaires with trainees, interviewing them and then reversing the roles, offering constructive feedback.

Project-staff arrived at the office approximately half an hour before the first appointment of each day and reviewed the schedule. This time was used to determine where everyone was to be stationed for the day and who was to cover each task. Interviewers set up their respective spaces, which were stocked with enough materials for the day, including: Breath test bags, urea cups, clips, clip boards, questionnaires, study information sheets, consent forms and pens. Spaces chosen for the interviews were separate from one another (for example two different exam rooms in the health center) and set up with two chairs of equal height approximately 3 feet from one another. Having the chairs set up in this manner assured the interviewer and participant would be at the same eye-level and positioned comfortably.

Unbiased probing techniques were used to encourage the respondent's provision of a complete answer, or a clarification or explanation of their response. In using these techniques, the interviewer aimed to solicit a clear and relevant answer without leading the participant or making assumptions about what the participant meant to say. For example, if when asked for their current occupation the participant stated their employment at the health center, an unbiased probe would ask what they do at the health center. Upon the completion of an interview, questionnaires were reviewed by the interviewer and one other member of the project-staff to confirm completion and correct documentation of responses. If the response to a question was missing, incomplete or difficult to interpret, project-staff made an effort to contact the participant and obtain or confirm their answer. In addition to identifying missing information, post-interview editing of questionnaires provided an opportunity for project-staff to advance their understanding of the questions.

Urea Breath Test Protocol

Test Preparation:

1. Prior to the appointment, the participant was instructed on how to prepare for the breath test. They were asked to refrain from consuming: any carbonated beverages for at least one hour before the test; any food or drink other than uncarbonated water for four hours before the test; and any acid suppressing medications or Pepto-Bismol for 24 hours before the test.
2. The collection bags were labeled with the participant's name, date of birth and project ID.
3. Each side of the collection bag was clearly marked T1 and T2 respectively, to denote samples taken at Time 1 (0 minutes) and Time 2 (30 minutes).
4. The following information was recorded in the UBT log for each participant: The participant's name, date of birth, project ID, time they last ate or drank anything other than water; Whether any PPIs, Pepto-Bismol or acid-suppressing medications had been taken in the past 7 days, name of the medication and the last time taken; Whether any antibiotics had been taken in the past 30 days, the name of the antibiotic and last time taken. For children 5 years of age or younger, the height and weight is recorded in inches and kilograms.

Test Administration

(Based on manufacturer instructions (http://www.helikit.com/en/physician-information/) and Gisbert and Parajes (2004)[29].

1. Mouthpieces were handled through the individual plastic wrap within which they were packaged to avoid touching them directly. The blue stopper from the end of the rubber tube on the side of the collection bag marked T1 was removed and the mouthpiece fully inserted into the tube.

2. Participants were provided with instructions for proper breath test technique in order to ensure the sample has an adequate CO_2 concentration, required for an accurate test result. The instructions provided to participants were as follows:
    a. Take a deep breath.
    b. Before blowing into the mouthpiece, release a small puff of breath (to release the air from the mouth and throat that did not go into the lungs).
    c. Without inhaling, blow the remainder of the breath from your lungs through the mouthpiece to fully inflate the bag.
3. Participants were asked to provide the first breath sample in the side of the bag marked T1, making sure the bag was as inflated as possible. If the participant did not provide a good sample of exhaled breath, the mouthpiece was removed using the plastic wrapper, so the bag could be deflated. Once the bag was deflated, the mouthpiece was replaced.
4. Once a good quality sample was collected in the first side of the bag, the time of the first baseline sample was recorded in the log.
5. A clip was attached to the rubber tube on side 1 of the bag, the mouthpiece was removed using the plastic wrapper and the blue cap replaced. The blue cap from the second side of the bag was removed and the mouthpiece inserted into the rubber tube. The plastic wrap was left around the mouthpiece and the bag was set aside.
6. 100mL of filtered water was mixed into a cup containing 50 mg of urea and citric acid. The participant was instructed to drink the entire volume of liquid. Once the urea was swallowed a timer was set for 30 minutes.
7. The volume of citric acid solution and time of consumption was recorded in the log.
8. When 30 minutes had passed, the participant was reminded of proper breath test technique and instructed to provide a second breath sample

in the side of the bag marked T2. The quality of the sample was monitored and the process repeated if necessary. The time of the second breath sample was recorded in the log.

9. Comments on the quality of the breath samples were recorded in the log, along with the number of attempts each participant required to provide a good sample and possible reasons for the difficulty (for example lung conditions).
10. A clip was attached to the rubber tube on the second side of the bag. The mouthpiece was removed and discarded and the blue cap placed back in the tube.

Endoscopy and Analysis of Biopsies

Endoscopies were offered to individuals aged 15 years or older from Aklavik in February of 2008 and Old Crow in January of 2012, irrespective of *H. pylori* infection status. In each community, a mobile endoscopy unit was set up in the health center and a medical team led by Dr. van Zanten, a gastroenterologist from the University of Alberta, performed transnasal (Aklavik) or transoral (Old Crow) upper endoscopies on consenting participants. Physicians examined each stomach for the presence of gastric lesions and took 7 biopsies from specified sections of gastric mucosa. In the event a lesion was present, physicians took a biopsy of the lesion for pathological examination. Of the 7 biopsies obtained, 2 were intended for microbiological examination and 5 for histopathological examination. The locations from which biopsies were sampled for histopathological examination were selected according to the updated Sydney protocol [189,190]. Tissue samples were carefully packaged to ensure preservation, guard against freezing in transit and in accordance with guidelines for the air transport of biohazardous material. Once packaged, the biopsies were shipped via cargo to the University of Alberta, at the laboratory addresses of Dr. Girgis (pathologist) and Dr. Keelan (microbiologist).

A team microbiologist, Dr. Keelan, cultured *H. pylori* from gastric biopsies in order to confirm diagnosis and estimate the prevalence of antibiotic resistant strains and virulence factors. Microscopic examination of the samples of gastric mucosa from several areas around the stomach confirmed the presence and density of the bacteria in *H. pylori*-positive participants. Microscopic stained samples were also evaluated for the presence of gastric neoplasms and to assess severity of inflammation. The biopsies were evaluated by a single pathologist, Dr. Girgis, who was blinded to endoscopic findings. Severity of inflammation in the stomach lining was graded as mild, moderate or severe according to the updated Sydney classification system [176,189,190].

Data Management and Analysis

Socio-environmental factors ascertained in questionnaire-based interviews were selected for analyses aimed at estimating the effects of relevant environmental exposures on *H. pylori* prevalence and severe gastritis prevalence in northern Canadian communities. Two analyses were completed to investigate the proposed research questions. The first analysis examined environmental exposures in all three communities. The second analysis investigated the relationship between untreated water consumption and severe gastritis prevalence in Aklavik, NT and Old Crow, YT, among participants with histopathology data.

Data Entry and Cleaning

A participant registry was created using Microsoft Access for electronic documentation of the participants, their contact information, whether they had a 13C-UBT, which questionnaires were completed and a record of each interaction (including phone calls for setting up appointments). This database was updated in the field every time a new individual enrolled in the project and completed any of the project components. Once the information was entered into the participant registry, the 13C-UBT logs and questionnaires were scanned

and saved in a project folder with restricted access. Two Microsoft Access databases were created for each questionnaire and data was entered into each database by two separate individuals. Using the program EpiInfo, developed by the Centers for Disease Control and Prevention, two entries of the same questionnaire were compared for differences. When the response varied between databases, the individual reconciling the two consulted the questionnaire to determine which entry was correct. Data was saved in the project folder with restricted access.

In order to obtain data relevant to the analyses, a data request form was submitted to the data manager for the project. This form outlined the purpose of the analysis, which variables were needed, which methods were to be used and the approximate timeline. Upon approval of this form, a database containing requested variables was created in a format compatible with the Statistical Package for the Social Sciences. Data from each community were cleaned in separate datasets. Variables representing exposures of interest were recoded (tables 1 and 2) and identified outliers and missing data were investigated. Investigation of outliers and missing data included a consultation of data from the original access database and the actual survey from which the variable was derived. If information on a given variable was not available in the questionnaire from which the variable was primarily extracted, data from other questionnaires the participant responded to were examined. For example, if information on NSAID use was not available in the clinical survey for that individual, the pre-endoscopy survey was examined for information about their use of anti-inflammatory medications.

Analysis 1: Environmental Exposures and Prevalent *H. pylori* Infection

The purpose of this analysis was to estimate the association between exposure to specified environmental sources of biological contamination and prevalence of *H. pylori* infection in residents of Aklavik, NT, Old Crow, YT and Tuktoyaktuk, NT.

The dependent variable was *H. pylori* infection status: infected (1); not infected (0).

Outcome Measurement

The breath test value indicated the difference in 13C measurement between the first and second breath sample. For participants aged 6 years and older a breath test value falling within the range of -1.99 to 2.49 was considered negative. A test value falling within the range of 2.50 to 3.99 was considered boderline. A borderline test was interpreted as meaning the participant might have the infection but another factor may have influenced the result, such as if the participant had taken a proton pump inhibiting medication. Individuals with a test result classified as borderline were advised to repeat the test for a more accurate result. The individual was also advised to repeat their test if the CO_2 concentration was too low in one or both of the samples or the breath test value was -2 or lower. A breath test value of 4.0 or higher was considered positive for *H. pylori* infection.

For children 5 years and younger, their height and weight influences their CO_2 production, which in turn changes what an appropriate cut point for a negative or positive test value would be. The methods used to correct for the influence of anthropomorphic differences on test outcome were adapted from Klein et al. (1999)[191]. The height and weight of the child was used to determine their CO_2 production, which was combined with the delta over baseline value estimated by the 13C-UBT to determine their height and weight corrected test value [191].

Exposure Ascertainment

Data on environmental factors were taken from responses provided in structure interviews (Table 1).

Pathways for Waterborne Transmission: Untreated Water. While the public health significance of reports pertaining to the ability of *H. pylori* to live in water is not clear, it is thought that certain conditions may facilitate survival. Conversely, it has been suggested that purification of the water using chemical additives, for example chlorine, is associated with death of microorganisms [105,118]. Therefore, individuals were considered exposed to a pathway for waterborne transmission if they reported consuming any untreated water in the past year. Untreated water was defined as including river water, melted snow or ice, or any source other than municipally supplied or otherwise chemically treated or boiled water.

Pathways for Waterborne Transmission: Contaminated Water (Sewage). Consumption of untreated water could result in exposure to *H. pylori* either because the water is a source of the bacteria, or the water is contaminated with infective digestive fluids. Because it is not generally possible to ascertain this exposure, a proxy variable was used to define exposure to water that is potentially contaminated with infective digestive fluids: individuals who reported having problems with their household sewage system were considered exposed.

Zoonotic transmission: Animals. As mentioned previously, the most relevant animal exposures for *H. pylori* in the Arctic are dogs, cats and mice. An individual was considered exposed to dogs or cats if they reported being a regular caretaker of a dog, which included feeding, grooming, petting, playing with or cleaning up after the animal. An individual was classified as exposed to mice if they reported having seen mice or mouse droppings around their house.

Zoonotic transmission: Animal Innards. Contact with the innards of animals has been suggested to have a relationship with the frequency of *H. pylori* infection observed in abattoir workers [89–91]. Individuals in Arctic communities continue to

follow a traditional lifestyle, which includes hunting live game. In the process of field dressing, individuals may come into contact with the blood and innards of a wide variety of animals. Therefore, an individual was classified as exposed if they reported cleaning fish or game.

Statistical Analysis

In order to estimate the effects of environmental exposures of interest on prevalence of *H. pylori* infection, prevalence odds ratios and 95% confidence intervals were estimated as measures of association. In order to account for lack of independence of response probabilities given a contagious outcome and participants clustered in households and communities, a mixed model was used, adjusting for clustering in communities as a fixed effect and in households as a random effect.

Table 1: Variable origin and coding for analysis of the effect of environmental exposures on prevalent infection.

Exposure of Interest	Question	Response Options (Coding)
Zoonotic Transmission		
Mice/Mouse Droppings	Do you ever have problems with mice getting into your house (have you seen mice or mouse droppings in your house)?	Yes (1) No (0) Unsure (.) Refused to Answer (.)
All Animals	Have you yourself ever regularly been the caretaker for one or more animals (such as pets or livestock), doing any of the following: feeding, grooming, cleaning up after, petting or playing with?	Yes (1) No (0) Unsure (.) Refused to Answer (.)
Dogs	Have you ever been the regular caretaker of a dog?	Yes (1) No (0) Unsure (.) Refused to Answer (.)
Cats	Have you ever been the regular caretaker of a cat?	Yes (1) No (0) Unsure (.) Refused to Answer (.)
Animal Innards	Have you ever cleaned fish or game?	Yes (1) No (0) Unsure (.) Refused to Answer (.)
Waterborne Transmission		
Untreated water (ever)	Did you ever, including when you were a child, drink river water that was not treated at the water treatment plant, for example water taken directly from a river, lake or creek?	Yes (1) No (0) Unsure (.) Refused to Answer (.)
Untreated water (past year)	According to your best estimate, how often in the past 12 months have you consumed: untreated, unboiled river water; melted river or lake ice; or melted snow?	1 or more times (1) Never (0) Unsure (.) Refused to Answer (.)
Contaminated water	Has your household ever had any problems with sewage?	Yes (1) No (0) Unsure (.) Refused to Answer (.)

Analysis 2: Untreated Water Consumption and Severe Gastritis Prevalence

The purpose of this analysis was to estimate the association between consumption of untreated water in the past year and the severity of gastritis among *H. pylori*-positive participants with histopathologically evaluated gastric biopsies in the Aklavik and Old Crow *H. pylori* projects. The dependent variable for this analysis was chronic inflammation severity: severe (1); moderate, mild, or none (0). Due to the extremely low prevalence of mild or absent gastritis in the study population with histopathology data, the reference category includes participants with moderate gastritis as well.

Outcome Measurement

A single pathologist examined the histopathological sections and graded the severity of gastric inflammation using the updated Sydney System [176,189,190]. This system employs a visual analogue scale, providing a schematic demonstration of the progression of inflammation [189]. Biopsies were obtained from 5 specified points in the gastric mucosa, in accordance with the updated Sydney System protocols. Severity of gastritis was graded in histopathological sections from each biopsy and an average for biopsies obtained from the same region in the stomach was calculated [189].

Exposure Ascertainment

Data on consumption of untreated water in the past year was collected using structured interviews (Table 2). As mentioned previously, consumption of chemical irritants can lead to the development of inflammation in the stomach lining [175]. As the purpose of this analysis was to investigate the hypothesis that chemical irritants in untreated drinking water from local polluted water sources increase the frequency of severe gastritis, an individual was considered exposed if they reported consuming water that had not been treated in any way.

Statistical Analysis

Prevalence odds ratios and 95% confidence intervals for the effect of untreated water consumption on the prevalence of severe gastritis were estimated using a logistic regression model. Clustering in communities was modeled as a fixed effect. The Likelihood-ratio (LR) test was used to determine the magnitude of the household effect and whether or not modeling clustering in households improved the fit of the model. Results of this test indicated the household effect was not strong and the final model did not include a random effect for household.

Table 2: Variable origin and coding for the analysis of the effect of untreated water consumption on severe gastritis prevalence

Exposure of Interest	Question	Response Options (Coding)
Untreated water (past year)	According to your best estimate, how often in the past 12 months have you consumed: untreated, unboiled river water; melted river or lake ice; or melted snow?	1 or more times (1) Never (0) Unsure (.) Refused to Answer (.)
Clinically Important Adjustment Variables		
Alcohol Consumption	Do you drink Alcohol?	Yes No (0) Unsure (.) Refused to Answer (.)
	If 'yes', how often do you drink?	Less than once a week (1) Once a week or more (2)
Cigarette Smoking	Do you smoke cigarettes?	Yes (1) No (0) Unsure (.) Refused to Answer (.)
NSAID use	Do you ever take any anti-inflammatory medications?	Yes No (0) Unsure (.) Refused to Answer (.)
	If 'yes', please specify which medications you take.	Any of the following: Ibuprofen (Advil, Motrin, Nuprin), Aspirin (ASA, Acetylsalicylic acid), Naproxen, Indocin, Celebrex, Indomethacin, Celebrex, Vioxx (1)

Bias Analysis

Misclassification of Outcomes

Outcome variables in both analyses were subject to misclassification resulting from imperfect accuracy of diagnostic methods used. The accuracy of the Sydney Classification for grading the severity of gastritis is subject to variability between pathologists. For this research, accuracy of the gastritis

classification was enhanced by the use of a single pathologist with specialized expertise in gastrointestinal pathology. The 13C-UBT has demonstrated high diagnostic accuracy in a variety of settings, with estimated sensitivity and specificity ranging from 90 to 100% [29]. While the test is considered accurate, false negatives might have occurred under certain circumstances, including a low density of bacteria in the stomach, quick emptying of the stomach, and recent intake of proton pump inhibiting medications or antibiotics [29]. Potential causes of false positives include timing the second breath sample when the labeled urea is in the oral cavity or intestines which are often colonized by other urease-producing bacteria, presence of other urease-producing bacteria in the stomach [29], and, when testing small children, failure to account for body-size dependent differences in CO_2 production. For this research, an optimized protocol was followed to enhance the accuracy of breath test results, and a correction was made to results of children 5 years of age and younger. Further, for individuals with biopsies evaluated, infection status was based on the results of culture and histopathology, as well as the 13C-UBT.

Analysis of Misclassification of Exposures

Misclassification of exposures may have occurred due to error introduced by poor construct validity of questionnaire data, if the variables that were proxies for an unmeasurable exposure of interest did not accurately substitute for the effect of the exposure. Methods for the analysis of the potential impacts of information bias on results were adapted from Greenland and Lash (2008) [192]. Equations designed to estimate the bias adjusted odds ratios for various levels of sensitivity and specificity were used in two methods for quantifying information bias proposed by Phillips (2003) [193]. These approaches were the bias-level and target adjustment sensitivity analyses. The bias-level sensitivity analysis involved the generation of a reasonable misclassification scenario to determine how much of the estimated association could be explained by the bias hypothesis. The target-adjustment sensitivity analysis generated a misclassification scenario

that would completely explain estimated effects from the present analysis followed by qualitative consideration of the plausibility of the corresponding error levels. The exposure variable subject to the greatest degree of misclassification was untreated water consumption, which was the focus of bias analysis pertaining to exposure misclassification for both the analysis of the effect of untreated water consumption on *H. pylori* infection prevalence and on severe gastritis prevalence.

Inaccuracies in questionnaire data might have arisen from respondents' imperfect recall or inclination to deviate from the truth due to social desirability. Methods employed in this research to ensure high quality of collected questionnaire data included using trained interviewers for in-person administration of questionnaires. This helped to ensure that respondents understood what they are being asked and minimized missing data. Proper probing techniques, the maintenance of a neutral tone and development of a rapport with the participant were methods employed by the interviewers to avoid inciting biased responses. Further methods to ensure accuracy of questionnaire data included having a separate interviewer review questionnaires to ensure completion, efforts to retrieve missing responses, and, to minimize data entry errors, reconciliation of double data entry was performed.

Analysis of Selection Bias

Estimates generated by the proposed analyses might have been subject to selection bias due to differential participation rates in relevant project components. For example, of the 384 individuals registered in the Aklavik *H. pylori* project, 90% completed a UBT, whereas only 52% consented to endoscopy. Further, this type of bias may have been introduced due to differential participation rates between communities. For example, of the approximately 590 residents of Aklavik in 2007, 65% participated in the project. Comparatively, 71% of the 250 residents of Old Crow and only 12% of the 870 residents of Tuktoyaktuk participated in the project. To assess the likely degree

of selection bias, the distribution of key demographic variables were compared between participants in each analysis and available census data for each community. Data from the census closest to the time of enrolment for each study were used. Given the reduction in collected information between the 2006 and 2012 census, data from the 2006 census was used for variables not available from the 2012 census for communities within which project data collection occurred closer to 2012. Variables taken from the 2011 census included population size, age and sex distribution, number of households and household size for Old Crow and Tuktoyaktuk. Data on remaining socio-demographic variables were taken from the 2006 census. A chi-square test was used to assess the similarity between the sample included in the analysis and the census population for each community. To assess the likely degree of selection bias for the second analysis, the distribution of key factors associated with exposures and outcomes were also compared across the subsets of participants contributing data to distinct relevant project components.

The potential impact of selection bias on estimated effects was assessed using methods adapted from Greenland and Lash (2008) [192], to employ target-adjustment sensitivity analysis as described by Phillips (2003) [193]. This assessment was performed by generating selection bias scenarios with disproportional selection fractions in exposed and unexposed cases and controls that would completely explain observed associations as being due to selection bias; this was followed by qualitative consideration of the plausibility of the corresponding error levels.

Confounder Adjustment

A key step for reducing error in the results is adjusting for potential confounders through multivariable logistic regression models using purposeful selection of covariates. Pearson's correlation coefficient was used to assess the degree of collinearity between pairs of independent variables. A value greater than or equal to 0.7 was considered highly correlated and in such cases model

results were examined to determine whether to include only one or the other of the correlated variables. If the correlated independent variables were exposures of interest, separate models were created, substituting the correlated variables for one another consecutively.

Purposeful selection, as proposed by Hosmer and Lemeshow (2000), [194] was used to develop the best model for each analysis. Given the large number of factors to consider, all potential confounders were assessed by estimating the crude odds ratio for the association with the dependent variable through univariate logistic regression. The variables from univariate analyses yielding a p-value <=0.25 were subsequently be included in a multivariable logistic regression model. Variables included in the multivariable model were subsequently removed one at a time. If the coefficient of any independent variable changed by greater than or equal to 10% upon the removal of a given variable, the removed variable was included as a confounder in the final model. Exposures of interest and clinically important variables were included regardless of statistical significance. Scientifically plausible interactions between independent variables were tested one at a time. The LR test was used to determine whether adding an interaction term improved the model fit. For plausible interactions, the interaction term was included in the final model if the LR test p-value was <=0.05.

Lowess plots were used to visually assess whether continuous variables had a linear relationship with the respective outcomes. If the relationship did not appear linear, appropriate transformations were tested. In order to faithfully adjust for the shape of the continuous data, cubic splines were fitted to the variable. The mathematical function used to create the cubic spline included terms which allowed the line to move up or down with the data, minimizing residual confounding caused by fitting a straight-line relationship to non-linear data. The number of knots was chosen based on the visual assessment of the data and locations of the knots generated by STATA were checked to ensure

adequate placement. The LR test was then used to statistically assess the fit of a model containing the continuous variable modeled as a cubic spline, relative to a model with the continuous variable as modeled as having a linear relationship with the outcome. If the resulting p-value was less than 0.05, the model containing the cubic spline was deemed a better fit for the data.

Missing Data

To assess the impact of missing data in some variables, estimates generated from models containing different adjustment variables with differing amounts of missing data were compared. Crude measures of association estimated using all subjects with data on the variable in question were compared to crude odds ratios estimated using only individuals with complete data on all variables to ass the potential degree of selection bias from missing data present in adjusted estimates. Finally, the comparison between the study population included in the analysis and the census population for each community also provided an assessment of whether missing data were missing at random.

Chapter 4: Results

Community Participation

Of 384 participants enrolled in the Aklavik *H. pylori* Project, 345 provided health history data; 285 provided individual-level socio-environmental data; 145 provided household-level socio-environmental data for 454 individuals; 333 completed a UBT (331 with classifiable results); 200 consented to endoscopy, and stomach biopsies were obtained from 194 (all 194 with a classification for gastritis severity). A total of 113 adults participated in a treatment trial comparing sequential and standard therapy (with 111 providing follow-up data). Community consultation on knowledge exchange strategies led to the production of a video documentary aimed at revealing the research process to the community.

Of 196 participants enrolled in the Old Crow *H. pylori* Project, 134 provided health history data; 125 provided individual-level socio-environmental data; 83 provided household-level socio-environmental data for 200 individuals; 188 completed a UBT (182 with a classifiable result); 65 consented to endoscopy and stomach biopsies were obtained from 63 (all 63 with a classification for gastritis severity). A total of 68 adults participated in a treatment trial comparing quadruple and sequential therapies (with 40 providing follow-up data to date).

Of 117 participants enrolled in the ISR *H. pylori* pilot project in Tuktoyaktuk, 85 provided health history data; 70 provided individual-level socio-environmental data; 77 provided household-level socio-environmental data for 231 individuals; and 103 completed a UBT (97 with a classifiable result). The endoscopy and treatment components have not yet been carried out in this community.

Prevalence of Infection and Gastric Lesions

Results from the Aklavik *H. pylori* Project and the Old Crow *H. pylori* Project support the perception of *H. pylori* as a major clinical problem. Of the 331 individuals with a classifiable UBT result from the Aklavik *H. pylori* Project, 58% were positive for *H. pylori* infection. Further, of the 194 individuals with stomach biopsies, 67% were positive for *H. pylori* on histological examination. The endoscopic findings for all Aklavik participants included 20 cases of esophagitis (10.3%), 5 cases of Barrett's esophagus (2.6%), 12 cases of gastric erosions (6.2%), 27 cases of gastritis (14%), 6 cases or gastric ulcer (3.1%), 1 case of duodenal erosions (0.5%), and 13 cases of duodenitis (6.7%). The histopathology findings from the Aklavik participants indicated that amongst 129 *H. pylori*-positive individuals: 43% had severe gastritis, 47% had moderate gastritis, 21% had atrophic changes, and 11% had intestinal metaplasia.

Of the 182 participants from the Old Crow *H. pylori* Project who completed a UBT, 70% were positive for *H. pylori* infection. The endoscopy phase of the Old Crow *H. pylori* project occurred in January 2012. Over the course of 4 days, 79 community members were seen by a member of the medical team, 64 individuals completed an endoscopy and 65 individuals were given one of two anti-*H. pylori* therapies. The histopathology findings from the Old Crow participants indicated that amongst 57 *H. pylori*-positive individuals: 65% had severe gastritis, 32% had moderate gastritis, 3% had mild gastritis, 74% had atrophic changes, and 35% had intestinal metaplasia.

Of the 103 participants in the ISR *H.pylori* project in Tuktoyaktuk who completed a UBT, 97 had a classifiable result and 57% were positive for *H.pylori* infection.

Environmental Exposures and Prevalent *H. pylori* Infection

Sample Size and Characteristics

Combined data from each of the 3 participating communities was used to examine the association between environmental exposures and *H. pylori* infection. The total sample size was 670. A combined total of 564 participants provided health history data; 580 individuals provided data on their own socio-environmental exposures; 279 households provided information on socio-environmental household exposures for 650 individual household members; 652 individuals were screened for *H. pylori* infection via urea breath test and 645 had classifiable results. The socio-demographic characteristics of the sample population are shown in Table 3. The total number of participants with complete data on all environmental exposures and *H. pylori* status was 368 (227 from Aklavik, 89 from Old Crow and 52 from Tuktoyaktuk).

Table 3: Socio-demographic Characteristics of Participants from Aklavik, NT, Old Crow, YT and Tuktoyaktuk, NT, 2008-2012

	n(%)
	Total (n= 670)
Age Range (Mean)	0-89 (37)
Sex	
Male	315 (47)
Female	355 (53)
Number of Households	228
Number of People Living in Household	
1	147 (65)
2	42 (18)
3	22 (9.6)
4	14 (6.1)
5	3 (1.3)
Average per House (Standard Deviation)	1.6 (0.98)
Ethnicity	
Non-Aboriginal	69 (12)
Inuvialuit	258 (46)
Gwich'in	205 (37)
Other Aboriginal	25 (4.5)
Proportion Missing: 16% (105/670)	
Education	
Still in School	22 (4.2)
Less than High School	267 (51)
High School or Equivalent	79 (15)
Trades Certificate	73 (14)
College or University	86 (16)
Proportion Missing: 16% (110/670)	
Household Income	
<$25,000	142 (29)
$25,000-$34,999	57 (12)
$35,000-$49,999	42 (8.5)
$50,000-$74,999	112 (23)
≥ $75,000	141 (29)
Proportion Missing: 21% (143/670)	

Socio-demographic Effects

Results of purposeful selection procedures for regression modeling indicated the most important adjustment variables were age, sex, household income, highest educational attainment, ethnicity and community. The likelihood-ratio test for the addition of a random effects parameter for clustering in households compared to a logistic regression model without this effect indicated a model containing the random effect had a better fit ($p<0.05$). Considerable variation between households was observed (standard deviation: 1.3; 95%CI: 0.69, 2.6). The estimated odds ratios and 95% CIs for the effects of socio-demographic characteristics on the prevalence odds of *H. pylori* infection in individuals with complete data on all variables are presented in Table 4. In order to more accurately adjust for the non-linear effect of age, a cubic spline with four knots was fitted. Therefore the effect of age on *H. pylori* prevalence could not be estimated.

The primary purpose of adding socio-demographic characteristics to the regression model was for adjustment of the effect estimates for the environmental exposures of interest, and for this reason the socio-demographic effects were carefully examined to select optimal category boundaries for confounder control. To capture meaningful variations in effects across categories, smaller categories were collapsed. The likelihood-ratio test was used to compare models with different categorizations of the same variable to the model without that variable to determine whether one method of categorization resulted in an improved model fit over the other. In each such instance examined in this analysis, the resulting p-values for the addition of the variable to the model in each of the selected alternate forms were equal, indicating that neither method of categorizing socio-demographic variables was statistically superior to the other.

In deciding how to categorize the socio-demographic adjustment variables, the change in standard errors between a model using more and

consequently smaller categories and a model with fewer and larger categories was assessed. For each socio-demographic variable, the reduction in standard error in response to using fewer categories in the overall model appeared small enough to justify using more categories in order to more faithfully adjust for the dose-response effect of each multilevel socio-demographic variable. For example, the standard error for the variable 'Sex' in the model with fewer categories was only reduced by 0.02 (0.30 to 0.28).

This approach led to re-categorization of ethnicity, household income, and educational attainment. Ethnicity was dichotomized and recoded as Aboriginal or non-Aboriginal, producing an odds ratio of 16 (95%CI: 3.5, 73) adjusting for all covariates in the model. The two smaller categories of household income were collapsed, creating a category representing households with an annual income of $25,000-49,999. Fully adjusted odds ratios for the three new categories of income, $25,000-49,999, $50,000- 74,999 and ≥ $75,000 compared to the reference category (<$25,000) were 0.75 (95%CI: 0.29, 2.0), 0.42 (95%CI: 0.16, 1.1) and 0.35 (95%CI: 0.13, 0.96), respectively. Finally, categories of educational attainment were collapsed into 'less than high school', 'high school' and 'some type of post-secondary school'. A similar effect was observed for having completed high school (OR: 0.73; 95%CI: 0.30, 1.8) and completing some type of post-secondary education (OR: 0.74; 95%CI: 0.33, 1.7), after adjusting for all covariates.

Pathways for Zoonotic Transmission

Distribution of Zoonotic Exposures and *H. pylori*-positivity

Of 580 individuals with data on individual-level socio-environmental exposures, 378 had complete data on all zoonotic exposures (263 from Aklavik, 63 from Old Crow and 52 from Tuktoyaktuk). The most common zoonotic exposures were contact with animal innards, followed by caring for

animals and specifically caring for dogs. The prevalence of *H. pylori* infection ranged from 42-75% across categories of zoonotic exposures in all three communities. The lowest prevalence was observed in individuals who reported caring for cats and the highest in those who reported exposure to mice/mouse droppings. The distribution of zoonotic exposures and associated prevalence of *H. pylori* infection in each community is shown in Table 5.

Table 4: Socio-Demographic Variables: Effects on Odds of Prevalent *H. pylori* Infection

Variable	n	OR	95%CI
Sex			
Male	177	1.0	
Female	191	1.0	(0.57, 1.8)
Ethnicity			
Non-Aboriginal	45	1.0	
Inuvialuit	173	11	(3.0, 39)
Gwich'in	134	6.8	(2.1, 22)
Other Aboriginal	16	20	(3.2, 128)
Household Income			
<$25,000	104	1.0	
$25,000-34,999	41	0.66	(0.24, 1.8)
$35,000-49,999	35	1.3	(0.41, 3.9)
$50,000-74,999	80	0.49	(0.22, 1.2)
≥ $75,000	108	0.41	(0.18, 0.95)
Education			
Less than High School	193	1.0	
High School	57	1.0	(0.46, 2.4)
Trades Certificate	53	0.68	(0.28, 1.7)
College or University	65	1.2	(0.46, 2.9)
Community			
Aklavik	227	1.0	
Old Crow	89	3.1	(1.2, 8.0)
Tuktoyaktuk	52	0.84	(0.33, 2.1)

Adjusted for age as a cubic spline, sex, ethnicity, income, education, all waterborne and zoonotic exposures, community and household as a random effect

Table 5: Pathways for Zoonotic Transmission: Distributions and Exposure-specific Prevalence of *HP* by Community

Variable	Aklavik, NT		Old Crow, YT		Tuktoyaktuk, NT	
	n (%)	*H*. pylori Prevalence	n (%)	*H*. *pylori* Prevalence	n (%)	*H*. pylori Prevalence
Mice / Mouse Droppings in Home						
No	270 (87)	162/270	130 (95)	87/130	74 (94)	37/74
Yes	41 (13)	31/41	7 (5.1)	6/7	5 (6.3)	3/5
Proportion Missing	*17% (64/375)*		*29% (57/194)*		*22% (22/101)*	
Cared for Any Animals/ Livestock						
No	78 (25)	53/78	34 (22)	25/34	40 (50)	24/40
Yes	229 (75)	136/229	121 (78)	83/121	40 (50)	18/40
Proportion Missing	*18% (68/375)*		*20% (39/194)*		*21% (21/101)*	
Cared for Dogs						
No	84 (27)	56/84	37 (24)	27/37	45 (56)	25/45
Yes	224 (73)	133/224	118 (76)	81/118	35 (44)	17/35
Proportion Missing	*18% (67/375)*		*20% (39/194)*		*21% (21/101)*	
Cared for Cats						
No	269 (90)	172/269	149 (97)	104/149	64 (80)	37/64
Yes	31 (10)	14/31	5 (3.2)	3/5	16 (20)	5/16
Proportion Missing	*20% (75/375)*		*21% (40/194)*		*21% (21/101)*	
Contact with Animal Innards						
No	80 (29)	51/80	16 (14)	9/16	13 (20)	3/13
Yes	199 (71)	126/199	96 (86)	71/96	53 (80)	30/53
Proportion Missing	*26% (96/375)*		*42% (82/194)*		*35% (35/101)*	

Logistic Regression Analysis

Results of logistic regression analysis for zoonotic exposures appear in Table 6. The largest effect observed was for the comparison of individuals who reported having seen mice or mouse droppings in their homes compared to those who reported that they had not. *H. pylori* positivity was 75% in individuals exposed to mice/mouse droppings, compared to 60% in those not exposed, producing a crude odds ratio of 2.3 (95%CI: 1.1, 5.0). The magnitude of this association became considerably larger when the selected socio-demographic characteristics (OR: 4.1; 95%CI: 1.2, 14) and other indicators of environmental transmission pathways (OR: 4.6; 95%CI: 1.2, 18) were included in regression models. The addition of one adjustment variable at a time to the model with mice/mouse droppings exposure variable showed that clustering in households (modeled as a random effect) resulted in the largest change in effect (OR: 3.5; 95%CI: 1.2, 10, adjusted only for household).

A notable effect was observed for caring for cats. The prevalence of *H. pylori* infection was 42% in individuals who reported owning caring for cats, compared to 65% in those who did not, producing a crude odds ratio of 0.40 (95%CI: 0.20, 0.81). Once adjusted for potential confounding variables and other environmental exposures, the direction of this association changed (OR: 1.4; 95%CI: 0.34, 5.4). In order to determine if a single adjustment variable was confounding the estimated crude association between caring for cats and *H. pylori* prevalence odds, the adjustment variables were added to the model one at a time. While ethnicity had the largest effect on the point estimate, producing an odds ratio of 0.88 (95%CI: 0.36, 2.1), a slight increase in the estimate was also observed following adjustment for income (OR: 0.48; 95%CI: 0.23, 0.99) and community (OR: 0.45; 95%CI: 0.22, 0.92). A change in the direction of this association was not observed upon adjustment for any covariate on its own.

Table 6: Pathways for Zoonotic Transmission: Results of Multivariable Logistic Regression Analysis of Effects on HP Prevalence Odds (n=368)

Variable	Unadjusted Estimates		Model 1 ❖		Model 2 ✚	
	OR	95%CI	OR	95%CI	OR	95%CI
Mice / Mouse Droppings in Home						
No	1.0		1.0		1.0	
Yes	2.3	(1.1, 5.0)	4.1	(1.2, 14)	4.6	(1.2, 18)
Cared for Any Animals/ Livestock						
No	1.0		1.0		1.0	
Yes	0.84	(0.51, 1.4)	0.78	(0.39, 1.6)	0.82	(0.38, 1.8)
Cared for Dogs						
No	1.0		1.0		1.0	
Yes	0.94	(0.59, 1.5)	0.76	(0.38, 1.5)	0.72	(0.33, 1.6)
Cared for Cats						
No	1.0		1.0		1.0	
Yes	0.40	(0.20, 0.81)	1.26	(0.37, 4.3)	1.36	(0.34, 5.4)
Contact with Animal Innards						
No	1.0		1.0		1.0	
Yes	1.2	(0.74, 1.9)	1.19	(0.57, 2.5)	1.58	(0.70, 3.6)

❖ Adjusted for age as a cubic spline, sex, ethnicity, income, education, community and household as a random effect

✚ Adjusted for age as a cubic spline, sex, ethnicity, income, education, all waterborne and zoonotic exposures, community and household as a random effect

Pathways for Waterborne Transmission

Distribution of Exposure to Pathways for Waterborne Transmission and *H.pylori*-positivity

Of 580 individuals with data on individual-level socio-environmental exposures, 361 had complete data on exposure to potential sources of waterborne pathogens or contaminated water (249 from Aklavik, 59 from Old Crow and 52 from Tuktoyaktuk). Having ever consumed untreated water was the most commonly reported exposure to a source of waterborne pathogens or contamination. Prevalence of *H. pylori* infection in different exposure categories ranged from 59-65%. *H. pylori* positivity was highest in those who reported consuming untreated water in the past year (65%), followed by ever consuming untreated water (63%) and the lowest prevalence was observed in individuals who reported having problems with sewage (59%). The distribution of exposure to pathways for waterborne transmission and associated prevalence of *H. pylori* infection in each community is shown in Table 7.

Table 7: Pathways for Waterborne Transmission: Distributions and Exposure-specific Prevalence of *HP* by Community

Variable	Aklavik, NT		Old Crow, YT		Tuktoyaktuk, NT	
	n (%)	*H. pylori* Prevalence	n (%)	*H. pylori* Prevalence	n (%)	*H. pylori* Prevalence
Ever Consumed Untreated Water						
No	112 (39)	63/112	29 (20)	20/29	5 (6.3)	5/5
Yes	203 (64)	131/203	117 (80)	82/117	75 (94)	37/75
Proportion Missing	*16% (60/375)*		*25% (48/194)*		*21% (21/101)*	
Consumed Untreated Water in the Past Year						
No	243 (81)	148/243	69 (53)	50/69	24 (36)	13/24
Yes	57 (19)	38/57	61 (47)	44/61	43 (64)	23/43
Proportion Missing	*20% (75/375)*		*33% (64/194)*		*34% (34/101)*	
Contami-nated Water (Sewage)						
No	231 (74)	143/231	84 (62)	62/84	58 (73)	29/58
Yes	81 (26)	50/81	51 (38)	29/51	21 (27)	11/21
Proportion Missing	*17% (63/375)*		*30% (59/194)*		*22% (22/101)*	

Logistic Regression Analysis

Results of logistic regression analysis for exposure to sources of waterborne pathogens or contamination are presented in Table 8. The largest effect was for the comparison of individuals who had consumed untreated water at some point in their life compared to those who had not. The unadjusted estimate denoted a slight increase in the odds of infection in those who consumed untreated water compared to those who did not, although the CI

spanned modest effect sizes on both sides of the null value (OR: 1.1; 95%CI: 0.57, 1.6). The direction of this association changed following adjustment for the selected socio-demographic variables (OR: 0.44; 95%CI: 0.20, 0.96) and other environmental exposures (OR: 0.14; 95%CI: 0.14, 0.94), consistent with a protective effect of having ever consumed untreated water on *H. pylori* infection odds. While adjustment for ethnicity induced the largest change from the crude estimate (OR: 0.72; 95%CI: 0.43, 1.27), the cumulative effect of adjusting for other socio-demographic variables increased the magnitude of the estimated protective effect.

Table 8: Pathways for Waterborne Transmission: Results of Multivariable Logistic Regression Analysis of Effects on HP Prevalence Odds (n=368)

	Unadjusted Estimates		**Model 1 ❖**		**Model 2 ✚**	
Variable	OR	95%CI	OR	95%CI	OR	95%CI
Ever Consumed Untreated Water						
No	1.0		1.0		1.0	
Yes	1.1	(0.57, 1.6)	0.44	(0.20, 0.96)	0.36	(0.14, 0.94)
Consumed Untreated Water in the Past Year						
No	1.0		1.0		1.0	
Yes	0.96	(0.61, 1.5)	0.77	(0.39, 1.5)	0.85	(0.40, 1.8)
Contaminated Water (Sewage)						
No	1.0		1.0		1.0	
Yes	0.83	(0.53, 1.3)	0.48	(0.25, 0.94)	0.49	(0.22, 1.1)

❖ Adjusted for age as a cubic spline, sex, ethnicity, income, education, community and household as a random effect

✚ Adjusted for age as a cubic spline, sex, ethnicity, income, education, all waterborne and zoonotic exposures, community and household as a random effect

Bias Analysis

Comparison with the Census Population

The distributions of key socio-demographic characteristics were compared between the sample of individuals with complete data on socio-environmental exposures and the census population for each community (Table 9). The chi-square test indicated that the distributions of ethnicity (Aboriginal versus non-Aboriginal) and sex were similar in the sample population in all three communities and the respective census populations. Individuals aged 0-19 were extremely underrepresented in the study population from all three communities, distorting the distribution of participation in adult age categories. For this reason, the youngest age category was removed from this comparison. The proportion of individuals in adult age categories who participated in the study reflected the proportion of individuals in those age groups in the census populations of both Old Crow and Tuktoyaktuk. However individuals from Aklavik aged 20-39 were underrepresented and those aged 40-59 were overrepresented in the study population ($p<0.001$). Study participants from both Aklavik and Old Crow had a higher median income than reported by the census population for each community.

Analysis of Misclassification of Exposure Status

A bias-level sensitivity analysis [193] was employed to assess the potential impact of misclassification on effect estimates for exposures thought to have the greatest degree of uncertainty regarding the accuracy of classification. The exposure chosen for this bias analysis was lifetime untreated water consumption (ever v. never).

The odds ratio for the effect of ever versus never consuming untreated water on the odds of prevalent *H. pylori* infection was 0.36 (95%CI: 0.14, 0.941.7), adjusting for socio-demographic variables and other indicators of exposure to environmental sources of the bacteria. The purpose of this analysis

was to determine how much of this inverse association could be explained by a reasonable bias hypothesis. For the purpose of this bias analysis, the assumption that there is no residual confounding influencing this estimate was made in order to examine the effects of one source of bias at a time.

A scenario of concern is poor sensitivity, due to imperfect recall and the absence of a plausible reason for participants to falsely report consuming untreated water when they did not. In order for the exaggerated inverse association to be influenced by misclassification, levels of error must differ between cases and noncases. A plausible differential misclassification of exposure scenario would be that *HP*-negative participants were more likely to report having consumed untreated water when they had, relative to *HP*-positive participants. The result of this scenario would be an exaggerated inverse association due to a higher false negative rate in noncases relative to cases. Plausible differential misclassification of exposure levels could be set at a false negative rate of 40% in *HP*-positive participants, relative to 30% in *HP*-negative participants. The corresponding exposure classification sensitivities of *HP*-positive and *HP*-negative participants would be 60% and 70%, respectively. A plausible explanation for the hypothesized difference in the accuracy of reporting exposure between infected and non-infected participants pertains to educational attainment. Since individuals with higher education are less likely to have the infection and it is reasonable to assume that higher education could correspond to more accurate responses, it is justifiable to postulate a difference in sensitivity for infected and non-infected participants.

In this scenario, it is reasonable to assume higher specificity in both infected and non-infected participants relative to sensitivity, as individuals would be less likely to report that they had consumed untreated water when they had not than to falsely report that they had not consumed untreated water. There is no apparent reason to assume differential specificity between infected and non-infected participants. In order to quantify bias caused by poor sensitivity in the

hypothesized scenario, specificity was set at 100% and sensitivity for cases and controls at 60% and 70%, respectively. The bias-adjusted odds ratio for this differential misclassification of exposure scenario is 0.37. While this bias-adjusted estimate moves in the expected direction (towards the null), it is approximately the same as the original estimate. In order for differential misclassification to explain a large portion of the inverse association, the sensitivities for cases and controls would need to differ by a large amount. For example, for approximately half of the estimated inverse association to be due to differential misclassification of exposure, the sensitivity in cases and controls would need to be 50% and 80%, respectively, with a corresponding bias-adjusted odds ratio of 0.63. While differential misclassification of exposure is likely to have had some influence on the estimated inverse association, results of this quantitative assessment indicate it is not likely that this type of bias is solely responsible for the estimated odds ratio.

Analysis of Selection Bias

The participatory nature of the research program invited all interested participants to enroll; as with all research requiring that people consent to participate, it is likely that some degree of selection bias influenced estimated associations. In order to assess whether it is likely that participants' self-selection for study participation led to a large amount of bias in the estimated effects, key demographic characteristics were compared (Table 9).

Target-adjustment sensitivity analysis was employed in the context of selection bias, in order to determine how much selection bias would be necessary to explain the estimated effect of exposure to mice on the odds of prevalent *H. pylori* infection. For the crude odds ratio of 2.3 (95%CI: 1.1, 5.0) for the effect of mice in the home on the odds of prevalent infection to be a product of selection bias, the ratio of the selection probabilities of exposed and unexposed cases from the source population who participated in the study would need to differ from the ratio of the selection probabilities of exposed and

unexposed noncases. A scenario that would explain this association completely would be a greater probability of participation in exposed cases relative to unexposed cases (e.g., 30% and 20%, respectively), compared to a lower probability of participation in exposed non-cases relative to unexposed non-cases (e.g. 23% and 35%, respectively).

Missing Data

In order to assess the impact of missing data on some of these effects, crude odds ratios were estimated from the subset of individuals with complete data on all variables as well as all individuals with data on the exposure of interest and infection status. The impact of missing data on the estimated effect of evidence of mice in the home on *H. pylori* was examined, given the 159-person reduction in sample size between crude estimates using the full data set and the subset of individuals with complete data. In all individuals with data on evidence of mice in the home and infection status, the crude odds ratio for the effect of having mice/mouse droppings in the home compared to not on the odds of *H. pylori* infection was 2.0 (95%CI: 1.1, 3.9), similar to the estimate from the subset with complete data of 2.3 (95%CI: 1.1, 5.0). This same comparison was carried out examining the effect of exposure to cats on odds of prevalent *H. pylori* infection. The unadjusted odds ratio for the effect of having cats compared to not on prevalent *H. pylori* infection was 0.39 (95%CI: 0.22, 0.71), nearly identical to the estimate from the subset with complete data of 0.40 (95%CI: 0.20, 0.81). These comparisons indicated that missing data did not have a large influence on the estimated effects.

Table 9: Comparison Between Individuals with Complete Data on Infection Status, Environmental Exposures and Socio-demographic Characteristics and the Census Population

	n(%)			n(%)		
	Aklavik *HP* Project	Aklavik Census Population [195]	p-value	Old Crow *HP* Project	Old Crow Census Population [196,197]	p-value
Population	227	594		89	245	
Age						
20-39	68 (27)	160 (42)	<0.01	31 (38)	70 (40)	0.79
40-59	137 (53)	135 (35)	<0.01	33 (40)	70 (40)	0.91
60-89	52 (20)	89 (23)	0.38	17 (21)	35 (20)	0.86
Sex						
Male	104 (46)	315 (53)		95 (50)	130 (53)	
Female	123 (54)	280 (47)	0.068	95 (50)	115 (47)	0.53
# Houses	128	220		60	110	
Average # of People/ Household (Standard Deviation)	1.8 (1.1)	2.7 (1.5)	<0.01	1.5 (0.8)	2.2 (1.2)	<0.01
Ethnicity						
Non-Aboriginal	23 (10)	40 (6.8)		13 (15)	35 (14)	
Aboriginal	205 (90)	545 (92)	0.12	76 (85)	215 (86)	0.89
Education (>15 years)						
< High School	150 (66)	270 (61)	<0.01	24 (27)	100 (50)	0.02
High School	30 (13)	60 (14)	0.20	18 (20)	20 (10)	<0.01
Trades Certificate	13 (5.7)	30 (4.5)	0.70	24 (27)	30 (15)	<0.01
Post-Secondary	34 (15)	60 (14)	0.05	23 (26)	50 (25)	0.29
Median Household Income	$50,000-$74,999	$34,944		$50,000-$74,999	36,352	

	n(%)		
	ISR *HP* Project (Tuktoyaktuk)	Tuktoyaktuk Census Population [198,199]	p-value
Population	52	854	
Age			
20-39	16 (31)	245 (44)	0.09
40-59	26 (51)	220 (39)	0.10
60-89	9 (18)	95 (14)	0.90
Sex			
Male	43 (45)	454 (53)	
Female	52 (55)	395 (46)	0.13
# Houses	40	270	
Average # of People/ Household (Standard Deviation)	1.3 (0.6)	3.1 (1.8)	<0.01
Ethnicity			
Non- Aboriginal	9 (17)	140 (16)	
Aboriginal	65 (83)	730 (84)	0.37
Education (>15 years)			
< High School	19 (37)	405 (62)	0.16
High School	9 (17)	65 (10)	0.01
Trades Certificate	16 (31)	45 (7.1)	<0.01
Post-Secondary	8 (14)	85 (14)	0.19
Median Household Income	$35,000-$49,999	40,064	

Untreated Water Consumption and Severe Gastritis Prevalence

Sample Size and Characteristic

Biopsies were obtained from 257 participants (194 from Aklavik and 63 from Old Crow), of which all 257 of which were graded for inflammation severity. The total number of participants with complete data on untreated water

consumption and gastritis severity from Old Crow and Aklavik is 226 (163 from Aklavik and 63 from Old Crow). Because nearly 100% of participants with chronic gastritis had *H. pylori* infection, the population for the analysis of effects on gastritis severity was restricted to individuals with the infection. The total number of *H. pylori* –positive participants with data on gastritis severity and untreated water consumption was 157 (108 from Aklavik and 52 from Old Crow). The total number of *H. pylori* –positive participants with complete data on clinically important adjustment variables (alcohol consumption, NSAID use and smoking) and socio-demographic variables (ethnicity, education, age and sex) was 153 (107 from Aklavik and 46 from Old Crow). The socio-demographic characteristics of the sample population are shown in Table 10.

Table 10: Socio-demographic Characteristics of Participants from Aklavik, NT, Old Crow, YT, 2008-2012

	n(%)
	Total (n= 226)
Age Range (Mean)	9-80 (43)
Sex	
Male	100 (44)
Female	126 (56)
Number of Households	161
Number of People Living in Household	
1	115 (71)
2	35 (22)
3	8 (5.0)
4	3 (1.9)
Average per House (Standard Deviation)	1.4 (0.67)
Ethnicity	
Non-Aboriginal	17 (48)
Inuvialuit	92 (41)
Gwich'in	108 (48)
Other Aboriginal	9 (4.0)
Educational Attainment	
Less than High School	118 (55)
High School or Equivalent	34 (16)
Some Type of Post Secondary School	62 (29)
Proportion Missing: 6.2% (14/226)	
Household Income	
<$25,000	33 (25)
$25,000-$34,999	15 (11)
$35,000-$49,999	18 (14)
$50,000-$74,999	33 (25)
≥ $75,000	34 (26)
Proportion Missing: 41% (93/226)	

Socio-demographic Effects

Purposeful selection of adjustment variables resulted in a model containing age, educational attainment, ethnicity and community. Despite not meeting the criteria of the purposeful selection approach, alcohol consumption,

smoking and NSAID use were included in the model due to clinical significance. The likelihood-ratio test for the addition of a random effects parameter for clustering in households indicated adjusting for clustering in households did not improve the fit of the model (p=1.00). Visual assessment of age indicated modeling it as having linear relationship with gastritis severity would not result in large residuals. To statistically assess this visual appraisal, a cubic spline with 3 knots was fitted. The likelihood-ratio test for the addition of age as a cubic spline compared to a model with linear age indicated the model containing the cubic spline was not a better fit (p=0.80). Since the cubic spline did not improve the fit of the model, it was decided that the addition of another term to model the effect of age was not appropriate given the small sample size.

Estimates of the association between socio-demographic characteristics and the development of severe gastritis are presented in Table 11. With most of these estimates having CIs that span moderate to strong effects on both sides of the null value, these effects on the development of severe gastritis could not be precisely estimated.

Table 11: Socio-demographic Effects

Variable	n	OR	95%CI
Age	153	0.99	(0.97, 1.0)
Sex			
Male	68	1.0	
Female	85	0.56	(0.28, 1.1)
Ethnicity			
Non-Aboriginal	7	1.0	
Inuvialuit	63	3.3	(0.51, 21)
Gwich'in	75	3.1	(0.50, 19)
Other Aboriginal	8	1.0	(0.09, 11)
Education			
Less than High School	84	1.0	
High School or Equivalent	25	2.0	(0.75, 6.1)
Some Type of Post Secondary School	44	1.1	(0.45, 2.5)
Community			
Aklavik	108	1.0	
Old Crow	45	2.9	(0.88, 9.3)

Distribution of Gastritis Severity

Of the total sample of individuals with biopsies evaluated (n=257), 73% had gastritis. The proportion with severe gastritis was 36%; 30% were graded as having moderate gastritis; and 6.2% had mild gastritis. Of the sample with biopsy data, 186 were positive for *H. pylori,* 98% of whom had gastritis. The proportion of *H. pylori* –positive individuals with severe gastritis was 48%; 42% had moderate gastritis and 2.6% had mild gastritis. The distribution of gastritis severity in *H. pylori* –positive participants from each community is presented in Table 12.

Table 12: Distribution of Chronic Inflammation Severity by Community

	n(%)	
Gastritis Severity	Old Crow *HP+* Participants (n=58)	*Aklavik HP+* Participants (n=107)
None	1 (1.7)	4 (3.7)
Mild	2 (2.5)	8 (7.5)
Moderate	18 (31)	49 (46)
Severe	37(64)	46 (43)

Logistic Regression Analysis

Of the 160 individuals with data on gastritis severity and untreated water consumption from both communities combined, 34% (55/160) reported consuming untreated water in the past year. The distribution of untreated water consumption and prevalence of severe gastritis by community is shown in Table 13. The prevalence of severe gastritis was 60% (33/55) in participants who reported consuming untreated water in the past year, compared to 43% (45/105) in those who did not. The unadjusted odds ratio for the effect of consuming untreated water in the past year compared to not on severe gastritis was 1.6 (95%CI: 0.85, 3.3). The magnitude of this association became slightly larger when important adjustment variables were accounted for. The odds of having severe gastritis were 1.8 (95%CI: 0.86, 3.8) times higher in individuals who consumed untreated water in the past year compared to those who did not, after adjusting for age, ethnicity, educational attainment, alcohol consumption, smoking, NSAID use and community. Given the potential for a large variation in water quality between communities due to basic differences in sanitation and water delivery infrastructure, this effect was examined in each community separately as well. Odds ratios and 95% CIs for the effect of untreated water consumption on severe gastritis in each community are presented in Table 14.

Table 13: Distribution of Untreated Water Consumption and Exposure-specific Prevalence of Severe Gastritis by Community.

	Aklavik, NT		Old Crow, YT	
Variable	n (%)	Prevalence of Severe Gastritis	n (%)	Prevalence of Severe Gastritis
Consumed Untreated Water in the Past Year				
No	73 (68)	27/73	32 (62)	18/32
Yes	35 (32)	19/35	20 (38)	14/20

Table 14: Results of Multivariable Logistic Regression Analysis of Effects of Untreated Water Consumption on Prevalence Odds of Severe Gastritis by Community

	Aklavik, NT Unadjusted Estimate		Aklavik, NT Adjusted Estimate §		Old Crow, YT Unadjusted Estimate		Old Crow, YT Adjusted Estimate §	
Variable	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI
Consumed Untreated H_20 in the past year		n= 108		n=108		n=45		n=45
No	1.0		1.0		1.0		1.0	(0.19,
Yes	2.0	(0.89, 4.6)	2.8	(1.1, 7.2)	1.0	(0.29, 3.6)	0.85	3.9)

§ Adjusted for age, ethnicity, education, alcohol consumption, smoking, NSAID use and community

Bias Analysis

Comparison with the Census Population

The distributions of key socio-demographic characteristics were compared between the sample with complete data on gastritis severity, untreated water consumption and important adjustment variables and the census population for each community (Table 15). The populations were

restricted to individuals aged 20 years and above, due to age restrictions on participation in endoscopy in Old Crow and low participation of young individuals from Aklavik. The distribution of ethnicity (Aboriginal versus non-Aboriginal) in both study populations was consistent with the census population. Whether remaining characteristics were representative of the respective census populations varied by community. The most striking differences between the subset of individuals with complete biopsy and socio-environmental data and the respective census populations were seen in the age distributions, household size and median income. Individuals aged 40-59 years were overrepresented in both community projects. Individuals aged 20-39 years were underrepresented in Aklavik. In both communities, the average household size for the subset of individuals who participated in endoscopy was much smaller than that reported by Statistics Canada. Finally, the median household income reported by Statistics Canada for the community of Aklavik was lower than the median income of participants.

Analysis of Misclassification of Exposure Status

In order to assess the potential impact of misclassification of exposure status, a bias-level sensitivity analysis approach was used to posit a reasonable bias hypothesis that could partially explain the estimated association. For the purposes of this bias analysis, it was assumed that the estimated odds ratio was not affected by residual confounding, in order to assess one bias at a time. The odds ratio for the effect of having consumed untreated water in the past year compared to not having done so was 1.8 (95%CI: 0.86, 3.8), adjusting for age, ethnicity, educational attainment, alcohol consumption, smoking, NSAID use and community.

If the true effect of untreated water consumption on the odds of severe gastritis were null, the estimated effect could be a product of misclassification of exposure. In order for misclassification to explain this effect, the probability of

correctly identifying an individual as exposed or unexposed would need to have differed between those with severe gastritis and those without. This would have occurred, for example, if individuals without severe gastritis were more likely to report not having consumed untreated water when they had, relative to individuals with severe gastritis. This differential misclassification scenario is not justifiable, given there is no reason to expect individuals without severe gastritis would be more likely to underreport exposure to untreated water.

While a differential misclassification of exposure scenario did not seem reasonable, error caused by non-differential misclassification likely influenced the estimated effect. A misclassification of exposure scenario in which sensitivity is low relative to specificity would be justifiable, with the reasoning that individuals would be more likely to report not having consumed untreated water when they had rather than report they had consumed untreated water when they had not. If the sensitivity and specificity were 80% and 100%, respectively, the corresponding bias-adjusted odds ratio would be 1.96. Thus, it does not seem likely that misclassification of exposure accounts for the observed positive association.

Analysis of Selection Bias

Selection bias may have distorted the estimated effect if factors that dictated which participants selected themselves for participation in endoscopy altered the observed relationship between the exposure and the outcome. Participation rates were observed to differ across relevant project components. In addition to comparing study participants to the census population for each community (Table 15), the distribution of key socio-demographic characteristics relevant to the exposure and the outcome were compared between individuals participating in the first phase of the project and those who selected themselves for participation in endoscopy (Table 16).

The estimated effect of untreated water consumption in the past year on severe gastritis (OR: 1.8; 95%CI: 0.86, 3.8) could be explained by a selection bias scenario wherein a higher fraction of the exposed cases from the source population participated in the study relative to exposed noncases. A quantitative example of this scenario that would completely explain the estimated association would be if the exposed and unexposed cases who participated represented 13% and 11% of their counterparts in the source population, respectively, along with selection fractions of exposed and unexposed noncases of 8% and 11%, respectively. A mechanism that would have produced the hypothesized differential participation is not obvious.

Missing Data

A total of seven individuals were missing data on socio-demographic and clinically relevant adjustment variables. All individuals with missing data were from Old Crow. Missing data were predominantly in the variable for educational attainment, with some individuals missing data on alcohol consumption. In order to quantify the potential amount of selection bias in the adjusted estimates due to missing data, the unadjusted estimates from the full sample and the subset of people with complete data on all adjustment variables were compared. The crude odds ratio estimated from the subset with complete data comparing individuals who reported consuming untreated water to individuals who did not consume untreated water was 1.6 (95%CI: 0.85, 3.3). The discrepancy between these estimates indicated that the estimate generated by this analysis might have been subject to a large degree of selection bias due to missing data.

Table 15: Comparison Between Individuals with Complete Data on Gastritis Severity, Socio-demographic Characteristics and Clinically Important Adjustment Variables and the Census Population

	n(%)			n(%)		
	Aklavik *HP* Project	Aklavik 2006 Census[195]	p-value	Old Crow *HP* Project	Old Crow Census Population[196,197]	p-value
Population	107	594		46	245	
Age						
20-39	53 (37)	160 (42)	<0.01	14 (30)	70 (40)	080
40-59	68 (47)	135 (35)	<0.01	25 (48)	70 (40)	0.001
60-89	23 (16)	89 (23)	0.09	7 (15)	35 (20)	0.87
Sex						
Male	45 (42)	315 (53)		23 (50)	130 (53)	
Female	63 (59)	280 (47)	0.031	23 (50)	115 (47)	0.70
# Houses	83	220		39	110	
Average # of People / Household (Standard Deviation)	1.3 (0.56)	2.7 (1.5)	<0.01	1.2 (0.43)	2.2 (1.2)	<0.01
Ethnicity						
Non-Aboriginal	5 (4.7)	40 (6.8)		2 (4.4)	35 (14)	
Aboriginal	102 (95)	545 (92)	0.40	44 (96)	215 (86)	0.069
Education (>15 years)						
< High School	70 (65)	270 (62)	<0.01	14 (30)	100 (50)	0.19
High School	17 (16)	60 (14)	0.078	8 (17)	20 (10)	0.051
Post-Secondary	20 (19)	90 (21)	0.35	24 (52)	80 (40)	0.011
Median Household Income	$50,000-$74,999	$34,944		$25,000-$34,999	36,352	

Table 16: Comparison Between Individuals with Complete Data who Participated in the First Component (UBT and Surveys) and those with Complete Data who Participated in Endoscopy

	n(%)			n(%)		
	Aklavik *HP* Project – UBT	Aklavik *HP* Project - Endoscopy	p-value	Old Crow *HP* Project - UBT	Old Crow *H.pylori* Project - Endoscopy	p-value
Population	227	107		89	46	
Age						
20-39	68 (27)	53 (37)	<0.01	31 (38)	14 (30)	0.61
40-59	137 (53)	68 (47)	0.58	33 (41)	25 (48)	0.055
60-89	52 (20)	23 (16)	0.77	17 (21)	7 (15)	0.58
Sex						
Male	104 (46)	45 (42)		95 (50)	23 (50)	
Female	123 (54)	63 (59)	0.48	95 (50)	23 (50)	1.000
# Houses	128	83		60	39	
Average # of People / Household (Standard Deviation)	1.8 (1.1)	1.3 (0.56)	<0.01	1.5 (0.8)	1.2 (0.43)	0.019
Ethnicity						
Non-Aboriginal	23 (10)	5 (4.7)		13 (15)	2 (4.4)	
Aboriginal	205 (90)	102 (95)	0.095	76 (85)	44 (96)	0.072
Education (>15 years)						
< High School	150 (66)	70 (65)	0.91	24 (27)	14 (30)	0.67
High School	30 (13)	17(16)	0.51	18(20)	8 (17)	0.69
Trades Certificate	47 (21)	20 (19)	0.67	47 (53)	24 (52)	0.94
Median Household Income	$50,000-$74,999	$50,000-$74,999		$50,000-$74,999	$25,000-$34,999	

Chapter 5: Discussion

Prevalence of *H. pylori* Infection

The prevalence of *H. pylori* infection observed in this analysis fell within the expected range for Aboriginal communities in the circumpolar region, who are disproportionately affected by the bacteria. *H. pylori* prevalence was 62% (414/670) in the study population. A review of the literature pertaining to *H. pylori* infection in the Aboriginal populations of the circumpolar regions of Canada, the United States, Greenland and Russia revealed prevalence estimates ranging from 51-95% [3-8]. In the context of the average across Canada, however, the observed frequency of infection is much greater than expected. Evidence from major urban centers across Canada reflects a decreasing frequency of transmission over time and an overall average prevalence of approximately 20-30% [1,2]. This comparison indicates that concerns about health risks from *H. pylori* infection brought forth by community leaders and health care providers in the participating communities were warranted.

Environmental Exposures and Prevalent *H. pylori* Infection

Household Effect

While not an aim of this analysis, and therefore not presented in the analysis results as such, *H. pylori* infection among household members represents an environmental exposure in and of itself. Because it was necessary to include a random effects parameter for household in multivariable logistic regression models to account for the lack of independence of the outcome probability (i.e., the probability of having *H. pylori* infection) among participants residing in the same household, some conclusions can be drawn about the effect

on *H. pylori* prevalence odds of the *H. pylori* status of household members. The observed variation of average outcome probabilities between households was considerable (standard deviation: 1.3; 95%CI: 0.69, 2.6). This variability indicated that there was a strong residual effect of the household on prevalence of *H. pylori*, beyond what was measured by the independent variables in the model. These results indicated that cohabitation with other study participants had a strong impact on the probability of having *H. pylori* infection.

For model checking in estimating the effects of environmental factors on *H. pylori* prevalence, the impact of adding the random effects parameter was assessed for all estimated effects; adding the household effect had a large impact on the estimated effects of two variables: having mice in the home and ethnicity. It should be noted that a model with a random effects parameter for household, to account for participants who live in the same household and are therefore not independent observations, assumes that most observations belong to clusters. However, approximately 65% (147/228) of the households in the study population had only one participant. Therefore, there was an imbalance in weighting of individuals counted as independent observations and those clustered with other members of their household. While this may have led to some distortion in the estimated effects on *H. pylori* prevalence adjusted for household, the model that included the household effect had a better statistical fit than the one that did not, and for this reason, the estimates adjusted for the household effect are considered more valid than estimates not adjusted for this effect.

Pathways for Zoonotic Transmission

Exposure to Mice or Mouse Droppings

In individuals who reported exposure to zoonotic pathways of transmission, the highest prevalence was observed in those who had seen mice

or mouse droppings in their home. In a comparison of participants with exposure to mice/mouse droppings and those without, having mice in the home was associated with an elevated odds of infection after adjusting for age as a cubic spline, sex, ethnicity, household income, educational attainment, community, and household as a random effect (OR: 4.1; 95%CI: 1.2, 14). The magnitude of the estimated association increased when exposure to other pathways for zoonotic and waterborne transmission were added to the model (OR: 4.6; 95%CI: 1.2, 18).

A review of the available literature pertaining to the role of mice in *H. pylori* transmission indicated the potential for mice to act as a source of the bacteria, with the pervasive use of mice in animal models and repeated demonstration of the ability to inoculate mice with the bacteria [95,172–174]. However, an epidemiologic assessment of whether or not there is an increased risk of *H. pylori* infection associated with having mice in the home was lacking. Thus, a direct comparison of the magnitude of the estimated association and what other investigators have reported was not possible.

Given the large amount of missing data in this analysis, the crude estimate from the full data set (OR: 2.0; 95%CI: 1.1, 3.9) and from the subset of individuals with complete data on all variables (OR: 2.3; 95%CI: 1.1, 5.0) were compared to quantify the potential amount of bias due to missing data. The small change in the point estimate and CI (which widened due to the reduction in sample size) indicated that missing data had a relatively small impact on the large effect estimated in the multivariable analysis.

In an assessment of which adjustment variable influenced the magnitude of the adjusted estimate the most, it was determined that the random effects for household induced the largest change in the point estimate. Further evaluation of the influence of adding a random effects parameter for household revealed a modification of the effect of exposure to mice on *H. pylori* prevalence odds by a combination of ethnicity and household cluster size. Among Aboriginal

participants who reported mice in their home, those with two or more participating household members had lower odds of infection relative to those who were the sole participating household member. Conversely, among non-Aboriginal participants who did not report mice in their home, those with two or more participating household members had higher odds of infection compared to those who were the sole participating household member. Therefore, the addition of the random effects parameter caused the magnitude of the odds ratio to increase substantially for both exposure to mice and ethnicity. Since this pattern of effect modification does not make intuitive sense, it may reflect error caused by selection bias. For example, a plausible selection bias scenario that could account for this is if having multiple members of Aboriginal families participate in the study is indicative of higher levels of engagement in their health.

Caring for Animals

Overall exposure to animals in relation to *H. pylori* infection was examined. The prevalence of *H. pylori* infection in individuals who reported being the regular caretaker of any livestock or animals was 68%, compared to 62% in those who had not cared for animals. The odds ratio for the effect of having cared for animals, compared to not having done so was 0.78 (95%CI: 0.39, 1.6), after adjusting for socio-demographic characteristics. There was a slight decrease in the magnitude of this association following inclusion of other environmental exposure variables to the model (OR: 0.82; 95%CI: 0.38, 1.8). While the adjusted odds ratios is in the direction of a protective effect, the CI spans a large range of values on both sides of the null, indicating inconclusiveness about the size and direction of this effect. To assess the potential for residual confounding by important socio-demographic characteristics, including ethnicity, household income and educational attainment, the distributions of these variables in those exposed to animals was examined. Participants who reported caring for animals

were distributed proportionally amongst categories of these socio-demographic characteristics. The estimated inverse association was consistent with most of the reviewed literature, which provided evidence of a null association or slightly decreased odds of infection in those who care for animals [36,46,143,159,160].

Caring for Dogs

Exposure to dogs is a specific zoonotic exposure commonly investigated in the literature [36,46,70,160] and pertinent to the study population given the large proportion of individuals in northern communities who own dogs. The odds ratio for the effect of regularly caring for dogs compared to not doing so was 0.76 (95%CI: 0.38, 1.5), adjusting for age as a cubic spline, sex, ethnicity, household income, educational attainment, community and household as a random effect. Inclusion of other indicators of exposure to pathways for zoonotic and waterborne transmission changed the estimate slightly away from the null (OR: 0.72; 95%CI: 0.33, 1.6). These findings were consistent with the predominant finding in the literature, suggesting there is an inverse association between caring for dogs and *H. pylori* prevalence [46,70,164].

Systematic review of the literature did not reveal any proposed biological explanations for a protective effect of contact with dogs on the prevalence of *H. pylori* infection; it is more plausible that the inverse association is due to the propensity for individuals of higher socioeconomic status to own pets or work animals [143,160]. While some indicators of socioeconomic status were measured and controlled for in this analysis, an uneven distribution of unmeasured aspects of socioeconomic status among infected and non-infected participants could have resulted in residual confounding. Also, misclassification of measured socioeconomic indicators could have resulted in residual confounding.

Caring for Cats

Of the examined zoonotic exposures, individuals exposed to cats had the lowest observed prevalence of *H. pylori* infection, resulting in a crude odds ratio denoting a protective effect of caring for cats (OR: 0.39; 95%CI: 0.22, 0.71). The direction of this association changed following adjustment for socio-demographic characteristics (OR: 1.3; 95%CI: 0.37, 4.3) and other indicators of exposure to pathways for zoonotic and waterborne transmission (OR: 1.4; 95%CI: 0.34, 5.4). While the adjusted estimates denote a modest harmful effect of caring for cats on *H. pylori*, wide confidence intervals leave room for doubt about the direction and magnitude of this effect.

In addition to socioeconomic factors that are likely to affect whether or not an individual cares for a cat, adjustment for ethnicity might have influenced the change in direction of the estimated effect in this population. Narratives from participants indicate that it is uncommon for Aboriginal people to own or care for cats, due to superstitious beliefs about the animals. These beliefs were most notably expressed amongst Gwich'in participants. Evidence of this cultural phenomenon was seen both in the small proportion of individuals who reported caring for cats overall and in the distribution of exposure to cats amongst categories of ethnicity. Of 534 individuals with data on caring for cats, only 10% had been the regular caretaker of a cat. Even though the proportion of the study population who were non-Aboriginal was 12%, the proportion of cat caretakers who were non-Aboriginal was 58% (30/52). While Gwich'in participants comprised 37% of all participants, only 5.6% (3/52) of cat caretakers were Gwich'in.

While the change in direction of the point estimate indicated successful adjustment for these effects, the low end of the 95% confidence interval left room for doubt. This might have been due in part to the uneven distribution of exposure amongst categories of ethnicity that could have resulted in residual confounding. Therefore, a large degree of uncertainty around the effect of

exposure to cats on the odds of prevalent *H. pylori* infection in this population remains. In the context of the reviewed epidemiologic literature on this association, these results were consistent with an overall lack of strong support for or against the potential role of cats in the transmission of *H. pylori* [35,160,165,170,171].

Contact with Animal Innards

Individuals who reported taking part in hunting-related activities including field dressing, cleaning or preparing local game animals for cooking were considered exposed to animal innards. Among participants exposed to animal innards, there was a slightly elevated odds of *H. pylori* infection relative to those who were not exposed to animal innards (OR: 1.2; 95%CI: 0.57, 2.5) following adjustment for socio-demographic characteristics. The magnitude of this association increased with the addition of other indicators of exposure to pathways for zoonotic and waterborne transmission (OR: 1.6; 95%CI: 0.70, 3.6). While the point estimates denote a detrimental effect of exposure to animal innards, the confidence intervals that span a sizable range in both directions from the null leave uncertainty about the direction and magnitude of this association. Thus, the present analysis does not provide strong support for zoonotic transmission through contact with animal innards.

A review of the literature revealed equally unconvincing results. In the epidemiologic literature examining this association, employment at an abattoir was used to define exposure [62,89,158]. In this group of studies, a slightly elevated prevalence of infection was reported in individuals who were in regular contact with animal innards [62,89,158]. However important confounding factors including age, educational attainment and income were not taken into account in these investigations. Therefore the present analysis and review of the literature do not provide solid evidence for or against transmission of *H. pylori* through contact with animal innards.

Pathways for Waterborne Transmission

Lifetime Consumption of Untreated Water

Untreated water was defined as including water taken directly from the river, melted snow or ice or any source other than municipally supplied or otherwise chemically treated or boiled water. A large proportion of participants reported having consumed untreated water at some point during their lifetime (73%; 395/541). *H. pylori* –positivity was 63% in those who had consumed untreated water at some point, compared to 60% in those who had not. Following adjustment for socio-demographic factors and other environmental exposures, the effect estimates indicated that individuals who had consumed untreated water during their lifetime had a relatively low frequency of infection (OR: 0.36; 95%CI: 0.14, 0.94).

In the context of the reviewed literature, the magnitude of the estimated inverse association was unexpected. While the scientific community is unable to definitively demonstrate whether or not *H. pylori* organisms are able to retain infectivity following exposure to water [56,91,101,200], epidemiologic evidence from investigations of the frequency of infection in individuals exposed to sources of untreated water suggests the potential for waterborne transmission[36–38,41,42,144–149,155]. However, the 95% confidence intervals from a large proportion of the estimates reported in the literature indicate the association may actually be closer to the null, or slightly inverse [36,38,145,146,148,155]. While some authors have reported a null association between untreated water consumption and prevalence of *H. pylori* infection [35,38,39], null findings were not the commonly reported in the literature. This may be due, in part, to a tendency for papers presenting positive results to be favoured for publication over those presenting null associations.

Ascertainment of this exposure was predicated on subject recall of untreated water consumption throughout their lifetime. Therefore, there was a great degree of uncertainty regarding accuracy of classification. The magnitude of the observed inverse association could have been a product of differential misclassification of exposure status if individuals with the infection were more likely to under report consumption of untreated water. Otherwise uncontrolled confounding might have influenced the estimate, or a combination of these effects.

Consumption of Untreated Water in the Past Year

A small proportion of individuals reported consuming untreated water in the past year (32%; 161/497), relative to those who reported consuming untreated water during their lifetime (73%; 395/541). *H.pylori*-positivity was similar between exposed and unexposed participants, with prevalence of 65% and 63%, respectively. Following adjustment for socio-demographic factors and other environmental exposures, the estimated odds ratio denoted a weak inverse association between having consumed untreated water in the past year compared to those who had not consumed untreated water and *H. pylori* infection, (OR: 0.85; 95%CI: 0.40, 1.8). This weak association is fairly consistent with the aforementioned investigations of the association between exposure to sources of water that are more likely to be contaminated with the bacteria and prevalent infection found in the literature.

Given evidence that *H. pylori* infection is most commonly acquired during early childhood, exposure to environmental sources of the bacteria occurring at that time would be most relevant to acquisition of the infection. However, it is reasonable to assume that adults are less likely to accurately recall frequency of exposure to potentially contaminated water during their childhood. Ascertainment of exposure in the past year can serve as a proxy for exposures occurring early in life, as individuals are more likely to recall recent consumption

of untreated water, if assumptions can be made about changes in exposure over the lifespan. It is likely that people who currently consume untreated water did so in childhood as well, but it cannot be assumed that people who currently avoid untreated water also did so in childhood. Therefore, it is plausible to consider frequency of consumption of unboiled and unfiltered water in the past year a proxy for frequency of consumption during childhood with high specificity but low sensitivity.

Exposure to Contaminated Water

Individuals were considered exposed to sources of biological contamination if they reported having had problems with their household sewage system. Of the indicators of exposure to waterborne transmission pathways, exposure to contaminated water was the least frequently reported (29%; 153/526). The prevalence of *H. pylori* infection was slightly lower in individuals who reported problems with their household sewage system relative to individuals who were not classified as exposed to sewage (59% and 63%, respectively), producing a crude odds ratio of 0.83 (95%CI: 0.53, 1.3). Following adjustment for important confounders and other investigated exposures, the magnitude of the estimated inverse effect increased substantially (OR: 0.49; 95%CI: 0.22, 1.1).

Exposure to sources of biological contamination such as sewage has been investigated in other settings by comparing individuals with pit latrines to those with flush toilets [36,37,39,45]. Estimates of the effect of this exposure generated from prospective investigations indicate that the association between sewage and acquisition of *H. pylori* infection is minimal or null [10,39]. Therefore the magnitude of the estimated inverse association was unexpected. Similar to untreated water consumption, classification of the frequency of sewage problems in the home was subject to a great deal of uncertainty. Differential misclassification of exposure status might explain the exaggerated association. A

scenario of concern is low sensitivity, in the absence of a reason to expect individuals to falsely report having problems with their sewage system when they have not. A differential exposure misclassification scenario that would result in an exaggerated inverse association would be if sensitivity were lower in infected individuals, relative to those without the infection. This scenario is plausible given socio-demographic characteristics, such as educational attainment, that are associated with the frequency of acquiring the infection and likely influence the accuracy of responses. Specifically, individuals with lower educational attainment are more likely to have the infection and provide less accurate responses.

Bias Analysis

Comparison with the Census Population

The comparison of the study sample with complete data on all socio-demographic and environmental variables with the census population for each community provided an assessment of selection bias and whether missing data were missing at random. Notable differences between the study and census populations were in categories of age, average household size, educational attainment and median household income. In all three community projects, individuals aged 0-19 years were extremely underrepresented. The average household size was considerably smaller than reported by Statistics Canada, indicating it was not common for a full household to participate. This comparison suggested that individuals with a higher household income were more likely to participate in the Aklavik and Old Crow *H. pylori* projects. Further, individuals with a high school education or less were underrepresented in the study populations from Old Crow and Tuktoyaktuk.

Analysis of Misclassification of Exposures

The potential impact of information bias was assessed for classification of untreated water consumption and exposure to sewage. The level of measurement error required to fully explain the crude odds ratios for the effects of these exposures on *H. pylori* prevalence was estimated.

This inverse effect of consumption of untreated water during the participant's lifetime might have resulted from individuals with the infection being less likely to report consuming untreated water when they had, relative to individuals without the infection. The justification for examining this differential misclassification scenario was that socio-demographic characteristics such as education influence an individual's risk of acquiring the infection and could also plausibly influence their provision of accurate responses to interview questions. Specifically, it is plausible that individuals with higher educational attainment are less likely to have the infection and more likely to provide accurate responses, relative to participants with lower levels of education.

The bias-adjusted sensitivity analysis yielded estimates of sensitivity necessary for the differential misclassification of exposure scenario to explain a portion of the estimated inverse association. In order for this scenario to explain a substantial portion of the estimated effect, sensitivity in cases and controls would have to differ by 30% (a sensitivity of 50% in cases and 80% in controls). The resulting bias-adjusted odds ratio was 0.63. The large discrepancy between cases and noncases with respect to accurate reporting of exposure status does not seem likely and as such, while differential misclassification of exposure is likely to have influenced this estimate to some degree, the present bias analysis indicated that this type of information bias is not solely responsible for the estimated effect.

Analysis of Selection Bias

The participatory nature of the research program allowed any interested individuals to enroll; as with results of all research requiring consent, estimates generated by this analysis are vulnerable to some degree of selection bias. The comparison with the census population for each community provided some insight into which categories of socio-demographic characteristics were over or under represented in the study population, reflecting the type of individuals more or less likely to participate, and informing estimates of subgroup-specific selection (i.e., participation) probabilities. Given that the socio-demographic factors that appeared to have had some influence on the probability of participation were measured on all study subjects and were not influenced by exposure or infection status, adjustment for these characteristics in the model helped to control for this type of bias [192].

In order to assess whether the estimated effect of exposure to mice on *H. pylori* prevalence could reasonably be explained completely by selection bias, a scenario with differential selection probabilities for exposed and unexposed cases and noncases that would falsely generate the effect was constructed. A low proportion of participating unexposed cases relative to the proportion of participating exposed cases, along with a relatively low proportion of exposed non-cases, would generate the exaggerated effect. While the comparison of the study populations to census data suggested that people exposed to mice may have been less likely to participate, assuming that such people would be of lower socioeconomic status on average, it does not seem likely that this differential participation would systematically differ between cases and noncases. While selection bias may have influenced the estimated effect, it is also likely that measurement error and unmeasured confounding played a role.

Missing Data

Of the 670 individuals who participated in one of the three community projects, 55% (368/670) had complete data on all environmental exposures and socio-demographic adjustment variables. Data were missing predominantly on household income (21%; 143/670), followed by educational attainment (16%; 110/670) and ethnicity (16%; 105/670). Patterns revealed in the comparison of the study data with census data were used to assess whether data were missing at random. The comparison with census data indicated that missing data from variables such as educational attainment (in Old Crow and Tuktoyaktuk) and household income (in Aklavik and Old Crow) were not missing at random. This was consistent with observations in the field, which suggested that participants with lower income and educational attainment were more likely to feel uncomfortable providing this information due to socio-cultural implications.

To assess the potential impact of missing data on adjusted estimates, crude odds ratios estimated from the full study population with data required for the effect of interest and from the subset of individuals with complete data on all variables were compared. Small differences in these crude estimates indicated that missing data is likely to have had a small impact on adjusted estimates.

Summary and Implications

Main Results from Environmental Exposures

This analysis indicates that people who report mice or mouse droppings in their home have a relatively high prevalence of *H. pylori* infection. However, it is not clear whether this reflects a role in transmission or an association with other transmission risk factors. The effect estimates for regular contact with animals on *H. pylori* prevalence do not provide strong evidence for or against a role for dogs and cats in *H. pylori* transmission in the study population. These findings were consistent with a body of literature that examined the prevalence

of non-pylori Helicobacter organisms in a variety of animals, with reported prevalence of 67 to 100% in some species [93–98]. While the prevalence of these other Helicobacter species is quite high in some animals, it is estimated that no more than 1% of humans are infected with these other species, indicating they are not readily transmitted between animals and humans [88,94]. Finally, the present analysis does not provide strong evidence for or against the role of contact with animal innards in transmission of this infection in the participating Canadian Arctic communities. The estimates for the effects of indicators of exposure to pathways for waterborne transmission on odds of prevalent infection show inverse associations. The direction of the estimated effects of exposure to sewage and having consumed untreated water during their lifetime on *H. pylori* prevalence were particularly unexpected.

Public Health Implications

Results from the Aklavik, Old Crow and ISR (Tuktoyaktuk) *H. pylori* Projects support the perception of *H. pylori* as a major clinical problem. Understanding transmission of *H. pylori* has important implications for the development of meaningful public health policy aimed at preventing the spread of the bacteria. While the science surrounding transmission remains unclear, evidence suggests likely routes include gastro-oral, fecal-oral and oral-oral, although the relative frequency of transmission through these person-to-person routes or the importance of spread through environmental intermediaries are not known [26,35]. The present analysis does not provide support for or against transmission through waterborne pathways or zoonotic pathways including dogs, cats, and animal innards. The public health importance of these findings includes the contribution to an understanding of the role of environmental exposures in transmission of *H. pylori* in the study populations. This is of particular importance given concern over contamination of the local environment with *H. pylori* commonly expressed in northern communities. In a

broader context, these findings contribute to an overall understanding of transmission of *H. pylori* in northern Canadian populations. Such information is invaluable for the eventual development of public health policy aimed at mitigating transmission in high-risk populations such as these. While many of the findings from this research are inconclusive, they provide a start at investigating the underlying community concerns.

The positive association observed between individuals reporting exposure to mice and *H. pylori* infection suggests the potential for mice to act as vectors of the bacteria. Because this association has not been addressed in the accessible literature, further research is needed to determine whether the estimated effect reflects a role in transmission or an association with other risk factors. In the context of the participating communities, given the small proportion of participants who reported exposure to mice (10%; 53/527), it is unlikely that this exposure plays a significant role in transmission, if at all.

Untreated Water Consumption and Severe Gastritis Prevalence

Distribution of Gastritis Severity

These data demonstrated a relatively high frequency of severe gastritis among *H. pylori*-positive residents of Aklavik and Old Crow (49%). The comparison of the observed distribution of gastritis severity in Canadian Arctic communities to the *H. pylori*-positive patient population from the University of Alberta in Edmonton suggested the frequency of severe gastritis in these communities is much greater than expected [201].

Consumption of Untreated Water in the Past Year

Having consumed untreated water in the past year was reported by 33% of *H. pylori*-positive participants with biopsies evaluated. A strong effect of consuming untreated water in the past year compared to not having done so on

the prevalence odds of severe gastritis was estimated in residents of Aklavik (OR: 2.8; 95%CI: 1.1, 7.2), after adjusting for age, ethnicity, educational attainment, alcohol consumption, smoking, NSAID use and community. However, the adjusted estimate from Old Crow showed an inverse association (OR: 0.85; 95%CI: 0.19, 3.9) resulting in a combined adjusted estimate of 1.8 (95%CI: 0.86, 3.8).

The rationale for examining this association was predicated on the understanding that chronic consumption of environmental toxins could contribute to the development of gastritis by irritating the stomach lining [175]. A review of the literature indicated inorganic mercury is an important environmental toxin in the development of gastrointestinal problems [184]. Distinct patterns of contamination in river water consumed by residents of Aklavik and Old Crow, due to the location of these communities in separate Arctic waterways, could lead to variation in the effect, if any, of untreated water consumption on gastritis severity; this analysis, however, does not have sufficient statistical precision to rule out random variation as the reason for different effect sizes observed in the two communities.

Bias Analysis

Comparison with the Census Population

Notable differences between the subset of project participants with biopsies evaluated and the census populations for Aklavik and Old Crow were observed in categories of age, number of people per household, educational attainment and household income. In both community projects, individuals aged 0-19 years were underrepresented and those aged 40-59 years were overrepresented among endoscopy participants. Underrepresentation of individuals aged 0-19 years was expected due to age restrictions on participation in the medical procedure. This comparison indicated that while individuals with a

higher household income appeared more likely to participate in endoscopy in Aklavik, median income for endoscopy participants in Old Crow was slightly lower than that reported by Statistics Canada. In categories of educational attainment, those with a higher level of education (high school or some type of post secondary school) were more likely to participate in this phase of the Old Crow *H. pylori* Project, or provide complete information. As previously mentioned, these findings are consistent with observations in the field, which suggested that patterns in participation probabilities or missing data were not random.

Analysis of Misclassification of Exposure Status

Misclassification of exposure to sources of untreated water may have occurred to due to error introduced by poor recall of the frequency of untreated water consumption in the past year. Differential misclassification might have occurred if reporting of exposures differed between individuals with severe gastritis and those without. Specifically, a positive association could arise from differential misclassification if individuals without severe gastritis were more likely to underreport untreated water consumption than individuals with severe gastritis. However, socio-demographic characteristics like educational attainment, which might influence accuracy of reported answers, would be expected to lead to reduced accuracy of exposure recall in individuals with severe gastritis. For example, a large proportion of individuals in the study population who reported being smokers, a known risk factor for the development of gastritis, had lower levels of education. In the absence of a reason to posit that individuals would falsely report having consumed untreated water when they had, it is unlikely that differential misclassification explains the estimated effect. A plausible non-differential exposure misclassification scenario is that of low sensitivity relative to specificity in participants with and without

severe gastritis. With a specificity of 100% and sensitivity of 80%, the bias-adjusted odds ratio would be 1.96.

Analysis of Selection Bias

A selection bias scenario of concern is lower participation of exposed individuals without severe gastritis, relative to those with severe gastritis. This may have occurred if factors that influenced the severity of gastritis also influenced the frequency of consuming untreated water as well as the probability of participation. This could have occurred, for example, if individuals who consumed alcohol regularly, a risk factor for the development of gastritis, had more digestive symptoms which motivated them to participate and regular alcohol drinkers were more likely to consume untreated water than people who were not regular alcohol drinkers due to different distributions of unmeasured socio-demographic characteristics in these two groups.

Missing Data

Another potential contributing factor to the apparent modification of effects estimated for Aklavik and Old Crow is missing data. Data were missing from participants of the Old Crow *H. pylori* Project on important adjustment variables including educational attainment and alcohol consumption. Of individuals with missing data, five had severe gastritis and reported consuming untreated water in the past year. The remaining two individuals did not have severe gastritis and reported that they had not consumed untreated water in the past year. The exposure and outcome status of all individuals left out of the full model due to missing data suggest that the reversal of the direction of association in the adjusted estimate for Old Crow is due to missing data.

Limitations of Bias Analysis Methods

While this approach provides a simple method for quantifying and assessing plausible levels of bias in epidemiologic studies, there are several limitations. First, this method does not allow for an assessment of the interaction of multiple errors likely to affect an estimated association [193]. However, employing this method does allow the incorporation of certain assumptions about other errors. Second, with the aim of determining the magnitude of bias necessary to completely explain the estimated effect, the goal becomes making the association go away rather than estimating a bias-corrected measure [193]. Finally, determining whether this bias hypothesis is plausible follows similar logic to frequentist decision making, that is, deciding whether or not the hypothesis is plausible rather than estimating how probable it is; thus it is not necessarily useful in understanding the range of probabilities associated with potential levels of bias [193].

The bias-level sensitivity analysis involves the formation of plausible scenarios that might influence the estimated effect and an evaluation of the probability of these scenarios occurring [193]. However a common criticism of this approach is that bias levels are generated based on the investigator's opinion of plausible scenarios and accepted or rejected on the same basis [193].

Summary and Implications

Main Results

Initial data collected by the CAN*Help* Working Group has demonstrated relatively high frequencies of severe gastritis among *H. pylori*-positive residents of Arctic communities. This analysis provides evidence of a possible link between having consumed untreated water in the past year compared to not having done so and the prevalence of severe gastritis.

Public Health Implications

This research represents a preliminary investigation of the potential for exposure to environmental contaminants to contribute to the development of severe gastritis in Canadian Arctic communities. Given the extensive documentation of high levels of environmental contaminants in the Arctic, the public health implications of an association between exposure to these contaminants and the development of severe gastritis or other precancerous conditions are substantial. This analysis, however, does not have the statistical power to provide a precise estimate of this effect; instead, it screened hypotheses for new lines of thinking regarding determinants of severe gastritis in the study populations.

Strengths and Limitations

Limitations

An important limitation of this analysis is misclassification of exposure and confounding variables caused by errors in questionnaire data, which likely occurred to some degree due to the respondents' imperfect recall or tendency to provide responses that deviate from the truth due to social desirability. For example, in the analysis of the effect of untreated water consumption on the prevalence of severe gastritis, an identified risk factor the development of gastritis is alcohol use. In communities where alcohol is not sold or permitted in large quantities, it is reasonable to expect some participants might not accurately report alcohol consumption. Other limitations of this analysis include potential selection bias due to described differences between the study population and the census information and missing data.

Strengths

This analysis used data generated by community projects set up by a comprehensive collaborative research program, recognized nationally and worldwide for conducting high-quality community-based research on *H. pylori* infection. Therefore, a major strength of this analysis was the population-based dataset. An important strength of this research is the high level of community engagement. Observations from the field revealed a high level of community interest, which facilitated recruitment and the provision of extensive information on demographics, individual and household level socio-environmental exposures and medical history from each participant. Comprehensive data collected from each participant allowed for the measurement and control of important confounding factors in the estimation of the effects of environmental exposures on digestive health outcomes in these communities.

Investigators from a variety of disciplines, including gastroenterology, microbiology and pathology provided expertise that contributed to an additional strength of this analysis. In addition to providing an assessment of the prevalence of severe gastritis, collection of biopsies and subsequent microbiological and pathological analysis allowed estimation of infection status to be based on the 13C-UBT, culture and histology. This strength reduced the potential for misclassification of infection status for the portion of participants who participated in endoscopy.

Chapter 6: Conclusion

This analysis aimed to investigate the relationship between environmental exposures and the digestive health of residents of three Canadian Arctic communities. Investigation of the associations between environmental sources of biological contamination and prevalent *H. pylori* infection indicated whether these sources play a role in transmission in these communities. Environmental exposures were grouped into pathways for zoonotic transmission (Evidence of mice in the home; caring for animals; specifically caring for dogs; specifically caring for cats; and contact with animal innards) and pathways for waterborne transmission (Ever consumed untreated water; consumed untreated water in the past year; exposure to contaminated water).

This analysis revealed a positive association between having reported evidence of mice in the home compared to not on prevalent infection. However, it is unclear whether the estimated effect reflects a role in transmission or a relationship between exposure to mice and other risk factors for transmission. The estimated associations between regular contact with animals or livestock do not provide strong support for or against the role of these exposures as risk factors for the infection. Further, results of this analysis did not provide evidence for or against the potential role of contact with animal innards on transmission of *H. pylori*.

The effect estimates for pathways for waterborne transmission show inverse associations. The direction of the estimated effects of exposure to sewage and having ever consumed untreated water on *H. pylori* prevalence were particularly unexpected. These estimates may reflect a combination of information biases, such as differential misclassification of exposure or selection probabilities. Further, residual confounding resulting from insufficient

measurement of other determinants of the infection is likely to have influenced the estimated associations.

Understanding transmission of *H. pylori* has important implications for the development of meaningful public health policy aimed at reducing transmission. These findings contribute to an overall understanding of how these bacteria are transmitted in northern Canadian communities, who experience an elevated frequency of the infection, relative to individuals from southern parts of the country.

The role of environmental exposures in the development of severe gastritis was investigated with the hypothesis that untreated water consumption may be a determinant of severe gastritis due to the potential presence of chemical irritants. This analysis provided evidence of a possible link between having consumed untreated water in the past year compared to not on prevalence of severe gastritis. Further investigation of the relationship between exposure to environmental sources of chemical contamination and severe gastritis prevalence is needed in order to more precisely estimate these effects.

Future Directions

Future research should include data from other Canadian Arctic communities, in order to more precisely estimate the effects of exposure to environmental sources of biological contamination on prevalent *H. pylori* infection. Further investigation of alternate transmission pathways for transmission in these communities is necessary. Additional exploration of the association between untreated water consumption and severe gastritis prevalence should include data from other communities. Further, local water sources should be tested for the presence of environmental contaminants hypothesized to play a role in the development of severe gastritis.

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H. pylori Project Information Sheets and Consent Forms

Principal Investigator: Karen Goodman, PhD, Gastroenterology & Public Health, University of Alberta

Research Team

Northwest Territories:

Leah Seaman, MD, Medical Health Officer, Beaufort-Delta Regional Health Authority, Inuvik

Kami Kandola, MD, MBA, Chief Public Health Officer, Government of the NWT

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University of Alberta, Edmonton:

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Other Scientists: Christopher Fletcher, PhD (Anthropology); Carl Phillips (Policy Sciences)

About *H. pylori*

"Helicobacter pylori" is the name of bacteria that infect the stomach lining. They are called "*H. pylori*" for short. *H. pylori* infection has no boundaries and is found all over the world. It is especially common in some Arctic communities. People usually become infected during childhood. Scientists are not sure exactly how people get infected. Most likely, it happens from contact with an infected person's germs, especially if that person is sick with vomiting or diarrhea. However, *H. pylori* may also spread by other means.

The infection irritates the stomach lining; it causes an inflammation called gastritis. The gastritis can be mild or severe. It may or may not make people feel sick. At first, some people may get stomach problems that go away after a few days. Often, the infection lasts many years, or even life-long, without symptoms. So, most people with *H. pylori* don't know they have it. Some people get long-lasting symptoms such as stomach pain, nausea or vomiting. Some people with long-lasting *H. pylori* get serious diseases. These diseases include stomach ulcers, and very rarely, stomach cancer. Many things other than *H. pylori* can cause stomach problems, so a medical exam is needed to find out the cause of long-lasting symptoms.

Usually, people are not tested for *H. pylori* unless they have long-lasting stomach problems. *H. pylori* infection cannot usually be cured with a single medication. Combinations of drugs cure *H. pylori* in some people, especially if the treatment is taken exactly as prescribed. Some infections, however, do not respond to treatment. And some people get re-infected after they are cured.

Study Purpose
This study was designed to address concerns about health risks from *H. pylori* infection raised by members of the communities that seek this research.

Health officials are aware that *H. pylori* is a major concern in many northern communities. Health officials also want research to learn how to reduce health risks from this infection. This study will investigate *H. pylori* in your community and others. The Fort McPherson *H. pylori* Project researchers want to help community members and health care providers find answers to their questions. The research aims to find out how *H. pylori* infection affects the health of people in your community, what can be done to reduce the risks, and how to help people understand this health problem.

Please note
If you are consenting for one or more children to participate in this research, "you" and "your" also means "your child" and "your child's (or "your children" and "your children's)."

Study Procedures
Several activities may be offered. The community planning committee will decide which activities will be offered. It is completely up to you which activities you participate in. You may be asked to do any or all of the following:

- Tell us some basic information about yourself
- Answer questions about your health and symptoms related to digestion (for about 20 minutes)
- Answer questions about your family, household environment, and diet (for about 60 minutes)
- Answer questions about what you know and think about *H. pylori* infection, at different times throughout the project (for about 10 minutes). The conversation may be tape-recorded if you agree.
- Participate in a group discussion to express your views on the research (for about one hour). The group discussion may be tape-recorded if you agree.
- Take one or more 13C-urea breath tests for *H. pylori*. 13C-labeled urea is a harmless powder that helps detect *H. pylori* in the stomach. For this test, you would: blow into a tube or bag; drink a harmless liquid that contains 13C-labeled urea; wait 30 minutes; blow into another tube or bag
- Give a sample of blood (a small 10ml tube), if you agree to tests for low iron levels or anemia
- Give a sample of stool (poop), if you agree to additional tests for *H. pylori* or fecal occult blood (hidden blood in poop)
- Have a scope test to examine the inside of your stomach or participate in a treatment trial. For these activities you will receive additional information and be asked to sign separate consent forms

- Allow us to look at your medical records to find out about health care visits, tests, diagnoses, and treatments of relevance to this research
- Allow us to store any blood, stool, or breath collected from you that is remaining from this study to use in future research; if you agree, your extra samples will be stored without any information that can identify you; allowing this will permit researchers to learn more about health in people from your community

You will be informed of your test results:

- If you have a breath test, you will receive a written report of the result; study staff will answer any questions you have about these results; if you wish, you can speak to a study doctor about the results.
- If you agree, we will give your test results to the local health centre so it becomes part of your medical record.

Possible Benefits

We will present study findings to northern health officials so they can decide how to manage *H. pylori* infection. If you agree, we will give your test results to the local health centre nurse; she can use this information in monitoring your health. You can also give your test results to other doctors you seek treatment from. Your participation will let us find out if you need more tests or treatment; if so, we will help arrange this for you.

Your participation will help researchers find out how *H. pylori* affects stomach health in your community. This will help health authorities know how important a problem this is so they can develop solutions for reducing health risks.

Possible Risks

We will respect your privacy, but may ask some questions you do not wish to answer. If we ask any questions that make you uncomfortable, you can tell us you prefer not to answer. We do not expect our tests to harm you. There is no known risk from the urea breath test. If you agree to a blood test, we will follow precautions to prevent injury. The blood test may cause mild pain, bleeding, or fainting, and/or bruising or infection where the needle is inserted. If you don't want to have a particular test, you can choose not to have it.

Confidentiality

All personal records relating to this study will be kept confidential. Any research data we collect will not identify you by name. We will not disclose your name outside the research project office. We will not identify you by name in any published report. We will use the health information we collect only for the purpose of this research study; we will keep it confidential unless release is required by law. In addition to the investigators, the Health Research Ethics Board may have access to your records; they may access your records to monitor the research and verify the accuracy of study data.

By signing the consent form you give permission to the study staff to: obtain health information about you from other health care professionals; and to access any such health information they deem necessary for the conduct of the research. By signing the consent form you give permission for the collection, use and disclosure of your medical records. The University of Alberta requires that study information be kept for 7 years. Even if you quit the study, the medical information obtained about you for study purposes will not be destroyed. You have a right to check your health records and request changes if your personal information is incorrect.

Voluntary Participation
Your participation in this study is strictly your choice. If you do not wish to participate, it will not affect the care you receive at your local Health Centre. If you enroll in the study, you can stop participating at any time, and it will not affect the care you receive at your local Health Centre.

Expenses
You will not have to pay for any tests or treatment done as part of this study. We do not pay you for your participation. Your participation is voluntary.

Contact Names and Telephone Numbers
If you have concerns about your rights as a study participant, you may contact the University of Alberta Health Research Ethics Board at 780-492-9724. This office is independent of the study investigators.

Please contact any of the individuals identified below if you have any questions or concerns:

Laura Aplin, University of Alberta Project Coordinator, 1-855-492-2525 (Toll-free)
Karen Goodman, PhD, Edmonton, Alberta, 780-492-1889
Sander van Zanten, MD, Edmonton, Alberta, 780-492-9840

Principal Investigator: Karen Goodman, PhD, Gastroenterology & Public Health, University of Alberta

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Other Scientists: Christopher Fletcher, PhD (Anthropology); Carl Phillips (Policy Sciences)

Purpose of Endoscopy

The Fort McPherson *H. pylori* Project researchers want to find out how *H. pylori* infection affects the stomachs of residents in your community. This infection irritates the stomach lining. It causes an inflammation called gastritis. The gastritis may be mild or severe. It may or may not cause pain or other symptoms. Doctors can't tell from your symptoms how healthy your stomach is.

H. pylori has different types. Some types cannot be treated with certain drugs. And some types may be more likely to cause serious disease. Doctors can't tell what type of *H. pylori* you have unless they take samples from your stomach.

They use endoscopy to examine your stomach. In this exam, a doctor uses a scope to see if the inside of your stomach looks normal. If they see an ulcer or other abnormality, they make a note of their diagnosis. The doctor also takes biopsies for lab tests. The biopsies are tiny samples of the stomach lining (only a few millimeters). This exam helps the doctor find out if you have a stomach abnormality that is not visible through the scope and also test to see what type of *H. pylori* you have.

Everyone in the community will be offered an endoscopy, whether or not they have *H. pylori* infection. To learn how *H. pylori* affects the stomach, it is important to compare people with and without the infection.

Please note

If you are consenting for one or more children to participate in this research, "you" and "your" also means "your child" and "your child's (or "your children" and "your children's)."

Study Procedures
The endoscopy will be done at the local health centre. Before the endoscopy, we will ask you to answer a few questions for the doctor to make sure you are eligible and cared for properly. For this exam, you will lie on a clinic bed. A nurse will spray the back of your throat with a numbing medicine. A doctor with special training will do the endoscopy. This doctor will insert a thin tube down into your stomach through your mouth. It reduces gagging and coughing; and it allows you to talk. The doctor will look through the scope and may take pictures. If the doctor sees anything abnormal, he or she will tell you and note the diagnosis in your endoscopy report.

The doctor will also take biopsies (very tiny samples of your stomach tissue). Usually, the biopsies do not hurt and they heal on their own. Lab techs will test some biopsies to see if they grow *H. pylori*; if so, other tests will find out what strain they are and which drugs act against them. A pathologist will examine other biopsies through a microscope. He will see if *H. pylori* organisms are visible. He will also see if there is mild or severe inflammation or other abnormalities. The pathologist will examine the biopsies in an Alberta Health Services lab. This lab will store personal information required for its own records, and will not share this information with anyone other than lab personnel.

The endoscopy will take 10-15 minutes. If you feel too uncomfortable, you can signal that you want to stop. If you can't complete the endoscopy, you may be offered the option of endoscopy with sedation. This will depend on the doctors' assessment and the availability of resources. After the endoscopy, we will ask you to answer a few questions to find out how it went for you. If follow up is required, the project doctors will advise your health care provider as needed.

The investigators would like to store any biopsies collected from you that remain after testing for this project, to use in future research. If you agree, your extra samples will be stored without any information that identifies you. Allowing this will permit researchers to learn more about health in people from your community.

Possible Benefits
If you have a scope test, one or more gastroenterologists (doctors specialized in digestive diseases) will examine you, explain the diagnosis at the time of the scope, and explain the pathology results later; at these times, you can consult the gastroenterologist about any stomach problems you have.

Possible Risks
The endoscopy may cause discomfort such as nausea, gagging, uncontrolled swallowing, mild sore throat or nose bleed. Some bleeding may occur from the site where the biopsies are taken; usually the blood loss is small. Very rarely, serious complications can occur: heavy blood loss may require a transfusion; a hole in the esophagus, stomach or intestine may require surgery. Another rare complication is getting fluid or stomach contents into your lungs.

The doctors who will perform the endoscopies are experienced specialists; they will be very careful to reduce risks. Sedation or general anesthesia reduces or eliminates discomfort during the endoscopy. However, there is a risk of adverse reaction to the sedation drug: possible effects include drowsiness, slow breathing, apnea (no breathing) or, extremely rarely, death. For this reason, we will first offer endoscopy without sedation. If you have sedation, however, you will be monitored carefully for any adverse reaction. In the very unlikely case that an adverse event occurs during your endoscopy, emergency measures will be taken as needed.

Confidentiality
All personal records relating to this study will be kept confidential. Any research data we collect will not identify you by name. We will not disclose your name outside the research project office. We will not identify you by name in any published report. We will use the health information we collect only for the purpose of this research study; we will keep it confidential unless release is required by law. In addition to the investigators, the Health Research Ethics Board may have access to your records; they may access your records to monitor the research and verify the accuracy of study data.

By signing the consent form you give permission to the study staff to: obtain health information about you from other health care professionals; and to access any such health information they deem necessary for the conduct of the research. By signing the consent form you give permission for the collection, use and disclosure of your medical records. At the University of Alberta, study information must be kept for 7 years. Even if you quit the study, the medical information obtained about you for study purposes will not be destroyed. You have a right to check your health records and request changes if your personal information is incorrect.

Voluntary Participation
Your participation in this study is strictly your choice. If you do not wish to participate, it will not affect the care you receive at your local Health Centre. If you enroll in the study, you can stop participating at any time, and it will not affect the care you receive at your local Health Centre.

Expenses
You will not have to pay for any tests or treatment done as part of this study. We do not pay you for your participation. Your participation is voluntary. If travel is required to receive an endoscopy, we will pay for those expenses

We will answer any questions you have about this study or about specific procedures at this time, or at any time during your participation.

Contact Names and Telephone Numbers
If you have concerns about your rights as a study participant, you may contact the University of Alberta Health Research Ethics Board, at 780-492-9724. This office is independent of the study investigators.

Please contact any of the individuals identified below if you have any questions or concerns:

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Karen Goodman, PhD, Edmonton, Alberta, 780-492-1889
Sander van Zanten, MD, Edmonton, Alberta, 780-492-98

Title of Project: *H. pylori* Project

Principal Investigator: Karen Goodman Phone Number: 780-492-1889

Project Coordinator: Laura Aplin Phone Number: 1-855-492-2525 (Toll-free)

Do you have questions about the project at this time? [ADDRESS ANY QUESTIONS]
Are you satisfied by the information you have received about the project at this time?
[ADDRESS ANY ADDITIONAL CONCERNS UNTIL THE ANSWER IS YES] **Yes ☐**

Part 2 (to be completed by the research participant)	Yes	No
Do you understand that you have been asked to be in a research study?	☐	☐
Have you read and received a copy of the attached Information Sheet?	☐	☐
Have I (study staff) answered your questions to your satisfaction?	☐	☐
Do you understand you can ask more questions later on if you like?	☐	☐
Do you understand the benefits and risks involved in taking part in this research study?	☐	☐
Do you understand that you are free to withdraw from the study at any time, without having to give a reason and without affecting your child's future medical care?	☐	☐
Has the issue of confidentiality been explained to you?	☐	☐
Do you understand that the project staff will have access to your health records?	☐	☐
Is it okay with you for us to give your tests results to the health center staff to include in your medical record?	☐	☐

Who explained this study to you? _____

I agree to take part in this study:
YES ☐ NO ☐
I consent to storage of remaining samples collected from me for use in future health research:

YES ☐ NO ☐

Signature of Participant _____ Date & Time _____

(Printed Name) _____

Signature of Witness _____ Date & Time _____

Signature of Investigator or Designee _____ Date & Time _____

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A COPY GIVEN TO THE RESEARCH PARTICIPANT

Title of Project: *H. pylori* Project

Principal Investigator: Karen Goodman Phone Number: 780-492-1889

Project Coordinator: Laura Aplin Phone Number: 1-855-492-2525 (Toll-free)

Do you have questions about the project at this time? [ADDRESS ANY QUESTIONS]
Are you satisfied by the information you have received about the project at this time?
[ADDRESS ANY ADDITIONAL CONCERNS UNTIL THE ANSWER IS YES] **Yes** ☐

Part 2 (to be completed by the research participant)	Yes	No
Do you understand that your child has been asked to be in a research study?	☐	☐
Have you read and received a copy of the attached Information Sheet?	☐	☐
Have I (study staff) answered your questions to your satisfaction?	☐	☐
Do you understand you can ask more questions later on if you like?	☐	☐
Do you understand the benefits and risks involved in taking part in this research study?	☐	☐
Do you understand that you are free to withdraw your child from the study at any time, without having to give a reason and without affecting your child's future medical care?	☐	☐
Has the issue of confidentiality been explained to you?	☐	☐
Do you understand that the project staff will have access to your child's health records?	☐	☐
Is it okay with you for us to give your child's tests results to the health center staff to include in your child's medical record?	☐	☐

Who explained this study to you? _____
Child's Name _____

I agree that my child can take part in this study:
YES ☐ NO ☐
I consent to storage of remaining samples collected from my child for use in future health research:

YES ☐ NO ☐

Signature of Parent or Guardian _____ Date & Time _____

(Printed Name) _____

Signature of Witness _____ Date & Time _____

Signature of Investigator or Designee _____ Date & Time _____

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A COPY GIVEN TO THE RESEARCH PARTICIPANT

Health Survey

Date: Day __ __ Month __ __ Year __ __ __ __ Interviewer Name: _____

Participant Name: _____ ID number: __ __ __ -__ __

Date of Birth: Day __ __ Month __ __ Year __ __ __ __ Gender: ❑ Male ❑ Female

Respondent: ❑ Participant ❑ Participant's mother ❑ Participant's father
❑ Other; specify relation to participant:
_____

Person assisting respondent in answering questions: ❑ None ❑ Participant ❑ Parent ❑ Interpreter
❑ Other; specify relation to participant: _____

Place of interview: ❑ Participant's home ❑ Fort McPherson Health centre
❑ Learning Center ❑ Other; specify _____

The purpose of this interview is to collect information about your health that we need to know for your participation in the project. Before we get started, please let me know if you would prefer to be interviewed by someone else (for example, someone of the opposite sex or someone who does not live in your community). If you are comfortable starting the interview now, we will start by assuring you that all of your answers to our questions will be strictly confidential. The project team will not reveal personal information about you to anyone.

1. When you are sick where do you go for care? ***(mark all that apply)***
   - ❑ Health Centre/Nursing Station
   - ❑ Regional Hospital; specify_____
   - ❑ Other; specify_____
   - ❑ Unsure
   - ❑ Refused to answer

Next, we would like to ask you some questions about *Helicobacter pylori* infection.

2. Have you heard of *Helicobacter pylori* or *H. pylori* infection?
   - ❑ Yes ➔ ➔ ➔ ➔
   - ❑ No
   - ❑ Unsure / don't remember
   - ❑ Refused to answer

If yes, answer 2a-2c

2a. Can you tell me what kind of illness it causes as far as you know or from what you have heard?
- ❑ Yes; specify all the illnesses: _____
- ❑ No ❑ Unsure ❑ Refused to answer

2b. Do you know or have an idea about how people get it?
- ❑ Yes; specify: _____
- ❑ No ❑ Unsure ❑ Refused to answer

2c. How did you first find out about *H. pylori* infection? *(mark all that apply)*
- ❑ TV/Radio ❑ Newspapers/magazines
- ❑ Nurse/Doctor told me ❑ School
- ❑ Had it myself ❑ Family members who had it ❑ Friends who had it
- ❑ Other; specify: _____
- ❑ Unsure/don't remember ❑ Refused to answer

3. Are you worried about how *H. pylori* infection might affect your health?
   - ❑ Yes: please explain why you are worried: _____
   - ❑ No ❑ Unsure ❑ Refused to answer

4. Are you worried about how *H. pylori* infection might affect the health of others?
   - ❑ Yes: please explain why you are worried: _____
   - ❑ No ❑ Unsure ❑ Refused to answer

5. How interested are you in learning about overall results from the Fort McPherson *H. pylori* project?
   ❑ Very interested ❑ Somewhat interested ❑ Neutral ❑ Not interested

5a. Is there something about H. pylori or H. pylori associated diseases that you are hoping to learn?
   - ❑ Yes,: please specify: _____
   - ❑ No ❑ Unsure ❑ Refused to answer

The next set of questions are about your health.

6. To your knowledge, has anyone in your family been told by a doctor they have *H. pylori* infection?
   - ❑ Yes ➔ ➔ ➔ ➔
   - ❑ No
   - ❑ Unsure / don't remember
   - ❑ Refused to answer

   6a. If yes, specify which relative or relatives ***(mark all that apply)***;
   ❑ Parents ❑ Grandparents ❑ Brother or sister
   ❑ Child ❑ Aunt, uncle or cousin
   ❑ Other relative, specify: _____

7. Have you ever seen a nurse or doctor about stomach or esophagus (the tube that food goes down to get to your stomach) problems?
   - ❑ Yes ➔
   - ❑ No
   - ❑ Unsure / don't remember
   - ❑ Refused to answer

   7a. If yes, how long ago in either years or months?
   _____years _____months ❑ Unsure/don't remember ❑ Refused to answer

8. Have you ever been told by a nurse or doctor you have peptic ulcer disease (an ulcer in your stomach or duodenum)?
   - ❑ Yes ➔
   - ❑ No
   - ❑ Unsure / don't remember
   - ❑ Refused to answer

   8a. If yes, how long ago in either years or months?
   _____years _____months ❑ Unsure/don't remember ❑ Refused to answer

9. Have you ever been diagnosed by a doctor with acid reflux (heartburn) disease by gastroscopy or pH probe study?
   - ❑ Yes ➔
   - ❑ No
   - ❑ Unsure / don't remember
   - ❑ Refused to answer

   9a. If yes, how long ago in either years or months?
   _____years _____months ❑ Unsure/don't remember ❑ Refused to answer

10. Has anyone in your family had stomach cancer?
    - ❑ Yes ➔
    - ❑ No
    - ❑ Other cancer ➔ 10b. Specify which relative or relatives ***(mark all that apply);***
    - ❑ Unsure / don't remember
    - ❑ Refused to answer

    10a. If yes, specify which relative or relatives ***(mark all that apply);***
    ❑ Parents ❑ Grandparents ❑ Brother or sister ❑ Child
    ❑ Aunt, uncle or cousin ❑ Other relative, specify: _____

    ❑ Parents ❑ Grandparents ❑ Brother or sister ❑ Child
    ❑ Aunt, uncle or cousin ❑ Other relative, specify: _____

11. Have you ever been tested for *H. pylori*, before this research project?
    - ❑ Yes ➔ ➔ ➔ ➔
    - ❑ No
    - ❑ Unsure / don't remember
    - ❑ Refused to answer

    If yes:

    11a. How many times were you tested for *H. pylori*?
    _____ ❑ Unsure/don't remember ❑ Refused to answer

    If tested more than once: 10a1. How long ago in years or months was your most recent test? _____years _____months ❑ Unsure/don't remember ❑ Refused to answer

    11b. How long ago were you first tested for *H. pylori*?
    _____years _____months ❑ Unsure/don't remember ❑ Refused to answer

    11c. Where were you tested for *H. pylori*? _____

    11d. What kind of test did you have for *H. pylori* (mark all that apply)
    ❑ Breath test ❑ Blood test ❑ Gastroscopy (camera/scope test)
    ❑ Other test; specify test: _____
    ❑ Unsure / don't remember ❑ Refused to answer

    11e. Have you ever tested positive for *H. pylori*?
    ❑ Yes ❑ No ❑ Unsure/don't remember ❑ Refused to answer
    If yes: 11e1. Was your first H. pylori test positive?
    ❑ Yes ❑ No ❑ Unsure/don't remember ❑ Refused to answer
    If tested more than once: 11e2. Was your most recent H. pylori test positive?
    ❑ Yes ❑ No ❑ Unsure/don't remember ❑ Refused to answer

12. Have you ever been treated with antibiotics for *H. pylori*?

❑ Yes ➔ ➔ ➔
❑ No
❑ Unsure / don't remember
❑ Refused to answer

If yes:

12a. How many times were you treated for *H. pylori*?
_____ ❑ Unsure/don't remember ❑ Refused to answer

If treated more than once:
12a1. How long ago in years or months was the most recent time that you were treated?
_____years _____months ❑ Unsure/don't remember ❑ Refused to answer
12a2. Where were you treated with antibiotics for *H. pylori* the most recent time that you were treated?
_____

12b. How long ago in years or months was the first time you were treated?
_____years _____months ❑ Unsure/don't remember ❑ Refused to answer
12c. Where were you treated with antibiotics for *H. pylori* the first time you were treated?
_____
12d. Did you complete the full course of antibiotics prescribed each time?
❑ Yes ❑ No ❑ Unsure/don't remember ❑ Refused to answer
12e. Did you ever get retested after being treated for *H. pylori* infection?
❑ Yes ❑ No ❑ Unsure/don't remember ❑ Refused to answer
12e1. Where were you retested for *H. pylori* infection?
_____

13. Have you ever had a gastroscopy (scope test with a camera to look inside your stomach) procedure before?

❑ Yes ➔ ➔ ➔ ➔
❑ No
❑ Unsure / don't remember
❑ Refused to answer

If yes:

13a. How many times did you have a scope test of the stomach?
_____ ❑ Unsure/don't remember ❑ Refused to answer

13b. How long ago in years or months was your most recent gastroscopy?
_____years _____months ❑ Unsure/don't remember ❑ Refused to answer
13c. Where did you have your most recent gastroscopy done?
_____

14. Do you ever take anti-inflammatory medications (for example, Advil, Motrin, Ibuprofen, Naproxen, Naprosyn, Indocid, Indomethacin, Celebrex, Vioxx)?
❑ Yes; specify which: _____
a. If yes, how many do you take in a typical day or a week? _____ per day_____ per week ❑ less often than weekly
❑ No ❑ Unsure ❑ Refused to answer

15. Do you ever take aspirin (ASA, acetylsalicylic acid)?

❑ Yes ➔ ➔ ➔
❑ No
❑ Unsure / don't remember
❑ Refused to answer

15a. If yes, how many aspirin do you take in a typical day or a week?
_____ per day _____ per week ❑ less often than weekly
❑ Unsure/don't remember ❑ Refused to answer

16. Do you ever take Plavix (clopidogrel)?

❑ Yes ➔ ➔ ➔
❑ No
❑ Unsure / don't remember
❑ Refused to answer

16a. If yes, how many plavix do you take in a typical day or a week?
_____ per day _____ per week ❑ less often than weekly
❑ Unsure/don't remember ❑ Refused to answer

17. Have you taken any medications for your stomach or heartburn in the last 30 days?

❑ Yes ➔ ➔ ➔
❑ No
❑ Unsure / don't remember
❑ Refused to answer

If yes: 17a. Specify which medications you take for your stomach or heartburn? ***(mark all that apply)***

❑ 1. Maalox _____ per day ____ per week ❑ less often than weekly
❑ 2. Pepto-Bismo _____ per day ____ per week ❑ less often than weekly
❑ 3. TUMS/Rolaids _____ per day ____ per week ❑ less often than weekly
❑ 4. Ranitidine (Zantac) _____ per day ____ per week ❑ less often than weekly
❑ 5. Famotidine (Pepcid) _____ per day ____ per week ❑ less often than weekly
❑ 6. Cimetidine (Tagamet) _____ per day ____ per week ❑ less often than weekly
❑ 7. Pantoprazole (Pantaloc)_____ per day ____ per week ❑ less often than weekly
❑ 8. Omeprazole (Losec) _____ per day ____ per week ❑ less often than weekly
❑ 9. Lansoprazole (Prevacid)_____ per day ____ per week ❑ less often than weekly
❑ 10. Esomeprazole (Nexium)_____ per day ____ per week ❑ less often than weekly
❑ 11. Rabeprazole (Pariet) _____ per day ____ per week ❑ less often than weekly
❑ 12. Vitamins _____ per day ____ per week ❑ less often than weekly
❑ 13. Other medications, specify:
_____ _____ per day ____ per week ❑ less often than weekly
❑ Unsure
❑ Refused to answer

Now, we will ask you about symptoms related to stomach problems.

18. Do you have difficulty swallowing solid food?
❑ Yes ❑ No ❑ Unsure ❑ Refused to answer

19. Do you have unexplained weight loss (more than 10% of your normal weight)?
❑ Yes ❑ No ❑ Unsure ❑ Refused to answer

20. Do you have recurrent vomiting?
❑ Yes ❑ No ❑ Unsure ❑ Refused to answer

Please take a few moments to think about any stomach problems you may have had in the past 6 months. Use the scale below to indicate the severity of your symptoms lasting longer than 3 months. Use the severity scale provided by the interviewer if it is helpful.

1. No problem
2. Minimal problem (can be easily ignored without effort)
3. Mild problem (can be ignored with effort)
4. Moderate problem (cannot be ignored but generally does not limit my daily activities)
5. Moderately severe problem (cannot be ignored and occasionally limits my daily activities)
6. Severe problem (cannot be ignored and often limits my daily activities)
7. Very severe problem (cannot be ignored and markedly limits my daily activities and often requires rest)

	1	2	3	4	5	6	7	Unsure	RTA
21. Upper abdominal symptoms, overall	❑	❑	❑	❑	❑	❑	❑	❑	❑
22. Epigastric (middle of abdomen just below breast bone) pain (or unpleasant sensation)	❑	❑	❑	❑	❑	❑	❑	❑	❑
23. Epigastric discomfort	❑	❑	❑	❑	❑	❑	❑	❑	❑
24. Epigastric burning	❑	❑	❑	❑	❑	❑	❑	❑	❑
25. Feeling full too long	❑	❑	❑	❑	❑	❑	❑	❑	❑
26. Feeling full even though you ate a small amount	❑	❑	❑	❑	❑	❑	❑	❑	❑
27. Heartburn (burning sensation under the lower part of the centre of the chest which rises towards or into the neck)	❑	❑	❑	❑	❑	❑	❑	❑	❑
28. Acid regurgitation (backward flow or sour or bitter fluid from the stomach into the food pipe)	❑	❑	❑	❑	❑	❑	❑	❑	❑
29. Upper abdominal bloating	❑	❑	❑	❑	❑	❑	❑	❑	❑
30. Excessive belching	❑	❑	❑	❑	❑	❑	❑	❑	❑
31. Nausea	❑	❑	❑	❑	❑	❑	❑	❑	❑
32. Other stomach or digestive	❑	❑	❑	❑	❑	❑	❑	❑	❑

symptoms									
32a. Specify symptom: _____ _	❑	❑	❑	❑	❑	❑	❑	❑	❑
32b. Specify symptom: _____ _	❑	❑	❑	❑	❑	❑	❑	❑	❑

33. ***(This question is for participants of ages 15 years and older).*** If the project were to offer endoscopy, which is a thin scope sent down into your stomach to test and look for *H. pylori* infection or other stomach problems, would you be willing to consider undergoing an endoscopy procedure?

❑ Yes; specify why: _____

❑ No; specify why not: _____

❑ Unsure; specify what you would need to make up your mind one way or another: _____ _____

Thank you for taking the time to tell us about your health. We will be contacting you about scheduling tests that will be offered as part of this study.

Participant Questionnaire – Individual Level Socio-environmental Exposures

Date: Day __ __ Month __ __ Year __ __ __ __ Interviewer Name: _____

Participant Name: _____ ID number: __ __ __ -__ __

Date of Birth: Day __ __ Month __ __ Year __ __ __ __

Respondent: ❑ Participant ❑ Participant's mother ❑ Participant's father ❑ Other; specify relation to participant: _____

Person assisting respondent in answering questions: ❑ None ❑ Participant ❑ Parent ❑ Interpreter ❑ Other; specify relation to participant: _____

Place of interview: ❑ Participant's home ❑ Health centre/Nursing Station ❑ Learning Centre ❑ Other; specify _____

A goal of the *H. pylori* Project is to see what we can learn about how people get *H. pylori*. To do this, we need to compare people with and without *H. pylori* to see how they might differ on things that might influence their risk of infection. For this reason, we are asking everyone the same set of questions. Achieving the project goals depends on getting accurate information from everyone. Please answer each question as accurately as you can. There are no right answers; no one will judge you; we just want to know what is true for you. You can tell me if you don't know or don't wish to answer. Before we get started, please let me know if you would prefer to be interviewed by someone else. If you are comfortable starting the interview now, we will start by assuring you that all of your answers to our questions will be strictly confidential. We will not reveal personal information about you to anyone.

We'll start by asking some basic questions about you.

1. Where did you live while you were growing up? Please list each place chronologically _____
_____

2. Where were these family members born and raised? You can be as general or specific as you know:
a. mother:_____
b. father: _____
c. mother's mother:_____
d. mother's father:_____
e. father's mother:_____
f. father's father: _____

3. If you don't mind saying, are these parents biological or adoptive? (If you have more than one set of parents, tell us about those who primarily raised you.)

a. Mother:	❑ Biological	❑ Adoptive	❑ Unsure	❑ Refused to answer
b. Father:	❑ Biological	❑ Adoptive	❑ Unsure	❑ Refused to answer

4. How much schooling did your parents complete? (If you have more than one set of parents, tell us about those who primarily raised you.)
a. Mother's grade or level completed:_____
b. Father's grade or level completed: _____

1. What is your employment status?
❑ Currently regularly employed→specify occupation_____
❑ Currently casually employed→specify usual occupation _____
❑ Seasonally employed →specify usual occupation _____
❑ Not employed→specify last occupation if any ***(if never employed, write "none")*** _____
❑ Unsure ❑ Refused to answer

What is your main source of financial support or income at present? ***(check one)***
❑ Parents/relatives (skip question 11)
❑ Employment
❑ Business/self employed
❑ Employment insurance (EI)
❑ Income support
❑ Pension
❑ Other; specify _____
❑ Unsure ❑ Refused to answer

2. How many people, including you, contribute income from any source to your household? _____

7a. ***(If respondent is the only person who contributes income)***: Can you tell me your monthly or yearly income? (check one)
❑ Yes → 11c
❑ No
❑ Unsure
❑ Refused to answer

7b. ***(If more than one person contributes income)***: Can you tell me your monthly or yearly income for yourself or your household or both? ***(check one)***
❑ Yes for self only → 11c
❑ Yes for household → 11d
❑ Yes for both → 11c and 11d
❑ Don't know either self or household
❑ Refused to answer both self and household

7c. Personal income
11c1. Approximately how much is your individual yearly income (produced by yourself alone)? (If you don't know yearly, tell me monthly and we will multiply by 12)
❑ <$10,000 ❑ $10,000 – 24,999
❑ $25,000 – 34,999 ❑ $35,000 - 49,999
❑ $50,000 -74,9999 ❑ >=$75,000
11c2. How many dependents does your individual income support (including you)?
_____ ❑ Unsure ❑ Refused to answer

7d. Household income
11d1. Approximately how much is the yearly income for your household? (If you don't know yearly, tell me monthly and we will multiply by 12)
❑ <$10,000
❑ $10,000 – 24,999
❑ $25,000 – 34,999 ❑ $35,000 - 49,999
❑ $50,000 -74,9999 ❑ >=$75,000

11d2. How many people does your household income support (including you)?
_____ ❑ Unsure ❑ Refused to answer

Diet
We would like to know how many servings of certain foods you ate in the last week, and how many you usually eat in a typical week in summer and winter.

	Number of servings					
		Do you eat these items seasonally or all year round? If all year, please fill out column b If seasonally, please fill out relevant columns c to f (indicate 0 if none eaten in that season)				
	In the past week	**Typically all year**	**Typical week: Spring**	**Typical week: summer**	**Typical week: Fall**	**Typical week: Winter**
(Approximate serving sizes specified)	*(If unsure enter "777", if refused to answer enter "888")*					
8. **Fresh fruit (1 whole or ½ cup diced)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
9. **Fruit juice (from real fruit) (½ cup = 4 oz, small glass), not Sunny D or anything from powder**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
10. **Raw vegetables (½ cup)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
11. **Cooked vegetables (½ cup)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
12. **Locally caught fish raw (6-8 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
13. **Locally caught fish cooked (6-8 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
14. **Locally caught fish smoked, salted, cured or dried (6-8 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____

15. **Store bought fish raw (6-8 oz)**						
16. **Store bought fish cooked (6-8 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
17. **Store bought fish smoked, salted, cured or otherwise processed (6-8 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
18. **Uncooked fish eggs (6-8 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
19. **Locally harvested meat or poultry raw (ex. muskox, caribou, polar bear, seal, whale, goose, duck, ptarmigan) (6-8 oz); specify: _____**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
20. **Locally harvested meat or poultry cooked (6-8 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
21. **Locally harvested meat or poultry smoked, salted, cured or dried (6-8 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
22. **Store bought meat or poultry raw (6-8 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
23. **Store bought meat or poultry cooked (6-8 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
24. **Store bought meat or poultry smoked, salted, cured or otherwise processed (6-8 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
25. **Muktuk raw (6-8oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
26. **Muktuk cooked (6-8oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
27. **Oil (ex. whale oil, seal oil) (1 Tbsp); specify: _____**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
28. **Locally harvested eggs (ex. goose eggs, duck eggs, seagull eggs) (1 egg)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
29. **Store bought eggs (1 egg)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
30. **Fresh milk (1 cup = 8 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
31. **Canned or packaged milk (1 cup = 8oz), specify: _____**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
32. **Yogurt (1 cup = 8 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
33. **Pop, non-diet (12 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
34. **Pop, diet (12 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
35. **Coffee (8 oz)**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
36. **Tea (8 oz), specify type: _____**	a. ____	b. ____	c. ____	d. ____	e. ____	f. ____
37. **Salty snacks (ex: chips, pretazels) (1oz = 28 g)**	**a.** ____	**b.** ____	**c.** ____	**d.** ____	**e.** ____	**f.** ____

38. Do you add salt to prepared food?

❑ Yes→ 40a: How often: ❑ Every meal ❑ Sometimes ❑ Rarely

40b: When you add salt, how many pinches or shakes do you usually add:_____

❑ No

❑ Unsure ❑ Refused to answer

Now we will ask some questions about hygiene or personal care. We know that this is personal, but we would like you to be as accurate as possible so the project can be successful. Everything you tell us will be kept strictly confidential. We would like to emphasize that there are no correct answers. When it comes to personal hygiene, people differ in their practices, and one way is not always healthier.

39. How often do you usually brush your teeth?

❑ Never

❑ Less than once per week

❑ A few times per week

❑ Once per day

❑ Twice per day

❑ After every meal

❑ Unsure ❑ Refused to answer

40. Do you share a toothbrush?

❑ Yes→ please indicate the number of people who also use this toothbrush: 40b. _____

❑ No ❑ Unsure ❑ Refused to answer

41. How often do you usually use mouthwash?

❑ Never

❑ Less than once per week

❑ A few times per week

❑ Once per day

❑ Twice per day

❑ After every meal

❑ Unsure ❑ Refused to answer

42. How often do you usually take a bath or shower?

❑ Never

❑ Less than once per week

❑ Once per week

❑ A few times per week

❑ Nearly every day

❑ Every day without fail

❑ Unsure ❑ Refused to answer

43. How often do you drink from the same containers as others without washing it first?

❑ Every day

❑ A few times per week

❑ A few times per month

❑ Very infrequently

❑ Never

❑ Unsure ❑ Refused to answer

44. How often do you eat from the same dish or bowl that others are eating from?

❑ Every day

❑ A few times per week

❑ A few times per month

❑ Very infrequently

❑ Never

❑ Unsure ❑ Refused to answer

45. How often do you wash/sanitize your hands:

47a: After using the washroom

❑ never/rarely ❑ usually ❑ always ❑ Unsure ❑ Refuse to answer

47b: Before eating

❑ never/rarely ❑ usually ❑ always ❑ Unsure ❑ Refuse to answer

47c: Before preparing food

❑ never/rarely ❑ usually ❑ always ❑ Unsure ❑ Refuse to answer

47d: After handling raw meat, poultry, fish, or muktuk
❑ never/rarely ❑ usually ❑ always ❑ Unsure ❑ Refuse to answer

47e: After handling raw eggs
❑ never/rarely ❑ usually ❑ always ❑ Unsure ❑ Refuse to answer

46. When you were a baby, as far as you know, were you fed food that was chewed for you first by the person feeding you?
❑ Yes ❑ No ❑ Unsure ❑ Refused to answer

47. When you were a baby, as far as you know, were you breastfed?
❑ Yes ❑ No ❑ Unsure ❑ Refused to answer

49a. If yes, until about what age in months or years were you breastfed?
_____ months _____ years ❑ Unsure ❑ Refused to answer

Now we would like to ask about some other practices that may affect a person's health.

48. ***[SKIP THIS QUESTION FOR CHILDREN IF A PARENT IS THE RESPONDENT]*** Do you smoke cigarettes?
❑ Yes ➔
❑ No
❑ Unsure / don't remember
❑ Refused to answer

50a. If yes, how many cigarettes do you smoke in either a day or in a week?
_____ per day _____ per week _____ less often
❑ Unsure/don't remember ❑ Refused to answer

49. ***[SKIP THIS QUESTION FOR CHILDREN IF A PARENT IS THE RESPONDENT]***
Do you ever smoke anything (such as a cigarette, pipe, joint - you don't have to say what) that has been passed around to one or more other people to smoke from; for example, someone starts it and passes it around to share?
❑ Yes; in a typical week:
a) On how many days do you share a smoke this way (one or more puffs from a shared cigarette, pipe, etc.):
_____ days / week ***(If unsure enter "777", if refused to answer enter "999")***
b) How many other people do you smoke with this way in a typical week:
_____ other people / week ***(If unsure enter "777", if refused to answer enter "999")***
❑ No ❑ Unsure ❑ Refused to answer

50. [***SKIP THIS QUESTION FOR CHILDREN IF A PARENT IS THE RESPONDENT]***
Do you drink alcohol?
❑ Yes ➔ ➔ ➔ ➔
❑No
❑Unsure / don't remember
❑Refused to answer

52a. If yes, what do you drink?
❑ Home Brew ❑ Store Bought
If Home brew, how long do you keep it for before you drink it? _____
52b. If yes to either, how many drinks do you have in a day or week?
52b1: Home brew _____ per day _____ per week less often, specify_____
❑ Unsure/don't remember ❑ Refused to answer
52b2: Store bought _____ per day _____ per week less often, specify_____
❑ Unsure/don't remember ❑ Refused to answer

51. Have you yourself ever regularly been the caretaker for one or more animals (such as pets or livestock), doing any of the following: feeding, grooming, cleaning up after, petting or playing with?
❑ Yes ❑ No ❑ Unsure ❑ Refused to answer

If Yes: List which type of animal	Indicate if you care for them now and during what age periods of your life you have done this* ***(If unsure enter "777", if refused to answer enter "888")***
a) _____	❑ Now _____start age _____end age _____total years
b) _____	❑ Now _____start age _____end age _____total years
c) _____	❑ Now _____start age _____end age _____total years
c) _____	❑ Now _____start age _____end age _____total years
d) _____	❑ Now _____start age _____end age _____total years
e) _____	❑ Now _____start age _____end age _____total years
f) _____	❑ Now _____start age _____end age _____total years
g) _____	❑ Now _____start age _____end age _____total years
h) _____	❑ Now _____start age _____end age _____total years
i) _____	❑ Now _____start age _____end age _____total years
j) _____	❑ Now _____start age _____end age _____total years
k) _____	❑ Now _____start age _____end age _____total years
l) _____	❑ Now _____start age _____end age _____total years
m) _____	❑ Now _____start age _____end age _____total years
n) _____	❑ Now _____start age _____end age _____total years

o) _____ ❑ Now _____start age _____end age _____total years

**if there were multiple periods: start age is at the start of the first time and end age is at the end of the last time or current age if this continues at present; total years is the total number of years spent caring for this animal not counting time in between periods*

52. Have you ever **fished**? ❑ Yes ❑ No ❑ Unsure ❑ Refused to answer

53. Have you ever **hunted or gone trapping**? ❑ Yes ❑ No ❑ Unsure ❑ Refused to answer

54. Have you ever **cleaned fish or game**? ❑ Yes ❑ No ❑ Unsure ❑ Refused to answer

54a. If you answered yes to 56, have you ever done field dressing?
❑ Yes ❑ No

54b. If you answered yes to 56, have you ever finished cleaning fish or game after field dressing?
❑ Yes ❑ No

55. Have you ever **smoked locally harvested fish or game**?
❑ Yes ❑ No ❑ Unsure ❑ Refused to answer

56. Have you ever **cooked, grilled, or barbecued locally harvested fish or game**?
❑ Yes ❑ No ❑ Unsure ❑ Refused to answer

If yes to any of the above (Q52-56):

List which type of fish or game	Indicate if you still fish, hunt, trap, clean or cook this fish/game now and during what age periods of your life have done this* ***(If unsure enter "777", if refused to answer enter "888")***			
a) _____	❑ Now	_____start age	_____end age	_____total years
b) _____	❑ Now	_____start age	_____end age	_____total years
c) _____	❑ Now	_____start age	_____end age	_____total years
d) _____	❑ Now	_____start age	_____end age	_____total years
e) _____	❑ Now	_____start age	_____end age	_____total years
f) _____	❑ Now	_____start age	_____end age	_____total years
g) _____	❑ Now	_____start age	_____end age	_____total years
h) _____	❑ Now	_____start age	_____end age	_____total years
i) _____	❑ Now	_____start age	_____end age	_____total years
j) _____	❑ Now	_____start age	_____end age	_____total years
k) _____	❑ Now	_____start age	_____end age	_____total years
l) _____	❑ Now	_____start age	_____end age	_____total years
m) _____	❑ Now	_____start age	_____end age	_____total years
n) _____	❑ Now	_____start age	_____end age	_____total years
o) _____	❑ Now	_____start age	_____end age	_____total years
p) _____	❑ Now	_____start age	_____end age	_____total years
q) _____	❑ Now	_____start age	_____end age	_____total years
r) _____	❑ Now	_____start age	_____end age	_____total years
s) _____	❑ Now	_____start age	_____end age	_____total years
t) _____	❑ Now	_____start age	_____end age	_____total years
u) _____	❑ Now	_____start age	_____end age	_____total years
v) _____	❑ Now	_____start age	_____end age	_____total years
w) _____	❑ Now	_____start age	_____end age	_____total years
x) _____	❑ Now	_____start age	_____end age	_____total years
y) _____	❑ Now	_____start age	_____end age	_____total years
z) _____	❑ Now	_____start age	_____end age	_____total years
1) _____	❑ Now	_____start age	_____end age	_____total years
2) _____	❑ Now	_____start age	_____end age	_____total years
3) _____	❑ Now	_____start age	_____end age	_____total years
4) _____	❑ Now	_____start age	_____end age	_____total years
5) _____	❑ Now	_____start age	_____end age	_____total years
6) _____	❑ Now	_____start age	_____end age	_____total years
7) _____	❑ Now	_____start age	_____end age	_____total years
8) _____	❑ Now	_____start age	_____end age	_____total years
9) _____	❑ Now	_____start age	_____end age	_____total years
10) _____	❑ Now	_____start age	_____end age	_____total years

**if there were multiple periods: start age is at the start of the first time and end age is at the end of the last time or current age if this continues at present; total years is the total number of years spent caring for this animal not counting time in between periods*

(Continued)

List which type of fish or game	Thinking across the periods of your life when you did this, during a typical year, how many of these animals would you*…			
	Catch, kill or trap	Clean, smoke or cook	Come into contact with its Blood	Insides
a)				
b)				
c)				
d)				
e)				
f)				
g)				
h)				
i)				
j)				
k)				
l)				
m)				
n)				
o)				
p)				
q)				
r)				
s)				
t)				
u)				
v)				
w)				
x)				
y)				
z)				
1)				
2)				
3)				
4)				
5)				
6)				
7)				
8)				
9)				
10)				

**fill numbers in grid*

57. Did you ever, including when you were a child ***(or a much younger child if respondent is a child)***, drink lake/river/creek water that was not treated at the water treatment plant - that is, taken directly from a river, lake or creek?

❑ Yes ❑ No ❑ Unsure ❑ Refused to answer

If yes: According to your best estimate how often did you drink:	***Please write down the frequencies per week, month or year (ex. write down 3 in the Week column for 3 times per week. (If participant took water from a certain source all the time, check "Always". If did not drink river water, enter "000", if unsure enter "777", if refused to answer enter "888")***							
	In the past 12 months				Between the age of 0-5 years			
	Week	Month	Year	Always	Week	Month	Year	Always
a) Untreated, unboiled lake/river/creek/pond water	____	____	____	❑	____	____	____	❑
b)Lake/river/creek/pond water that was boiled	____	____	____	❑	____	____	____	❑
c) Lake/river/creek/ pond water otherwise treated; specify: _____ __	____	____	____	❑	____	____	____	❑
d) Melted ice from lake/river/creek/pond (ice water, Immaq) {Do not include snow}	____	____	____	❑	____	____	____	❑
e) Snow	____	____	____	❑	____	____	____	❑

58. Have you ever taken a food safety course? ❑ Yes ❑ No ❑ Unsure ❑ Refused to answer

59. How often do you attend feasts?

❑ A few times per month ❑ Once per month ❑ A few times per year ❑ Once or twice per year
❑ Less than once per year ❑ Never ❑ Unsure ❑ Refused to answer

Household Questionnaire – Household Level Socio-environmental Exposures

Date ___/___/___ Interviewer _____ Household ID _____

Household Respondent Name: _____ ID number: __ __ __ -__ __

Person(s) assisting respondent with answering questions: ❑ None ❑ Interpreter
❑ Other(s); specify relation to respondent: _____

Place of interview: ❑ Respondent's home ❑ Fort McPherson Health centre
❑ Learning Centre ❑ Other; specify _____

To achieve the goals of the *H. pylori* Project we need to compare households of people with and without *H. pylori* to see how they differ. The purpose of our household survey is to ask a set of questions about each household. Some of these questions are similar to those we ask individuals, but it is important for the research to find out about families as well as individuals.

[*If respondent completed individual survey, the interviewer can say, "Remember…" and select from the following statements as needed*:] Achieving the project goals depends on getting accurate information. Please answer each question as accurately as you can. There are no right answers; no one will judge you; we just want to know what is true for you. You can tell me if you don't know or don't wish to answer. Before we get started, please let me know if you would prefer to be interviewed by someone else. If you are comfortable starting the interview now, we will start by assuring you that all of your answers to our questions will be strictly confidential. We will not reveal personal information about your household to anyone.

We are interested in looking for patterns in families, so we would like to know about each member of your immediate family and each person who lived in your household at least part of the time during the last year.

Name	Relation to respondent	Lives away	DOB dd/mm/yy	Older sibs*	Place born & raised**	Years in Community	School level***
1 Respondent	Respondent	❑			*Check participant registry questions*		
2		❑					
3		❑					
4		❑					
5		❑					
6		❑					
7		❑					
8		❑					
9		❑					
10		❑					
11		❑					
12		❑					
13		❑					

*Number of older siblings born to the mother who raised this family member;
**Place where this person's family was living when this person was born
***Highest grade or level completed

Do some of your family members usually spend part of the year away from the community (including on the land or other surrounding areas)?

❑ Yes; If yes:

Who?	Where?	Which months?
1) _____	_____	_____
2) _____	_____	_____
3) _____	_____	_____
4) _____	_____	_____
5) _____	_____	_____
6) _____	_____	_____

❑ No ❑ Unsure ❑ Refused to answer

We would like to ask some questions about conditions and practices in your household.

1. How long have members of your household lived at your current address?

____ years ____ months ❑ Unsure ❑ Refused to answer

2. How many times has your household moved to a new residence in the past 5 years?____ times ❑ Unsure
❑ Refused to answer

3. Does one of your household members own your home or is it rented?
❑ Own ❑ Rent public housing ❑ Rent private housing
❑ Other; specify: _____
❑ Unsure ❑ Refused to answer

4. Does someone in your household currently own a vehicle that runs?
❑ Yes ❑ No ❑ Unsure ❑ Refused to answer

4a. If the answer to 4 is yes, what type(s) and how many of each type?

Type?	Number?
1) _____	_____
2) _____	_____
3) _____	_____
4) _____	_____
5) _____	_____
6) _____	_____

❑ Unsure ❑ Refused to answer

5. How many of the following does your home have?
____ bedrooms ❑ Unsure ❑ Refused to answer
____ indoor bathrooms ❑ Unsure ❑ Refused to answer
____ living/dining/den ❑ Unsure ❑ Refused to answer
____ beds (any size) ❑ Unsure ❑ Refused to answer
____ sinks ❑ Unsure ❑ Refused to answer
____ showers ❑ Unsure ❑ Refused to answer
____ bathtubs ❑ Unsure ❑ Refused to answer
____ toilets ❑ Unsure ❑ Refused to answer

6. What type of floor is in your home? (Check all that apply)
❑ Carpet ❑ Lino/ Tile ❑ Wood
❑ Other (including combinations; specify:
_____
❑ Unsure ❑ Refused to answer

7. Where does your household usually get **drinking** water? (Check one)
❑ Treated water trucked to water tank ❑ Bottled water ❑ Lake/river/creek water
❑ By melting ice from a lake/river/creek ❑ Store filtered water
❑ Other; specify: _____
❑ Unsure ❑ Refused to answer

8. How often is your household **drinking** water treated or purified in your home?
❑ Always ❑ Usually ❑ Sometimes ❑ Not usually ❑ Never
❑ Unsure ❑ Refused to answer

8a. If the answer to 8 is always, usually, sometimes or not usually
How is your household **drinking** water treated or purified in your home? (Check all that apply)
❑ Boiling ❑ Chemical additive (iodine, bleach) ❑ Filter (example, Brita)
❑ Other; specify: _____
❑ Unsure ❑ Refused to answer

If filtered, what type of filter do you use? _____ How often do you change it? _____

9. How often do members of your household take water directly from the lake/river/creek for:
(Check one response for a-d and e if applicable)

	Always	Usually	Occasionally	Rarely	Never	Unsure	Refused to answer
a. Drinking	❑	❑	❑	❑	❑	❑	❑
b. Bathing	❑	❑	❑	❑	❑	❑	❑
c. Washing dishes	❑	❑	❑	❑	❑	❑	❑
d. Washing clothes	❑	❑	❑	❑	❑	❑	❑

e. Other uses; specify: _____	❑	❑	❑	❑	❑	❑	❑

10. How often does your household's water tank run out of water?
_____ times/year _____ times/month _____ times/week ❑ Very rarely/never

If the household ever runs out of water:
10a. When your household runs out of water, how long is it until you typically have the tank refilled?
_____ hours _____ days ❑ Unsure ❑ Refused to answer

11. How often does your household's water tank get cleaned?
❑More than once a year ❑Once a year ❑Every 2-3 years ❑Less than every 3 years
❑Unsure ❑Refused to answer

12. Has your household ever had any problems with the water or sewage?
❑ Yes ❑ No ❑ Unsure ❑ Refused to answer
If yes, please describe: _____
_____

13. How many of the following animals do you have living in or around your home? (enter 0 if none)
a. Dogs _____ ❑ Unsure ❑ Refused to answer
b. Cats _____ ❑ Unsure ❑ Refused to answer
c. Other; specify number: _____ and type: _____❑ Unsure ❑ Refused to answer
d. Other; specify number: _____ and type: _____❑ Unsure ❑ Refused to answer
e. Other; specify number: _____ and type: _____❑ Unsure ❑ Refused to answer
f. Other; specify number: _____ and type: _____❑ Unsure ❑ Refused to answer

14. Do you ever have problems with mice getting in your house (seen mice or mice droppings in your house)?
❑ Yes ❑ No ❑ Unsure ❑ Refused to answer
14a. If yes, how often in past 12 months?
_____ times/year _____ times/month _____ times/week

We would like to ask your household income for research purposes only. We will not report your income to anyone. (If you answered household income on the individual survey, this question can be marked as answered on individual survey.*)*

15. What is your best estimate (or guess) of the current combined annual income for everyone who lives in your household?
❑ Answered on individual survey
❑ <$10,000 ❑ 10,000-$24,999 ❑ $25,000-34,999 ❑ $35,000-49,999
❑ $50,000-74,999 ❑ >=$75,000
❑ No idea ❑ Refused to answer

We would like to know about your family's health practices.
16. Does your family seek traditional medicines for illnesses?
❑ Yes ❑ No ❑ Unsure❑ Refused to answer

16a. (If yes to 16) We would like to know which traditional medicines your family uses, if you don't mind telling us, and what you use them for.
(If you prefer not to tell us the names of traditional medicine, we would like to know the reasons or illnesses you use it for and if it depends on the season – please fill out reason for use and season of use only.)

Name of traditional medicine	Reason for use	Season of use or year-round
1)		
2)		
3)		
4)		
5)		
6)		