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## Epidemiological Study of Contributing Factors in the Development of Peptic Ulcer and Gastric Cancer Initiated by Helicobacter Pylori Infection in India

Rahul Suresh Mhaskar  
*University of South Florida*

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Epidemiological Study of Contributing Factors in the Development of Peptic Ulcer and Gastric  
Cancer Initiated by *Helicobacter Pylori* Infection in India.

by

Rahul Suresh Mhaskar

A dissertation submitted in partial fulfillment  
of the requirements for the degree of  
Doctor of Philosophy  
Department of Global Health  
College of Public Health  
University of South Florida

Major Professor: Boo Kwa, Ph.D.  
Ricardo Izurieta, M.D., DrPH, MPH, DTM&H  
Azliyati Azizan, Ph.D.  
Lakshminarayan Rajaram, Ph.D.

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## **Dedication**

I am thankful to all the study participants for their participation.

Successful completion of this project was made possible by the dedicated efforts of many people. Dr. Ricardo Izurieta has supported me as a professional mentor and guide throughout the four years it took me to complete my Doctor of Philosophy in Public Health degree. Dr. Djulbegovic and Dr. Kumar's foresight and experience, who are my mentors at work, were invaluable through this journey. The high standards of professionalism they exemplify will be a model for me throughout my career. As Chair of my department, Dr. Boo Kwa provided a strong source of support from classroom to project completion. Dr. Azizan and Dr. Rajaram, whose technical support as microbiology and statistics advisors were invaluable.

I am thankful to my father, mother and brother and other members of my family, including my in-laws for their support and encouragement. Finally, special thanks to my wife, Asmita, for her unshakable faith, constancy in presence, and willingness to travel with me through this journey and many more to come. Last but not the least, I am grateful to the most important person in my life my daughter, Rama, without her this world means nothing to me. But with her presence everything that I experience in reality and even imagine in my dreams has relevance and meaning. This project is dedicated to my family who has never failed to give me moral support, and teaching me that even the largest task can be accomplished with self belief.

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**Epidemiological Study of Contributing Factors in the Development of Peptic Ulcer and  
Gastric Cancer Initiated by *Helicobacter Pylori* Infection in India**

**Rahul Suresh Mhaskar**

**ABSTRACT**

Background: *Helicobacter pylori* (*H. pylori*) infection is a significant risk factor for peptic ulcer (PU) and gastric cancer (GC). Apart from the virulent *CagA* genotype of *H. pylori* environmental and dietary factors influence disease outcomes. There have been no studies addressing these factors in Western India. Hence, we conducted a case control study enrolling PU, GC patients and controls at Pune, India.

Methods: Risk factors for PU and *H. pylori* infection were assessed in participant interview. *H. pylori* status was assessed from stool by monoclonal antigen detection. To understand treatment effect, we followed 100 *H. pylori* positive patients.

Results: We enrolled 190 PU patients, 125 Controls and 35 GU patients. Prevalence of *H. pylori* was 61% among symptomatic patients and 45% among controls. *H. pylori* infection (OR: 1.70, 95% CI: 1.03-2.89), meat (OR: 1.10, 95% CI: 1.02-1.75), fish (OR: 1.05, 95% CI: 1.02-1.89) consumption, and family history of ulcer (OR: 1.20, 95% CI: 1.08-1.60) were risk factors for PU. Consumption of snacks with alcohol (OR: 0.32, 95% CI: 0.13-0.78) and history of anti-parasite treatment (OR: 0.51, 95% CI: 0.30-0.86) were protective factors against PU. Lower socioeconomic status (SES) (OR: 1.10, 95% CI: 1.02-1.39), meat consumption (OR: 2.35, 95% CI: 1.30-4.23), smoking (OR: 2.23, 95% CI: 1.24-4.02), eating restaurant food thrice per week (OR: 3.77, 95% CI: 1.39-10.23) and drinking non-filtered or non-boiled water (OR: 1.05, 95% CI: 1.01-1.23) were risk factors for *H. pylori* infection. Consumption of chili peppers (OR: 0.20,

95% CI: 0.10-0.37) and concurrent parasite infestation (OR: 0.44, 95% CI: 0.24-0.80) were protective against *H. pylori* infection. *H. pylori* infection was eradicated only in 53% (40/75) of treated patients.

Conclusion: This study indicates that *H. pylori* infection is associated PU. Consumption of meat, fish and family history of PU are risk factors for PU. Lower SES, consumption of restaurant food, meat, non filtered water and smoking are risk factors for *H. pylori* infection. Consumption of chili peppers and concurrent parasite infestation are protective against *H. pylori* infection while history of anti parasite treatment protects against PU. *H. pylori* were eradicated only in 53% of patients.

## Chapter One

### Introduction

#### Background

The Medicine Nobel Prize of 2005 was awarded to an observant pathologist Robin Warren and an enterprising physician Barry Marshal, both from Australia, for the discovery of *Helicobacter pylori* (*H. pylori*) and its role in peptic ulcer disease and gastritis in 1983.[1] Since its discovery, the organism has generated tremendous interest among the medical researchers. *H. pylori* are a micro-aerophilic, highly motile, gram-negative spiral organisms with 4-6 flagella at one end. The organism has the striking biochemical characteristic of abundant urease enzyme production. *H. pylori* have a special affinity for gastric mucosa and is etiologically associated with chronic active gastritis, peptic ulcer (duodenal and gastric) and gastric cancer.[1] Chronic gastritis induced by *H. pylori* is usually not symptomatic but is considered to be the background of several diseases, *i.e.*, peptic ulcer and gastric malignancies that typically occur in adulthood.[2] *H. pylori* infection is almost always acquired in early childhood and usually persists throughout life unless a specific treatment is given (spontaneous eradication is rare). *H. pylori* infects at least 50% of the world's human population [3] and poor socio-economic condition is regarded as the most important risk factor for acquisition of the infection.[2, 4]

The inside of the stomach is bathed in about half a gallon of gastric juice every day. Gastric juice is composed of digestive enzymes and concentrated hydrochloric acid, which can readily tear apart the toughest food or microorganism. Bacteria, viruses, and ingested food are all consumed in this deadly bath of chemicals. It used to be thought that the stomach contained no bacteria and

was actually sterile, but *H. pylori* changed that. [5]

The stomach is protected from its own gastric juice by a thick layer of mucus that covers the stomach lining. *H. pylori* take advantage of this protection by living in the mucus lining. Once *H. pylori* is safely ensconced in the mucus, it is able to fight the stomach acid that does reach it with an enzyme it possesses called Urease. Urease converts urea, of which there is an abundant supply in the stomach (from saliva and gastric juices), into bicarbonate and ammonia, which are strong bases. This creates a cloud of acid neutralizing chemicals around the *H. pylori*, protecting it from the acid in the stomach. The reaction of urea hydrolysis is important for diagnosis of *H. pylori* by the breath test (UBT). Another defense *H. pylori* has is that the body's natural defenses cannot reach the bacterium in the mucus lining of the stomach. The immune system will respond to an *H. pylori* infection by sending white cells, killer T cells, and other infection fighting agents. However, these potential *H. pylori* eradicators cannot reach the infection, because they cannot easily get through stomach lining. They do not go away either and lead to strengthened immune response. Polymorphs die, and spill their destructive compounds (superoxide radicals) on stomach lining cells. Extra nutrients are sent to reinforce the white cells, and the *H. pylori* can feed on this. This process results in gastritis and may eventually lead to peptic ulcer and / or stomach cancer. It may not be *H. pylori* itself which causes peptic ulcer, but the inflammation of the stomach lining; i.e. the response to *H. pylori*. [6]

### **Transmission of *Helicobacter pylori***

The reservoirs of *H. pylori* appear to be numerous, but the exact mode of transmission still remains unproven. In developing countries, poor sanitation makes the feco-oral route the most common route of transmission.[7] Dirty water, milk adulterated by dirty water, or consumption of uncooked vegetables irrigated with or washed in water contaminated by human excrement is believed to be responsible. In the developed world, oral-oral transmission is likely to be the

dominant route, especially in situations where there is overcrowding, and more so among children. [8]

In addition, communal eating habits and the parental practice of masticating food for children at weaning could be vehicles of infection transmission.[9] Young children also experience repeated colonization of the oral cavity by regurgitation, emphasizing the importance of the gastro-oral route. [10] Saliva and dental plaque can harbor *H. pylori*, and saliva could act as a vehicle of transmission. One of the most proven modes of transmission is via gastric intubation experiments or gastroscopy and removal of biopsy tissue from the stomach by non-disposable forceps.[10, 11] The conversion of *H. pylori* into viable but non-culturable coccoid form during adverse conditions and re-growth into the spiral vegetative form when conditions are favorable is a particularly intriguing concept. [12]

### **Changing prevalence of *H. pylori* infection**

The prevalence of *H. pylori* is different worldwide. In the United States infection is low among white and economically advantaged Americans. However, minority populations have a higher frequency of infection: United States data indicate that African American children aged 5 to 9 years have an overall infection frequency of 30%. Around the world, infection among children ranges from approximately 35% in Russia to 20% in China and Poland, 12% in Korea and America to <10% in France, Belgium and Finland.[13] [14] [15] [16-20] Despite high rates of infection in certain pockets of the globe, the frequency of *H. pylori* infection is declining worldwide. For instance, in one study conducted in Matsumoto, Japan, the rate of infection declined by up to 20% between 1986 and 1994 among individuals aged 9 to 70 years of age. [14, 21]

A recent study from Russia examined the effect of recent improvement in standards of living on the prevalence of *H. pylori* in Russian children by conducting 2 cross-sectional studies among

children in St. Petersburg. The first study was undertaken in 1995 and the second a decade later. *H. pylori* status was evaluated using the same ELISA method for anti *H. pylori* IgG (HM-CAP). The research found that the initial overall prevalence of *H. pylori* infection was 44% and decreased to 13% ten years later. In both studies, the prevalence increased with age. In 1995 the prevalence was 30% among children younger than 5 years. A decade later the prevalence in the same age group is 2%. The authors concluded that improvements in the standard of living in Russia have resulted in a marked reduction in *H. pylori* transmission. Different rates of acquisition *H. pylori* form the basis for the differences in prevalence of infection between and among populations. The changes in Russia are a dramatic example of how sensitive *H. pylori* acquisition is to improvement in standards of living.[14]

This finding regarding socioeconomic status (SES) during childhood holds true for all subgroups in the United States. For instance, in a pediatric study conducted in Arkansas, children coming from a family with an income of <\$5,000 had up to a 60% rate of infection between the ages of 11 and 20 years, whereas those with a family income of >\$25,000 had only a 15% rate of infection.[4] This phenomenon has also been observed in other countries. In recently developed countries, such as Korea although approximately 80% of individuals >20 years of age are infected; the prevalence of infection in young children is inversely related to the socioeconomic class of their family. Those aged 10 to 19 years who are in a high socioeconomic class had a 20% frequency of *H. pylori* infection, while those of the same age who are in a low socioeconomic class have a 60% frequency. [15]

A study from Kazakhstan was conducted among unrelated asymptomatic individuals between the ages 10 and 60 years and examined various aspects of the local household environment and access to water. [22] The study reported that transmission of *H. pylori* is largely opportunistic and can be water borne. If the rate of *H. pylori* transmission is to be reduced it will require an improvement in overall sanitation including waste disposal, clean water, and safe food as well as

in household hygienic practices. A cross sectional study conducted in children residing in industrial and rural areas in Italy reported that the sero-prevalence of *H. pylori* infection was significantly higher in children residing in rural areas compared to those in industrial areas. [23] The study also found that in rural areas, children having dogs were at greatest risk for *H. pylori* acquisition but this effect was not observed among children from the industrial areas.

#### Prevalence of gastric cancer in Asian countries

There is a marked international variation in gastric cancer incidence with highest rates reported from Japan. It is interesting to note that despite Japan being a developed country with a lower frequency of *H. pylori* infection; it has highest frequency of gastric cancer. Similarly, frequency of gastric cancer is quite high in China despite a lower frequency of *H. pylori* infection. In contrast, people living in less developed countries of Asia with high frequency of *H. pylori* infection [24-31] that is acquired at an earlier age have the lowest risk of developing gastric cancer.[32] It has also been observed that frequency of gastric cancer differs in different parts within many countries; for example, in Japan [33], variation in gastric cancer risk has been well documented in different regions and has been presumed to be related to variation in nutrient consumption. In China [34], gastric cancer mortality in Changle county is about 10-fold higher than that in Hong Kong and has been attributed to variation in frequency of *H. pylori* infection in the two regions. In India the prevalence of *H. pylori* has been reported in the range of 60 % to 80%. [27, 35-38] In India, southern [39] and eastern parts (personal observation) of the country experience somewhat higher frequency of gastric cancer than the western and northern parts of the country. This may be attributed to the underlying variation in the diet, host genetics and genotype of *H. pylori* between these diverse populations. Interestingly, similar epidemiological observations were made long ago in India in respect of another *H. pylori* related gastro-duodenal ailment, i.e., peptic ulcer disease. [40]

## Peptic ulcer and gastric cancer in India

Studies from India have shown an association between *H. pylori* infection and peptic ulcer.[31, 41, 42] However, studies from India failed to show an association between *H. pylori* infection and gastric cancer.[43-45] In a study on 50 patients with gastric cancer and 50 controls with non-ulcer dyspepsia, *H. pylori* infection was detected less frequently in gastric cancer (38%, 19/50) than those with non-ulcer dyspepsia (68%, 34/50).[43] Another study demonstrated that 64.7% (33/51) patients with gastric carcinoma and 74.4% (32/43) with non-ulcer dyspepsia had infection with *H. pylori*.[44] These studies can be criticized due to small sample size with a consequent type II statistical error. However, these studies had inadequate reporting of follow up time. Similarly, in most of these studies, endoscopy-based tests were used to diagnose *H. pylori* infection. Since *H. pylori* is less likely to survive in a stomach lining with metaplasia. Endoscopy-based tests can be false negative in patients with gastric cancer due to gastric atrophy and intestinal metaplasia .[46] However, studies from China and Japan showed association between *H. pylori* infection and gastric cancer. [47, 48] In essence, studies from India support the role of *H. pylori* as the most important risk factor for peptic ulcers while studies elucidating association between *H. pylori* and stomach cancer suffer from poor methodological quality.

There are some countries like India with highest frequency of *H. pylori* infection have the lowest risk of gastric cancer in contrast to the countries like Japan and China where gastric cancer risk is highest in the world despite a lower occurrence of *H. pylori* infection. This casts major objection to some of the simplified model of gastric carcinogenesis resulting from *H. pylori* infection that stated that if the infection is acquired at an early age particularly in presence of malnutrition, it may reduce gastric acid secretion, pangastritis and gastric cancer may be the likely outcome. In contrast, infection acquired later in life and in person with good nutritional status and normal gastric acid secretion would result in hyperchlorhydria and duodenal ulcer disease.[49] It is well documented in the literature that patients with duodenal ulcer infrequently or never develop



gastric cancer.[50] If this simplified model of gastric carcinogenesis would have been true, India, Bangladesh, Pakistan would have higher frequency of gastric cancer than Japan and China.[48] However, this is certainly not the case; there are certain factors apart from *H. pylori* infection such as dietary and environmental factors which modulate the prevalence of these diseases. In summary there are various factors along with *H. pylori* infection which determine the disease outcome. These factors are broadly classified into agent factor, host factors and environmental factors.

### **Risk factors for *H. pylori* infection**

Agent factors: *Cag* pathogenicity island and *CagA*

The *Cag* pathogenicity island (PAI) qualifies as an important virulence factor. Its presence is associated with an increased risk of peptic ulcer and gastric cancer. Biologically, the *Cag* PAI is associated with enhanced mucosal inflammation both in vivo and in vitro. The *Cag* PAI encodes a type IV secretory apparatus (a molecular syringe) that injects the *CagA* protein and other bacterial products into eukaryotic cells. [51] Most *H. pylori* in the world contain the *Cag* PAI, and the risk of a clinical outcome is significantly increased in association with its presence.

However at times peptic ulcer and gastric cancer can occur in individuals infected with *Cag* PAI negative *H. pylori*. Even within regions where essentially all *H. pylori* contain the *Cag* PAI, there are marked regional differences in cancer risk, which confirms that disease outcome involves an interaction among host, environment, and bacterial factors. [51]

So it appears that all strains of *H. pylori* are not pathogenic. Is it possible that people living in countries with lower frequency of gastric cancer are infected with non-pathogenic strains of *H. pylori* than people living in China or Japan? Peptic ulcer disease, which is associated with infection by pathogenic strains of *H. pylori*, has been reported a common problem in India and Bangladesh.[48]

Genotypic analysis of *H. pylori* strains from India showed pathogenic strains to be present in more than 80% of adults and children with gastro-duodenal diseases as well as in control population.[52, 53] Studies that used *CagA* antibody in patients with non-ulcer dyspepsia have shown that *CagA* antibody is detected in sera of most patients.[54] A recently completed large study on 279 patients with gastric neoplasms (263 gastric cancer and 16 primary gastric lymphoma) and controls (101 non-ulcer dyspepsia and 355 healthy subjects) showed that frequency of *CagA* IgG antibody was similar among the patients with gastric carcinoma and the controls, suggesting that difference in virulence factor of *H. pylori*, at least *CagA* alone, is unlikely to explain the variation in outcome of *H. pylori* infection.[55]

So, some environmental factors and host genetic factors along with bacterial virulence factors such as *CagA* are interplaying leading to diseases. In a study from US, Korea and Colombia [56] in which the first-degree relatives of patients with gastric cancer were evaluated to know whether similar strains of *H. pylori* or similar environmental factors are responsible for pattern of gastritis. However, this study failed to show any relationship between specific virulence factors or *H. pylori* strains and specific histologic pattern or outcome even among those sharing the same environment in childhood. [56] However, several studies from Japan and China [57, 58] showed that virulence factors of *H. pylori* are strongly associated with gastric carcinoma. Based on the available evidence, one cannot conclude that in Asian countries, despite high frequency of *H. pylori* infection, low frequency of gastric cancer is related to infection with only non-pathogenic strains. We are not aware of any studies that document the prevalence of the most virulent East Asian compared to less virulent Western *CagA* genotype in population from India. Indeed there are other factors such as host genetic factors and environmental factors at play which may explain this dilemma. [59]

## Host genetic factors

Host's genetic make-up determines in a major way response to any infection, including that to *H. pylori*. This is evidenced by the fact that relatives of patients with gastric cancer infected with *H. pylori* developed precancerous abnormalities like gastric atrophy and hypochlorhydria more often than those with non-ulcer dyspepsia. [60] Patients with duodenal ulcer, which is also caused by *H. pylori*, do not develop gastric cancer in contrast to other conditions associated with *H. pylori* infection, such as gastric ulcer, non-ulcer dyspepsia and hyperplastic gastric polyp. [61] These also depict variations in host's response despite infection with the same organism. Japanese immigrants to the United States have higher gastric cancer risk than native born Americans, though lesser than Japanese living in Japan [48]; this suggests the importance of the genetic factors with additive effects of environmental factors. Difference in carcinogenic risk in people living in different geographical areas might be related to variation in genetic make-up among different races. Specific allelic variation of different genes (polymorphism) present in a proportion of general population may determine variation in carcinogenic potential in different populations in response to environmental carcinogenic exposure, including that to *H. pylori* infection.[62] Genetic susceptibility of a person may be important in a number of carcinogenic processes that include: 1) mucosal protection against *H. pylori* infection and injury by other carcinogens; 2) mucosal inflammatory response to infection with *H. pylori*; 3) degree of apoptotic cell death; 4) carcinogen activation and detoxification by various enzyme systems of the hosts; 5) variability in the repair of mutated DNA; and 6) ability of the cell to proliferate in a controlled manner to repair the damage.

Several studies have been carried out on single nucleotide polymorphism in relation to gastric carcinogenesis. [62] However, many of these studies did not take into account the role of *H. pylori* infection and dietary factors in addition to the genetic factors. Therefore, there is need of more data on genetic polymorphism in relation to *H. pylori* infection and dietary factors.

It is impossible entirely to separate environmental factors from genetic influences. An early twin study on Swedish population suggested that there was a genetic component for acquiring *H. pylori* infection and a follow-up study on the same twins' population has questioned if there are genetic influences for peptic ulcer disease in common with genetic influences for *H. pylori* infection. [63] Comparisons of mono-zygotic and di-zygotic cross-twin and cross-trait correlations in that study demonstrated that despite the similarity in heritability for the two traits (peptic ulcer disease and *H. pylori* infection), the genetic influences for liability to peptic ulcer disease are independent of such genetic effects for acquiring *H. pylori* infection. It is feasible that the relationship between *H. pylori* and peptic ulcer disease could be mediated by familial environmental factors, (i.e. environmental experiences or situations that are shared by family members). Examples of some familial environmental factors that may mediate the association between *H. pylori* and peptic ulcer disease are diet, smoking, or drug consumption (e.g., alcohol, caffeine, non steroidal anti inflammatory drug consumption). [14]

#### Dietary and environmental factors

Diet may play a major role in gastric carcinogenesis. In India, southern [39] and eastern parts (personal observation) of the country experience somewhat higher frequency of gastric cancer than the northern parts of the country. Rice is the staple cereal in eastern India. Non-vegetarian foods, particularly fish, are very common in eastern Indian diet, which is also spicy with more salts. Diet in southern India is somewhat similar to that in eastern India with rice, fish, excess spice and salt being commonly eaten. In contrast, northern Indian diet is mainly wheat-based and a greater proportion of people are vegetarian. Tobacco smoking, high-temperature food intake, spicy food and rice eating have been shown to be risk factors for gastric cancer in India.[64] In another study, consumption of dry fish has been shown to be a risk factor for gastric cancer in India.[65] Diet has been considered to be a major factor for increased frequency of gastric and esophageal cancer in Kashmir province of India.[41] Similar observations have also been made in

several countries, including Japan where northern districts have reported a higher frequency of gastric cancer than southern district and this has been related to increased dietary intake of salts in northern districts.[32] Tobacco use and alcohol consumption are the other factors that may influence the international variation in frequency of gastric cancer.[66]

There are certain protective factors against *H. pylori* infection and diseases caused by it.

Interestingly there are certain elements in the diet which are noted be a protective factor against *H. pylori* and in turn lowering the risk of peptic ulcers and gastric cancer. Some examples are ginger, chili peppers, and turmeric.[67-70]

Another protective factor against disease caused by *H. pylori* infection which is recently shown promise in the research is concurrent helminth infection. Interestingly concurrent enteric helminth infection has shown to modulate inflammation and gastric immune responses and reduces helicobacter-induced gastric atrophy.[71] It is well know that the progression of helicobacter-induced gastritis and gastric atrophy mediated by type 1 T-helper cells may be modulated by concurrent parasitic infection. [71] Here, in mice with concurrent helminth infection, helicobacter-associated gastric atrophy was reduced considerably despite chronic inflammation and high helicobacter colonization. [71] Thus, concurrent enteric helminth infection can attenuate gastric atrophy, a premalignant lesion. Moreover in a study conducted among children in Columbia suggest that intestinal helminthiasis in children promotes Th2-polarizing responses to *H. pylori* and may decrease gastric cancer risk in these individuals later in life. [72] Concurrent helminthiasis may alter inflammatory responses to *H. pylori* and thus affect the progression of gastritis to gastric atrophy, dysplasia, and cancer. [72]

Summary of risk factors for *H. pylori* infection

In summary, it is estimated that 50% of the world’s population is infected with *H. pylori* and approximately 20% will develop peptic ulcer disease.[73] Factors that may influence the acquisition of *H. pylori* infection are geographic area, age, race, ethnicity, and SES.[74] The prevalence of *H. pylori* infection in developing countries can be >80% in children under 10 years old and in developed countries it can be up to 50% of children living in poor socioeconomic conditions.[75] High prevalence of infection have been reported in India, Africa, Latin America, and eastern Europe.[73] A study of a population from, the US-Mexico border, found a higher *H. pylori* sero-prevalence among preschool children in a developing country than in a developed country location indicating that geographic variation is an important risk factor.[76] Other risk factors that have been associated with high prevalence include having an infected mother [77], an infected older sibling [78], low SES, household crowding, migration from high prevalence regions, urban residence [78], indicators of poor nutritional status, drinking water source [79], consumption of raw vegetables [80], and low maternal education. [76, 81] Birth order has also been identified as a risk factor for *H. pylori*. A study done in Japan showed that younger siblings had a higher risk for *H. pylori* infection than older siblings.[82] We have summarized the risk factors for *H. pylori* infection in Table 1.

Table 1: Risk factors for *H. pylori* infection

Risk factor		Association with <i>H. pylori</i>	Reference
Gender	Male (adults)	Increased risk	Martel et al 2006 [83]
Age	Increasing age (in children)	Increased risk	Cullen et al 1993 [84]; Mendall et al 1992 [85]; McCollian et al 1997 [86]
	Increasing age (in adults)	Increased risk	Parsonnet et al 1992 [87]; Cullen et al 1993 [84];

Risk factor		Association with <i>H. pylori</i>	Reference
Animals	pets at home	increased risk	Rothenbacher 1998 [88]; Moreira et al, 2004 [81]; Herbarth et al, 2001 [89]
		no association	Webb et al, 1994 [90]; Luzzza et al, 1998 [91]; Bode et al, 1998 [92]; Bazzoli et al, 2001 [93]; Brown et al, 2001 [94]
	Sheep	higher risk	Goodman et al, 1996 [95]; Dore et al, 1999 [96]
Occupation	Endoscopists	increased risk	Liu et al, 1996 [97]; Lin et al, 1994 [98]; Su et al, 1996 [99]; Mitchell et al, 1999 [5]
		no association	Braden et al, 1997 [100]; Nishikawa et al, 1998[101]
	Dentists	no association	Lin et al, 1998 [102]; Banatvala et al, 1995 [103]
		increased risk	Matsuda et al, 2005 [104]
	endoscopy and dentistry nurses	no association	Lin et al, 1998 [102]; Banatvala et al, 1995 [103]
	direct contact with patients	Increased risk	Mastromarino et al, 2005 [105]
Water source		increased risk	Nurgalieva et al, 2002 [22]; Fujimura et al, 2004 [106]; Rolle-Kampczyk et al, 2004 [107]; Herbarth et al, 2001 [89]
		no association	Yamashita et al, 2001 [108]; Reshetnikov et al, 2001 [109]
Diet	consumption of fruit and vegetables	decreased risk	Goodman et al,1996 [95]; Goodman et al,1997 [80]; Fontham et al, 1995 [110]
	Garlic, ginger	decreased risk	Jonkers et al [67]
	Curcumin	decreased risk	Foryst-Ludwig et al [68]; Mahaday et al [69]
	Chili peppers	decreased	Jones et al [111]; Lopez Carillo et al [112]

Risk factor		Association with <i>H. pylori</i>	Reference
		risk	
	milk and fat	increased risk	Guo et al, 2002 [113]
	vitamin C	decreased risk	Jarosz et al. 1998 [114]; Goodman et al, 1997 [80]; Fontham et al, 1995 [110]
		increased risk	Malaty et al, 1998 [115]
	Beta-carotines	increased risk	Goodman et al, 1997 [80]
	Meat and fish	increased risk	Qasim, Webberly, Phukan, Olfasson [116-119]
	Salt	increased risk	Kato, Fox, Gamboa [120-123]
Previous antibiotic treatment		no influence	Rothenbacher et al, 1997 [124]; Tindberg 2001b [125]; Leung et al, 2002 [126]
		decreased risk	Rothenbacher et al, 1998 [127]; Bures et al, 2006 [128]
Socio-economic status	lower SES	increased risk	Reploge et al, 1995 [129]; EUROGAST study group, 1993 [130]; Malaty et al, 1992 [131]; 1994 [132]; 1996 [16]; Murray et al, 1997 [133]; Peach et al, 1997 [134]; Hopkins et al, 1993 [135]; Chow et al, 1995 [136]; Buckley et al, 1998 [137]; Rotbenbacher et al, 1998 [138]; Webb et al, 1994 [90]; Souto et al, 1998 [139]; Torres et al, 1998 [140]; Reshetnikov et al, 2003 [30]; Bani-Hani et al, 2006 [141]
		no association	Teh et al, 1994 [142]; Mendall et al, 1992 [85]; Koch et al, 2005 [143]
Hygiene practices	poor hygiene conditions	increased risk	Peach et al, 1997 [134]; Goodman et al, 1996 [95]
		no association	Breuer et al, 1996 [144]; Mendall et al, 1992 [85]



Risk factor		Association with <i>H. pylori</i>	Reference
	sharing a cup or toothbrush	decreased risk	Moreira et al, 2004 [81]; Al-Shamahy et al, 2005 [145]; Bani Hani et al, 2006 [141]; Peach et al, 1997 [134]; Breuer et al, 1996 [144]; Rodrigues et al, 2004 [146]
	raw vegetables	increased risk	Goodman et al, 1996 [95]; Hopkins et al, 1993 [135]
Attendance at a day care centre		increased risk	Parsonnet, 1995 [147]; Dore et al, 2002 [23]
		no association	Tindberg et al, 1999 [148]; Wizla-Derambure et al, 2001 [149]
Institutionalized persons		increased risk	Nessa et al, 2001 [150]; Wallace et al, 2002 [151]
Mouth-to-mouth contact		increased risk	Chow et al, 1995 [136]; Moreira et al, 2004 [81]
Living density	crowded environment	increased risk	Peach et al, 1997 [134]; Kikuchi et al, 2002 [152]; Goodman et al, 1996 [95]; Breuer et al, 1996 [144] Rothenbacher et al, 1997 [153] and 1998 [138]; Kyriazanos 2001 [154]; Grimm 2003 [155]; Rodrigues et al, 2004 [146]; Bani Hani et al, 2006 [141];
		no association	Tindberg, 2001[125]
Educational status of parents	higher educational status	decreased risk	Moreira et al, 2004 [81]; Bani Hani et al, 2006 [141]; Bures et al, 2006 [128]
Family	<i>H. pylori</i> positive family member	increased risk	Ma et al, 1998 [156]; Brenner et al, 1998 [157]; Goodman et al, 2000 [158]; Dominici et al, 1999 [159]; Rothenbacher et al, 1999 [160]; 2002 [161]; Tindberg, 2001 [125]; Rocha 2003 [162]; Kivi et al, 2005 [163]; Aguemon et al, 2005 [164]; Rowland et al, 2006 [77]
	siblings	increased risk	Kikuchi et al, 2002 [152]; Breuer et al, 1996 [144]; Glynn et al, 2002 [165]; Selimoglu et al, 2002 [166]; Farrell et al, 2005 [167]; Kivi et al, 2005 [163]
Parasites	Helminthes	Decreased risk	Fox et al [71, 168, 169]
CagA genotype		Increased risk	Azuma, Atherton, Al Marhoon ,Alarcon, Akimoto [170-176] Evans, Handa, Salih [177-179]

In summary, possible explanation of the reported low prevalence of peptic ulcer and stomach cancer with high prevalence of *H. pylori* in some parts of world such as India, might be related to difference in diet, environment factors (including helminthes co infection) and prevalence of *H. pylori* genotype between different countries. Hence to address this discrepancy we undertook a cross sectional study in Ecuador and Panama followed by a case control study in India.

## Chapter Two

### Study Methods Description

#### **Assessment of *H. pylori* prevalence and its risk factors among healthy individuals in Ecuador and Panama**

We conducted a cross sectional study in Ecuador and Panama to assess risk factors contributing to prevalence of *H. pylori* and its genotypes in healthy individuals. The study was conducted in Ecuador and Panama from May 2007 to August 2007. All participants were screened for age and medical history in order to select asymptomatic adult individuals. In this study, asymptomatic individuals were defined as those who have not had any subjective symptoms of gastric disorders or have not been diagnosed with any stomach diseases by a medical doctor within the last 6 months. Those individuals who had taken antibiotics within 6 months were excluded. A total of 90 and 75 participants living in Ecuador and Panama, respectively, were enrolled in the study. All of the participants gave their informed consent. The research protocols were approved by the ethics committees of the University of South Florida (Tampa, FL), Universidad Central del Ecuador (Quito, Ecuador), and Instituto Gorgas de Estudios de la Salud (Panama City, Panama).

#### Data Collection

##### *Clinical specimens:*

The study used stool samples. After signing the informed consent the participants were given an empty container for stool sample collection. The participants were requested to collect the stool sample and bring it back to the clinic the next day. The participants were notified of the parasitic

infections if detected. We carried out the tests in a laboratory in capital of Ecuador. The stool specimens were delivered to this laboratory at 4°C and stored at -20°C until used.

#### *Participant Interview:*

After getting the informed consent from the participants, local physicians participating in this research interviewed the participants using a structured questionnaire. The questionnaire dealt with issues of food habits, alcohol and cigarette consumption etc. Each interview lasted for on average of 20 minutes (range: 15 minutes - 45minutes). The interviewer marked the responses in the survey questionnaire. The form had a unique center and subject ID marked on it.

#### *Detection of Helminthes*

Stool samples were emulsified in Protifix, ALPHA TEC, Canada. The identification of helminthes was made morphologically following the guidelines of the American Society of Parasitology.

#### *Detection of Helicobacter pylori Antigen*

*H. pylori* antigen (catalase) in stool specimens was detected using a rapid test kit (Testmate Rapid Pylori Antigen, BD, Tokyo, Japan) by following the manufacturer's instructions. Briefly, a 20-mg stool specimen was taken and suspended in the sample dilution buffer (a component of the kit). One drop of the stool suspension was used for the immunochromatography test. The reaction was carried out at room temperature for 10 min.

The rapid test kit (Testmate Rapid Pylori Antigen, BD, Tokyo, Japan) uses monoclonal antibodies that react specifically to native catalase *H. pylori* antigens.[180, 181] If these antigens are present in the stool sample, they form immune complexes with the red latex-labeled anti-*H. pylori* mouse monoclonal antibodies and migrate by capillary action and then are captured by solid-

phase anti-*H. pylori* monoclonal antibodies to form a red test line. Red latex-labeled anti-*H. pylori* monoclonal antibodies not forming immune complexes migrate further up to be captured by solid-phase IgG rabbit polyclonal antibodies forming a red control line. The results were read in the test window as follows: the result was considered positive if both control and test lines appeared, negative if only the control line appeared, and uncertain if only the test line appeared.

Since the sensitivity and specificity of the antigen detection method used in this study were high, bacterial DNA was extracted from *H. pylori* antigen-positive stool specimens. The DNA extraction was performed using a QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The bacterial DNA extraction method we used has been previously used in *H. pylori* detection with good results. Approximately 1 g of each stool specimen was suspended in 3.0 mL ASL buffer (a component of the kit). After mixing the suspension, approximately 1.2 mL of supernatant was used for the extraction. The extracted DNA was dissolved in a volume of 200  $\mu$ L AE buffer. All extracted DNA was stored at  $-20^{\circ}\text{C}$  until used. [182]

As a template, 50 ng/ $\mu$ L or a 10-fold diluted solution of the extracted DNA was used for PCR in consideration of the reducing inhibitors present in stool specimens. For detecting the *16S rRNA* of *H. pylori*, real-time PCR was performed using TaqMan Universal PCR Master Mix (ABI, Foster City, CA) according to the method described by Yamazaki and colleagues using the "ABI PRISM 7700 Sequence Detection System" (Applied Biosystems). [183]

The conditions for real-time PCR were as follows:  $95^{\circ}\text{C}$  for 15 min, followed by 50 cycles consisting of  $95^{\circ}\text{C}$  for 15 seconds and  $60^{\circ}\text{C}$  for 1 minute.

For genotyping of *CagA*, nested PCR was adopted. The first-round PCR was performed with 2 primer sets: Forward/Reverse-1 and Forward/Reverse-2.

The conditions for the first-round PCR were as follows: 95°C for 2 minutes, followed by 40 cycles consisting of 94°C for 15 Seconds, 55°C for 30 Seconds, and 68°C for 1 minute.

The second-round PCR for genotyping of *CagA* was performed with 1 µL of the first-round PCR products as a template.

The second-round PCR conditions were as follows: 94°C for 2 minutes, followed by 50 cycles of 98°C for 10 Seconds and 65°C for 2 Seconds.

PCR products of the predicted size were obtained as visualized by 2% agarose gel electrophoresis with ethidium bromide staining. The specificity of the PCR was determined in several samples by sequencing of the PCR products. The discriminatory power of the genotyping method was validated using reference strains. The sensitivity of the method was assessed by a spike test. That is, serial 10-fold dilutions of *H. pylori* TK1023 (an East-Asian genotype *CagA*-positive strain) suspension in phosphate-buffered saline were added to an *H. pylori*-negative stool followed by DNA extraction and PCR. The sensitivity of the method used was  $215 \pm 14$  colony forming unit/g stool for *16S rRNA* by real-time PCR. [182]

#### Data Entry

Data entry was done in an MS Access database. The database was secured with a password known to only principal investigator and the co- investigators.

#### Data Analysis

The association between various risk factors and *H. pylori* status was analyzed by chi-square test or Fisher's exact test. The level of statistical significance was set at  $P < 0.05$ .

## **Assessment of risk factors for peptic ulcer and gastric cancer along with *H. pylori* infection in India**

We conducted a case control study in India enrolling patients diagnosed with peptic ulcer, stomach cancer and controls. We assessed the prevalence of *H. pylori* along with its risk factors among healthy individuals, and patients diagnosed with peptic ulcer and stomach cancer in Pune, India. We also followed a cohort of 100 *H. pylori* positive individuals to assess the effect of treatment.

Study location: We conducted our study at Pune city in the state of Maharashtra in India at Deenanath Mangheskar Hospital and Research Center (DMH) between Spring 2008 – Fall 2010. DMH caters to all strata in the Pune population and is central mutli-speciality hospital. In this multidisciplinary tertiary level DMH, over 60 research projects are being conducted by young, highly qualified, abroad-trained, experienced consultants and medical and paramedical men & women, social workers, clinical psychologists, counsellors and so on. The unique location and services offered with a strong commitment to clinical research made DMH the hospital of choice for our study.

### **Study Methods**

#### Study design

Hospital based case control study. We carried out the case control study with three groups. 1) Peptic ulcer group: patients diagnosed with peptic ulcer (as defined by hospital record using international classification of diseases (ICD) 10 codes) 2) Stomach cancer group: patients diagnosed with gastric cancer (as defined by hospital record using ICD 10 codes) and 3) Controls: patients obtaining medical care at DMH hospital but not diagnosed with either peptic ulcer or stomach cancer

We also followed a cohort of *H. pylori* positive patients (n =100) during their first visit to DMH. We enrolled consecutive patients with *H. pylori* positive patients during their first visit to DMH until we accrued 100 patients.

We obtained the approval from the institutional review board at University of South Florida and from the DMH Institutional Ethics Committee (IEC). We revised the questionnaire which was used in Panama and Ecuador studies by Sasaki and colleagues to suit to the population in Pune, India.[184] The questionnaire was pilot tested at the DMH in a small focus group enrolling some affiliated physicians and their patients (n=15). Based on their feedback the questionnaire was revised at least 5 times (specifically the questions related to food, diet, smoking habits etc.). We translated the questionnaire into the native Marathi language and back translated it in English language to assure consistency in terms of content and criterion validity.

The cases were identified via the DMH database using the ICD10 coding. Any patient more than 18 years of age and listed in the DMH database as having diagnosis of either peptic ulcer or stomach cancer was eligible for enrollment. The cases which were enrolled from the satellite clinics were enrolled if they were diagnosed (as being either suffering from peptic ulcer or stomach cancer) by their respective attending physicians. We used SAS software random number generation code to generate a list of potential eligible patients. We recruited the cases (patients diagnosed with either peptic ulcer or stomach cancer) at the beginning of the study enrollment followed by controls. The study personnel contacted the patients in the hospital and requested them to participate in the study. If the potential participant agreed to participate he / she were requested to sign the informed consent form. Each participant was given a copy of the signed informed consent form. The study was explained in details to each potential participant in their native “Marathi” language by the study personnel.

Data Collection



We trained 2 local physicians (RAs) to collect data for this project. They were involved in the project since the revision of the data collection questionnaire. Since they are practicing physicians in Pune city they had adequate experience and knowledge of local customs and traditions (especially related to dietary habits etc.). They sent the completed questionnaires every weekend to principal investigator. We discussed the collected data and resolved any discrepancies by consensus. Methods for data collection and analysis of stool samples were standardized with all researchers participating in this study.

#### *H. pylori antigen detection*

All the tests were performed by the trained lab technicians at the DMH pathology lab. We detected the *H. pylori* antigen from stool specimens using the ImmunoCard STAT HpSA antigen detection kit. The details of the ImmunoCard STAT HpSA test and the standard operating procedures that were followed by the DMH pathology laboratory staff are listed below.

#### **ImmunoCard STAT HpSA test**

ImmunoCard STAT HpSA is a rapid lateral flow immunoassay that utilizes a monoclonal anti-*H. pylori* antibody as the capture and detector antibodies. A diluted patient stool sample is dispensed into the sample port of the test device and the appearance of a pink-red line in the reading window next to the letter T after five minutes of incubation at room temperature indicates a positive result. (*Figure 1*)

#### Specimen

##### *Preferred sample types*

Only solid, semi-solid or liquid samples were accepted. We rejected samples in transport media and swab samples or samples mixed with preservatives.

### *Interfering substances*

The following substances have shown to have no effect on results when present in stool at the concentrations indicated in the parenthesis.[185]

Tums® Antacid (5 mg/mL), Tagamet® (5 mg/mL), Prilosec® (5 mg/mL), Mylanta® Antacid (1:20), Pepto-Bismol® (1:20), Barium sulfate (5%), Whole Blood (50%), Leukocytes (50%), Mucin (3.4%), Stearic acid/palmitic acid (fecal fat) (4%), Hemoglobin (tarry stool) (12.5%).[185]

### Specimen Collection

The specimen were transported in an airtight container and stored at 2°-8° C until tested. The specimen were tested as soon as possible, but were at times held up to 72 hours at 2°-8°C prior to testing. If testing was not performed within this time frame, specimens were frozen immediately upon receipt and stored frozen ( $\leq -20^{\circ}\text{C}$ ) until tested. Specimens were frozen and thawed twice if required (in case of transportation or other delays in analysis were expected). Stool sample was thoroughly (regardless of consistency) mixed before testing.

### Materials

1. ImmunoCard STAT HpSA Test Devices, in individual foil pouches with a desiccant.
2. Sample Diluent, in a plastic dropper vial.
3. Positive Control, in a plastic dropper vial.
4. 100  $\mu\text{L}$  transfer pipettes.

### Preparation and Performance considerations

Specimens and reagents were brought to room temperature (20°-26° C) before testing. As patient specimens may contain infectious agents, they were handled and disposed of as potentially biohazardous. We instructed the lab technicians not to interchange reagents from different kit lot numbers. We allowed kit components and specimens to reach the room temperature (20°-26° C) before performing a test, as cold reagents and/or specimens may decrease assay sensitivity. Reagents took 20-30 minutes to warm following refrigeration. We mixed the stool samples thoroughly (regardless of consistency) to ensure a representative sample prior to sampling.

*Following were the instructions given to the DMH pathology laboratory personnel to ensure fair and unbiased testing of the stool samples.*

- Lab technicians were instructed to inspect test devices before removing the foil pouch. They were also told not to use test devices that had holes in the foil pouch or where the pouch has not been completely sealed. (False negative reactions may result due to deterioration of the improperly stored Test Device.)
- Similarly, lab technicians were instructed not to use the sample diluent or positive control if turbid. Turbidity may be a sign of microbial contamination. The positive control was handled as potentially infectious even though it contains inactivated *H. pylori*.
- Lab technicians were instructed to hold reagent vials vertically when dispensing drops to ensure consistent drop size and delivery.
- Lab technicians were requested to not deviate from the method described here or falsely positive or falsely negative results may occur.
- Lab technicians were instructed to thoroughly read the test instructions before performing any testing and to not use a device if its pouch was punctured prior to use.
- Lab technicians were made aware that at times particulate matter may initially interfere with sample flow. In cases where the test device does not readily absorb the diluted specimen, gently touch the bottom of the sample port with an applicator stick, moving the stool solid

particle that might prevent the absorption. Alternatively, a new aliquot of the sample can be withdrawn from the diluent and retested.

- Lab technicians were instructed to store kit refrigerated at 2° to 8° C and return the test kit to the refrigerator promptly after each use. Lab technicians were instructed to not freeze the kit.

#### Quality control

The reactivity of ImmunoCard STAT test devices was verified upon receipt using the external positive and negative control reagents provided in the kit. We evaluated the test in the following manner.

##### *Internal controls*

Internal controls are contained within the test device and therefore were evaluated with each test.

1. A colored band appearing at the control line served as a positive control and indicated that the test has been performed correctly, that sample was added, that it flowed properly, and that the test reagents were active at the time of use.
2. A clear background around the control or test lines served as a negative control. A background that obscures the reading of results invalidates the test and can be an indication of reagent deterioration, inappropriate sample or improper test performance.

*External controls:* The reactivity of ImmunoCard STAT HpSA Test Devices were verified upon opening each new kit using the external Positive and Negative Control reagents provided in the kit. Each operator was strictly advised to test a positive and negative control at least once with each 20-test kit.

## Procedure

*(Each lab technician was given a print out of the following standard operating procedure for performing this test.)*

### A. Test

1. Bring all test devices, reagents and samples to room temperature (20°-26° C) before testing.
2. Use 1 ImmunoCard STAT Test Device for each patient sample.
3. Remove the ImmunoCard STAT Test Device from its foil pouch. The Test Device is marked to indicate where test and control lines will appear. The round window marked with an arrow is the test window where sample is added.
4. Label the device with the patient's name. Prepare the specimen according to the instructions in the specimen collection and preparation section above.
5. Hold the diluted specimen vial upright and tap the bottom gently on the countertop before proceeding.
6. Cover the top of the diluted sample vial with absorbent paper to avoid splatter.
7. Break off the red tip on the outside of the red cap. (Do not break off the white applicator stick on the inside of the cap.)
8. Hold the vial upside down and dispense 4 drops of diluted sample into the round window (at arrow) of the Test Device. Do not touch the tip of the vial to the Test Device.
9. Set a timer and incubate the test at 20°-26° C for 5 minutes.
10. At the end of 5 minutes, read the results within 1 minute. See the Interpretation of results

section below for a description of positive and negative test results.

*B. Alternate Test Procedure with Simple Sample*

Simple sample is an alternate sampling unit that can be used in place of the Sample Diluent vial provided with ImmunoCard STAT HpSA test kit.

1. Bring all test devices and Simple Sample dilution vials to room temperature (20°-26° C) before testing.
2. Use 1 ImmunoCard STAT Test Device for each patient sample.
3. Remove the ImmunoCard STAT Test Device from its foil pouch. The Test Device is marked to indicate where test and control lines will appear. The round window marked with an arrow is the test window where sample is added.
4. Label the device with the patient's name. Prepare the specimen according to the instructions in the Simple Sample package insert.
5. Mix specimen by inverting the Simple Sample several times. Remove the translucent screw cap from the tip of the Simple Sample.
6. Hold the Simple Sample vial upside down and dispense 4 drops of diluted sample into the round window (at arrow) of the Test Device. Do not touch the tip of the vial to the Test Device.
7. Set a timer and incubate the test at 20°-26° C for 5 minutes.
8. At the end of 5 minutes, read the results within 1 minute. See the interpretation of results section below for a description of positive and negative test results.

### C. Controls

Positive and Negative Controls are designed to show all reagents are reactive, specific, and capable of producing the expected results.

- a. Bring all control reagents to 20°-26°C before testing.
- b. Use 1 ImmunoCard STAT Test Device each for a Positive and Negative Control.  
Label each device with the control to be tested.
- c. Hold reagent vials upside down to dispense reagents.
- d. Add 4 drops of the Positive Control to the test window (at arrow) of 1 device. *Do not allow the tip of the vial to touch the sample port.*
- e. Break off the red tip on the outside of the red cap of an unused vial of Sample Diluent.
- f. Dispense 4 drops of the Sample Diluent to the test window (at arrow) of another Test Device.
- g. Set a timer and incubate the tests at 20°-26° C for 5 minutes.
- h. After 5 minutes, read the results within 1 minute of test completion.

### Interpretation of results

#### *Negative test result*

Only one BLUE colour band (Control Line) appears across the central window of the device close to the letter “C”. (*H. pylori* antigens are absent or below the level of detection.) No other bands should be seen.

### *Positive test result*

In addition to the BLUE band (Control Line), a distinguishable PINK-RED band (Test Line) also appears across the central window of the device close to the letter “T”. The intensity of the band will vary depending on the antigen concentration in the specimen. Any pink-red line, even very weak, must be considered as a positive result. (A positive test line indicates that *H. pylori* antigens are in the specimen.) The background should not interfere with reading the test. (*Figure1*)

### *Invalid test results*

1. The BLUE band (Control Line) is absent, with or without a visually detectable PINK-RED band (Test Line),
2. A PINK-RED band appears at the letter “T” in the window after six minutes, or there is a line at this position of another colour other than pink-red,
3. No Control Line band appears close to the letter “C”. (The test is invalid since a shift in or absence of the control line indicates that the test procedure was performed improperly or that deterioration of the reagents has occurred.)

*If any test is difficult to interpret, the test should be repeated with the same sample to eliminate the potential for error. Obtain a new sample and retest when the original sample repeatedly produces unreadable results.*



## Reporting of results

### *Negative for H. pylori*

Report test result as: “*H. pylori* antigen is absent or below the level of detection.”

### *Positive for H. pylori*

Report test result as: “*H. pylori* antigen detected.”

## Expected values

Studies on the epidemiology of *H. pylori* have shown that this organism is present worldwide. Gastritis caused by *H. pylori* has been shown to correlate with age, ethnic background, family size and socioeconomic class. The prevalence of *H. pylori* infection in a given population can vary from 20% to 90%. In patients diagnosed with duodenal ulcers, however, it has been shown in every age group to be approximately 80%. Currently recommended eradication treatments have an efficacy rate between 75% and 90%. The ImmunoCard STAT HpSA test detects the presence of *H. pylori* antigens in human stool.

## Limitations of the test

1. The test is qualitative and no quantitative interpretation should be made with respect to the intensity of the positive line when reporting the result.
2. Test results are to be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures.
3. Failure to add sufficient stool to the Specimen Diluent may result in a falsely negative test result. Addition of too much stool may result in invalid test results due to the inhibition of proper sample flow.

- Over incubation of tests may lead to false-positive test results. Incubating tests at reduced temperatures or times may lead to falsely negative results.
- Performance characteristics have not been established for watery diarrheal stools. Watery stools composed mainly of fluid with little or no solid matter may give false negative test results.

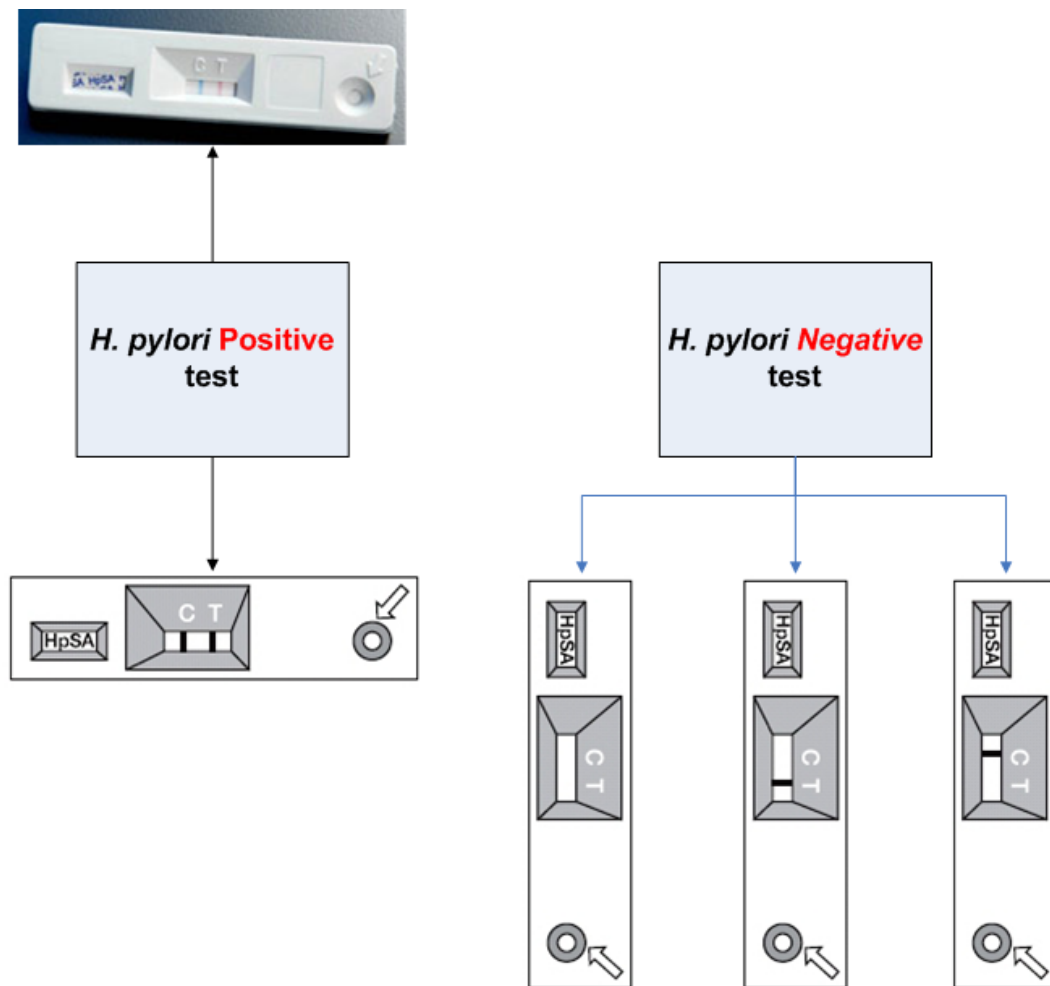


Figure 1 ImmunoCard STAT HpSA test results

## Sample Size Calculations

We based our sample size calculations assuming following parameters: alpha error = 0.05, power (beta error) = 0.90, Effect size (odds ratio) = 2.1, proportion of controls with the exposure = 0.40 (*H. pylori*), proportion of cases with the exposure (*H. pylori*) = 0.50. The required sample size was 314 (157 cases and 157 controls). Based on the current literature and research conducted in India the average odds ratio addressing the relationship of *H. pylori* between peptic ulcer cases and controls was 4.2. We have utilized a much conservative OR in our sample size calculations. We created an MS access database for entering the data. The database was pilot tested in India and then revised to accommodate the suggestions by the RAs.

## Data Analysis

The association between each potential risk factor and the outcome (*H. pylori* status, chronic gastric disease status, disease status) was measured using logistic regression models containing only the risk factors of interest. All risk factors associated with (*H. pylori* status, chronic gastric disease status, disease status) (at  $p < .10$ ) were then entered into a multivariable logistic regression with backward elimination ( $p < .05$  to retain) to select the final set of independent risk factors. The same multivariable model was chosen using a forward stepwise selection with an entry level  $p = .10$  and a stay level  $p = .05$ . The multiple logistic regression model fit was determined by the Hosmer–Lemeshow test statistic. A model was considered a fit for the data if the Hosmer–Lemeshow statistics was with  $p \text{ value} > .05$ . Continuous variables were checked in the models for linearity in the logit, using the method of fractional polynomials. We also check all the models for the presence of effect modification due to age and gender. Statistical analyses were conducted using SAS 9.1.3 (SAS Institute, Cary, NC, USA) and STATA 9.0 (STATA Corporation, College Station, TX, USA). All  $p$ -values are two-sided and a  $p < .05$  was considered statistically significant.

## Chapter Three

### Assessment of *H. pylori* prevalence and its risk factors among healthy individuals in Ecuador and Panama

#### Background

One of the most important virulence factors of *H. pylori* is *CagA*, which is located in the pathogenicity island region. [184] *CagA* is further classified into 2 major types, East-Asian type and Western type. The East-Asian type *CagA* is considered to be more virulent than the Western type by virtue of its more potent stimulation of host signal transduction pathways. [184] [186] Hence, many researchers have studied *CagA* genotype extensively from stomach biopsies of individuals suffering from gastric ailments. [184] [187, 188] However, since it is tedious to collect gastric biopsy samples from asymptomatic individuals, the genotype of the virulence factors of *H. pylori* isolated from asymptomatic people have been insufficiently analyzed, both qualitatively and quantitatively. [184] Analysis of *H. pylori* infection in asymptomatic people in areas with different prevalence of stomach cancer may provide important information regarding the possible factors contributing to the development of stomach cancer. [184] So, to assess the risk factor contributing to prevalence of *H. pylori* and its genotypes in healthy individuals in Ecuador and Panama we undertook a cross sectional study with following specific aims:

- 1) To determine the prevalence of *H. pylori* among healthy individuals living in Ecuador and Panama
- 2) To determine the prevalence of *CagA* positive *H. pylori* infection in healthy individuals in Ecuador and Panama and
- 3) To determine various risk factors for prevalence of *H. pylori* infection.

## Methods

The study was conducted in Ecuador and Panama from May 2007 to August 2007. All participants were screened for age and medical history in order to select asymptomatic adult individuals. Asymptomatic individuals were defined as those who have not had any subjective symptoms of gastric disorders or have not been diagnosed with any stomach diseases by a medical doctor within the last 6 months. Those individuals who had taken antibiotics within 6 months were excluded. A total of 90 and 75 participants living in Ecuador and Panama, respectively, were enrolled in the study. All of the participants gave their informed consent. The research protocols were approved by the ethics committees of the University of South Florida (Tampa, FL), Universidad Central del Ecuador (Quito, Ecuador), and Gorgas de Estudios de la Salud (Panama City, Panama).

We collected data on risk factors for *H. pylori* infection in one on one interview conducted by a bilingual (English-Spanish) interviewer using a structured questionnaire. *H. pylori* antigen (catalase) in stool specimens was detected using Testmate Rapid Pylori Antigen kit (BD, Tokyo, Japan) by following the manufacturer's instructions. Briefly, a 20-mg stool specimen was taken and suspended in the sample dilution buffer. One drop of the stool suspension was utilized for the immunochromatography test. The reaction was carried out at room temperature for 10 min. *H. pylori* virulence factor *cagA* was genotyped by polymerase chain reaction. The identification of helminth was made morphologically following the guidelines of the American Society of Parasitology. The association between various risk factors and *H. pylori* status was analyzed by chi-square test or Fisher's exact test. Statistical significance was set at  $P < 0.05$ .

## Results

The demographic characteristics of 90 participants from Ecuador are listed in Table 2. There were 68 (76%) study participants between 30 to 55 years of age. The number of females and males in the study were approximately equal (48 and 42 respectively). The percentage of participants who have primary or secondary school education was 69, while only 23% of the sample had university level education and 8% had a diploma. Majority (77%) of the subject's ethnicity was Mestizo.

Table 2 Demographic characteristic of the participants in Ecuador (n =90).

Characteristics	N (%)
Age group	
30-55 years	68 (76)
> 55 years	22 (24)
Gender	
Female	48 (53)
Male	42 (47)
Education	
Primary	36 (44)
High School	21 (25)
University	21 (23)
Other (diploma)	12 (8)
Ethnicity	
Mestizo	70 (77)
African descent	6 (7)
Spanish descent	7 (8)
Indian descent	7 (8)
Length of stay at current residence	
0.5-20 years	53 (59)
21-40 years	27 (30)
>40 years	10 (11)

The demographic characteristics of 75 participants from Panama are presented in Table 3. There were 34 (45%) study participants between 20 to 55 years of age. The number of females (37 % (28 /75)) were less compared to males (63 % ( 47/75)). The percentage of participants who have primary or high school education was 84 (63/75), while only 16% (12/75) were uneducated. Majority (46% (32/75)) of the subject's ethnicity was Spanish decent.

Table 3 Demographic characteristic of the participants in Panama (n= 75)

Characteristics	N (%)
Age group	
20-55 years	34 (45)
> 55 years	41 (55)
Gender	
Female	28 (37)
Male	47 (63)
Education	
None	12 (16)
Primary / High School	63 (84)
Ethnicity	
Mestizo	18 (26)
African descent	3 (4)
Spanish descent	32 (46)
Indian descent	17 (24)
Length of stay at current residence	
0.5-20 years	8 (11)
21-40 years	15 (20)
>40 years	52 (69)

The frequency distribution of participant characteristics by three study sites in Ecuador is listed in Table 4. A total of 71% (65/90) of subjects were infected with *H. pylori*. These 65 subjects were evenly distributed over the three study regions. One third of our study participants had history of chronic gastric disease. We did not confirm this by examining the subject for diagnosis of chronic gastric disease in our study. The prevalence of intestinal parasitic infection and the drugs used for its treatment along with drugs used for general parasitic infection was similar in three study regions. We did not find any regional differences in the prevalence of protozoa infestation. But malaria was significantly associated with different regions (P-Value=0.00). Compared to cases of malaria in highlands of Quito, there were significantly higher number of cases in subtropical area of La Concordia and tropical Atacames.

Table 4: Frequency of study variables by regions in Ecuador

Participant characteristics	Quito N (%)	La Concordia N (%)	Atacames N (%)	P-Value
<i>H. pylori</i>				
Positive	20 (31)	23 (35)	22 (34)	0.66
Negative	9 (36)	10 (40)	6 (24)	
Gastric disease				
Yes	12 (39)	11 (36)	8 (25)	0.59
No	17 (29)	22 (37)	20 (34)	
Intestinal Parasite				
Yes	19 (34)	20 (35)	17 (31)	0.92
No	10 (30)	13 (38)	11 (32)	
Malaria				
Yes	3 (7)	26 (56)	17 (37)	0.00
No	26 (59)	7 (16)	11 (25)	
Anti parasitic drug use				
Yes	22 (30)	29 (40)	22 (30)	0.49
No	7 (41)	4 (23)	6 (36)	
Intestinal parasite drug use				
Yes	11 (35)	8 (26)	12 (39)	0.27
No	18 (30)	25 (42)	16 (28)	
Protozoa infestation				
Yes	9 (24)	17 (45)	12 (31)	0.26
No	20 (38)	16 (31)	16 (31)	

The relationship between selected participant characteristics and their *H. pylori* status in Ecuador are given in Table 5. Demographic characteristics such as gender, length of stay at current residence and age were not associated with *H. pylori* status. However, overall educational attainment was significantly associated with *H. pylori* status (P-Value=0.03). Participants who had lower education were more likely to have *H. pylori* infection compared to participants who had higher education. Study participant's current tobacco use and the age at which they started smoking were not associated with *H. pylori* (P-Value=1.00 and 0.15 respectively). Amount of whiskey and beer consumption per day were not associated with *H. pylori* status (P-Value=0.17 and 0.58). Prevalence of gastric disease was not associated with *H. pylori* status (P-Value=0.32). In this study, the amount of chili peppers consumed were not associated with *H. pylori* (P-Value=0.34). Furthermore, anti-parasitic drug use and protozoa infestation were not associated



with *H. pylori* status (P-Value=1.00 and 0.81 respectively).

Table 5: Relationship between participant characteristics and *H. pylori* in Ecuador

Participant characteristics	<i>H. pylori</i>		P-Value
	Present N (%)	Absent N (%)	
Gender			
Female	33 (69)	15 (31)	0.48
Male	32 (76)	10 (24)	
Education			
Primary School	22 (61)	14 (39)	0.03
High School	19 (91)	2 (9)	
University	13 (62)	8 (38)	
Other	11 (92)	1 (8)	
Smoking Age			
<20 years	24 (80)	6 (20)	0.15
21-35 years	4 (44)	5 (64)	
>36 years	37 (72)	14 (28)	
Alcohol amount (whiskey)			
≤ 50 mL / day	51 (76)	16 (24)	0.17
> 50 mL / day	14 (60)	9 (40)	
Chili peppers amount			
≤ 1.5 chili peppers/day	36 (68)	17 (32)	0.34
> 1.5 chili peppers/day	29 (78)	8 (22)	
Current Tobacco use			
Yes	30 (73)	11 (27)	1.00
No	35 (71)	14 (29)	
Beers per day			
≤ 1 can / bottle	39 (70)	16 (30)	0.58
2-4 cans / bottles	16 (80)	4 (20)	
≥ 5 cans / bottles	10 (66)	5 (34)	
Gastric disease			
Yes	20 (65)	11 (35)	0.32
No	45 (76)	14 (24)	
Anti-parasitic drug use			
Yes	53 (73)	20 (27)	1.00
No	12 (71)	5 (29)	
Protozoa infestation			
Yes	28 (74)	10 (26)	0.81
No	37 (71)	15 (29)	

The relationship between selected participant characteristics and their *H. pylori* status in Panama are presented in Table 6. Demographic characteristics such as length of stay at current residence

and age were not associated with *H. pylori* status. However, male participants were more likely to have *H. pylori* infection compared to female participants (P-Value=0.05). Study participant's smoking status and the age at which they started smoking were not associated with *H. pylori* (P-Value=0.10). However, alcohol amount was associated with *H. pylori* status. Participants who were consuming more than 50ml of alcohol per day were more likely to have *H. pylori* infection compared to participants consuming 50ml or less alcohol per day. (P-Value=0.02). Chronic gastric disease was not associated with *H. pylori* (P-Value=0.40). The amount of chili peppers consumed were not associated with *H. pylori* (P-Value= 0.79). Furthermore, anti-parasitic drug use and protozoa infestation were not associated with *H. pylori* status (P-Value=0.23 and 1.00 respectively).

Table 6: Relationship between participant characteristics and *H. pylori* in Panama

Participant characteristics	<i>H. pylori</i>		P-Value
	Present N (%)	Absent N (%)	
Gender			
Female	11 (39)	17 (61)	0.05
Male	30 (64)	17 (36)	
Education			
None	8 (67)	4 (33)	0.26
Primary	31 (55)	25 (45)	
High School / Technical	2 (29)	5 (71)	
Smoking			
Yes	20 (67)	10 (33)	0.10
No	21 (47)	24 (53)	
Alcohol amount			
<= 50 mL / day	13 (39)	20 (61)	0.02
> 50 mL / day	28 (67)	14 (33)	
Chili peppers amount			
<= 1.5 chili peppers/day	13 (59)	9 (41)	0.79
> 1.5 chili peppers/day	28 (53)	25 (47)	
Chronic gastric disease			
Yes	7 (44)	9 (56)	0.40
No	34 (58)	25 (42)	
Anti-parasitic drug use			
Yes	25 (56)	20 (44)	0.23
No	14 (54)	12 (46)	
Protozoa infestation			
Yes	2 (50)	2 (50)	1.00
No	39 (55)	32 (45)	

The relationship between participant characteristics and chronic gastric disease in Ecuador are presented in Table 7. Education and gastric disease were not associated with each other (P-Value=0.13). The p-value suggests that education has a weak association with chronic gastric disease. Participants who had lower education were found to have chronic gastric disease compared to participants who had higher education. Antiparasitic drug use for intestinal parasites was associated with having chronic gastric disease (P-Value=0.06). Individuals having history of antiparasitic drugs use for intestinal parasites were found to have chronic gastric disease. Hypertension was significantly associated with having chronic gastric disease (P-Value=0.01). Hypertensive individuals seemed to have chronic gastric disease. Individuals who reported current medication use were also found to have chronic gastric disease (P-Value=0.08).

Table: 7 Relationship between participant characteristics and chronic gastric disease in Ecuador

Participant characteristics	Chronic gastric disease		P-Value
	Present N (%)	Absent N (%)	
Center			
Quito	12 (41)	17 (59)	0.59
La Concordia	11 (33)	22 (67)	
Atacames	8 (29)	20 (71)	
Gender			
Female	19 (40)	29 (60)	0.37
Male	12 (29)	30 (71)	
Education			
Primary School	14 (38)	23 (62)	0.13
High School	9 (43)	12 (57)	
University	5 (25)	15 (75)	
Other	0 (0)	7 (100)	
First Food Choice			
Vegetarian	15 (45)	18 (55)	0.11
Non-Vegetarian	16 (28)	41 (72)	
Intestinal anti-parasitic drug use			
Yes	15 (48)	16 (52)	0.06
No	16 (27)	43 (73)	
Hypertension			
Yes	9 (64)	5 (36)	0.01
No	22 (29)	54 (71)	
Current Medication use			
Yes	13 (48)	14 (52)	0.08
No	17 (28)	44 (72)	

Participant characteristics	Chronic gastric disease		P-Value
	Present N (%)	Absent N (%)	
Beers per day			0.11
<= 1 can / bottle	24 (43)	22 (57)	
2-4 cans / bottles	4 (20)	16 (80)	
>= 5 cans / bottles	3 (21)	11 (79)	
Alcohol consumption			0.15
<= 1 drink/month	27 (38)	43 (62)	
> 1 drinks/month	3 (17)	15 (83)	
Everyday	1 (50)	1 (50)	

The relationship between selected participant characteristics and chronic gastric disease in Panama is listed in Table 8. Education, gender and ethnicity were not associated with chronic gastric disease. Similarly, alcohol amount and amount of chili peppers consumed were not associated with chronic gastric disease. However, smoking status was associated with chronic gastric disease (P-Value=0.04). Antiparasitic drug use and protozoa infestation were not associated with chronic gastric disease.

Table: 8 Relationship between participant characteristics and chronic gastric disease in Panama

Participant characteristics	Chronic gastric disease		P-Value
	Present N (%)	Absent N (%)	
Gender			0.38
Female	4 (14)	24 (86)	
Male	12 (25)	35 (75)	
Education			0.43
None	4 (33)	8 (67)	
Primary	10 (18)	46 (82)	
High School / Technical	2 (29)	5 (71)	
Ethnicity			0.06
Mestizo	0 (0)	18 (100)	
African descent	1 (33)	2 (67)	
Spanish descent	8 (25)	24 (75)	
Indian descent	6 (35)	11 (65)	
Smoking			0.04
Yes	10 (33)	20 (67)	
No	6 (13)	39 (87)	
Alcohol amount			0.27
<= 50 mL / day	5 (15)	28 (85)	
> 50 mL / day	11 (26)	31 (74)	

Participant characteristics	Chronic gastric disease		P-Value
	Present N (%)	Absent N (%)	
Chili peppers amount			
<= 1.5 chili peppers/day	5 (23)	17 (73)	1.00
> 1.5 chili peppers/day	11 (21)	42 (79)	
Anti-parasitic drug use			
Yes	25 (56)	20 (44)	0.23
No	14 (54)	12 (46)	
Protozoa infestation			
Yes	10 (22)	35 (73)	1.00
No	5 (19)	21 (81)	

Assessment of *H. pylori* antigen in the stool specimens obtained from participants revealed an *H. pylori* infection rate of 72% in Ecuador, and that this positive rate was significantly higher than the rate detected in Panama (54%) (*Figure 2*).

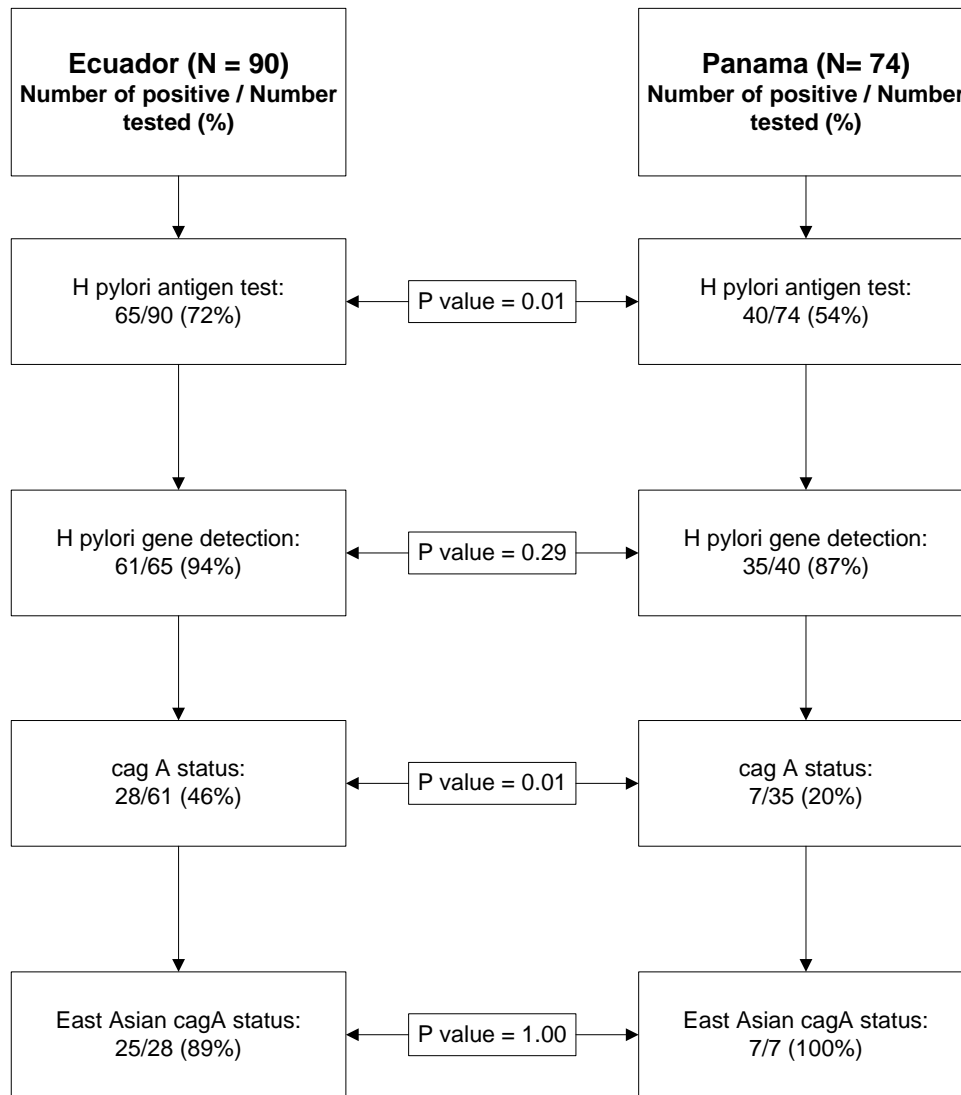


Figure 2 *H. pylori* genotype assessment

Bacterial DNA was extracted from *H. pylori* antigen-positive stool specimens and the extracted DNA was subjected to PCR for the detection of *H. pylori* genes. The *H. pylori* 16S rRNA gene was detected by real-time PCR for confirmation of the existence of *H. pylori* DNA in the extracted DNA. As shown in Figure 1, *H. pylori* genes were detected in 94% of *H. pylori* antigen-positive Ecuador specimens and in 87% of the Panama specimens.[182]

The *CagA* gene was detected in 46% and 20% of the *H. pylori* DNA-positive specimens from

Ecuador and Panama, respectively, and this difference was demonstrated to be statistically significant (*Figure 1*). Furthermore, analysis of the *CagA* genotype revealed that the East-Asian type was predominant in both countries (89% in Ecuador and 100% in Panama) (*Figure 1*).[182]

### **Discussion:**

This was the first study to document the prevalence of *H. pylori* among healthy individuals in Ecuador and Panama. Previously, it was reported that the prevalence of *H. pylori* infection in dyspeptic patients in Ecuador was 89.5% [189] and 79.6% to 82.9% in patients with chronic dyspepsia, gastric or duodenal ulcer, gastritis, or gastric cancer in Panama. [190-192] The results obtained in the present study are slightly lower than the published data for patients with chronic gastric disease in Ecuador and Panama. However, the infection rates in our study are higher than those in Western countries (32.7% in the United States, 25.4% in France, and 39.2% in Germany), which have a lower incidence of gastric cancer. [66] The reasons for the high *H. pylori* infection rates in asymptomatic people in both Ecuador and Panama are not clear. It is speculated that the poor public health conditions in both countries may be a contributory factor in the facilitating transmission of *H. pylori*.

We hypothesize that certain prevalent food habits such as pre mastication of food by parents while feeding infants (person to person transmission via saliva) [193], consumption of restaurant food and possible contaminated water consumption (feco-oral transmission) might explain the high prevalence of *H. pylori* in Ecuador and Panama. In Ecuador and Panama the drinking water is chlorinated but the wastewater is not treated and is used for irrigation. Some researchers have recently shown that *H. pylori* can thrive in a non culturable coccoid form in biofilms even after chlorination. [194-197] Hence *H. pylori* may be able to tolerate disinfectants in distribution systems and, therefore, may be transmitted by a waterborne route.

The detection of *CagA* in extracted DNA revealed that 46% of *H. pylori* DNA-positive samples

were *CagA*-positive in Ecuador, but that only 20% of similar samples were *CagA*-positive in Panama. Thus, a significant number (28%) of asymptomatic people in Ecuador are infected with the more pathogenic East-Asian genotype *CagA*-positive *H. pylori*. We hypothesize that this might be due to the racial differences between people from Ecuador and Panama. The high incidence rate of gastric cancer in Ecuador (24 per 100,000) compared to Panama (11.6 per 100,000) can be attributed to these differences in *CagA* positive *H. pylori* prevalence.[198]

The results obtained in the present study indicated that the methodology used is suitable for assessing the status of *H. pylori* infection in asymptomatic people. In addition, these results suggested that the prevalence of *H. pylori* infection and/or the occurrence of *CagA* in the *H. pylori* detected in asymptomatic people may be related to the risk of stomach cancer.

Most of the risk factors of interest were not significantly associated with *H. pylori* and chronic gastric disease. Our study concluded that gender was not associated with either *H. pylori* or history of gastric disease for a long duration. This finding is consistent with studies done in India and Northwest region of Ethiopia. [199-201] Education attainment was weakly associated with chronic gastric disease. However the contingency table analysis suggested that chronic gastric disease was more prevalent among participants having primary school education than participants having secondary school education and higher. In case of *H. pylori* prevalence; primary educated individuals had fewer than expected *H. pylori* cases than university level education. But at the same time we found that secondary level educated individuals had higher than expected *H. pylori* prevalence. This finding is not consistent with studies done in Saudi Arabia and Brazil where lower education was associated with high prevalence of *H. pylori*. [81, 202-207]

So far in many of the studies published we found that *H. pylori* incidence was increasing with age. In our study age not associated with *H. pylori*. (P value = 1.00) Our finding is not consistent with other studies probably due to the small sample size (n = 90) Owing to our inclusion criteria



the youngest participant in our study was 39 years old. After categorizing the participant's age into only two groups we did not find any association with *H. pylori*.

Tobacco and alcohol use was found to be associated with *H. pylori* in many of the studies published so far. [74, 130, 208-210] In our study the current tobacco use, age at which the participant's started to smoke cigarettes and alcohol consumption (whiskey, beer and home-made alcohol) was not associated with *H. pylori* status.

Dietary choices such as being vegetarian and eating chili peppers were not associated with *H. pylori* status (P value  $\geq 0.10$ ). Anti-parasitic drug use and protozoa infestation were not associated with *H. pylori* status.

Entamoeba coli was the most prevalent infection (n=20) followed by Entomeba Histolytica (n = 13). Eight study participants were found to have Endolimax nana infection where as only six study participants were found infected with Blastocistis Hominis.

In this cross sectional study we used prevalence of chronic gastric disease as a surrogate measure of gastric pathology in general. In this study we tried to shed more light on the relationship between gastric disease and risk factors such as education, spicy food consumption, consumption of high temperature food, intestinal parasite drug use, and hypertension. These risk factors were associated with having chronic gastric disease.

Our study had some limitations. This was a cross sectional study. Hence we did not expect to prove cause – effect relationship between various risk factors of *H. pylori* and its prevalence. Owing to the financial constraints we recruited ninety participants from three areas of Ecuador. During the data analysis; as evident from the contingency tables overview this sample of ninety individuals proved to be small as most of the variables that we assessed were having cell size less than five. Overall the power of the Fisher exact test used to test the association between risk

factors and *H. pylori* was poor (power = 0.20). We collected information regarding history of chronic gastric disease. In our data analysis we have used chronic gastric disease as an indicator variable for gastric pathology. One of the study aims was to elucidate the relationship between helminth and *H. pylori* co-infection. We collected the stool specimens from all the study participants and we analyzed it for presence of helminthes. Only one study participant was diagnosed to have a helminth infection (*Strongyloides stercoralis*). Our study participants were from a lower / moderate socio-economic status; we believe that the data we have is an indicator of the success of the mass de-worming campaign implemented by the government of Ecuador.

Based on above mentioned limitations and findings we revised the data collection instrument and study design to conduct a hospital based case control study in India.

## Chapter Four

### Assessment of risk factors for peptic ulcer and gastric cancer along with *Helicobacter pylori* infection in India

#### Background

It is estimated that the gastric pathogen *Helicobacter pylori* (*H. pylori*), first isolated in 1982 by Warren and Marshall, infects more than 50% of the world's population. [130, 211-214] The infection is usually acquired in childhood, with low socioeconomic status being associated with a high prevalence of infection. There is variation in the prevalence of *H. pylori* among various countries in the world. [38, 128, 130, 211, 214-217] Previous studies from India have reported a high, at times as high as 80% prevalence of *H. pylori* in India. [66] Peptic ulcer disease is frequent in India but there is paucity of data on its prevalence. [41, 218] The prevalence of peptic ulcer is noted to be on average around 8.0 per 100,000 in India. [41] The age adjusted incidence rate of gastric cancer in urban registries in India is reported in the range of 3.0 per 100,000 which is on the lower side among those reported worldwide (14 per 100,000). [219] Previous Indian studies on the prevalence of peptic ulcer were not community based and may have been subject to selection bias. Indeed, some researchers have noted the association between *H. pylori* infection and peptic ulcer in India but at times no association between stomach cancer and *H. pylori* infection. [31, 220-224] However, these studies are criticized to have small sample size and inadequate methodological details such as inadequate description of sample size calculations, case definitions etc. [35, 225-227] Nonetheless, no studies have been conducted in Western India

enrolling adequate number of patients and having sound methodological quality to assess the relationship between peptic ulcer and *H. pylori* and their various risk factors. Indeed, some researchers have noted that apart from the virulent genotype of *H. pylori* environmental and dietary factors influence the outcome of disease. [117, 221, 228, 229] However, these risk factors for prevalence of *H. pylori* especially the environmental and dietary factors in Indian context have received minimal attention.

Hence to address these issues we conducted a case control study in Pune, Maharashtra, India with following specific aim. 1) To determine various risk factors for peptic ulcer, stomach cancer and prevalence of *H. pylori* infection in Pune, India.

## **Methods**

We conducted our study at Pune city in the state of Maharashtra in India at Deenanath Mangheskar Hospital and Research Center (DMH) between Spring 2008 – Fall 2010. Our hospital based case control study had three groups: 1) peptic ulcer patients 2) stomach cancer patients 3) controls. The peptic ulcer and stomach cancer cases were identified via the DMH database using the ICD10 coding. Any patient more than 18 years of age and listed in the DMH database as having diagnosis of either peptic ulcer or stomach cancer was eligible for enrollment. We also followed a cohort of *H. pylori* positive patients (n =100) during their first visit to DMH. We enrolled consecutive patients with *H. pylori* positive patients during their first visit to DMH until we accrued 100 patients.

We collected data on risk factors for *H. pylori* infection in one on one participant interview. All interviews were conducted by the bilingual (English – Marathi) speaking native study research associates (RA). *H. pylori* antigen in stool specimens was detected using ImmunoCard STAT HpSA antigen detection kit by following the manufacturer's instructions. (Meridian Diagnostics, Inc, USA). Briefly, a small portion (5-6 mm diameter) of stool specimen was transferred into the

sample diluent vial using the applicator stick, vortexed for 15 s, and then 4 drops were dispensed into the round window at the lower end of the device. The results were read 5 min later.

We based our sample size calculations assuming following parameters: alpha error = 0.5, power (beta error) = 0.90, Effect size (odds ratio) = 2.1, proportion of controls with the exposure = 0.40 (*H. pylori*), proportion of cases with the exposure (*H. pylori*) = 0.50). The association between each potential risk factor and the outcome (*H. pylori* status, chronic gastric disease status, disease status) was measured using logistic regression models containing only the risk factors of interest. All *p*-values are two-sided and a *p* < .05 was considered statistically significant. We obtained the approval for this project from the institutional review board at University of South Florida and from the DMH ethics committee.

### **Results:**

We contacted a total of 491 potential participants. We were able to enroll 190 patients diagnosed with peptic ulcer and only 35 patients diagnosed with stomach cancer. We then were able to enroll 125 controls in our study. (*Figure 3*)

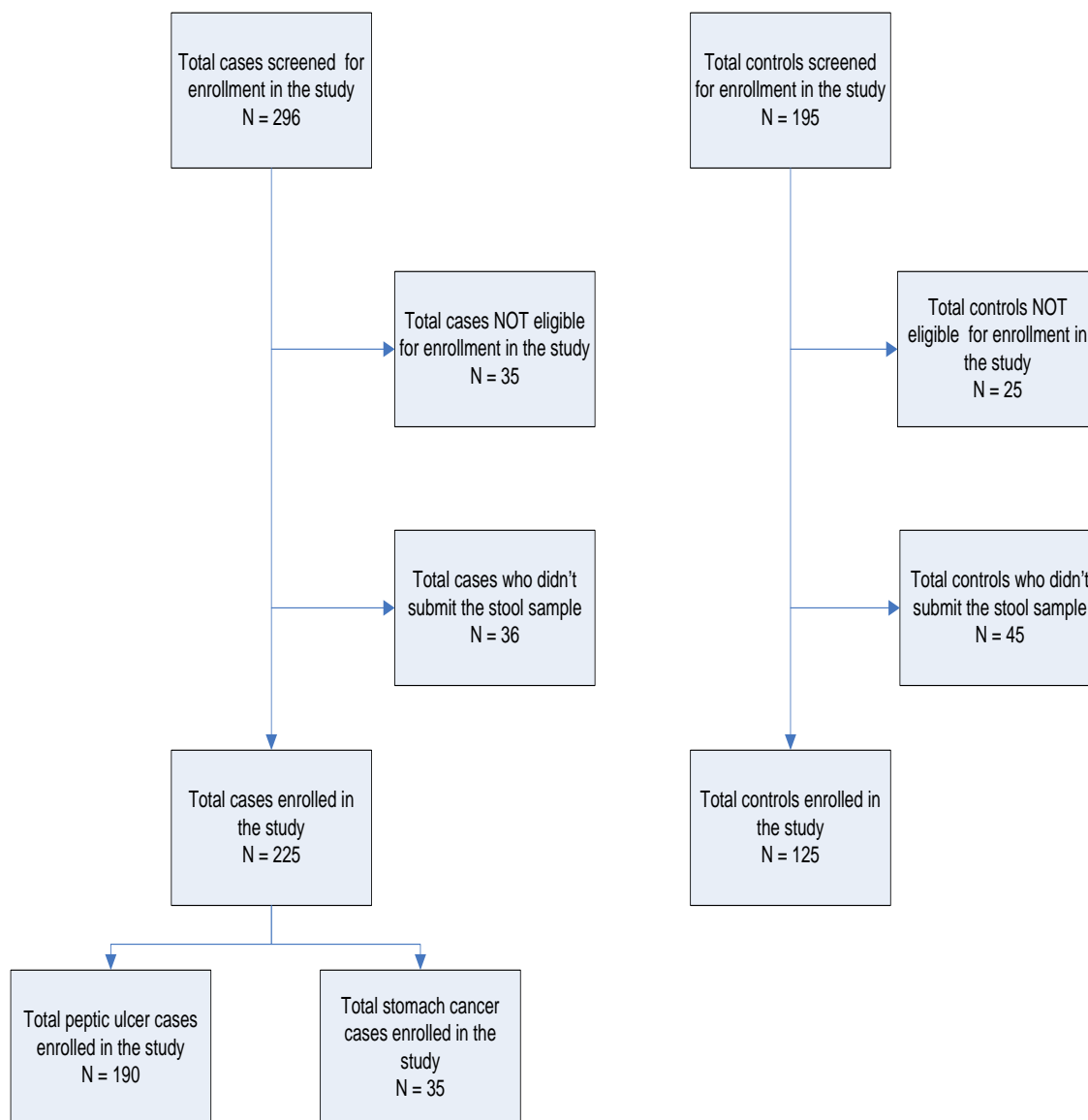


Figure 3 Study participant recruitment flow chart

The median age of the enrolled participants was 56 years (range: 18 – 83 years). Fifty three percent (187/350) of participants were females while 47% (163/350) were males. Forty percent (139 /350) of participants had primary education while 32% (111/350) had completed high school. Only 29% (100/350) of participants had university level education. (Table 9)

Table 9 Demographic characteristics of study participants Pune, India

Characteristics	N (%)
Disease	
Controls	125 (36)
Peptic ulcer	190 (54)
Stomach cancer	35 (10)
Median age (Range)	56 years (range:18-83)
Gender	
Female	187 (53)
Male	163 (47)
Education	
Primary School	139 (40)
High School	111 (32)
University	100 (29)

In the initial univariate analysis, demographic characteristics such as gender (P-Value=0.74), education (P-Value=0.12), age were not associated with *H. pylori* status. Study participant's disease status (having peptic ulcer versus not having any gastric ailment) was associated with *H. pylori* (P-Value=0.00). Study participant's current tobacco use was associated with *H. pylori* (P-Value=0.01). Participants' consumption of snacks while drinking alcohol was not associated with *H. pylori* status (P-Value=0.10). However, consumption of chili peppers (P-Value=0.00) and meat (P-Value=0.02) had a statistically significant association with *H. pylori* status. But, fish consumption (P-Value=0.31) and consuming the food items while they were hot (in terms of food temperature) (P-Value=0.07) were not associated with *H. pylori* status. Interestingly, frequency of outside food consumption was highly associated with *H. pylori* status (P-Value=0.00). The participant's chronic gastric status (P-Value=0.00) and its frequency (P-Value=0.02) were associated with their *H. pylori* status. Similarly, family history of peptic ulcer was associated with *H. pylori* status (P-Value=0.00). Moreover, parasite (P-Value=0.00) and protozoa infestations (P-Value=0.02) were associated with *H. pylori* status. But, helminth infection was not associated with *H. pylori* status (P-Value=0.55). However, anti parasitic drug use was associated with *H. pylori* status (P-Value=0.04). But, prior treatment of *H. pylori* was not associated with current *H. pylori* status (P-Value=0.80). Participant's *H. pylori* status was associated their spouses' *H. pylori*

infection status (P-Value=0.00). (Table 10)

The final multivariate logistic regression analysis results for the association of participant characteristics and *H. pylori* status are shown in Table 11. As noted in the univariate analysis, the SES as indicated by the car ownership remained associated with *H. pylori* status in the multivariate analysis (OR: 1.10 95%CI: 1.02-1.39). Prevalence of *H. pylori* was highly associated with chronic gastric ailments. (OR: 3.73 95%CI: 1.69-8.26). Smoking was also highly associated with prevalence of *H. pylori* in this population (OR: 2.23 95%CI: 1.24-4.02). Similarly, consumption of meat was a high risk factor of *H. pylori* infection (OR: 2.35 95%CI: 1.30-4.23). As seen in the univariate analysis consumption of chili peppers was an important protective factor against *H. pylori* infection (OR: 0.20 95%CI: 0.10-0.37). Drinking filtered or boiled water versus non filtered or boiled water use was associated with *H. pylori* status. (OR: 1.05 95%CI: 1.01-1.23). Consumption of restaurant food was also associated with prevalence of *H. pylori*. (Table 11)

Table 10: Relationship between participant characteristics and *H. pylori* in Pune, India

Participant characteristics	<i>H. pylori</i> (n= 350)		Univariate P-Value
	Present N (%)	Absent N (%)	
Gender			
Female	98 (52)	89 (48)	0.74
Male	82 (50)	81 (50)	
Education			
Primary School	78 (56)	61 (54)	0.12
High School	59 (53)	52 (47)	
University	43 (43)	57 (57)	
Car ownership			
Yes	99 (45)	118 (55)	0.00
No	81 (61)	52 (39)	
Drinking water			
Filtered / boiled	104 (45)	125 (55)	0.00
Not filtered / boiled	76 (63)	45 (37)	
Disease status			
Peptic ulcer	115 (61)	75 (39)	0.00
Stomach cancer	8 (23)	27 (77)	
Controls	57 (46)	68 (54)	



Participant characteristics	<i>H. pylori</i> (n= 350)		Univariate P-Value
	Present N (%)	Absent N (%)	
Smoking			
Yes	110 (57)	82 (43)	0.01
No	70 (44)	88 (56)	
Snack with Alcohol			
Yes	69 (57)	52 (43)	0.10
No	35 (56)	27 (44)	
Not applicable	76 (46)	91 (54)	
Chili pepper Consumption			
Yes	87 (42)	122 (58)	0.00
No	93 (66)	48 (34)	
Meat Consumption			
Yes	87 (61)	55 (39)	0.00
No	93 (45)	115 (55)	
Fish Consumption			
Yes	52 (56)	41 (44)	0.31
No	128 (50)	129 (50)	
Consumption of food while it is hot			
Yes	90 (57)	68 (43)	0.07
No	90 (47)	102 (53)	
Frequency of outside food consumption			
Almost never	99 (42)	137 (58)	0.00
1 / week	18 (56)	14 (44)	
2 / week	32 (76)	10 (24)	
3 / week	31 (77)	9 (23)	
Chronic gastric disease			
Yes	127 (59)	89 (41)	0.00
No	53 (40)	81 (60)	
Frequency of gastric disease			
Never	76 (46)	88 (54)	0.02
1 /month	52 (67)	26 (33)	
1 /week	33 (47)	37 (53)	
≥ 2 / week	19 (50)	19 (50)	
Family history of ulcer			
Yes	62 (63)	37 (37)	0.00
No	118 (47)	133 (53)	
Parasite infestation			
Yes	69 (42)	94 (58)	0.00
No	111 (59)	76 (41)	
Protozoa infestation			
Yes	86 (46)	102 (54)	0.02
No	94 (58)	68 (42)	
Helminth infestation			
Yes	46 (48)	49 (52)	0.55
No	134 (53)	121 (47)	

Participant characteristics	<i>H. pylori</i> (n= 350)		Univariate P-Value
	Present N (%)	Absent N (%)	
Anti-parasitic drug use			
Yes	107 (57)	82 (43)	0.04
No	73 (45)	88 (55)	
<i>H. pylori</i> treatment kit used			
Yes	46 (50)	46 (50)	0.80
No	134 (52)	124 (48)	
Spouses' <i>H. pylori</i> infection status			
Yes	29 (78)	8 (22)	0.00
No	40 (75)	13 (25)	
Don't know	111 (43)	149 (57)	

Table 11: Multivariate model: Relationship between participant characteristics and *H. pylori* In Pune, India

Participant characteristics	Adjusted Odds ratio	95% Confidence interval	P value
Car ownership			
Yes	1		0.05
No	1.10	1.02 – 1.39	
Parasite infestation			
Yes	0.44	0.24 – 0.80	0.00
No	1		
Chronic gastric disease			
Yes	3.73	1.69 – 8.26	0.00
No	1		
Chili pepper Consumption			
Yes	0.20	0.10 – 0.37	0.00
No	1		
Meat Consumption			
Yes	2.35	1.30 – 4.23	0.00
No	1		
Smoking			
Yes	2.23	1.24 - 4.02	0.00
No	1		
Eating outside			
3 / week	3.77	1.39 - 10.23	0.01
2 / week	2.69	1.18 - 7.42	
1 / week	1.61	0.65 – 3.98	
Almost never	1		
Water source			
Filtered / boiled	1		0.05
Not filtered / boiled	1.05	1.01 – 1.23	

We could enroll only 35 gastric cancer patients in this study. Also, the probability of *H. pylori* thriving in the stomach lining with the metaplasia is low. Hence due to their inadequate sample size and to avoid bias, we have eliminated these 35 patients from the analysis investigating association between participants' disease status and various risk factors.

In the initial univariate analysis, demographic characteristics such as gender (P-Value=0.42), education (P-Value=0.23), age were not associated with disease status. Study participant's disease status (having peptic ulcer versus not having any gastric ailment) was associated with *H. pylori* (P-Value=0.01). Study participant's current tobacco use was not associated with disease status (P-Value=0.16). Participants' consumption of snacks while drinking alcohol was associated with disease status (P-Value=0.00). Consumption of chili peppers (P-Value=0.06) did not have a statistically significant association with disease status. But, consuming the food items while they were hot (in terms of food temperature), meat (P-Value=0.02) and fish (P-Value=0.01) was associated with disease status (P-Value=0.04). Frequency of outside food consumption was not associated with disease status (P-Value=0.30). Participants' Family history of peptic ulcer was associated with disease status (P-Value=0.04). Parasite (P-Value=0.56), protozoa (P-Value=0.20) and helminth (P-Value=0.25) infestations were not associated with disease status. Prior treatment of *H. pylori* was associated with current disease status (P-Value=0.03). Participant's disease status was associated their spouses' *H. pylori* infection status (P-Value=0.00). (Table 12)

The final multivariate logistic regression analysis results for the association of participant characteristics and disease status are shown in Table 13. As noted in the univariate analysis, participants *H. pylori* status remained associated with disease status in the multivariate analysis (OR: 1.70 95%CI: 1.03-2.89). Participants disease status was associated with consumption of meat (OR: 1.10 95%CI: 1.02-1.75). Similarly, participants disease status was associated with consumption of fish (OR: 1.05 95%CI: 1.02-1.89). Similarly, participants' positive family history of ulcer was a risk factor of their peptic ulcer status (OR: 1.20 95%CI: 1.08-1.60). As seen in the

univariate analysis history of anti parasite drug use was an important protective factor against peptic ulcer prevalence (OR: 0.51 95%CI: 0.30-0.86). Similarly, consumption of snacks with alcohol remained an important protective factor against peptic ulcer in this study population (OR: 0.32 95%CI: 0.13-0.78). (Table 13)

Table 12: Relationship between participant characteristics and disease status (peptic ulcer vs. control) in Pune, India

Participant characteristics	Disease status (n = 315)		Univariate P-Value
	Control N (%)	Peptic ulcer N (%)	
Gender			
Female	62 (37)	104 (63)	0.42
Male	63 (42)	86 (53)	
Education			
Primary School	45 (35)	85 (65)	0.23
High School	40 (41)	58 (59)	
University	40 (46)	47 (54)	
<i>H. pylori</i> status			
Positive	57 (33)	115 (67)	0.01
Negative	68 (47)	75 (52)	
Smoking			
Yes	63 (36)	112 (64)	0.16
No	62 (44)	78 (56)	
Snack with Alcohol			
Yes	44 (39)	68 (61)	0.00
No	9 (17)	45 (83)	
Not applicable	72 (48)	77 (52)	
Chili peppers Consumption			
Yes			0.06
No	65 (35) 60 (46)	119 (65) 71 (54)	
Meat Consumption			
Yes	45 (39)	94 (61)	0.02
No	80 (42)	96 (58)	
Fish Consumption			
Yes	11 (12)	78 (88)	0.01
No	114 (51)	112 (49)	
Consumption of food while it is hot			
Yes	50 (34)	99 (66)	0.04
No	75 (45)	91 (55)	
Outside food consumption			
Almost never	90 (43)	119 (57)	0.30
1 / week	12 (39)	19 (61)	
2 / week	13 (32)	28 (68)	
3 / week	10 (29)	24 (71)	

Participant characteristics	Disease status (n = 315)		Univariate P-Value
	Control N (%)	Peptic ulcer N (%)	
Family history of ulcer			
Yes	31 (31)	68 (69)	0.04
No	94 (44)	122 (56)	
Parasite infestation			
Yes	55 (38)	91 (62)	0.56
No	70 (41)	99 (59)	
Protozoa infestation			
Yes	61 (36)	107 (64)	0.20
No	64 (44)	83 (56)	
Helminth infestation			
Yes	30 (34)	58 (66)	0.25
No	95 (42)	132 (58)	
<i>H. pylori</i> treatment kit used			
Yes			0.03
No	42 (49)	43 (51)	
	83 (36)	147 (64)	
Spouses' <i>H. pylori</i> infection status			
Yes	25 (68)	12 (32)	0.00
No	0 (0)	43 (100)	
Don't know	100 (43)	135 (57)	

Table 13: Multivariate model: Relationship between participant characteristics and disease status (peptic ulcer vs. control) in Pune , India

Participant characteristics	Adjusted Odds ratio	95% Confidence interval	P value
<i>H. pylori</i> status			
Positive	1.70	1.03 – 2.89	0.04
Negative	1		
Snack with Alcohol			
Yes	0.32	0.13 - 0.78	0.00
No	1		
Meat Consumption			
Yes	1.10	1.02 – 1.75	0.05
No	1		
Fish Consumption			
Yes	1.05	1.02 – 1.89	0.05
No	1		
Family history of ulcer			
Yes	1.20	1.08 – 1.60	0.04
No	1		
Anti-parasitic drug use			
Yes	0.51	0.30 - 0.86	0.01
No	1		

In this study we used prevalence of chronic gastric disease as a surrogate measure of gastric pathology in general. In this study we tried to shed more light on the relationship between gastric disease and risk factors such as education, spicy food consumption, consumption of high temperature food, intestinal parasite drug use, etc.

In the initial univariate analysis, demographic characteristics such as gender (P-Value=0.80), education (P-Value=0.10), age were not associated with chronic gastric disease status. Study participant's disease status (having peptic ulcer or stomach cancer versus not having any of these gastric ailments) was associated with chronic gastric disease (P-Value=0.03). Study participant's *H. pylori* status was associated with chronic gastric disease (P-Value=0.00). Study participant's current tobacco use was not associated with chronic gastric disease (P-Value=0.58). Participants' consumption of snacks while drinking alcohol was not associated with chronic gastric disease status (P-Value=0.73). However, consumption of meat (P-Value=0.04) and fish (P-Value=0.03) had a statistically significant association with chronic gastric disease status. But, chili peppers consumption (P-Value=0.57) was not associated with chronic gastric disease status. However, consuming the food items while they were hot (in terms of food temperature) was associated with chronic gastric disease status (P-Value=0.00). Interestingly, frequency of outside food consumption was not associated with chronic gastric disease (P-Value=0.67). Family history of peptic ulcer was associated with chronic gastric disease status (P-Value=0.00). Parasite (P-Value=0.23), protozoa (P-Value=0.51) and helminth infestations (P-Value=0.34) were not associated with chronic gastric disease status. (*Table 14*)

The final multivariate logistic regression analysis results for the association of participant characteristics and chronic gastric disease status are shown in *Table 15*. As noted in the univariate analysis, participants *H. pylori* status remained strongly associated with chronic disease status in the multivariate analysis (OR: 2.48 95%CI: 1.24-4.96). Participants chronic gastric disease status was highly associated with consumption of meat (OR: 1.05 95%CI: 1.03-1.78). Similarly,

participants disease status was highly associated with consumption of fish (OR: 1.09 95%CI: 1.06-1.90). Similarly, participants' positive family history of ulcer was a high risk factor of their chronic disease status (OR: 4.45 95%CI: 1.95-10.15). As seen in the univariate analysis history of anti parasite drug use was a protective factor against peptic ulcer prevalence (OR: 0.43 95%CI: 0.21-0.89). (Table 15)

Table 14: Relationship between participant characteristics and chronic gastric disease status in Pune, India

Participant characteristics	Chronic gastric disease (n=350)		Univariate P-Value
	Present N (%)	Absent N (%)	
Gender			
Female	114 (61)	73 (39)	0.82
Male	102 (63)	61 (37)	
Education			
Primary School	90 (65)	49 (35)	0.10
High School	73 (66)	38 (34)	
University	53 (53)	47 (47)	
Disease status			
Peptic ulcer	126 (66)	64 (34)	0.03
Stomach cancer	15 (43)	20 (57)	
Controls	75 (60)	50 (40)	
<i>H. pylori</i> status			
Positive	127 (70)	53 (30)	0.00
Negative	89 (52)	81 (48)	
Smoking			
Yes	121 (63)	71 (37)	0.58
No	95 (60)	63 (40)	
Snack with Alcohol			
Yes	78 (65)	43 (35)	0.73
No	38 (61)	24 (39)	
Not applicable	100 (60)	67 (40)	
Chili peppers Consumption			
Yes	126 (60)	83 (40)	0.57
No	90 (64)	51 (36)	
Meat Consumption			
Yes	97 (68)	45 (31)	0.04
No	119 (57)	89 (43)	
Fish Consumption			
Yes	72 (88)	21 (22)	0.03
No	144 (56)	113 (44)	

Participant characteristics	Chronic gastric disease (n=350)		Univariate P-Value
	Present N (%)	Absent N (%)	
Consumption of food while it is hot			0.00
Yes	116 (73)	42 (27)	
No	100 (52)	92 (48)	
Frequency of outside food consumption			0.67
Almost never	141 (60)	95 (40)	
1 / week	21 (66)	11 (34)	
2 / week	29 (69)	13 (31)	
3 / week	25 (63)	15 (37)	
Family history of ulcer			0.00
Yes	84 (85)	15 (15)	
No	132 (53)	119 (47)	
Parasite infestation			0.23
Yes	95 (58)	68 (42)	
No	121 (65)	66 (35)	
Protozoa infestation			0.51
Yes	113 (60)	75 (40)	
No	103 (64)	59 (36)	
Helminth infestation			0.34
Yes	63 (66)	32 (34)	
No	153 (60)	102 (40)	

Table 15: Multivariate model: Relationship between participant characteristics and chronic gastric disease in Pune, India

Participant characteristics	Adjusted Odds ratio	95% Confidence interval	P value
<i>H. pylori</i> status			0.00
Positive	2.48	1.24 – 4.96	
Negative	1		
Meat Consumption			0.05
Yes	1.05	1.03 – 1.78	
No	1		
Fish Consumption			0.05
Yes	1.09	1.06 – 1.90	
No	1		
Family history of ulcer			0.04
Yes	4.45	1.95 – 10.15	
No	1		
Anti-parasitic drug use			0.02
Yes	0.43	0.21 - 0.89	
No	1		



We followed a cohort of 100 participants who were *H. pylori* positive for two years. Eighty percent (80/100) of these individuals were suffering from peptic ulcer and 20 % (20/100) were from the control group. The median age was 45 years (range: 28-63years). Forty five percent (45/100) were females while 55% (55/100) were males. All the participants of this cohort took medications for the treatment of *H. pylori* infection. However, only 75% (75/100) opted for a test to check their *H. pylori* status after finishing their treatment. Interestingly, treatment against *H. pylori* status was changed from initial positive to negative in only 53% of these (40/75) individuals. *H. pylori* infection was eradicated in 50% (35/70) of individuals who opted for the antibiotic therapy. (Figure 4) However, 40% (40/100) of individuals reported alleviation of initial gastric ailments within 6 months of completing the treatment. [Median time: 2 month (range: 1 month – 6 month)]. Seventy percent (70/100) of individuals took the anti *H. pylori* triple therapy (*H. pylori* treatment kit) while 20% (20/100) of participants opted for traditional herbal treatment and only 10 % (10/100) choose other remedies. Interestingly, only 10% (10/100) individuals that were diagnosed of *H. pylori* sought a specialist (gastro-enterologist) consultation. (Table 16)

Table 16: Prospective cohort participant characteristics in Pune, India (N=100)

Characteristics	N (%)
Disease status	
Peptic ulcer	80 (80)
Controls	20 (20)
Median age (Range)	45 years (range: 28-63)
Gender	
Female	45 (45)
Male	55 (55)
<i>H. pylori</i> treatment taken	
Yes	100 (100)
No	0 (0)
<i>H. pylori</i> diagnostic test taken after treatment	
Yes	75 (75)
No	25 (25)
<i>H. pylori</i> status changed from positive to negative after treatment	
Yes	40 (53)
No	35 (47)

Characteristics	N (%)
Gastric complaints are alleviated after treatment	
Yes	40 (40)
No	60 (60)
Type of treatment taken	
Antibiotics ( <i>H. pylori</i> treatment kit)	70 (70)
Herbal	20 (20)
Other	10 (10)
Sought gastro-enterologists' consultation	
Yes	10 (10)
No	90 (90)

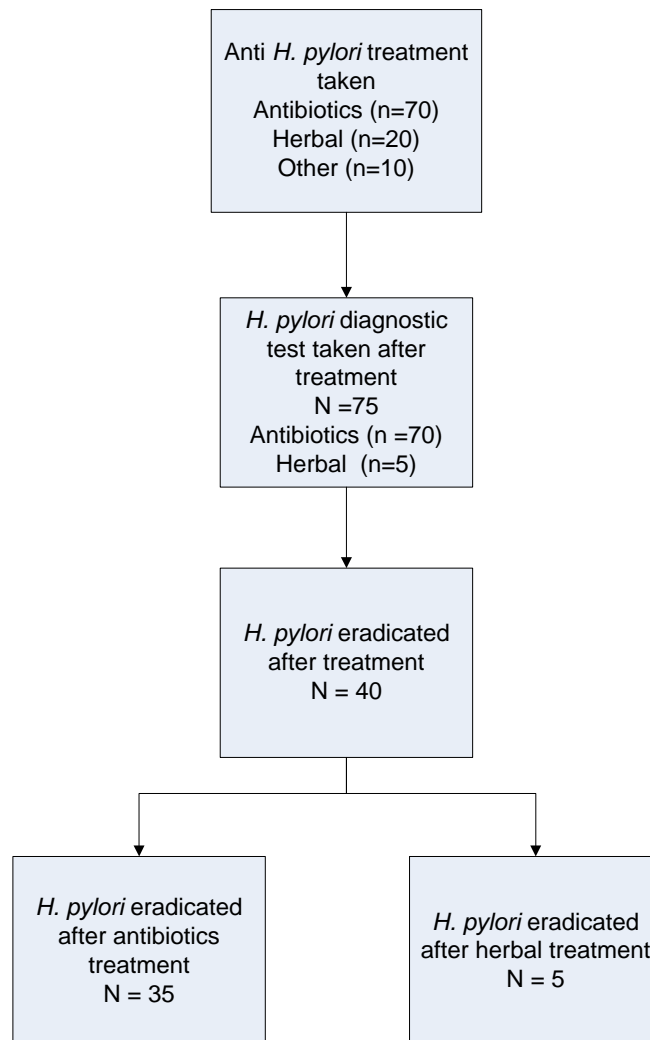


Figure 4 Anti *H. pylori* treatment success in Pune, India

## Discussion

This is the first study to document the prevalence of *H. pylori* among patients with peptic ulcer, stomach cancer and healthy individuals in Pune, Maharashtra. To our knowledge this is also the largest study (n=350) investigating risk factors of *H. pylori* prevalence in the state of Maharashtra, India.

*H. pylori* infection is one of the most common infections worldwide. Epidemiological studies from India have shown 70%, [230] 77.2%, [223] 78% [231] and 79% [24] prevalence of *H. pylori* infection. In the present study, the prevalence of *H. pylori* infection was 61% in symptomatic and 45 % in asymptomatic individuals. The prevalence of *H. pylori* infection was independent of gender in the present study, which is consistent with previous reports. [24] Similarly, we found no association between age and prevalence of *H. pylori* infection. However, in most of the studies from developing countries it's noted that within first 10 years of life the prevalence of *H. pylori* increases with age. [77, 232-238] It is important to note that we enrolled only adults in our study in India. Hence we were unable to note the association between age and prevalence of *H. pylori* in our study. In some studies, lower education has shown to be associated with high prevalence of *H. pylori*. [130, 239, 240] In our study education was not associated with prevalence of *H. pylori*. This association should be explored in a larger and more diverse cohort of individuals, perhaps recruiting individuals from villages near to Pune city as none of the participants in our study were uneducated. We recruited participants from the city of Pune and the educational attainment in our study cohort represents the education level of general population of Pune city.

There is no doubt that improvements in the standards of living are an important factor in changing the risk factors related to *H. pylori* infection. A recent study has shown a sharp decrease of prevalence of *H. pylori* infection in St. Petersburg, Russia, comparing the prevalence in children in 1995 and 2005. [14] Another example that improvements in the standards of living are related

to *H. pylori* infection was reported by Parente et al.,[241] who compared the prevalence in children of low and high socioeconomic situation living in the same neighborhood in Terezina, Brazil. While in children 5–6 years old from low socioeconomic class, the prevalence reached 70%, in the high socioeconomic class it was 17.4% .[241] *H. pylori* infection is related to social class, being higher in low social class. The leading hypothesis for *H. pylori* transmission is person to person via contact with either gastric or fecal contents. The increasing prevalence of *H. pylori* infection with age in most of the places probably reflects the infection acquired at different ages as a result of poor sanitation facilities and hygienic practices as well as crowding during childhood. Water supply seems to be an important source of *H. pylori* infection. Klein et al. have shown in Lima, Peru that children whose homes had an external water source were three times more likely to be infected than children living in homes provided with internal water source. [79] Nurgalieva et al. reported that drinking river water had highest risk for *H. pylori* infection compared to tap water, suggesting that transmission of *H. pylori* infection can be water borne. [242]

The importance of drinking river water as a transmission route of *H. pylori* was emphasized by Fujimura et al. in Japan, who collected samples from four rivers (upper middle and downstream). *H. pylori* was detected by nested polymerase chain reaction in the water of middle and downstream, suggesting that river water in the natural environment could be a risk factor for *H. pylori* transmission.[106] Using a bivariate analysis, Rodrigues et al. showed in children from an urban community in northeast Brazil a positive correlation between type of drinking water and *H. pylori* infection.[146] Boiling the water seems to be an effective way to prevent the infection, as suggested by Sung .[243] Our results confirm that source of drinking water is a risk factor for *H. pylori* infection. Prevalence was higher when the source of drinking water was a river and lower when the water was filtered or boiled [OR 1.05 (95% CI = 1.01–1.23)].

We also found that, the increasing frequency of eating outside was significantly associated with

prevalence of *H. pylori* in our study population. (Table 11) In developing countries, it is well known that eating restaurant food favors a feco-oral route transmission. SES has been considered as an important risk factor for *H. pylori* infection, probably because SES relates to hygienic practices. The prevalence of *H. pylori* infection has been shown to be influenced by SES in previous studies; [24, 244, 245] similarly, it was dependent on SES in the present study.[31] Car ownership is a significant indicator of SES in state of Maharashtra and in Pune city. Specifically, in our study, individuals who owned a car were less likely to have *H. pylori* infection compared with participants who did not own a car (OR: 1.10 95% CI: 1.02-1.39).

Meat has shown to be a risk factor for *H. pylori* infection in numerous studies. [117, 246-248] Consumption of meat was a significant risk factor for *H. pylori* infection in our study as well. [OR 2.35 (95% CI = 1.30–4.23)]. On the other hand, consumption of chili peppers was shown to have a protective effect against *H. pylori* infection. In Maharashtra and in Pune chili peppers are common ingredients of most of the recipe. The chili peppers are added with turmeric (*Curcuma longa*) and cooking oil while preparing food items from produce. Previous studies have shown the protective effect of turmeric, chili peppers and some spices against *H. pylori* infection. [70] Several in vitro studies have looked at the effect of plant extracts on *H. pylori*. Anti-microbial effects have been reported for garlic [67], green tea [249], honey [250], thyme [251], some Iranian plants [252] and the essential oils from several species of mint [252]. Some of these studies have been validated in animals and confirmed the potential benefit of using plants as the source of anti-microbial agents against *H. pylori*. [70] However, the exact mechanism behind the bactericidal action of chili peppers has not been validated. Certainly, more work needs to be done in determining the active ingredients of chili peppers as well as performing studies on a larger number of patients.

In our study 27 % participants were infected with helminth and 54% with protozoa and 47 % had some kind of parasite infection. Parasite infection was the only protective factor that remained

statically significant in the multivariate model. Parasite infestation is seen as another protective factor against diseases caused by *H. pylori* infection. In our study individuals who suffered from parasite infestations were less likely to have *H. pylori* infection. Moreover, individuals who had history of an anti parasite drug were less likely to have a peptic ulcer or a chronic gastric ailment. Indeed its hypothesized that concurrent enteric helminth infection can attenuate gastric atrophy via immune modulation. [71, 169]

It is well known that the immune response to infectious agents leads to the expansion of particular CD4+ve T-helper (Th) cell subsets. Th1 cells are reported to produce Interleukin (IL)-2, IL-12 and interferon  $\gamma$  (IFN $\gamma$ ), and are associated with cell-mediated immunity, while Th2 cells have been reported to secrete IL-4, IL-5, IL-6, IL-10 and IL-13, and are responsible for strong antibody responses, including IgE-dependent allergies of the immediate type.[253] In general, Th1 responses are associated with intracellular micro-organisms including bacteria, protozoa and fungi, whereas extracellular pathogens induce Th2 responses. Studies to date have shown that natural infection with *H. pylori*, which is by and large an extracellular infection, leads to a Th1-predominant response, with IL-2, IL-12, TNF $\alpha$  and IFN $\gamma$  reported to be present in the gastric mucosa of *H. pylori*-positive subjects. [254-256] In contrast, the Th2 cytokines IL-4 and IL-5 have been found to be virtually absent in *H. pylori* infected subjects although a number of studies have reported IL-10 to be present in the gastric mucosa of subjects with *H. pylori*-related active gastritis.[256-259]

Recently, Fox *et al.*[169] showed that mice infected with *Helicobacter felis* alone showed a Th1 response, but in mice co-infected with *H. felis* and the helminth *H. polygyrus*, there was a shift to a pattern of cytokine expression consistent with a Th2 immune response.[71] This corresponded to a significant reduction in mucosal hyperplasia, mucosal metaplasia and glandular atrophy. Thus, associated with the Th2 immune response, there was a marked reduction in

*Helicobacter*-associated corpus atrophy, despite chronic inflammation and high *Helicobacter* colonization. If this is extrapolated to man, then intestinal helminth infection may provide a protective effect against development of gastric atrophy and gastric cancer. [71] Given that animal models do not necessarily replicate the human situation, Fox's study, indicates the complexity of biological actions associated with *H. pylori* which merits further investigation.

In our study, *H. pylori* were seen as a significant risk factor for peptic ulcer disease. [OR: 1.70 (95% CI: 1.03- 2.89)] as well as chronic gastric disease [OR: 2.48 (95% CI: 1.24- 4.96)]. The recognition of *H. pylori* infection as the main cause of ulcer [260] was undoubtedly the most important achievement in the past 50 years in gastroenterology. Showing that ulcer was a curable disease with a short treatment schedule represented a blessing to millions of people. [261-264] Lately, it was recognized that not only ulcer was related to *H. pylori* infection, but also the majority of gastric cancer [1, 265-267] and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [268, 269]. Indeed eradication of *H. pylori* would probably be a very important tool in the management of upper digestive disease, particularly looking on high-risk cancer population.

In our study, consumption of meat is suggested to be an important risk factor peptic ulcer. [OR: 1.10 (95% CI: 1.02- 1.75)] and chronic gastric disease [OR: 1.05 (95% CI: 1.03- 1.78)].

Consumption of fish has also shown to be an important risk factor peptic ulcer. [OR: 1.05 (95% CI: 1.02- 1.89)] and chronic gastric disease [OR: 1.09 (95% CI: 1.06- 1.90)]. It is well known that individuals consume smoked, dried salted meat and fish mostly outside homes (in restaurants) in Pune. These put them at increased risk of acquiring *H. pylori* infection which may contribute to development of gastric pathologies such as peptic ulcer and or stomach cancer. Our findings are line with many other researchers who showed that consumption of smoked meat, salted fish and meat are risk factors for various gastric ailments including stomach cancer. Smoke-drying and preservation leads to formation of *N*-nitroso compounds. Nitrite reacts with amines and amides found in meats and other proteins to form *N*-nitroso compounds, which are animal carcinogens

and possible human carcinogens.[270] Furthermore, although salt is not an independent carcinogen, it is thought to increase the risk of gastric cancer through direct damage to the gastric mucosa, which results in gastritis, increased DNA synthesis, and cell proliferation.[271] This indirectly contributes to the development of chronic atrophic gastritis, leading to the development of stomach cancer. [121, 123, 272-276] Because of the presence of both salt and nitrite in processed fish and meats, its role in the development of stomach cancer cannot be ignored, as was found in the present study. Studies in the past have also shown positive associations of high intake of processed meats as a group or for individual cured meats. [277-282]

Family history of ulcer has also shown to be a statistically significant risk factor for peptic ulcer [OR: 1.20 (95% CI: 1.08- 1.60)] and chronic gastric disease [OR: 4.45 (95% CI: 1.95- 10.15)] in our study. Positive family history of peptic ulcer has been well recognized as significant risk factor for peptic ulcer. [283-285]

Alcohol and tobacco consumption did not emerge as a risk factor for peptic ulcer and chronic gastric disease in our study. However, consumption of snacks with alcohol was a statistically significant protective factor against peptic ulcer [OR: 0.32 (95% CI: 0.13- 0.78)]. This can be partially be explained by the link of type, amount of alcohol consumed by individuals from various socio economic strata. Usually individuals from higher SES strata consume snacks with alcohol; these individuals may be drinking in moderation and may be opting for treatment for their ailments compared to individuals from lower SES who don't snack with their alcohol. These factors may be interlinked and may play a role in protection of gastric mucosal layer in people who drink but consume snacks while drinking. However, this hypothesis needs to be formally investigated.

In our study we also followed a cohort of *H. pylori* positive individuals for approximately 2 years. All the 100 individuals opted for some kind of treatment to treat their *H. pylori* infection.



However, only 75% (75/100) opted for a test to check their *H. pylori* status after finishing their treatment. Interestingly, treatment against *H. pylori* status was changed from initial positive to negative in only 53% of these (40/75) individuals. This may reflect the poor success of *H. pylori* eradication by the triple therapy or by the herbal medicines that were used to treat *H. pylori*. The antimicrobial resistance of *H. pylori* is on the rise across the globe and is well documented in India. [28, 286-299] It is frequently observed that patients buy only a part of the *H. pylori* treatment kit or use only the half and once their symptoms are alleviated they share the rest of the kit with their friends and family members (personal observation). These facts certainly merit attention and patient education should be made a mandatory part while prescribing anti microbial agents.

Our study has some limitations. First we were not successful in enrolling the desired number of patients (n=50) diagnosed with stomach cancer. We believe this is owing to the facts related to high mortality rate among individuals diagnosed with stomach cancer in India and also to low proportion of patients diagnosed with stomach cancer seeking active treatments. Future studies need to utilize innovative strategies such as individual patient counseling and organizing patient education seminars to boost enrollment of patients diagnosed with stomach cancer into research studies. We came across a lot of missing data for the amount of cigarettes and alcohol consumed by the participants. We believe owing to the stigma attached to smoking and alcohol consumption individuals participating in this study were reluctant to share the amount of alcohol and tobacco consumed by them. Hence, we were unable to investigate the dose response of tobacco and alcohol consumption. We also made an attempt to elucidate the intra-familial transmission pathways of *H. pylori* by enquiring about spouses' *H. pylori* status. Unfortunately 74% of our participant did not know *H. pylori* status of their spouse. Almost all of them reported that their spouse was never tested for presence of *H. pylori*. Lastly, owing to the financial restraints and non availability of technical expertise required for isolation of DNA from stool samples and

genotyping were unable to conduct the genotyping of *H. pylori* to detect the prevalence of *CagA* positive and furthermore East Asian *CagA H. pylori* infection in Pune. This area certainly needs attention as previous studies from India have shown high prevalence of *CagA* positive *H. pylori* among symptomatic population.

## Chapter Five

### Conclusions

Our studies in Ecuador and Panama documented the prevalence of virulent East Asian *CagA* positive *H. pylori* among healthy individuals. This finding is of utmost importance in understanding the patho-physiology of stomach cancer in Ecuador and Panama. It is well documented that the grades of inflammation, activity of gastritis, and atrophy are significantly higher in gastritis patients infected with the East Asian *CagA*-positive strain than in gastritis patients infected with the *CagA*-negative or Western *CagA*-positive strains. [300-303] The prevalence of the East Asian *CagA*-positive strain is associated with the mortality rate of gastric cancer in Asia. Endemic circulation of *H. pylori* populations carrying biologically more active *CagA* proteins in East Asian countries, where the mortality rate of gastric cancer is among the highest in the world, may be involved in increasing the risk of gastric cancer in populations such as of Ecuador and Panama. [304-306] This can explain the difference between the incidence rate of stomach cancer in Ecuador and Panama. Our study from Pune, India underscores *H. pylori* along with family history of peptic ulcer, meat and fish consumption as significant risk factors for peptic ulcer and chronic gastric disease. Our study in India also confirms low SES along with meat consumption and tobacco smoking as one of the risk factors for *H. pylori* infection. We also highlight the statistically significant association of increased frequency of eating restaurant food and drinking non filtered water as risk factors for *H. pylori* infection. These may suggest the feco-oral and water-born transmission of *H. pylori* in Pune, India. Interestingly we also bring to light the role of chili peppers consumption as protective factor against *H. pylori* infection. Last but not the least we emphasize the protective influence of concurrent parasite infestation on *H. pylori*

infection and development of chronic gastric ailments. This underscores the need of research to investigate the relationship of various dietary factors especially chili peppers and other protective factors such as parasite infestation and *H. pylori* infection in developing countries.

In summary, the speculations in this paper are based on objective scientific evidence that fits with the changing patterns of peptic ulcer and gastric cancer that we have seen. We recommend studies in countries like India and Ecuador to investigate the epidemiology of specific types of parasite infections as well as the influence of diet, host genetic susceptibility, *H. pylori* genotype and other co-morbidity states in determining gastric cancer rates. Moreover, the protective effect of concurrent parasite infection on the development of pre-neoplastic lesions such as gastric atrophy should be extended to studies on gastric cancer. We have highlighted the protective role of chili peppers against *H. pylori* infection. Although the bactericidal and anti-adhesive effects of chili peppers, ginger, cumin, and turmeric, have been shown *in vitro*, further studies are needed to be carried out to investigate their effects *in vivo*, to see whether the extracts are able to remain effective despite the harsh process of digestion. Lastly, the issues of treatment compliance leading to anti microbial resistance are also equally vital and need to be studied in larger population based studies.

## References

1. IARC Monographs of the Evaluation of Carcinogenic Risks to Humans. Available from: <http://www.iarc.fr/IARCPress/mono/index.php>.
2. Poddar, U. and S.K. Yachha, *Helicobacter pylori* in children: an Indian perspective. *Indian Pediatr*, 2007. **44**(10): p. 761-70.
3. Ernst, P.B. and B.D. Gold, *Helicobacter pylori* in childhood: new insights into the immunopathogenesis of gastric disease and implications for managing infection in children. *J Pediatr Gastroenterol Nutr*, 1999. **28**(5): p. 462-73.
4. Fiedorek, S.C., et al., *Factors influencing the epidemiology of Helicobacter pylori infection in children*. *Pediatrics*, 1991. **88**(3): p. 578-82.
5. Mitchell, H.M., *The epidemiology of Helicobacter pylori*. *Curr Top Microbiol Immunol*, 1999. **241**: p. 11-30.
6. Lehours, P. and O. Yilmaz, *Epidemiology of Helicobacter pylori infection*. *Helicobacter*, 2007. **12 Suppl 1**: p. 1-3.
7. Leverstein-van Hall, M.A., et al., *Transmission of Helicobacter pylori via faeces*. *Lancet*, 1993. **342**(8884): p. 1419-20.
8. Stone, M.A., *Transmission of Helicobacter pylori*. *Postgrad Med J*, 1999. **75**(882): p. 198-200.
9. Axon, A.T., *The transmission of Helicobacter pylori: which theory fits the facts?* *Eur J Gastroenterol Hepatol*, 1996. **8**(1): p. 1-2.
10. Goodman, K.J. and P. Correa, *The transmission of Helicobacter pylori. A critical review of the evidence*. *Int J Epidemiol*, 1995. **24**(5): p. 875-87.
11. Abraham, P. and S.G. Kulkarni, *Transmission routes of Helicobacter pylori*. *Indian J Gastroenterol*, 2000. **19 Suppl 1**: p. S7-9; discussion S9-10.
12. Mendall, M.A., *Transmission of Helicobacter pylori*. *Semin Gastrointest Dis*, 1997. **8**(3): p. 113-23.
13. Megraud, F., *Epidemiology of Helicobacter pylori infection*. *Gastroenterol Clin North Am*, 1993. **22**(1): p. 73-88.
14. Malaty, H.M., *Epidemiology of Helicobacter pylori infection*. *Best Pract Res Clin Gastroenterol*, 2007. **21**(2): p. 205-14.
15. Malaty, H.M., et al., *Prevalence of Helicobacter pylori infection in Korean children: inverse relation to socioeconomic status despite a uniformly high prevalence in adults*. *Am J Epidemiol*, 1996. **143**(3): p. 257-62.
16. Malaty, H.M., et al., *Helicobacter pylori and socioeconomic factors in Russia*. *Helicobacter*, 1996. **1**(2): p. 82-7.
17. Mitchell, H.M., et al., *Epidemiology of Helicobacter pylori in southern China: identification of early childhood as the critical period for acquisition*. *J Infect Dis*, 1992. **166**(1): p. 149-53.
18. Yamaoka, Y., et al., *Conservation of Helicobacter pylori genotypes in different ethnic groups in Houston, Texas*. *J Infect Dis*, 2000. **181**(6): p. 2083-6.

19. Matysiak-Budnik, T., et al., *Helicobacter pylori* infection in Eastern Europe: seroprevalence in the Polish population of Lower Silesia. *Am J Gastroenterol*, 1996. **91**(12): p. 2513-5.
20. Ashorn, M., et al., *Helicobacter pylori* infection in Finnish children and adolescents. A serologic cross-sectional and follow-up study. *Scand J Gastroenterol*, 1995. **30**(9): p. 876-9.
21. Kumagai, T., et al., *Acquisition versus loss of Helicobacter pylori* infection in Japan: results from an 8-year birth cohort study. *J Infect Dis*, 1998. **178**(3): p. 717-21.
22. Nurgalieva, Z.Z., et al., *Helicobacter pylori* infection in Kazakhstan: effect of water source and household hygiene. *Am J Trop Med Hyg*, 2002. **67**(2): p. 201-6.
23. Dore, M.P., et al., *Risk Factors Associated with Helicobacter pylori* Infection among Children in a Defined Geographic Area. *Clin Infect Dis*, 2002. **35**(3): p. 240-5.
24. Graham, D.Y., et al., *Seroepidemiology of Helicobacter pylori* infection in India. Comparison of developing and developed countries. *Dig Dis Sci*, 1991. **36**(8): p. 1084-8.
25. Ahmad, M.M., et al., *Prevalence of Helicobacter pylori* in asymptomatic population--a pilot serological study in Bangladesh. *J Epidemiol*, 1997. **7**(4): p. 251-4.
26. Perez-Perez, G.I., et al., *Seroprevalence of helicobacter pylori* infection in couples. *J Clin Microbiol*, 1991. **29**(3): p. 642-4.
27. Mazumder, D.N. and U.C. Ghoshal, *Epidemiology of Helicobacter pylori* in India. *Indian J Gastroenterol*, 1997. **16 Suppl 1**: p. S3-5.
28. Gill, H.H., et al., *Epidemiology of Helicobacter pylori: the Indian scenario*. *Indian J Gastroenterol*, 1993. **12**(1): p. 9-11.
29. Abasiyanik, M.F., M. Tunc, and B.A. Salih, *Enzyme immunoassay and immunoblotting analysis of Helicobacter pylori* infection in Turkish asymptomatic subjects. *Diagn Microbiol Infect Dis*, 2004. **50**(3): p. 173-7.
30. Reshetnikov, O.V., et al., *Helicobacter pylori* seropositivity among adolescents in Novosibirsk, Russia: prevalence and associated factors. *J Pediatr Gastroenterol Nutr*, 2003. **36**(1): p. 72-6.
31. Singh, V., et al., *Epidemiology of Helicobacter pylori* and peptic ulcer in India. *J Gastroenterol Hepatol*, 2002. **17**(6): p. 659-65.
32. Miwa, H., M.F. Go, and N. Sato, *H. pylori* and gastric cancer: the Asian enigma. *Am J Gastroenterol*, 2002. **97**(5): p. 1106-12.
33. Tsubono Y, T.T., Iwase Y, Itoi Y, Akabane M, Tsugane S. , *Nutrient consumption and gastric cancer mortality in five regions of Japan*. . *Nutr Cancer*, 1997. **27**: p. 310-315.
34. Wong, B.C., et al., *Differential Helicobacter pylori* infection rates in two contrasting gastric cancer risk regions of South China. *China Gastric Cancer Study Group*. *J Gastroenterol Hepatol*, 1999. **14**(2): p. 120-5.
35. Abraham, P. and S.J. Bhatia, *Position paper on Helicobacter pylori* in India. *Indian Society of Gastroenterology*. *Indian J Gastroenterol*, 1997. **16 Suppl 1**: p. S29-33.
36. Batmanabane, V., V. Kate, and N. Ananthkrishnan, *Prevalence of Helicobacter*

- pylori in patients with portal hypertensive gastropathy--a study from south India. Med Sci Monit, 2004. 10(4): p. CR133-6.*
37. Bhasin, D.K., et al., *Helicobacter pylori in gastric cancer in India. Trop Gastroenterol, 1999. 20(2): p. 70-2.*
  38. Choudhuri, G. and S. Mohindra, *Epidemiology of Helicobacter pylori in India. Indian J Gastroenterol, 2000. 19 Suppl 1: p. S3-5; discussion S5-6.*
  39. Mohandas, K.M., *Helicobacter pylori and gastric lymphoma. N Engl J Med, 1994. 331(11): p. 746.*
  40. Tovey, F.I., *Helicobacter pylori infection. Trop Doct, 1995. 25(2): p. 49.*
  41. Khuroo MS, Z.S., Mahajan R, Banday MA. , *High incidence of oesophageal and gastric cancer in Kashmir in a population with special personal and dietary habits. Gut, 1992. 33: p. 11-15.*
  42. Tovey, F.I., et al., *Helicobacter pylori and peptic ulcer recurrence. Gut, 1992. 33(9): p. 1293.*
  43. Kate, V. and N. Ananthkrishnan, *Helicobacter pylori and gastric carcinoma: evidence for the link. Natl Med J India, 2000. 13(6): p. 329.*
  44. Kate, V., et al., *Prevalence of Helicobacter pylori infection in disorders of the upper gastrointestinal tract in south India. Natl Med J India, 1998. 11(1): p. 5-8.*
  45. Khanna, A.K., et al., *Correlation of Helicobacter pylori and gastric carcinoma. J Postgrad Med, 2002. 48(1): p. 27-8.*
  46. Karnes, W.E., Jr., et al., *Elevation of meal-stimulated gastrin release in subjects with Helicobacter pylori infection: reversal by low intragastric pH. Rev Infect Dis, 1991. 13 Suppl 8: p. S665-70.*
  47. Asaka, M., et al., *Relationship between Helicobacter pylori infection, atrophic gastritis and gastric carcinoma in a Japanese population. Eur J Gastroenterol Hepatol, 1995. 7 Suppl 1: p. S7-10.*
  48. Singh, K. and U.C. Ghoshal, *Causal role of Helicobacter pylori infection in gastric cancer: an Asian enigma. World J Gastroenterol, 2006. 12(9): p. 1346-51.*
  49. Graham, D.Y., *Helicobacter pylori infection in the pathogenesis of duodenal ulcer and gastric cancer: a model. Gastroenterology, 1997. 113(6): p. 1983-91.*
  50. Hansson, L.E., et al., *The risk of stomach cancer in patients with gastric or duodenal ulcer disease. N Engl J Med, 1996. 335(4): p. 242-9.*
  51. Lu, H., Y. Yamaoka, and D.Y. Graham, *Helicobacter pylori virulence factors: facts and fantasies. Curr Opin Gastroenterol, 2005. 21(6): p. 653-9.*
  52. Mukhopadhyay, A.K., et al., *Distinctiveness of genotypes of Helicobacter pylori in Calcutta, India. J Bacteriol, 2000. 182(11): p. 3219-27.*
  53. Singh, M., et al., *Genotypes of Helicobacter pylori in children with upper abdominal pain. J Gastroenterol Hepatol, 2003. 18(9): p. 1018-23.*
  54. Kumar, S., et al., *Antibodies to Cag A protein are not predictive of serious gastroduodenal disease in Indian patients. Indian J Gastroenterol, 1998. 17(4): p. 126-8.*
  55. Ghoshal, U.C., et al., *Frequency of Helicobacter pylori and CagA antibody in patients with gastric neoplasms and controls: the Indian enigma. Dig Dis Sci, 2008. 53(5): p. 1215-22.*
  56. Li, L., et al., *Helicobacter pylori strain and the pattern of gastritis among first-degree relatives of patients with gastric carcinoma. Helicobacter, 2002. 7(6): p.*

- 349-55.
57. Nomura, A.M., et al., *Helicobacter pylori CagA seropositivity and gastric carcinoma risk in a Japanese American population*. J Infect Dis, 2002. **186**(8): p. 1138-44.
  58. Yang, G.F., et al., *Expression of nuclear factor-kappa B and target genes in gastric precancerous lesions and adenocarcinoma: association with Helicobacter pylori cagA (+) infection*. World J Gastroenterol, 2004. **10**(4): p. 491-6.
  59. Bravo, L.E., et al., *Virulence-associated genotypes of Helicobacter pylori: do they explain the African enigma?* Am J Gastroenterol, 2002. **97**(11): p. 2839-42.
  60. El-Omar, E.M., et al., *Increased prevalence of precancerous changes in relatives of gastric cancer patients: critical role of H. pylori*. Gastroenterology, 2000. **118**(1): p. 22-30.
  61. Uemura, N., et al., *Helicobacter pylori infection and the development of gastric cancer*. N Engl J Med, 2001. **345**(11): p. 784-9.
  62. Gonzalez, C.A., J.M. Sanz, and A. Agudo, [Risk factors for gastric cancer]. Gastroenterol Hepatol, 1997. **20**(5): p. 239-47.
  63. Malaty, H.M., et al., *Are genetic influences on peptic ulcer dependent or independent of genetic influences for Helicobacter pylori infection?* Arch Intern Med, 2000. **160**(1): p. 105-9.
  64. Gajalakshmi CK, S.V., *Lifestyle and risk of stomach cancer: a hospital-based case-control study*. Int J Epidemiol 1996. **25**: p. 1146-1153.
  65. Rao DN, G.B., Dinshaw KA, Mohandas KM., *A casecontrol study of stomach cancer in Mumbai, India*. Int J Cancer 2002. **99**: p. 727-731.
  66. Lunet, N. and H. Barros, *Helicobacter pylori infection and gastric cancer: facing the enigmas*. Int J Cancer, 2003. **106**(6): p. 953-60.
  67. Jonkers, D., et al., *Antibacterial effect of garlic and omeprazole on Helicobacter pylori*. J Antimicrob Chemother, 1999. **43**(6): p. 837-9.
  68. Foryst-Ludwig, A., et al., *Curcumin blocks NF-kappaB and the motogenic response in Helicobacter pylori-infected epithelial cells*. Biochem Biophys Res Commun, 2004. **316**(4): p. 1065-72.
  69. Mahady, G.B., et al., *Turmeric (Curcuma longa) and curcumin inhibit the growth of Helicobacter pylori, a group I carcinogen*. Anticancer Res, 2002. **22**(6C): p. 4179-81.
  70. O'Mahony, R., et al., *Bactericidal and anti-adhesive properties of culinary and medicinal plants against Helicobacter pylori*. World J Gastroenterol, 2005. **11**(47): p. 7499-507.
  71. Fox, J.G., et al., *Concurrent enteric helminth infection modulates inflammation and gastric immune responses and reduces helicobacter-induced gastric atrophy*. Nat Med, 2000. **6**(5): p. 536-42.
  72. Whary, M.T., et al., *Intestinal helminthiasis in Colombian children promotes a Th2 response to Helicobacter pylori: possible implications for gastric carcinogenesis*. Cancer Epidemiol Biomarkers Prev, 2005. **14**(6): p. 1464-9.
  73. Torres, J., et al., *A comprehensive review of the natural history of Helicobacter pylori infection in children*. Arch Med Res, 2000. **31**(5): p. 431-69.
  74. Brown, L.M., *Helicobacter pylori: epidemiology and routes of transmission*. Epidemiol Rev, 2000. **22**(2): p. 283-97.



75. Imrie, C., et al., *Is Helicobacter pylori infection in childhood a risk factor for gastric cancer?* Pediatrics, 2001. **107**(2): p. 373-80.
76. O'Rourke, K., et al., *Determinants of geographic variation in Helicobacter pylori infection among children on the US-Mexico border.* Am J Epidemiol, 2003. **158**(8): p. 816-24.
77. Rowland, M., et al., *Age-specific incidence of Helicobacter pylori.* Gastroenterology, 2006. **130**(1): p. 65-72; quiz 211.
78. Muhsen, K., et al., *Prevalence and risk factors of Helicobacter pylori infection among healthy 3- to 5-year-old Israeli Arab children.* Epidemiol Infect, 2006. **134**(5): p. 990-6.
79. Klein, P.D., et al., *Water source as risk factor for Helicobacter pylori infection in Peruvian children.* Gastrointestinal Physiology Working Group. Lancet, 1991. **337**(8756): p. 1503-6.
80. Goodman, K.J., et al., *Nutritional factors and Helicobacter pylori infection in Colombian children.* J Pediatr Gastroenterol Nutr, 1997. **25**(5): p. 507-15.
81. Moreira, E.D., Jr., et al., *Risk factors for Helicobacter pylori infection in children: is education a main determinant?* Epidemiol Infect, 2004. **132**(2): p. 327-35.
82. Ueda, M., et al., *Helicobacter pylori risk associated with childhood home environment.* Cancer Sci, 2003. **94**(10): p. 914-8.
83. de Martel, C. and J. Parsonnet, *Helicobacter pylori infection and gender: a meta-analysis of population-based prevalence surveys.* Dig Dis Sci, 2006. **51**(12): p. 2292-301.
84. Cullen, D.J., et al., *When is Helicobacter pylori infection acquired?* Gut, 1993. **34**(12): p. 1681-2.
85. Mendall, M.A., et al., *Childhood living conditions and Helicobacter pylori seropositivity in adult life.* Lancet, 1992. **339**(8798): p. 896-7.
86. McCallion, W.A., et al., *Helicobacter pylori infection in children: relation with current household living conditions.* Gut, 1996. **39**(1): p. 18-21.
87. Parsonnet, J., et al., *Symptoms and risk factors of Helicobacter pylori infection in a cohort of epidemiologists.* Gastroenterology, 1992. **102**(1): p. 41-6.
88. Rothenbacher, D., et al., *Prevalence and determinants of Helicobacter pylori infection in preschool children: a population-based study from Germany.* Int J Epidemiol, 1998. **27**(1): p. 135-41.
89. Herbarth, O., et al., *Helicobacter pylori prevalences and risk factors among school beginners in a German urban center and its rural county.* Environ Health Perspect, 2001. **109**(6): p. 573-7.
90. Webb, P.M., et al., *Relation between infection with Helicobacter pylori and living conditions in childhood: evidence for person to person transmission in early life.* Bmj, 1994. **308**(6931): p. 750-3.
91. Luzzi, F., et al., *Suggestion against an oral-oral route of transmission for Helicobacter pylori infection: a seroepidemiological study in a rural area.* Dig Dis Sci, 1998. **43**(7): p. 1488-92.
92. Bode, G., et al., *Pets are not a risk factor for Helicobacter pylori infection in young children: results of a population-based study in Southern Germany.* Pediatr Infect Dis J, 1998. **17**(10): p. 909-12.
93. Bazzoli, F., *Key points from the revised Maastricht Consensus Report: the impact*

- on general practice. Eur J Gastroenterol Hepatol, 2001. **13 Suppl 2**: p. S3-7.
94. Brown, L.M., et al., *Helicobacter pylori* infection in rural China: exposure to domestic animals during childhood and adulthood. Scand J Infect Dis, 2001. **33**(9): p. 686-91.
  95. Goodman, K.J., et al., *Helicobacter pylori* infection in the Colombian Andes: a population-based study of transmission pathways. Am J Epidemiol, 1996. **144**(3): p. 290-9.
  96. Dore, M.P., et al., *High prevalence of Helicobacter pylori* infection in shepherds. Dig Dis Sci, 1999. **44**(6): p. 1161-4.
  97. Liu, W.Z., et al., *Seroprevalence of Helicobacter pylori* infection in medical staff in Shanghai. Scand J Gastroenterol, 1996. **31**(8): p. 749-52.
  98. Lin, S.K., et al., *Helicobacter pylori* prevalence in endoscopy and medical staff. J Gastroenterol Hepatol, 1994. **9**(4): p. 319-24.
  99. Su, Y.C., et al., *High seroprevalence of IgG against Helicobacter pylori* among endoscopists in Taiwan. Dig Dis Sci, 1996. **41**(8): p. 1571-6.
  100. Braden, B., et al., *Endoscopy is not a risk factor for Helicobacter pylori* infection--but medical practice is. Gastrointest Endosc, 1997. **46**(4): p. 305-10.
  101. Nishikawa, J., et al., *Seroprevalence of immunoglobulin G antibodies against Helicobacter pylori* among endoscopy personnel in Japan. Gastrointest Endosc, 1998. **48**(3): p. 237-43.
  102. Lin, S.K., et al., *The prevalence of Helicobacter pylori* in practising dental staff and dental students. Aust Dent J, 1998. **43**(1): p. 35-9.
  103. Banatvala, N., et al., *Helicobacter pylori* infection in dentists--a case-control study. Scand J Infect Dis, 1995. **27**(2): p. 149-51.
  104. Matsuda, R. and T. Morizane, *Helicobacter pylori* infection in dental professionals: a 6-year prospective study. Helicobacter, 2005. **10**(4): p. 307-11.
  105. Mastromarino, P., et al., *Does hospital work constitute a risk factor for Helicobacter pylori* infection? J Hosp Infect, 2005. **60**(3): p. 261-8.
  106. Fujimura, S., S. Kato, and T. Kawamura, *Helicobacter pylori* in Japanese river water and its prevalence in Japanese children. Lett Appl Microbiol, 2004. **38**(6): p. 517-21.
  107. Rolle-Kampczyk, U.E., et al., *Well water--one source of Helicobacter pylori* colonization. Int J Hyg Environ Health, 2004. **207**(4): p. 363-8.
  108. Yamashita, Y., et al., *Epidemiology of Helicobacter pylori* infection in children: a serologic study of the Kyushu region in Japan. Pediatr Int, 2001. **43**(1): p. 4-7.
  109. Reshetnikov, O.V., et al., *Seroprevalence of Helicobacter pylori* infection in Siberia. Helicobacter, 2001. **6**(4): p. 331-6.
  110. Fontham, E.T., et al., *Determinants of Helicobacter pylori* infection and chronic gastritis. Am J Gastroenterol, 1995. **90**(7): p. 1094-101.
  111. Jones, N.L., S. Shabib, and P.M. Sherman, *Capsaicin as an inhibitor of the growth of the gastric pathogen Helicobacter pylori*. FEMS Microbiol Lett, 1997. **146**(2): p. 223-7.
  112. Lopez-Carrillo, L., et al., *Capsaicin consumption, Helicobacter pylori* positivity and gastric cancer in Mexico. Int J Cancer, 2003. **106**(2): p. 277-82.
  113. Guo, Z., et al., *A case-control study on risk factors of helicobacter pylori* infection in out-patients with stomach diseases. Zhonghua Yu Fang Yi Xue Za Zhi, 2002.

- 36(3): p. 187-90.
114. Jarosz, M., et al., *Effects of high dose vitamin C treatment on Helicobacter pylori infection and total vitamin C concentration in gastric juice*. Eur J Cancer Prev, 1998. **7**(6): p. 449-54.
  115. Malaty, H.M., et al., *Co-twin study of the effect of environment and dietary elements on acquisition of Helicobacter pylori infection*. Am J Epidemiol, 1998. **148**(8): p. 793-7.
  116. Olafsson, S. and A. Berstad, *Changes in food tolerance and lifestyle after eradication of Helicobacter pylori*. Scand J Gastroenterol, 2003. **38**(3): p. 268-76.
  117. Phukan, R.K., et al., *Dietary habits and stomach cancer in Mizoram, India*. J Gastroenterol, 2006. **41**(5): p. 418-24.
  118. Qasim, A. and C.A. O'Morain, *Review article: treatment of Helicobacter pylori infection and factors influencing eradication*. Aliment Pharmacol Ther, 2002. **16 Suppl 1**: p. 24-30.
  119. Webberley, M.J., et al., *Seroepidemiology of Helicobacter pylori infection in vegans and meat-eaters*. Epidemiol Infect, 1992. **108**(3): p. 457-62.
  120. Bergin, I.L., B.J. Sheppard, and J.G. Fox, *Helicobacter pylori infection and high dietary salt independently induce atrophic gastritis and intestinal metaplasia in commercially available outbred Mongolian gerbils*. Dig Dis Sci, 2003. **48**(3): p. 475-85.
  121. Fox, J.G., et al., *High-salt diet induces gastric epithelial hyperplasia and parietal cell loss, and enhances Helicobacter pylori colonization in C57BL/6 mice*. Cancer Res, 1999. **59**(19): p. 4823-8.
  122. Gamboa-Dominguez, A., et al., *Salt and stress synergize H. pylori-induced gastric lesions, cell proliferation, and p21 expression in Mongolian gerbils*. Dig Dis Sci, 2007. **52**(6): p. 1517-26.
  123. Kato, S., et al., *High salt diets dose-dependently promote gastric chemical carcinogenesis in Helicobacter pylori-infected Mongolian gerbils associated with a shift in mucin production from glandular to surface mucous cells*. Int J Cancer, 2006. **119**(7): p. 1558-66.
  124. Rothenbacher, D., et al., *Use of commonly prescribed antibiotics is not associated with prevalence of Helicobacter pylori infection in adults*. Scand J Gastroenterol, 1997. **32**(11): p. 1096-9.
  125. Tindberg, Y., et al., *Helicobacter pylori infection in Swedish school children: lack of evidence of child-to-child transmission outside the family*. Gastroenterology, 2001. **121**(2): p. 310-6.
  126. Leung, W.K., et al., *Follow up of serial urea breath test results in patients after consumption of antibiotics for non-gastric infections*. World J Gastroenterol, 2002. **8**(4): p. 703-6.
  127. Rothenbacher, D., et al., *History of antibiotic treatment and prevalence of H. pylori infection among children: results of a population-based study*. J Clin Epidemiol, 1998. **51**(3): p. 267-71.
  128. Bures, J., et al., *Epidemiology of Helicobacter pylori infection in the Czech Republic*. Helicobacter, 2006. **11**(1): p. 56-65.
  129. Replogle, M.L., et al., *Biologic sex as a risk factor for Helicobacter pylori infection in healthy young adults*. Am J Epidemiol, 1995. **142**(8): p. 856-63.

130. *Epidemiology of, and risk factors for, Helicobacter pylori infection among 3194 asymptomatic subjects in 17 populations. The EUROGAST Study Group. Gut, 1993. 34(12): p. 1672-6.*
131. Malaty, H.M., et al., *Helicobacter pylori in Hispanics: comparison with blacks and whites of similar age and socioeconomic class. Gastroenterology, 1992. 103(3): p. 813-6.*
132. Malaty, H.M. and D.Y. Graham, *Importance of childhood socioeconomic status on the current prevalence of Helicobacter pylori infection. Gut, 1994. 35(6): p. 742-5.*
133. Murray, L.J., et al., *Epidemiology of Helicobacter pylori infection among 4742 randomly selected subjects from Northern Ireland. Int J Epidemiol, 1997. 26(4): p. 880-7.*
134. Peach, H.G., D.C. Pearce, and S.J. Farish, *Helicobacter pylori infection in an Australian regional city: prevalence and risk factors. Med J Aust, 1997. 167(6): p. 310-3.*
135. Hopkins, R.J., et al., *Seroprevalence of Helicobacter pylori in Chile: vegetables may serve as one route of transmission. J Infect Dis, 1993. 168(1): p. 222-6.*
136. Chow, T.K., et al., *Helicobacter pylori in Melbourne Chinese immigrants: evidence for oral-oral transmission via chopsticks. J Gastroenterol Hepatol, 1995. 10(5): p. 562-9.*
137. Buckley, M., et al., *Management of dyspepsia in primary care. Antibiotic resistance is a problem. Bmj, 1998. 316(7141): p. 1388.*
138. Rothenbacher, D., et al., *Active infection with Helicobacter pylori in an asymptomatic population of middle aged to elderly people. Epidemiol Infect, 1998. 120(3): p. 297-303.*
139. Souto, F.J., et al., *Prevalence of Helicobacter pylori infection in a rural area of the state of Mato Grosso, Brazil. Mem Inst Oswaldo Cruz, 1998. 93(2): p. 171-4.*
140. Torres, J., et al., *A community-based seroepidemiologic study of Helicobacter pylori infection in Mexico. J Infect Dis, 1998. 178(4): p. 1089-94.*
141. Bani-Hani, K.E., et al., *Prevalence and risk factors of Helicobacter pylori infection in healthy schoolchildren. Chin J Dig Dis, 2006. 7(1): p. 55-60.*
142. Teh, B.H., et al., *Seroprevalence and associated risk factors of Helicobacter pylori infection in Taiwan. Anticancer Res, 1994. 14(3B): p. 1389-92.*
143. Koch, A., et al., *Seroprevalence and risk factors for Helicobacter pylori infection in Greenlanders. Helicobacter, 2005. 10(5): p. 433-42.*
144. Breuer, T., et al., *Prevalence of and risk factors for Helicobacter pylori infection in the western part of Germany. Eur J Gastroenterol Hepatol, 1996. 8(1): p. 47-52.*
145. Al-Shamahy, H.A., *Seroprevalence of Helicobacter pylori among children in Sana'a, Yemen. Ann Saudi Med, 2005. 25(4): p. 299-303.*
146. Rodrigues, M.N., et al., *Prevalence of Helicobacter pylori infection in children from an urban community in north-east Brazil and risk factors for infection. Eur J Gastroenterol Hepatol, 2004. 16(2): p. 201-5.*
147. Parsonnet, J., *Bacterial infection as a cause of cancer. Environ Health Perspect, 1995. 103 Suppl 8: p. 263-8.*
148. Tindberg, Y., M. Blennow, and M. Granstrom, *Clinical symptoms and social factors in a cohort of children spontaneously clearing Helicobacter pylori*

- infection*. Acta Paediatr, 1999. **88**(6): p. 631-5.
149. Wizla-Derambure, N., et al., *Familial and community environmental risk factors for Helicobacter pylori infection in children and adolescents*. J Pediatr Gastroenterol Nutr, 2001. **33**(1): p. 58-63.
  150. Nessa, J., et al., *Human serum antibody response to Helicobacter pylori whole cell antigen in an institutionalized Bangladeshi population*. J Appl Microbiol, 2001. **90**(1): p. 68-72.
  151. Wallace, M.R., *Risk factors for Helicobacter pylori resistance*. Curr Gastroenterol Rep, 2002. **4**(4): p. 277.
  152. Kikuchi, S., *Epidemiology of Helicobacter pylori and gastric cancer*. Gastric Cancer, 2002. **5**(1): p. 6-15.
  153. Rothenbacher, D., et al., *Helicobacter pylori in out-patients of a general practitioner: prevalence and determinants of current infection*. Epidemiol Infect, 1997. **119**(2): p. 151-7.
  154. Kyriazanos, I., et al., *A cohort study on Helicobacter pylori serology before and after induction in the Hellenic Navy*. Mil Med, 2001. **166**(5): p. 411-5.
  155. Grimm, W. and W. Fischbach, *[Helicobacter pylori infection in children and juveniles: an epidemiological study on prevalence, socio-economic factors and symptoms]*. Dtsch Med Wochenschr, 2003. **128**(37): p. 1878-83.
  156. Ma, J.L., et al., *Helicobacter pylori infection and mode of transmission in a population at high risk of stomach cancer*. Int J Epidemiol, 1998. **27**(4): p. 570-3.
  157. Brenner, H., et al., *Parental history of gastric or duodenal ulcer and prevalence of Helicobacter pylori infection in preschool children: population based study*. Bmj, 1998. **316**(7132): p. 665.
  158. Goodman, K.J. and P. Correa, *Transmission of Helicobacter pylori among siblings*. Lancet, 2000. **355**(9201): p. 358-62.
  159. Dominici, P., et al., *Familial clustering of Helicobacter pylori infection: population based study*. Bmj, 1999. **319**(7209): p. 537-40.
  160. Rothenbacher, D., et al., *Helicobacter pylori among preschool children and their parents: evidence of parent-child transmission*. J Infect Dis, 1999. **179**(2): p. 398-402.
  161. Rothenbacher, D., G. Bode, and H. Brenner, *Dynamics of Helicobacter pylori infection in early childhood in a high-risk group living in Germany: loss of infection higher than acquisition*. Aliment Pharmacol Ther, 2002. **16**(9): p. 1663-8.
  162. Rocha, G.A., et al., *Transmission of Helicobacter pylori infection in families of preschool-aged children from Minas Gerais, Brazil*. Trop Med Int Health, 2003. **8**(11): p. 987-91.
  163. Kivi, M., et al., *Helicobacter pylori status in family members as risk factors for infection in children*. Epidemiol Infect, 2005. **133**(4): p. 645-52.
  164. Aguemon, B.D., et al., *Prevalence and risk-factors for Helicobacter pylori infection in urban and rural Beninese populations*. Clin Microbiol Infect, 2005. **11**(8): p. 611-7.
  165. Glynn, M.K., et al., *Seroincidence of Helicobacter pylori infection in a cohort of rural Bolivian children: acquisition and analysis of possible risk factors*. Clin Infect Dis, 2002. **35**(9): p. 1059-65.

166. Selimoglu, M.A., V. Ertekin, and T. Inandi, *Seroepidemiology of Helicobacter pylori infection in children living in eastern Turkey*. *Pediatr Int*, 2002. **44**(6): p. 666-9.
167. Farrell, S., et al., *Risk factors for Helicobacter pylori infection in children: an examination of the role played by intrafamilial bed sharing*. *Pediatr Infect Dis J*, 2005. **24**(2): p. 149-52.
168. Fox, J.G. and T.C. Wang, *Reply to "The 'African enigma' - another explanation"*. *Nat Med*, 2000. **6**(12): p. 1297-8.
169. Fox, J.G., T.C. Wang, and C. Nagler-Anderson, *The African enigma: the parasite's perspective*. *Gut*, 2001. **49**(1): p. 156-7.
170. Akimoto, M., et al., [*Correlation between serum anti CagA antibody and gastric disease*]. *Nippon Shokakibyō Gakkai Zasshi*, 1996. **93**(1): p. 69.
171. Alarcon, T., et al., *Prevalence of CagA and VacA antibodies in children with Helicobacter pylori-associated peptic ulcer compared to prevalence in pediatric patients with active or nonactive chronic gastritis*. *Clin Diagn Lab Immunol*, 2000. **7**(5): p. 842-4.
172. Al-Marhoon, M.S., S. Nunn, and R.W. Soames, *The association between cagA+ H. pylori infection and distal gastric cancer: a proposed model*. *Dig Dis Sci*, 2004. **49**(7-8): p. 1116-22.
173. Al-Marhoon, M.S., S. Nunn, and R.W. Soames, *Effects of cagA+ and cagA- strains of Helicobacter pylori on the human gastric mucus layer thickness*. *J Gastroenterol Hepatol*, 2005. **20**(8): p. 1246-52.
174. Atherton, J.C., *CagA, the cag pathogenicity island and Helicobacter pylori virulence*. *Gut*, 1999. **44**(3): p. 307-8.
175. Atherton, J.C., *CagA: a role at last*. *Gut*, 2000. **47**(3): p. 330-1.
176. Azuma, T., et al., *Correlation between variation of the 3' region of the cagA gene in Helicobacter pylori and disease outcome in Japan*. *J Infect Dis*, 2002. **186**(11): p. 1621-30.
177. Evans, D.J., Jr. and D.G. Evans, *Helicobacter pylori CagA: analysis of sequence diversity in relation to phosphorylation motifs and implications for the role of CagA as a virulence factor*. *Helicobacter*, 2001. **6**(3): p. 187-98.
178. Handa, O., Y. Naito, and T. Yoshikawa, *CagA protein of Helicobacter pylori: a hijacker of gastric epithelial cell signaling*. *Biochem Pharmacol*, 2007. **73**(11): p. 1697-702.
179. Salih, B.A., *The role of the putative virulence markers (cagA and vacA ) of Helicobacter pylori in peptic ulcer disease*. *Saudi Med J*, 2004. **25**(7): p. 830-6.
180. Suzuki, N., et al., *Catalase, a specific antigen in the feces of human subjects infected with Helicobacter pylori*. *Clin Diagn Lab Immunol*, 2002. **9**(4): p. 784-8.
181. Suzuki, N., et al., *Production and application of new monoclonal antibodies specific for a fecal Helicobacter pylori antigen*. *Clin Diagn Lab Immunol*, 2002. **9**(1): p. 75-8.
182. Hirai, I., et al., *A method for assessment of Helicobacter pylori genotype using stool specimens*. *FEMS Immunol Med Microbiol*, 2009. **56**(1): p. 63-6.
183. Yamazaki, S., et al., *Identification of Helicobacter pylori and the cagA genotype in gastric biopsies using highly sensitive real-time PCR as a new diagnostic tool*. *FEMS Immunol Med Microbiol*, 2005. **44**(3): p. 261-8.

184. Sasaki, T., et al., *Analysis of Helicobacter pylori Genotype in Stool Specimens of Asymptomatic People*. Lab Medicine, 2009. **40**(7): p. 412-414.
185. Li, Y.H., et al., *Clinical value of Helicobacter pylori stool antigen test, ImmunoCard STAT HpSA, for detecting H pylori infection*. World J Gastroenterol, 2004. **10**(6): p. 913-4.
186. Higashi, H., et al., *Biological activity of the Helicobacter pylori virulence factor CagA is determined by variation in the tyrosine phosphorylation sites*. Proc Natl Acad Sci U S A, 2002. **99**(22): p. 14428-33.
187. Chattopadhyay, S., et al., *Virulence genes in Helicobacter pylori strains from West Bengal residents with overt H. pylori-associated disease and healthy volunteers*. J Clin Microbiol, 2002. **40**(7): p. 2622-5.
188. Molnar, B., et al., *Significantly elevated Helicobacter pylori density and different genotype distribution in erosions as compared with normal gastric biopsy specimen detected by quantitative real-time PCR*. Eur J Gastroenterol Hepatol, 2008. **20**(4): p. 305-13.
189. Debets-Ossenkopp, Y.J., et al., *Characteristics of clinical Helicobacter pylori strains from Ecuador*. J Antimicrob Chemother, 2003. **51**(1): p. 141-5.
190. Garcia, H., et al., *[Prevalence of Helicobacter pylori in patients with gastric cancer in Panama]*. Rev Med Panama, 1992. **17**(3): p. 203-7.
191. Perez Ferrari, R., et al., *[Helicobacter pylori at Hospital Santo Tomas]*. Rev Med Panama, 1993. **18**(3): p. 229-32.
192. Sierra Lopez, R., F. Bertoli, and E.F. Penafiel, *[Helicobacter pylori infection and the risk of gastric carcinoma. Report of a case]*. Rev Med Panama, 1994. **19**(1): p. 48-54.
193. Axon, A.T., *Review article: is Helicobacter pylori transmitted by the gastro-oral route?* Aliment Pharmacol Ther, 1995. **9**(6): p. 585-8.
194. Baker, K.H., et al., *Effect of oxidizing disinfectants (chlorine, monochloramine, and ozone) on Helicobacter pylori*. Appl Environ Microbiol, 2002. **68**(2): p. 981-4.
195. Johnson, C.H., E.W. Rice, and D.J. Reasoner, *Inactivation of Helicobacter pylori by chlorination*. Appl Environ Microbiol, 1997. **63**(12): p. 4969-70.
196. Moreno, Y., et al., *Survival and viability of Helicobacter pylori after inoculation into chlorinated drinking water*. Water Res, 2007. **41**(15): p. 3490-6.
197. Giao, M.S., et al., *Effect of chlorine on incorporation of Helicobacter pylori into drinking water biofilms*. Appl Environ Microbiol, 2010. **76**(5): p. 1669-73.
198. Ferlay J, S.H., Bray F, Forman D, Mathers C and Parkin DM. (2008) *GLOBOCAN 2008, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]*.
199. Asrat, D., et al., *Prevalence of Helicobacter pylori infection among adult dyspeptic patients in Ethiopia*. Ann Trop Med Parasitol, 2004. **98**(2): p. 181-9.
200. Lindkvist, P., et al., *Age at acquisition of Helicobacter pylori infection: comparison of a high and a low prevalence country*. Scand J Infect Dis, 1996. **28**(2): p. 181-4.
201. Lindkvist, P., et al., *Risk factors for infection with Helicobacter pylori--a study of children in rural Ethiopia*. Scand J Infect Dis, 1998. **30**(4): p. 371-6.
202. Melo, E.T., et al., *Seroprevalence of Helicobacter pylori antibodies in medical*

- students and residents in Recife, Brazil.* J Clin Gastroenterol, 2003. **36**(2): p. 134-8.
203. Rodrigues, M.N., et al., *Helicobacter pylori infection in adults from a poor urban community in northeastern Brazil: demographic, lifestyle and environmental factors.* Braz J Infect Dis, 2005. **9**(5): p. 405-10.
  204. Santos, I.S., et al., *Prevalence of Helicobacter pylori infection and associated factors among adults in Southern Brazil: a population-based cross-sectional study.* BMC Public Health, 2005. **5**: p. 118.
  205. Abdullah, A.M., et al., *Helicobacter pylori infection in children in Saudi Arabia.* Trop Gastroenterol, 1997. **18**(2): p. 63-5.
  206. Mohamed, A.E., et al., *Helicobacter pylori: incidence and comparison of three diagnostic methods in 196 Saudi patients with dyspepsia.* Hepatogastroenterology, 1994. **41**(1): p. 48-50.
  207. Novis, B.H., G. Gabay, and T. Naftali, *Helicobacter pylori: the Middle East scenario.* Yale J Biol Med, 1998. **71**(2): p. 135-41.
  208. Baena, J.M., et al., *Relation between alcohol consumption and the success of Helicobacter pylori eradication therapy using omeprazole, clarithromycin and amoxicillin for 1 week.* Eur J Gastroenterol Hepatol, 2002. **14**(3): p. 291-6.
  209. Battaglia, G., et al., *Helicobacter pylori infection, cigarette smoking and alcohol consumption. A histological and clinical study on 286 subjects.* Ital J Gastroenterol, 1993. **25**(8): p. 419-24.
  210. Brenner, H., et al., *Alcohol consumption and Helicobacter pylori infection: results from the German National Health and Nutrition Survey.* Epidemiology, 1999. **10**(3): p. 214-8.
  211. Banatvala, N. and R. Feldman, *The epidemiology of Helicobacter pylori: missing pieces in a jigsaw.* Commun Dis Rep CDR Rev, 1993. **3**(4): p. R56-9.
  212. Cave, D.R., *Transmission and epidemiology of Helicobacter pylori.* Am J Med, 1996. **100**(5A): p. 12S-17S; discussion 17S-18S.
  213. Crespo, A. and B. Suh, *Helicobacter pylori infection: epidemiology, pathophysiology, and therapy.* Arch Pharm Res, 2001. **24**(6): p. 485-98.
  214. Everhart, J.E., *Recent developments in the epidemiology of Helicobacter pylori.* Gastroenterol Clin North Am, 2000. **29**(3): p. 559-78.
  215. Asaka, M., *[Epidemiology of Helicobacter pylori infection in Japan].* Nippon Rinsho, 2003. **61**(1): p. 19-24.
  216. Caballero Plasencia, A.M., et al., *Epidemiology of dyspepsia in a random mediterranean population. Prevalence of Helicobacter pylori infection.* Rev Esp Enferm Dig, 2000. **92**(12): p. 781-92.
  217. Edwards, C.N., et al., *Epidemiology of Helicobacter pylori infection in Barbados.* West Indian Med J, 1997. **46**(1): p. 3-7.
  218. Tovey, F., *Peptic ulcer in India and Bangladesh.* Gut 1979. **20**: p. 329-347.
  219. Keechilat Pavithran, D.C.D., and Kamal K. Pandey, *Gastric cancer in India.* Gastric Cancer, 2002. **5**: p. 240-243.
  220. Gill, H.H. and H.G. Desai, *Helicobacter pylori and gastroduodenal disorders in India--lessons from epidemiology.* J Clin Gastroenterol, 1993. **16**(1): p. 6-9.
  221. Jain, A., et al., *Risk factors for duodenal ulcer in north India.* Trop Gastroenterol, 1999. **20**(1): p. 36-9.



222. Jain, A.K. and V.M. Dayal, *Helicobacter pylori* recolonization and ulcer relapse after its eradication in India. *Indian J Gastroenterol*, 1997. **16 Suppl 1**: p. S22-4.
223. Katelaris, P.H., et al., *Dyspepsia, Helicobacter pylori, and peptic ulcer in a randomly selected population in India*. *Gut*, 1992. **33**(11): p. 1462-6.
224. Tovey, F.I., et al., *Duodenal gastric metaplasia and Helicobacter pylori infection in high and low duodenal ulcer-prevalent areas in India*. *J Gastroenterol Hepatol*, 2004. **19**(5): p. 497-505.
225. Abraham, P., *Helicobacter pylori: a review of practices and research in India*. *Indian J Gastroenterol*, 1997. **16 Suppl 1**: p. S1-2.
226. Ahuja, V., *The case for Helicobacter pylori eradication in India: sensationalism, skepticism and scientific salesmanship*. *Indian J Gastroenterol*, 2006. **25**(1): p. 20-4.
227. Mohandas, K.M., *Problems with epidemiologic studies on Helicobacter pylori in India*. *Indian J Gastroenterol*, 1997. **16 Suppl 1**: p. S6-8.
228. Jedrychowski, W., et al., *Effect of Helicobacter pylori infection, smoking and dietary habits on the occurrence of antrum intestinal metaplasia. Clinico-epidemiological study in Poland*. *Pol J Pathol*, 1999. **50**(4): p. 289-95.
229. Jenkins, D.J., *Helicobacter pylori and its interaction with risk factors for chronic disease*. *Bmj*, 1997. **315**(7121): p. 1481-2.
230. Singh, V., et al., *Helicobacter pylori: evidence for spouse-to-spouse transmission*. *J Gastroenterol Hepatol*, 1999. **14**(6): p. 519-22.
231. Misra, V., et al., *Point prevalence of peptic ulcer and gastric histology in healthy Indians with Helicobacter pylori infection*. *Am J Gastroenterol*, 1997. **92**(9): p. 1487-91.
232. Bago, J., et al., *Relationship of gastric metaplasia and age, sex, smoking and Helicobacter pylori infection in patients with duodenal ulcer and duodenitis*. *Coll Antropol*, 2000. **24**(1): p. 157-65.
233. Bateson, M.C., *Helicobacter pylori infection with age*. *Lancet*, 1992. **339**(8801): p. 1121.
234. Camargo, M.C., et al., *Age at acquisition of Helicobacter pylori infection: comparison of two areas with contrasting risk of gastric cancer*. *Helicobacter*, 2004. **9**(3): p. 262-70.
235. Choi, J.W., *Does Helicobacter pylori infection relate to iron deficiency anaemia in prepubescent children under 12 years of age?* *Acta Paediatr*, 2003. **92**(8): p. 970-2.
236. Malaty, H.M., et al., *Age at acquisition of Helicobacter pylori infection: a follow-up study from infancy to adulthood*. *Lancet*, 2002. **359**(9310): p. 931-5.
237. Sinha, S.K., et al., *Age at acquisition of Helicobacter pylori in a pediatric Canadian First Nations population*. *Helicobacter*, 2002. **7**(2): p. 76-85.
238. Tiwari, S.K., et al., *Prognostic significance of genotyping Helicobacter pylori infection in patients in younger age groups with gastric cancer*. *Postgrad Med J*, 2008. **84**(990): p. 193-7.
239. Celinski, K., et al., *The effects of environmental factors on the prevalence of Helicobacter pylori infection in inhabitants of Lublin Province*. *Ann Agric Environ Med*, 2006. **13**(2): p. 185-91.
240. Chang, W.K., et al., *Association between Helicobacter pylori infection and the*

- risk of gastric cancer in the Korean population: prospective case-controlled study.* J Gastroenterol, 2001. **36**(12): p. 816-22.
241. Parente, J.M., et al., *Helicobacter pylori infection in children of low and high socioeconomic status in northeastern Brazil.* Am J Trop Med Hyg, 2006. **75**(3): p. 509-12.
  242. Nurgalieva Z, M.H., Graham DY, Almuchambetova R, Machmudova A, Kapsultanova D, Osato MS, *Helicobacter pylori infection in Kazakhstan: effect of water source and household hygiene.* Am J Trop Hyg, 2003. **67**: p. 201–8.
  243. Sung, J., ed. *Factors associated with disappearance of Helicobacter pylori in the Far East.* . Helicobacter pylori: Basic Mechanisms to Clinical Cure., ed. T.G. Hunt RH, eds. Vol. 53. 2000, Dordrecht: Kluwer.
  244. Sitas, F., et al., *Helicobacter pylori infection rates in relation to age and social class in a population of Welsh men.* Gut, 1991. **32**(1): p. 25-8.
  245. *An international association between Helicobacter pylori infection and gastric cancer. The EUROGAST Study Group.* Lancet, 1993. **341**(8857): p. 1359-62.
  246. Buckley, M.J., et al., *A community-based study of the epidemiology of Helicobacter pylori infection and associated asymptomatic gastroduodenal pathology.* Eur J Gastroenterol Hepatol, 1998. **10**(5): p. 375-9.
  247. Palli, D., et al., *Diet, Helicobacter pylori, and p53 mutations in gastric cancer: a molecular epidemiology study in Italy.* Cancer Epidemiol Biomarkers Prev, 1997. **6**(12): p. 1065-9.
  248. Shah, S., et al., *Evaluation of the factors influencing stomach-specific delivery of antibacterial agents for Helicobacter pylori infection.* J Pharm Pharmacol, 1999. **51**(6): p. 667-72.
  249. Matsubara, S., et al., *Suppression of Helicobacter pylori-induced gastritis by green tea extract in Mongolian gerbils.* Biochem Biophys Res Commun, 2003. **310**(3): p. 715-9.
  250. al Somal, N., et al., *Susceptibility of Helicobacter pylori to the antibacterial activity of manuka honey.* J R Soc Med, 1994. **87**(1): p. 9-12.
  251. Tabak, M., et al., *In vitro inhibition of Helicobacter pylori by extracts of thyme.* J Appl Bacteriol, 1996. **80**(6): p. 667-72.
  252. Nariman, F., et al., *Anti-Helicobacter pylori activities of six Iranian plants.* Helicobacter, 2004. **9**(2): p. 146-51.
  253. Bellinghausen, I., et al., *Signals involved in the early TH1/TH2 polarization of an immune response depending on the type of antigen.* J Allergy Clin Immunol, 1999. **103**(2 Pt 1): p. 298-306.
  254. D'Elis, M.M., A. Amedei, and G. Del Prete, *Helicobacter pylori antigen-specific T-cell responses at gastric level in chronic gastritis, peptic ulcer, gastric cancer and low-grade mucosa-associated lymphoid tissue (MALT) lymphoma.* Microbes Infect, 2003. **5**(8): p. 723-30.
  255. Ernst, P., *Review article: the role of inflammation in the pathogenesis of gastric cancer.* Aliment Pharmacol Ther, 1999. **13 Suppl 1**: p. 13-8.
  256. Bamford, K.B., et al., *Lymphocytes in the human gastric mucosa during Helicobacter pylori have a T helper cell 1 phenotype.* Gastroenterology, 1998. **114**(3): p. 482-92.
  257. D'Elis, M.M., et al., *Gastric autoimmunity: the role of Helicobacter pylori and*

- molecular mimicry*. Trends Mol Med, 2004. **10**(7): p. 316-23.
258. D'Elcios, M.M., et al., *Helicobacter pylori* and gastric autoimmunity. Microbes Infect, 2004. **6**(15): p. 1395-401.
  259. D'Elcios, M.M., et al., *T helper 1 effector cells specific for Helicobacter pylori in the gastric antrum of patients with peptic ulcer disease*. J Immunol, 1997. **158**(2): p. 962-7.
  260. Marshall, B., *Helicobacter pylori: 20 years on*. Clin Med, 2002. **2**(2): p. 147-52.
  261. Bazzoli, F. and P. Pozzato, *Therapy of H. pylori infection*. J Physiol Pharmacol, 1997. **48 Suppl 4**: p. 39-46.
  262. Lind, T., et al., *Eradication of Helicobacter pylori using one-week triple therapies combining omeprazole with two antimicrobials: the MACH I Study*. Helicobacter, 1996. **1**(3): p. 138-44.
  263. Malhotra, S. and P. Pandhi, *Eradication of Helicobacter pylori: current perspectives*. Expert Opin Pharmacother, 2002. **3**(8): p. 1031-8.
  264. Unge, P., *Antibiotic treatment of Helicobacter pylori infection*. Curr Top Microbiol Immunol, 1999. **241**: p. 261-300.
  265. Watanabe, T., et al., *Helicobacter pylori infection induces gastric cancer in mongolian gerbils*. Gastroenterology, 1998. **115**(3): p. 642-8.
  266. Uemura, N., S. Okamoto, and S. Yamamoto, *H. pylori infection and the development of gastric cancer*. Keio J Med, 2002. **51 Suppl 2**: p. 63-8.
  267. Wong, B.C., et al., *Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial*. Jama, 2004. **291**(2): p. 187-94.
  268. Wotherspoon, A.C., et al., *Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori*. Lancet, 1993. **342**(8871): p. 575-7.
  269. Stolte, M. and M. Vieth, *Gastritis and gastric cancer: which morphological type of Helicobacter gastritis is a precancerous risk?* Chin J Dig Dis, 2005. **6**(3): p. 110-1.
  270. Correa, P., *Helicobacter pylori and gastric carcinogenesis*. Am J Surg Pathol, 1995. **19 Suppl 1**: p. S37-43.
  271. Shikata, K., et al., *A prospective study of dietary salt intake and gastric cancer incidence in a defined Japanese population: the Hisayama study*. Int J Cancer, 2006. **119**(1): p. 196-201.
  272. Kountouras, J., C. Zavos, and D. Chatzopoulos, *Salt intake and Helicobacter pylori infection*. J Hypertens, 2004. **22**(12): p. 2397.
  273. Nozaki, K., et al., *Synergistic promoting effects of Helicobacter pylori infection and high-salt diet on gastric carcinogenesis in Mongolian gerbils*. Jpn J Cancer Res, 2002. **93**(10): p. 1083-9.
  274. Tsugane, S., *Salt, salted food intake, and risk of gastric cancer: epidemiologic evidence*. Cancer Sci, 2005. **96**(1): p. 1-6.
  275. Tsugane, S., et al., *Salt and salted food intake and subsequent risk of gastric cancer among middle-aged Japanese men and women*. Br J Cancer, 2004. **90**(1): p. 128-34.
  276. Willis, P., et al., *Cell proliferation in the post-surgical stomach, dietary salt, and the effect of H pylori eradication*. J Clin Pathol, 1999. **52**(9): p. 665-9.

277. Buiatti E, P.D., DeCarli A. , *A case-control study of gastric cancer and diet in Italy*. Int J Cancer, 1989. **44**: p. 611.
278. Gonzalez, C.A., *Vegetable, fruit and cereal consumption and gastric cancer risk*. IARC Sci Publ, 2002. **156**: p. 79-83.
279. Gonzalez, C.A., et al., *Meat intake and risk of stomach and esophageal adenocarcinoma within the European Prospective Investigation Into Cancer and Nutrition (EPIC)*. J Natl Cancer Inst, 2006. **98**(5): p. 345-54.
280. Nomura, A., *Searching for the causes of gastric cancer*. Hawaii Med J, 2002. **61**(2): p. 33-4.
281. Nomura, A. and G.N. Stemmermann, *Helicobacter pylori and gastric cancer*. J Gastroenterol Hepatol, 1993. **8**(3): p. 294-303.
282. Kneller, R.W., et al., *Risk factors for stomach cancer in sixty-five Chinese counties*. Cancer Epidemiol Biomarkers Prev, 1992. **1**(2): p. 113-8.
283. Tarpila, S., et al., *Morphology and dynamics of the gastric mucosa in duodenal ulcer patients and their first-degree relatives*. Hepatogastroenterology, 1983. **30**(5): p. 198-201.
284. Tarpila, S., et al., *Endoscopic and clinical findings in first-degree relative of duodenal ulcer patients and control subjects*. Scand J Gastroenterol, 1982. **17**(4): p. 503-6.
285. Valle, J., et al., *Helicobacter pylori and duodenal ulcer. A study of duodenal ulcer patients and their first-degree relatives*. Scand J Gastroenterol Suppl, 1991. **186**: p. 45-51.
286. Weil, J., et al., *Helicobacter pylori and metronidazole resistance*. Lancet, 1990. **336**(8728): p. 1445.
287. Cattoir, V., et al., *Update on fluoroquinolone resistance in Helicobacter pylori: new mutations leading to resistance and first description of a gyrA polymorphism associated with hypersusceptibility*. Int J Antimicrob Agents, 2007. **29**(4): p. 389-96.
288. Fischbach, L. and E.L. Evans, *Meta-analysis: the effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for Helicobacter pylori*. Aliment Pharmacol Ther, 2007. **26**(3): p. 343-57.
289. Hu, C.T., et al., *Resistance rate to antibiotics of Helicobacter pylori isolates in eastern Taiwan*. J Gastroenterol Hepatol, 2007. **22**(5): p. 720-3.
290. Khurana, R., et al., *Meta-analysis: Helicobacter pylori eradication treatment efficacy in children*. Aliment Pharmacol Ther, 2007. **25**(5): p. 523-36.
291. Latifi-Navid, S., et al., *Antimicrobial effectiveness of ketoconazole against metronidazole-resistant Helicobacter pylori isolates from Iranian dyspeptic patients*. J Antimicrob Chemother, 2007. **59**(1): p. 160-1.
292. Morales-Espinosa, R., et al., *Susceptibility profile to common antimicrobials used for eradication of Helicobacter pylori Infection in Mexico by agar dilution method*. J Chemother, 2007. **19**(1): p. 108-9.
293. Raymond, J., et al., *Clarithromycin resistance in Helicobacter pylori strains isolated from French children: prevalence of the different mutations and coexistence of clones harboring two different mutations in the same biopsy*. Helicobacter, 2007. **12**(2): p. 157-63.
294. Lee, C.C., et al., *Levofloxacin-resistant Helicobacter pylori in Hong Kong*.

- Chemotherapy, 2008. **54**(1): p. 50-3.
295. Miehle, S., et al., *One-week once-daily triple therapy with esomeprazole, moxifloxacin, and rifabutin for eradication of persistent Helicobacter pylori resistant to both metronidazole and clarithromycin*. *Helicobacter*, 2008. **13**(1): p. 69-74.
296. Broussard, C.S., et al., *Exposure to antibiotics in a United States-Mexico border birth cohort*. *Pediatrics*, 2010. **125**(6): p. e1468-74.
297. Bhatia, S.J. and S.G. Kulkarni, *Cost-effectiveness of Helicobacter pylori eradication in India: to live and let live ... expensively?* *Indian J Gastroenterol*, 1997. **16 Suppl 1**: p. S25-8.
298. Mishra, K.K., et al., *Antibiotic susceptibility of Helicobacter pylori clinical isolates: comparative evaluation of disk-diffusion and E-test methods*. *Curr Microbiol*, 2006. **53**(4): p. 329-34.
299. Ramteke, S. and N.K. Jain, *Clarithromycin- and omeprazole-containing gliadin nanoparticles for the treatment of Helicobacter pylori*. *J Drug Target*, 2008. **16**(1): p. 65-72.
300. Azuma, T., et al., *Diversity of vacA and cagA genes of Helicobacter pylori in Japanese children*. *Aliment Pharmacol Ther*, 2004. **20 Suppl 1**: p. 7-12.
301. Fu, H.Y., et al., *East Asian-type Helicobacter pylori cytotoxin-associated gene A protein has a more significant effect on growth of rat gastric mucosal cells than the Western type*. *J Gastroenterol Hepatol*, 2007. **22**(3): p. 355-62.
302. Kanada, R., et al., *Genotyping of the cagA gene of Helicobacter pylori on immunohistochemistry with East Asian CagA-specific antibody*. *Pathol Int*, 2008. **58**(4): p. 218-25.
303. Kim, S.Y., et al., *Helicobacter pylori CagA transfection of gastric epithelial cells induces interleukin-8*. *Cell Microbiol*, 2006. **8**(1): p. 97-106.
304. Satomi, S., et al., *Relationship between the diversity of the cagA gene of Helicobacter pylori and gastric cancer in Okinawa, Japan*. *J Gastroenterol*, 2006. **41**(7): p. 668-73.
305. Uchida, T., et al., *Immunohistochemical diagnosis of the cagA-gene genotype of Helicobacter pylori with anti-East Asian CagA-specific antibody*. *Cancer Sci*, 2007. **98**(4): p. 521-8.
306. Zhou, W., et al., *The diversity of vacA and cagA genes of Helicobacter pylori in East Asia*. *FEMS Immunol Med Microbiol*, 2004. **40**(1): p. 81-7.