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Prevalence of *Helicobacter pylori* infection in children in Ho Chi Minh city in Vietnam and risk factors

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Prevalence of *Helicobacter pylori* infection in children in Ho Chi Minh city in Vietnam and risk factors

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SUMMARY

Helicobacter pylori (*H. pylori*) infection in children has shown to be a threat in Asian cities, reaching 77% in Hanoi in 2016. Such information was not available in Ho Chi Minh city (HCMC), despite it is one of the most overcrowded cities globally. The overall objective of our thesis was to contribute to improving the control of *H. pylori* infection by investigating the prevalence of *H. pylori* infection among children in HCMC, its geographical distribution, and risk factors.

We conducted a school-based cross-sectional study among school-aged children in HCMC. A total of 1476 children aged 6 – 15 years from 48 public schools (216 classes) cross 24 districts of HCMC were erolled in our study. We found that *H. pylori* infection in children in HCMC is highly prevalent (87.7%), and it increased with population density or employees density. Younger age, crowded living areas, larger family size, and poor hygienic practices were found to be positively associated with a higher prevalence of *H. pylori* infection. High rates of antimicrobial resistance among the common antibiotics used for *H. pylori* treatment also observed in clinical studies. These findings highlight a fecal-oral route for transmission. Crowded living conditions and high rates of antibiotic resistance to the high prevalence of *H. pylori* in children in HCMC. Efficient preventive programs should be set up with a focus on education of the whole population for good hygiene practices. National actions against antibiotic resistance should be prioritized in public health policies.

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LIST OF ABBREVIATIONS

AMO	:	Amoxicilline
CagA	:	Cytotoxin-associated gene A
CHCs	:	Commune health centers
CHSs	:	Community health stations
CLA	:	Clarithromycin
DHS	:	Demographic Health Survey
ED	:	Employees density
EIA	:	Enzyme-immunoassay
EPZs	:	Export processing zones
ESPGHAN	:	The European Society for Paediatric Gastroenterology Hepatology and Nutrition
EUCAST	:	The European Committee on Antimicrobial Susceptibility
FDI	:	Foreign Direct Investment
GDP	:	Gross Domestic Product
H. pylori	:	*Helicobacter pylori*
HAV	:	Hepatitis A virus
HBV	:	Hepatitis B virus
HPV	:	Human papillomavirus
HVC	:	Hepatitis C virus
IARC	:	The International Agency for Research on Cancer
ICC	:	Intracluster correlation coefficient
IgG	:	Immunoglobulin G
IL-8	:	Interleukin 8

LEV	:	Levofloxacin
MALT	:	Mucosa-associated lymphoid tissue
MET	:	Metronidazole
MOET	:	Ministry of Education and Training
MOH	:	Ministry of Health
NASPGHAN	:	The North American Society for Pediatric Gastroenterology, Hepatology and Nutrition
OD	:	Optical density
OipA	:	Outer inflammatory proteins
OLGIM	:	Operative Link for Gastric Intestinal Metaplasia
PCR	:	Polymerase Chain Reaction
PD	:	Population density
PHC	:	Primary health care
PMH	:	Phu My Hung
PPIs	:	Proton pump inhibitors
RUT	:	Rapid Urease test
SAT	:	Stool Antigen test
SAWACO	:	Saigon Water Corporation
SSD	:	Sample size determination
SEZs	:	Special economic zones
T4SS	:	Type IV secretion system
TET	:	Tetracycline
UBT	:	Urea breath test
VacA	:	Vacuolating Cytotoxin
WHO	:	The World Health Organization

CHAPTER 1: INTRODUCTION

Helicobacter pylori (H. pylori) is commonly acquired during childhood, and tends to persist if untreated (1). It is well-known as a pathogenic bacteria that causes gastroduodenal diseases such as gastritis and gastroduodenal ulcers. In some cases, it can progress to stomach cancer decades later (2,3). Although rare, *H. pylori* infection also leads to gastric mucosa-associated lymphoid tissue (MALT) lymphoma, particularly in children. Furthermore, its role in extra-intestinal disorders such as chronic idiopathic thrombocytopenic purpura, iron deficiency anemia, Henoch-Schonlein purpura, and nutritional status is also well documented in recent reports (1,3,4). These *H. pylori*-related gastric diseases and other-related disorders together cause over a million deaths each year, making *H. pylori* infection one of the most severe issues to public health worldwide.

1.1 Global burden of *Helicobacter* pylori infection

The incidence of *H. pylori* infection has been changing over the years since its discovery in 1983, with a decline in most Western areas (Europe, Northern America, and Oceania) but it remains high in Asia and in Latin America and Caribbean (Figure 1.1) (5,6).

A systematic review conducted by Hooi et al. reported that approximately 50% of the world's population have been infected with *H. pylori* infection in 2015, but with a wide variation in *H. pylori* distribution across regions (Figure 1.2) (5). Regions with the highest *H. pylori* burden were Africa (70.1%), South America (69.4%), and Western Asia (66.6%), whereas regions with the lower prevalence of *H. pylori* were Oceania (24.4%), Western Europe (34.3%), and Northern America (37.1%) (5).

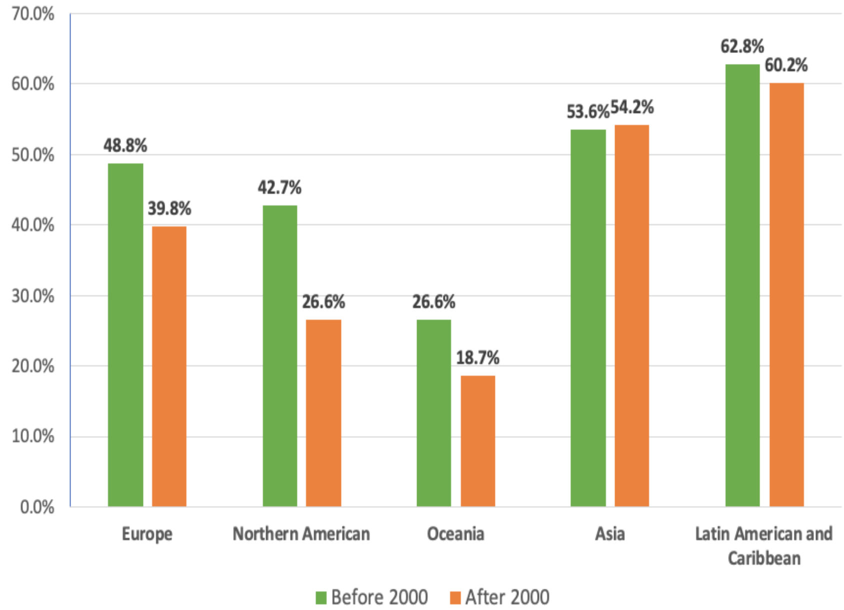


Figure 1.1 Time trend in *H. pylori* infection by United Nations geoscheme regions
H. pylori infection is decreased in most Western regions (Europe, Northern America, and Oceania) but it remains high in Asia and in Latin America and Caribbean
Adapter from: James K. Y. Hooi et al, Gastroenterology 2017 (5)

A huge variation in *H. pylori* infection is also observed between countries within a region, particularly in industrialized countries with similar standard of living, like Europe. For example, the prevalence of *H. pylori* infection in Portugal (84.2%) and Turkey (82.5%) was higher than in other European countries, despite improvements in sanitation, living conditions and development of economy across all Europe in recent decades (6,7).

Differences in prevalence of *H. pylori* infection can also be found even within the same country. Different ethnic groups have different *H. pylori* prevalence. For instance, several studies carried out in Malaysia, a Southeast

Asian country with three main ethnic groups – Malays (61.8%), Chinese (21.4%), and Indians (6.4%) – reported the infection rate of H. pylor infection was lower in Malays (18.3% – 19.6%) compared to Chinese (36.8% – 40%), and Indians (45.4% – 50.7%) (6,8).

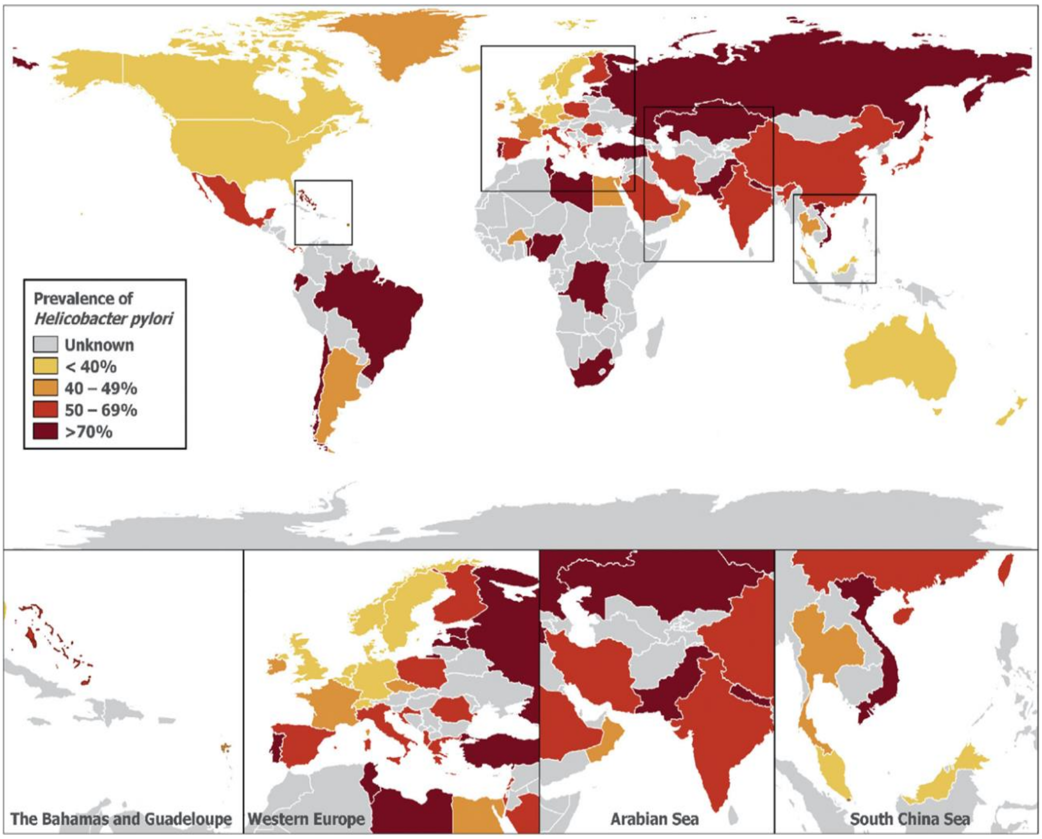


Figure 1.2 Global prevalence of *H. pylori* infection
Source: James K. Y. Hooi et al, Gastroenterology 2017. https://people.ucalgary.ca/%7Eggkaplan/HP2016.html

A recent systematic review and meta-analysis conducted by James K. Y. Hooi et al. to investigate changing in the global prevalence of *H. pylori* in 2015 showed several of wide variations in *H. pylori* prevalence between regions, countries, and even within the same country (5). Moreover, they also suggested that *H. pylori* infection continues to be an important health issue in most of the world, with approximately 4.4 billion individuals with *H. pylori* globally (5). Even in the country with the lowest infection rate (Switzerland, 18.9%), there were still over 1.6 million infected individuals . All these data are reported at the whole population level.

1.2 ***Helicobacter* pylori infection in children**

Data on *H. pylori* infection in children versus adults remains incomplete due to most infected children showing no symptoms and limited studies. A recent review and meta-analysis of studies performed on healthy children, estimated an overall prevalence of 33% (9). The prevalence varies widely across different geographic regions and within specific regions, influenced by socioeconomic factors. The highest rates were observed in the Africa region (44.1%), while the lowest rates were found in Western Pacific region (20.0%) (Figure 1.3) (9). Moreover, the infection rates among children in low-income and middle-income countries (43.2%) was almost twice that children in high-income countries (21.7%), emphasizing the impact of birth country on the infection rates (9).

The prevalence of *H. pylori* in children is also decreasing in developed countries (9,10). For instance, Iceland reported a decline in the infection rate in children from 10-15% in 1996 to 3.4% in 2012 (9,11,12). Similarly, Japan also reported a significant drop in childhood infection from 13.6% in 1996 to 3.1% in 2013 (13). However, this decrease trend has not been observed in developing countries from Eastern and Southern Europe and Asia (7,9,10). Reasons behind this differential trend have not been fully understood, possibly due to improved education, nutrition, better hygiene practices, and standards of living in developed countries compared to developing countries. Taken together, despite the declining prevalence of *H. pylori* infection in children, this prevalence still remains high, particularly in devloping countries, requesting further attention from health sciences.

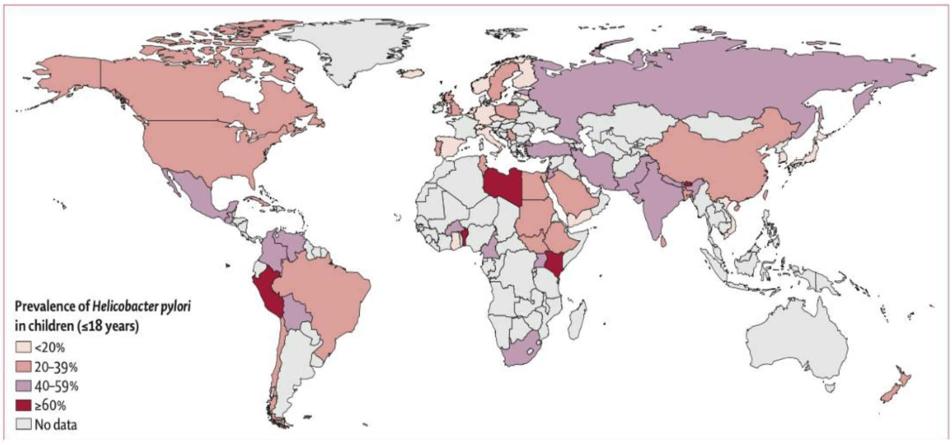


Figure 1.3 Estimated prevalence of *H. pylori* infection among children in 60 countries
Source: Yuan C et al, Lancet Child & Adolescent Health 2022 (9).
https://www.thelancet.com/journals/lanchi/article/PIIS2352-464200400-4/fulltext

1.3 Risk factors for *Helicobacter pylori* infection

There have been several studies on risk factors of *H. pylori*. The major independent risk factors associated with *H. pylori* infection were low socioeconomic status, increasing number of siblings, having an infected parent, particularly a mother (9,10). However, the risk factors varied greatly among different populations.

No association between *H. pylori* infection and gender has been reported both in adults and in children (5,6,9). However, a report from South Korea showed a slightly higher rate of *H. pylori* infection in men compared to women in adults (14). Similarly, no association between *H. pylori* infection and age has been reported in adult population. However, there is evidence of a birth cohort effect with a higher rate of infection in individuals born before 1920 (73%) compared to those born after 1980 (22%) (15).

Several studies pointed that factors related to living conditons such as the presence of infected family members, larger siblings, crowded living conditions, or living in a urban area were positively associated with *H. pylori* infection (3,9,14). Poor sanitation and hygiene accessibility also showed higher *H. pylori* prevalence. Individuals with low socioeconomic status, measured as family income level, were more likely to *H. pylori* infected. Indeed, people with low socioeconomic status are more likely to live in crowded conditions and have poor sanitation, which increases the risk of *H. pylori* infection. Moreover, an inverse association was found between education level and *H. pylori* infection (16). These findings support that improved socioeconomic conditions in developed countries contribute to a decline in the rate of infection, while the infection remains high in developing countries due to poor socioeconomic conditions.

The relationship between *H. pylori* infection and lifestyle habits such as smoking and alcohol consumption is not consistent across studies (5,10). While most studies found no association between these habits and *H. pylori* infection, some studies showed a positive association (7,17,18). In contrast, regular alcohol consumption was found to be a protective factor for *H. pylori* infection in a study conducted in Turkey in 2013 (19). Therefore, further research is needed to better understand the relationship between these lifestyle factors and *H. pylori* infection.

1.4 Transmission routes of *Helicobacter pylori* infection

The transmission of *H. pylori* is not completely understood, but it is believed that the bacteria mainly transmitted from person to person through three possible routes: oral-oral, fecal-oral, or gastro-oral routes (3,20–22).

Oral-oral transmission

H. pylori has been successfully isolated from saliva, subgingival biofilm, and dental plaque of infected individuals indicating that the mouth might be a reservoir of *H. pylori* (23). Furthermore, several studies pointed that close contact with infected individuals (mother, grandmother, or sibling) increases the possibility of *H. pylori* infection. Indeed, sharing eating utensils, and kissing with infected individuals, especially infected mothers, were also found to increase risk of *H. pylori* infection (9,20,22,24,25). These data support the hypothesis of oral-oral spearding of *H. pylori*.

Fecal-oral transmission

Fecal-oral transmission may occur directly from infected individual or from crowded or poor sanitary conditions or indirectly from contaminated environment (foods, water, or surfaces). A strong association between *H. pylori* and hepatitis A virus (HAV), an indicator for fecal-oral exposure, was found in a study conducted by Bui et al. supporting the potential for fecal-oral transmission of *H. pylori* (26). Moreover, several reports have detected *H. pylori* in human feces by culture or PCR (7,27).

Gastro-oral transmission

H. pylori has been also detected in vomitus samples of all infected patients in a clinical series (28). In addition, it was also cultured from air sampled during vomiting of infected patients. These findings support that gastro-oral pathway may be one of the routes of *H. pylori* transmission.

Waterborne transmission

Water may play a role as a medium in the fecal-oral transmission of *H. pylori* infection. However, water might be also an environmental reservoir of *H. pylori*. Indeed, water was first suggested as a source of *H. pylori* infection in 1991 by Klein et al., who observed that the infection rate was 12-fold higher in children using municipal water than in children using water from community wells (29). Another study conducted in Pakistan in 2012 reported that treated municipal drinking water samples were positive for *H. pylori* further supporting water as a reservoir of *H. pylori* (30). A recent study conducted in Iran also detected *H. pylori* in various water sources such as tap water and bottled mineral water (31).

Food-borne transmission

Similar to water, food might also play a role as an environmental reservoir. Several studies found the presence of *H. pylori* in various types of food such as milk, meat, or vegetables. Among these foods, milk products were the most studied maybe because *H. pylori* is already acquired during childhood, and milk is mostly consumed during this period (32). In Greece, for instance, Angelidis et al. found *H. pylori* in 20% of raw cow's milk samples (33). Similarly, in 2018, Ranjbar et al. detected *H. pylori* in 11.53% of cheese samples, 5% of ice cream samples, 4% of butter samples, and 1.11% of yoghurt samples (34).

1.5 Pathogenesis

Mechanisms of *H. pylori* infection

The stomach lining has effective defenses against bacterial infections. However, *H. pylori* has various mechanisms to avoid responses of the immune system, allowing it to colonize in the stomach mucus, and eventually cause gastric inflammation. The specialized mechanisms of *H. pylori* is complex and involves multiple processes (figure 1.4), including the following ones (1,35):

(1) **Penetration of the mucous layer and attachement to the surface of epithelial cells:** *H. pylori* uses its flagella to move towards the mucus layer, and reach the epithelial cells. Ultimately, it attaches tightly to the epithelial cells by using multiple outer-membrane proteins, also known as adhesion.

(2) **Production of virulence factors:** *H. pylori* secretes various virulence factors, which help *H. pylori* to survive and cause damage to the epithelial cells of the stomach. Urease is one of the major virulence factor of *H. pylori*. It breaks down urea into ammonia and carbon

dioxide, which neutralized the acidity of the stomach environment, enabling *H. pylori* to survive longer in the stomach environment. Other virulence factors and their roles will be further described and explained in the next section.

(3) Colonization, proliferation, and migration of *H. pylori*: once the acidic environment of the stomach has been neutralized, more and more *H. pylori* can migrate, proliferate, and start to colonize in this particular area. These things allow *H. pylori* to expand its population and establish a stable colonization in that neutralized area.

(4) Development of gastritis and ulcers: *H. pylori* infection triggers an inflammatory response, leading to the recruitment of immune cells and the production of certain cytokines inside the stomach lining. Over time, this chronic inflammation causes damage to the gastric epithelium, and leads to the development of gastritis and ulcers.

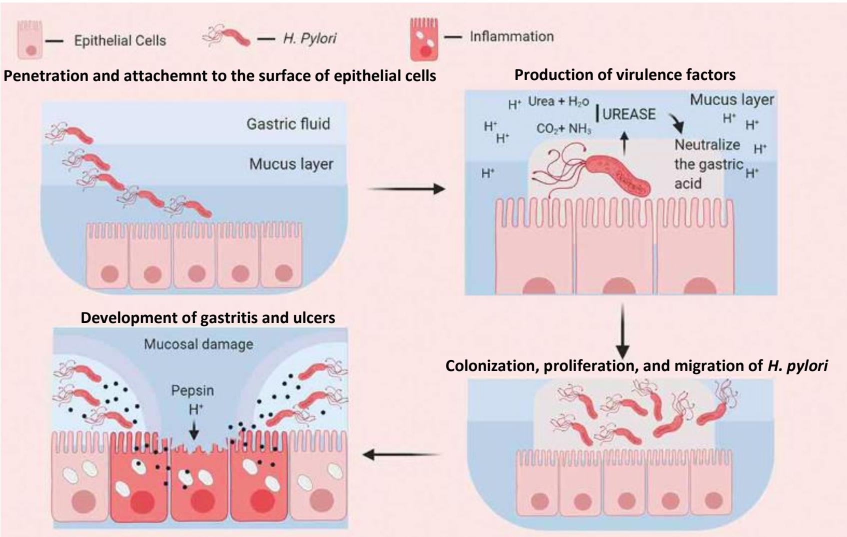


Figure 1.4 **Pathogenesis of *H. pylori***

Source: Khan S et al., Expert Rev Anti Infect Ther 2022 (34)

Virulence factors of *H. pylori*

The severity of clinical outcomes in *H. pylori* infection is influenced by several bacterial virulence factors. The bacterial virulence is bacterial products that enter host cells, or factors secreted by the bacteria that have effects on host cells (1,36,37), including:

(1) Flagella: *H. pylori* have multiple, polar sheathed flagella responsible for its motility. These flagella aid the bacterium in navigating through the viscous mucus layer that covers the gastric epithelial cells. Additionally, they also contribute to the bacterium's ability to evade and colonize the stomach.

(2) Urease: Urease helps to neutralize the acidic environment of the stomach by converting urea to ammonia and carbon dioxide.

(3) Adhesins: Adhesins are a group of molecules located on the surface of *H. pylori*, which aid the bacterium to attach to and to persist within the gastric epithelium. There are various adhesions (babA, babB,…), each of which adheres to specific receptors at the surface of gastric epithelium.

(4) Outer inflammatory proteins (OipA): OipA is an outer member protein that plays a role in adhesion and IL-8 induction. It induces inflammation and actin dynamics by activating multiple signaling pathways that interact with cag PAI (Cag-A)-related pathways.

(5) Vacuolating Cytotoxin (VacA): VacA is a toxin produced by *H. pylori* that plays a critical role in development of *H. pylori*-associated gastric diseases. It induces vacuolization in eukaryotic cells, attachment to cell-membrane receptors which initiates inflammatory responses, disrupts the epithelial barrier, releasing cytochrome c from mitochondria which leads to apoptosis, and also inhibits T-cell activity.

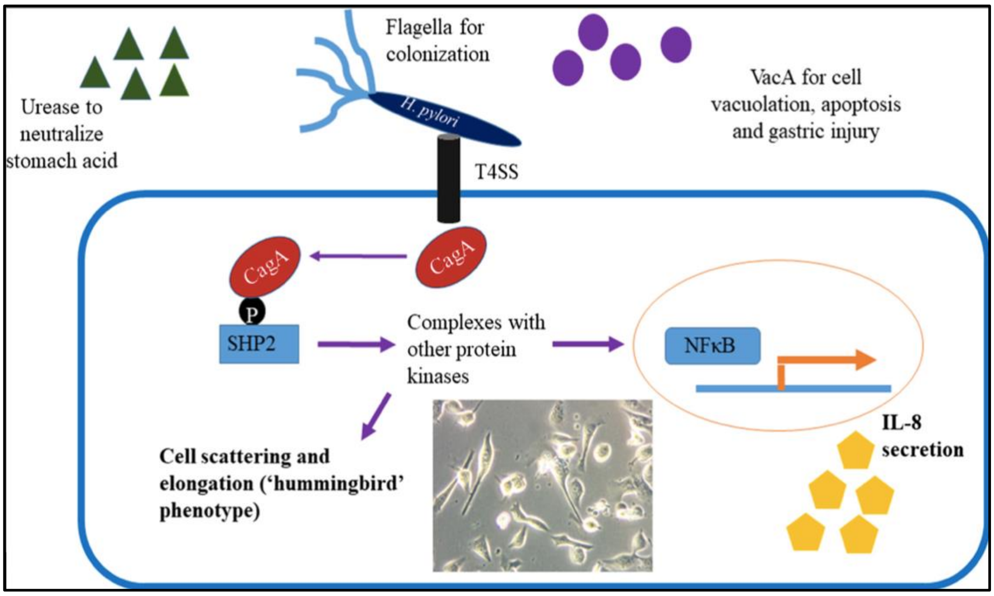


Figure 1.5 Virulence factors of *H. pylori*
Source: Sukri A et al., APMIS 2020 (36). https://onlinelibrary.wiley.com/doi/10.1111/apm.13034#

(6) Cytotoxin-associated gene A (CagA) and type IV secretion system (T4SS): Cag is a protein produced by *H. pylori* which infects virulence factors into host cells by a pilus structure known as T4SS. Once inside the host cell, CagA can modify intracellular signal transduction pathways and disrupt normal cellular functions, leading to the malignant transformation of gastric epithelial cells. Additionally, CagA and T4SS also increase gastric inflammation and secretion of IL-8, which may contribute to genetic instability and promote the development of cancer.

(7) **Secretory enzymes:** *H. pylori* secretes various enzymes such as mucinase, protease, and lipase which may cause inflammation and damage to gastric tissues.

1.6 Natural history of *Helicobacter pylori* infection

H. pylori infection is mainly acquired in the first few years of life and tends to persist if untreated. Acute infection with *H. pylori* causes temporary hypochloremia, and is rarely detected because there are no symptoms in most cases. However, chronic *H. pylori* infection potentially leads to the progression of various upper gastrointestinal diseases, including duodenal or gastric ulcers (1 to 10% of infected patients), gastric cancer (0.1 to 3% of infected patients), and gastric mucosa-associated lymphoid-tissue (MALT) lymphoma (< 0.01% infected patients) (38).

The risk of these outcomes varies greatly among infected patients, depending on both bacterial and host factors. Patients with higher acid production are more prone to have antral-predominant gastritis, the most common form of *H. pylori* gastritis, which predisposes them to duodenal ulcer. Conversely, patients with lower acid production are more likely to develop corpus-predominant gastritis and multifocal atrophy, which predisposes them to gastric ulcers, gastric atrophy, intestinal metaplasia, and potentially leading to gastric carcinoma. In addition, *H. pylori* could induce the formation of MALT in gastric mucosa, which can lead to malignant lymphoma (1).

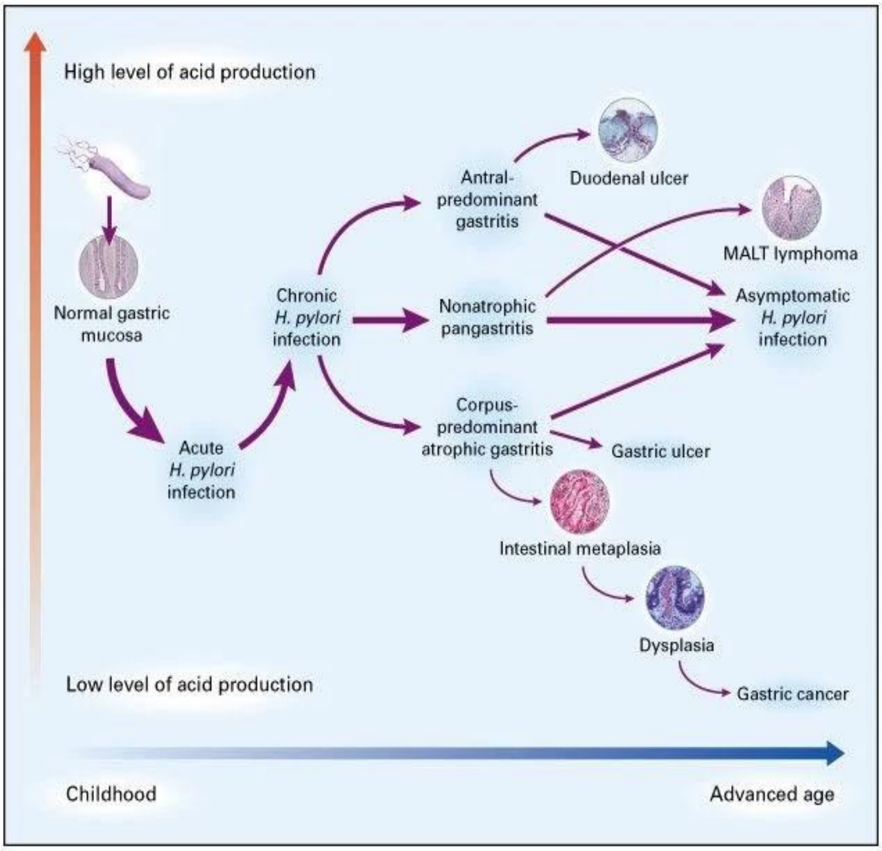


Figure 1.6 Nature history of *H. pylori* infection
Source: Sebastian Suerbaum et al. NEJM 2002.
https://www.nejm.org/doi/full/10.1056/nejmra020542

1.7 *Helicobacter pylori* infection and gastric cancer

H. pylori has been classified as a class I carcinogen by the World Health Organization (WHO) and is the most important risk factor for gastric cancer (39,40). It was estimated that nearly 75% of all gastric cancers were attributable to *H. pylori* infection (41). *H. pylori* is also recognized as bacterial carcinogen that contributes to the development of gastric cancer by the International Agency for Research on Cancer (IARC) (39). Indeed, numerous studies have also reported a strong relationship between *H. pylori* infection and development of gastric cancer (3,40,41). Regions with a high incidence of gastric cancer were prone to high *H. pylori* infection rates. A randomized, controlled trial showed that eradication of *H. pylori* reduced the incidence of gastric cancer, with effects seen even in persons with a first-degree family history of gastric cancer (42).

Moreover, *H. pylori* is also an important infectious cause of cancer worldwide. Of the 11 infectious bacteria classified as pathogens-associated cancer by IARC, *H. pylori* was responsible for more than 35% (810000/2100000) of all new infection-related cancers in 2018 (41,43).

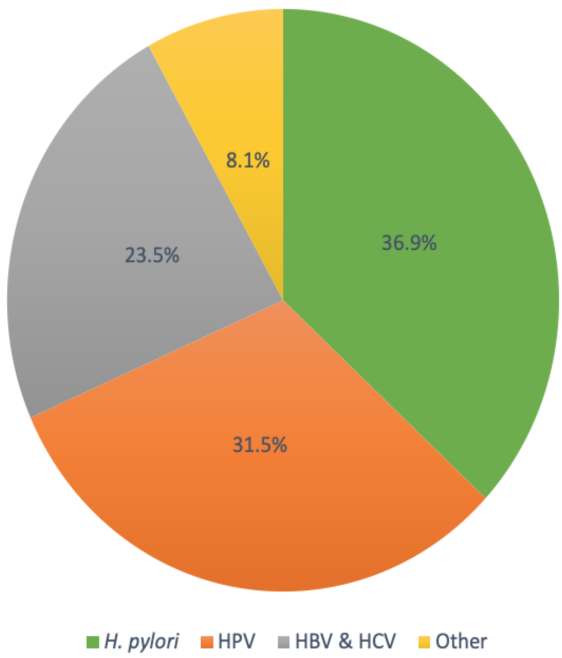


Figure 1.7 Proportion of cancer cases attributable to infection in 2018
Adapter from: Plummer M et al.the Lancet Global Health 2020

With more than 1 million new cases per year, gastric cancer is the fifth most commonly diagnosed cancer and the fourth most common cause of cancer-related death worldwide. The age-standardized incidence rates are the highest in East Asian, corresponding also to a high prevalence of *H. pylori* (41). Therefore, reducing *H. pylori* infection rate is crucial for decreasing the burden of gastric cancers in the future in Asian countries.

1.8 Diagnostic tests of *Helicobacter pylori* infection

H. pylori can be detected by invasive tests or non-invasive tests. Invasive methods require endoscopic biopsy of gastric mucosa, comprising histology, culture, polymerase chain reaction (PCR), rapid urease test (RAP). Non-invasive methods include stool antigen test (SAT), urea breath test (UBT), and serologic test. The choice of an appropriate test depends on the clinical situation. Generally, the invasive tests are more commonly used in clinical pratice, while non-invasive tests are mainly used to monitor the outcome of treatment and in epidemilogical studies. However, the current guidelines recommended that confirming *H. pylori* infection should be based on either positive culture or the combination of histological evidence with at least one other biopsy-based test (UBT or PCR) (3).

Histology

The existence of *H. pylori* can be detected through histological examination of gastric biopsies using special stains with hematoxylin and eosin or Giemsa. This examination is widely performed, and allows the scoring of gastritis by using the updated classification of Sydney or OLGIM. To increase sensitivity of the test, it is recommended to obtain a minimum of two samples from the antrum at the level of large curvature, one from the small curvature, and two at the fundus. To evaluate atrophy, a biopsy from the small curvature should also be obtained (1).

Rapid Urease Test

The rapid urease test works by detecting the activity of the urease enzyme produced by *H. pylori*. The test is performed by placing a gastric biopsy into a solution containing urea and a pH indicator. If *H. pylori* is present, it urease breaks down urea into ammonia and carbon dioxide, causing a change in colour of the pH indicator. This change in colour can be observed within 30 minutes, making the test rapid and convenient (1,44).

Culture

Culturing of *H. pylori* is often challenging because of the fragility of *H. pylori* and its requirement for microaerophilic conditions. It is not commonly used as a primary method for diagnosing *H. pylori* infection, but it is recommended after the failure of second-line therapy. This method has several advantages, including ability to evaluate the antibiotic sensitivity, adjust antimicrobial therapy, and enhance patient's eradication rates. Because of the potential for mixed infections with susceptible and resistant strains, the current guidelines are recommending to take multiple biopsies from different stomach sites (at leat one in the antrum and one in the fundus) (1,45).

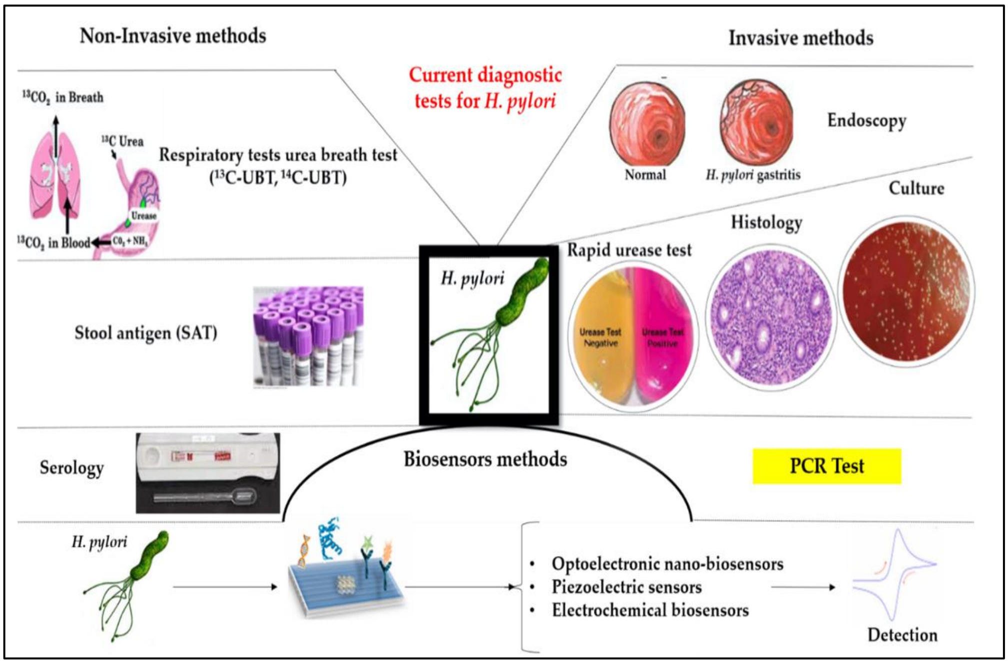


Figure 1.8 Invasive and non-invasive for diagnostic tests for *H. pylori*
Source: Cardos AI et al. Diagnostics (Basel) 2022 (44)

Polymerase Chain Reaction (PCR)

Molecular biology techniques can substitute culture in diagnosing *H. pylori* infection in case of lacking of the technical capability or if being unable to send frozen samples to a microbiology department with the necessary expertise. PCR can also facilitate the identification of mutations responsible for antibiotic resistance (1).

Serology

Serologic test is a low-cost and widely utilized method for detecting *H. pylori* infection. This test detects immunoglobulin G (IgG) antibodies produced by the immune system in response to *H. pylori* infection, which can persist in the blood for an extended period even after the infection has been eliminated. Therefore, serological testing for *H. pylori* infetion cannot distinguish between an active and a recent infection, limiting its usefulness in determining success of therapy (1,46).

Table 1.1 Advantages and disadvantages of different tests for *H. pylori* infection (47)

Test	Advantages	Disadvantages
Invasive methods		
Histology	Good sensitivity and specificity	Requires trained personnel
Culture	Excellent specificity; provides opportunity to test for antibiotic sensitivity	Variable sensitivity; requires trained staff and properly equipped facilities
Rapid Urease test	Rapid, inexpensive, and accurate in selected patients	False negative results possible in the presence of PPIs or with recent use of antibiotics or bismuth preparations
Non-invasive methods		
Stool Antigen test	High sensitivity (89%-98%) and high specificity (90%) with monoclonal-antibody test (48); useful before and after treatment	Process of stool collection may be distasteful to patient, false negative results possible in the presence of PPIs or with recent use of antibiotics or bismuth preparations
Urea breath test	High sensitivity and specificity of 95% (47); useful before and after treatment	False negative results possible in the presence of PPIs or with recent use of antibiotics or bismuth preparations; considerable resources and personnel required to perform test
Serologic test	Widely available; the least expensive of available tests	Positive result may reflect previous rather than current infection; not recommended for confirming eradication in a patient.

PPIs: proton pump inhibitors

Urea breath test

Urea breath test involves the administration of carbon-labeled urea to patient, followed by the measurement of this stable isotope in the expired CO_2. If *H. pylori* is present in the stomach, it produces urease that breaks down urea into carbon dioxide and ammonia. The carbon dioxide is then absorbed into the bloodstream and exhaled through the lungs, causing an elevation of CO_2 in expired breath. A mass spectrometro can detect this increase in CO_2, which is indicative of the presence of *H. pylori* infection. The urea breath test is non-invasive, safe, and a highly accurate method to diagnose *H. pylori* infection. However, false negative results can occur if the patient has recently taken antibiotics or PPI, since these medications may suppress the infection and reduce the sensitity of testing (47).

Stool antigen test

H. pylori infection can also be detected through the identification of *H. pylori*-specific antigens in a stool sample using either polyclonal or monoclonal antibodies. The monoclonal-antibody test is more accurate than the polyclonal antibody test with a sensitivity ranging from 89% to 98% and a specificity over 90% (48). The antigen test can use for screening purposes in epidemiological studies, and also for monitoring patients for an eradication treatment (47).

1.9 Treatment of symptomatic *Helicobacter pylori* infection

Indications for treatment in children

Studies have shown that the detect-treat approach should be avoided. Indications for paediatric *H. pylori* treatment are symptomatic duodenal or gastric ulcers patients with biopsy-proven *H. pylori* infection, individuals with *H. pylori*-associated mucosa-associated lymphoid tissue (MALT) lymphoma (table 1.2) (49–52). However, it is important to consider the regional infection rate and pathogenicity of the bacterium when determining these indications.

Table 1.2 Indications for treatment of *H. pylori* infection in children

Definite indicators
Duodenal or gastric ulcers with biopsy-proven *H. pylori* infection
Mucosa-associated lymphoid tissue (MALT) lymphoma with *H. pylori* infection
Relative indicators
Unexplained iron-deficiency anemia (other causes have been excluded)
Not recommended
Recurrent abdominal pain
Cancer prevention
Asymptomatic
Infected family member

Treatment regimens for *H. pylori* infection in children

According to the Joint ESPGHAN and NASPGHAN 2016 guidelines, the standard triple therapy (PPI–amoxicillin–clarithromycin or metronidazole) is used as the first-line therapy recommended (49). However, if culture and antibiotic susceptibility testing for *H. pylori* are unavailable, the quadruple therapy with bismuth should be first-line therapy (table 2.3).

Table 1.3 Recommended options for first-line therapy for *H. pylori* infection in children

CLA	MET	Suggested treatment
Patients without penicillin allergy		
Susceptible	Susceptible	PPI-AMO-CLA
Resistant	Susceptible	PPI-AMO-MET
Susceptible	Resistant	PPI-AMO-CLA
Resistant or unknown	Resistant or unknown	Bismuth-PPI-MET-tetracycline (if ≥8 years), **or** Bismuth-PPI-AMO-MET (if <8 years), **or** PPI-AMO-MET with high-dose AMO*
Patients with penicillin allergy		
Susceptible	Susceptible	PPI-MET-CLA
Resistant	Susceptible	Bismuth-PPI-MET-tetracycline (if ≥8 years)¶
Susceptible	Resistant	Bismuth-PPI-CLA-tetracycline (if ≥8 years)
Resistant or unknown	Resistant or unknown	Bismuth-PPI-MET-tetracycline (if ≥8 years)¶

CLA: clarithromycin; MET: metronidazole; PPI: proton pump inhibitor; AMO: amoxicillin.
* Where bismuth is available, bismuth-based quadruple therapy is preferred due to better eradication rates compared with high-dose AMO.
¶ If child is <8 years old and the allergy may be mild, refer to an allergist to determine whether AMO-based treatment is feasible.

After the initial therapy, it is recommended to perform follow-up testing at least four weeks later to confirm the successful eradication of the *H. pylori* infection. Non-invasive tests like the urea breath or the stool antigen test are preferred for this purpose. Serologic tests are not recommended for diagnosis or follow-up due to poor sensitivity and specificity as well as inability to distinguish between active and past infection. If the follow-up test is negative, further testing is unnecessary.

If the initial therapy is unsuccessful, it is recommended to perform culture with antibiotic sensitivity testing or polymerase chain reaction (PCR) to guide the selection of subsequent therapy. However, it is important to note that treatment failure can be influenced by several factors, including poor compliance, insufficient treatment duration, and bacterial resistance to antibiotics. Therefore, the following strategies should be considered when managing a child who did not respond to the initial therapy: assessing bacterial resistance, choosing an alternative regimen that avoids previously used antibiotics and agents with known resistance, and providing counseling to the child and caregiver on the importance of adhering to the complete treatment. By considering these factors and implementing appropriate strategies, the chances of successful treatment can be improved.

CHAPTER 2: SETTINGS

2.1 Vietnam

2.1.1 Geography

Vietnam is a country situated on the Eastern Indochina Peninsula in Southeast Asia, with a form as the letter S. The S-shaped country has a north-to-south distance of 1,650 kilometers, with a narrowest width of about 50 kilometers. It is bordered by China in the North, Laos and Cambodia in the West, and has a long coastline stretching over 3,200 kilometers in the East (53). Its total land area is approximately 331,212 km^2, with nearly 40% of the land used for agriculture (54). The country can be divided into six distinct regions: Northern midlands and mountain, Red River Delta, North Central and Central Coastal, Central Highlands, Southeast, and Mekong River Delta (figure 2.1). The Northern midlands and mountain mainly consists of medium mountains, while the Red River Delta is a flat region with fertile soil and is home to the capital city, Ha Noi. The North Central and Central Coastal area is located in the middle of Vietnam and play a role as an economic exchange area between the North and the South economic regions. The Central Highlands is a mountainous region with peaks reaching up to 2,500 meters above sea level, and is famous for its coffee and tea plantations. The Southeast is a narrow strip of land along the eastern coastline, and is home to some of the country's largest cities, including Ho Chi Minh City (HCMC). The Mekong River Delta is a lush region in the South, where the Mekong River empties into the South East Asia Sea, and is known for its rice paddies and fruit orchards (55–57).

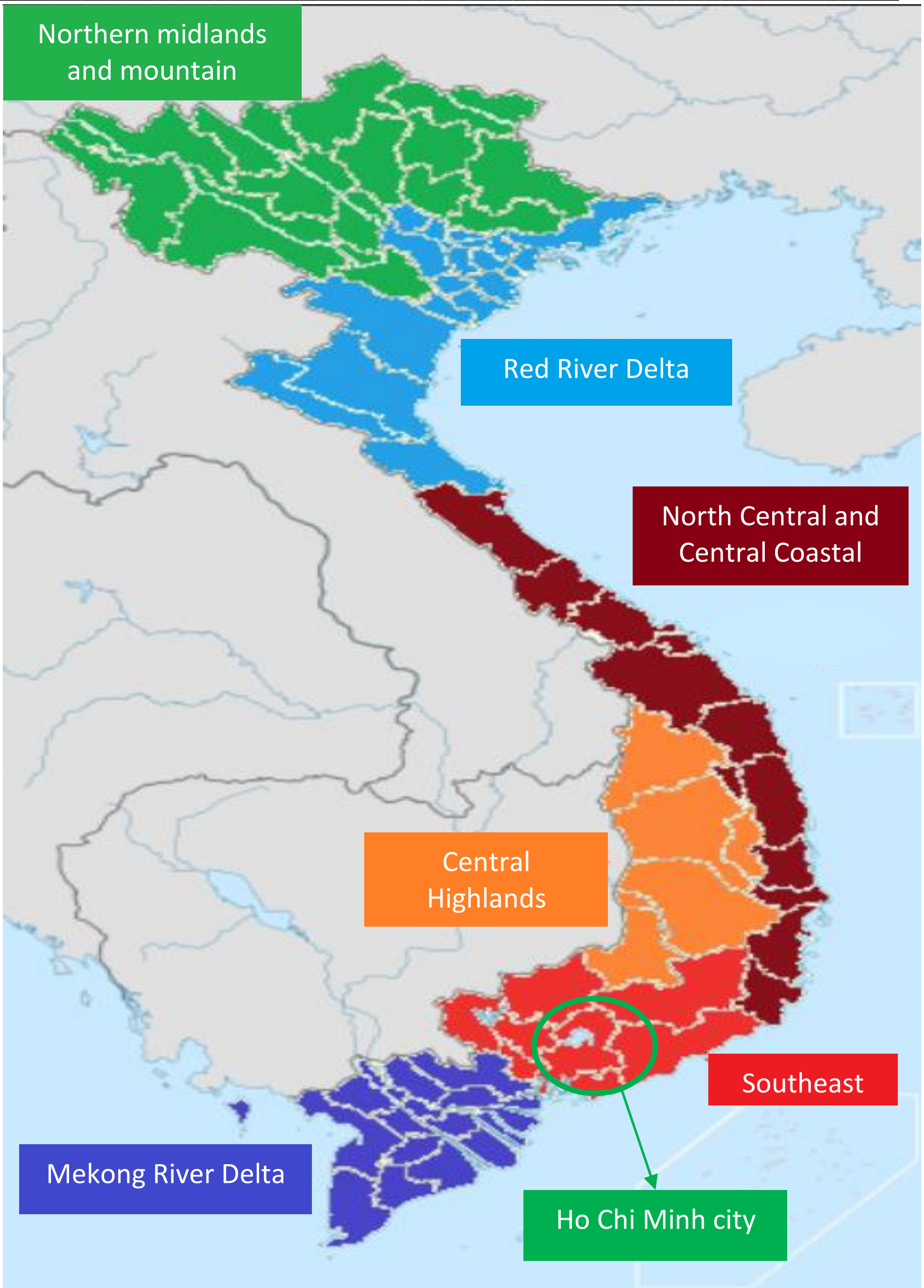


Figure 2.1 **Map of Vietnam by regions**

Source: https://xaydungso.vn/bai-viet-khac/tong-hop-ban-do-7-vung-kinh-te-viet-nam-day-du-nhat-vi-cb.html

Vietnam has a tropical climate in the South with two distinct seasons – a rainy season from mid-May to mid-September, and a dry season from mid-October to mid- March. In the North, there is a monsoon climate with four seasons – spring, summer, autumn, and winter. Located along the coast, the climate in Vietnam is regulated by the ocean currents and brings many maritime climate factors such as seasonal temperature variation, increased precipitation due to more moist air. Average relative humidity is 84% throughout the year. The annual rainfall ranges from 1,200 to 3,000 mm, and the annual temperature varies from 5° C to 37° C (58,59).

2.1.2 Population

Vietnam's population has grown rapidly from 79.9 million in 2000 to 98.5 million persons in 2021, making it the 15th most populous country worldwide. Population density in the entire country is 308 persons/km^2 (60), which is lower than Belgium (377 person/km^2, www.statbel.fgov.be). However, the population is not evenly distributed, with 36.6 million persons (37.1%) residing in urban areas and 61.9 million persons (62.9%) living in rural areas. The Red River Delta and the Mekong River Delta regions have the highest population densities due to the favorable conditions for important economic activities such as agriculture and fisheries. After the Doi Moi economic reform in the 1986, these areas became the most populated areas in Vietnam with a heavy urbanization.

In 2019, children aged 6-15 years accounted for 15.8% (15 million) of the whole population, with 53.3% (8 million) being male, and 47.7% (7 million) being female (61).

Table 2.1 Population and population density (World Bank 2021)

	Population (million persons)	Population density (persons/km^2)
Vietnam	98.5	308
Ho Chi Minh city	9.3	4,363
Ha Noi city	8.1	2,398
Da Nang city	1.4	2,126
China	1,455.1	153
Beijing	21.5	1,300
Shanghai	24.3	3,900
Belgium	11.7	377
Brussels	1.2	7,300

2.1.3 Educational system

The education system in Vietnam has five levels: preschool, primary, secondary, high, and tertiary school. Preschool education is not compulsory, but it is recommended for children aged three to five years old. Primary school is mandatory for all children, and covers 5 grades (1st – 5th) with expected ages from 6 to 10 years. Secondary education is divided into lower secondary education (junior high school) and upper secondary education (high school). Lower secondary education covers four grades (6th – 9th), with expected ages from 11 to 14 years, while upper secondary education consists of 3 grades (6th – 9th) for pupils with range of 15 to 18 years. Tertiary level is divided into undergraduate education and graduate education (62).

There are two co-existing education systems in Vietnam: public schools (92%) and private schools (8%). Public schools are overseen by the Ministry of Education and Training (MOET), and is provided free of charge to all pupils up to the end of lower secondary school. After that, pupils have to pay for their education. However, the government also provides financial support to needy pupils. One of the challenges faced by the public education system in Vietnam is the large class sizes, which can sometimes exceed 50 pupils per class. This can make it difficult for teacher to provide individualized attention to each pupil. Additionally, the quality of education can vary widely across different regions of the country, with some schools in rural areas lacking resources and trained teachers (62).

Private education has been growing rapidly in recent years, particularly in larger cities such as Ha Noi and Ho Chi Minh city. Private schools in Vietnam cater to all levels of education, ranging from preschool to tertiary education, and offer a wide range of curricula, including Vietnamese, British, American, Australian, and Singaporean. One of the main advantages of private schools in Vietnam is their smaller class sizes (20 – 25 pupils/class), which enables a more personalized attention provided to pupils, as compared to public shools. However, private education in Vietnam is generally expensive, making it inaccessible to low-income or even middle income families (63,64).

The school year usually spans nine months, begins in September and ends in May. Public shcools typically have a half-day schedule with morning or afternoon sessions that last for 3 to 4 hours, held 5 days a week. Private schools generally have a longer school day that can last up to 6 to 8 hours a day, 6 days a week. In addition to regular classes, public schools also offer additional half-day courses each day of the week that require additional fees, which most pupils attend (62).

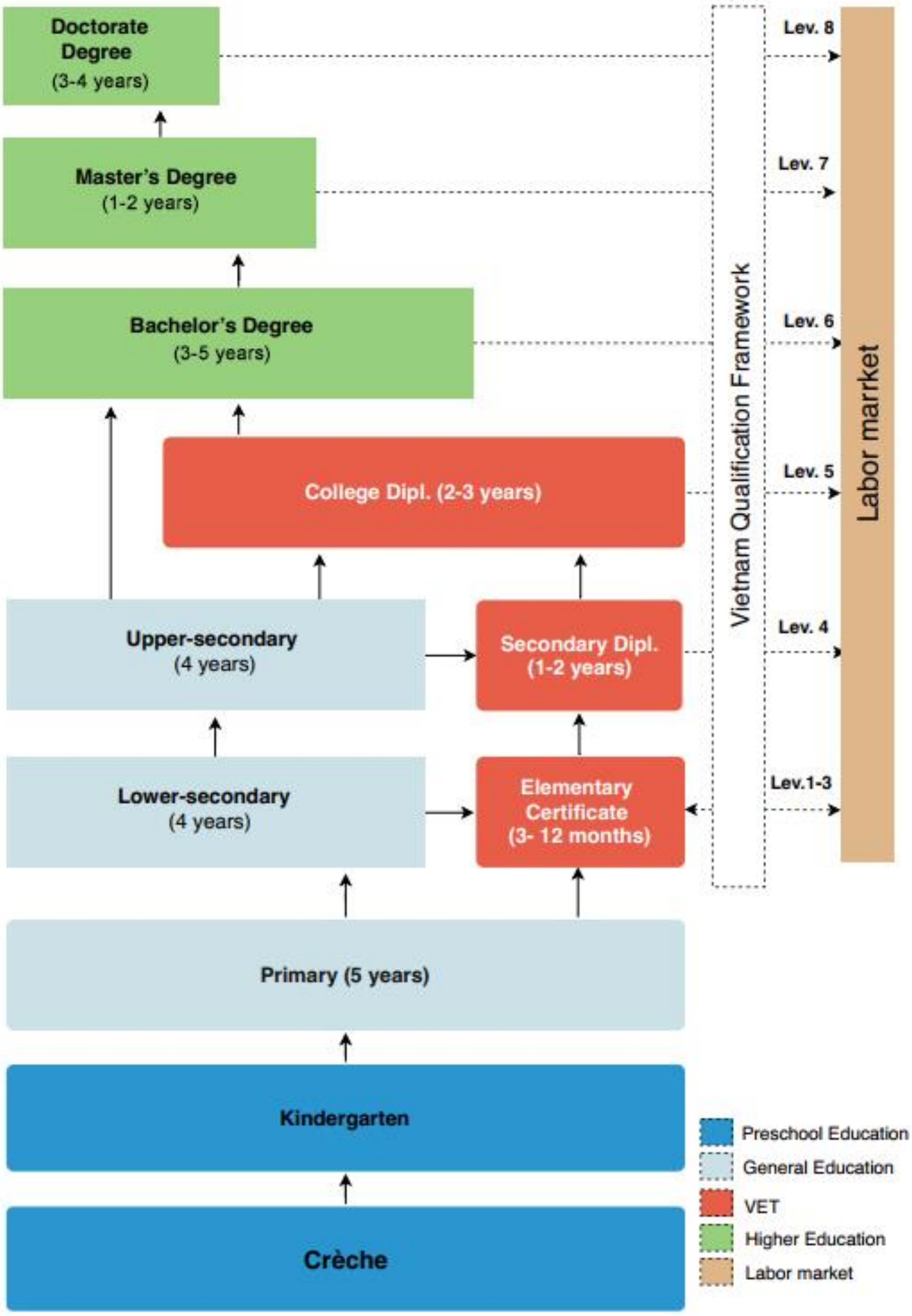


Figure 2.2 Education system of Vietnam
Source: https://daotaocq.gdnn.gov.vn/en/vocational-education-and-training-system/

2.1.4 Healthcare system

Vietnam's healthcare system is organized according to the pyramidal structure with four administrative levels: the central (the national), the provincial, the district, and the commune. The People's Committee holds a central role in the administrative supervision at each level. The Ministry of Health (MOH) is responsible for formulating and implementing health policies in Vietnam (65).

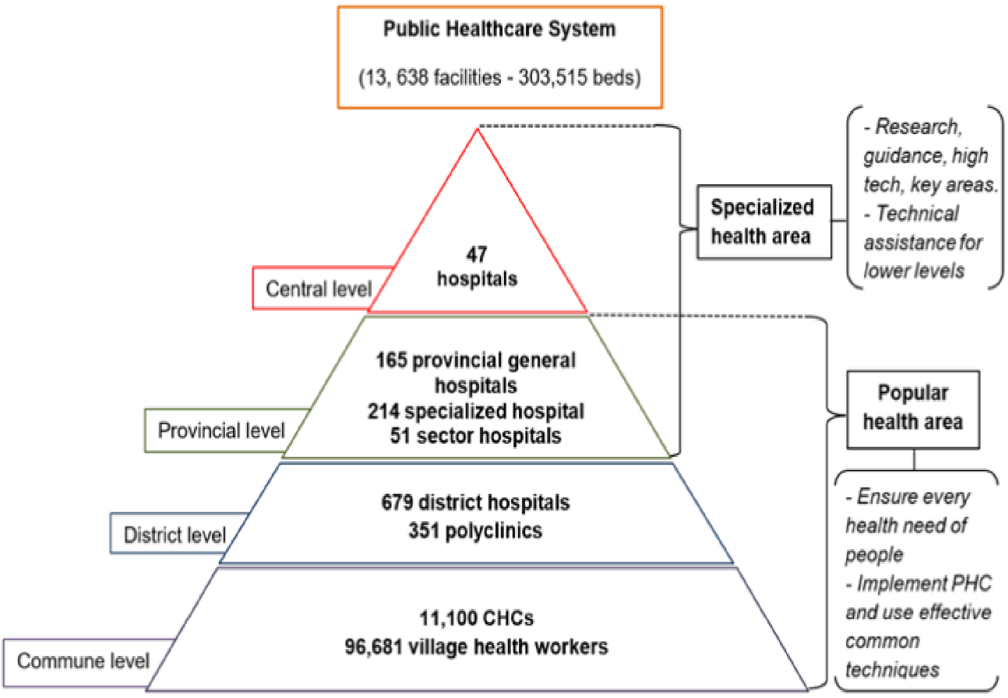


Figure 2.3 Healthcare system of Vietnam
CHCs: commune heath centers , PHC: primary health care
Source: Nguyen HV et al. Asia Pacific Journal of Health Management, 2021 .

Each level is responsible for different aspects of healthcare services. Central-level hospitals focus on specialist services, technical support and training for the lower levels. Provincial and district health facilities provide general, less technical services (65,66). At the commune level, community health stations (CHSs) have missions of providing primary healthcare services, detecting epidemic outbreaks, treating common diseases, and handling normal deliveries. In CHSs, an obstetrician is always available, but a pediatrician is often lacking.

In Vietnam, the health care system is still on the way completing (67). The number of doctors reached 0.78 per 1,000 inhabitants in 2020, while the number of nurses reached 1.4 per 1,000 inhabitants. As a comparison, Belgium has 3.1 doctors and 11.7 nurses per 1,000 inhabitants (68). Access to medical services is influenced by geographic, economic (ability to pay), cultural, and social factors. To improve geographic accessibility, the MOH has prioritized the development of grassroots healthcare network nationwide. Presently, 100% communes have CHSs. However, the doctors are not available at all health centers, particularly the pediatricians. Just 76% of CHSs have a medical doctor. The children are not followed up every month by doctors up to 2 years of age like in the USA or in Europe.

Health insurance

In order to reduce the financial burden related to healthcare, the government of Vietnam has implemented policies aimed to expanding the health insurance coverage, particularly in children. Children under the age of 6 receive free care services, while those above 6 years old can benefit from two main health insurance programs (69). Free care services mean no payment for physician but family has to organize and manage for foods, cloths, beds. These programs are operated by Vietnam Health Insurance Organization on a non-profit and public basis. The first is student health insurance, which is provided for school children on a voluntary basis. The second program is free health insurance for the poor children, and children in ethnic minorities and other policy households such as households with war merit or invalid members (65,69). According to Vietnam Household Living Standard Surveys, 62.8% of children age above 6 years were enrolled in at least a health insurance program in 2006 and this rate increased to 96% in 2022.

2.1.5 *Helicobacter pylori* infection in Vietnam

Despite rapid economic growth and improvements in the health system over the past few decades, Vietnam still has a high burden of *H. pylori* infection. A recent study published on Asian Pacific Journal of Cancer Prevention pointed that *H. pylori* infection is prevalent in Vietnam, with an overall prevalence of 60% . The prevalence of *H. pylori* in Vietnam was higher than into other countries in Asia, such as China (44.2%), or Thailand (43.6%) (70,71).

There was also a wide variation in *H. pylori* distribution across different geographic regions in Vietnam: Mekong delta (36.7%), Northern midlands and mountain area (46.8%), North Central and Central Coastal area (61.5%), and Red River Delta area (76.8%) (21,72–74). Interestingly, the Red River Delta region, which has a highest population density of 1,091 persons/km^2, has a higher prevalence of *H. pylori* compared to other regions (61). This suggests that living in crowded areas may contribute to an increased risk of *H. pylori* infection in Vietnam. However, it is important to note that crowded living conditions are not necessarily related to population density. Other factors contributing to crowding, such as number of people in household, number of children in household, sharing a bed are also warrant investigation beyond just population density.

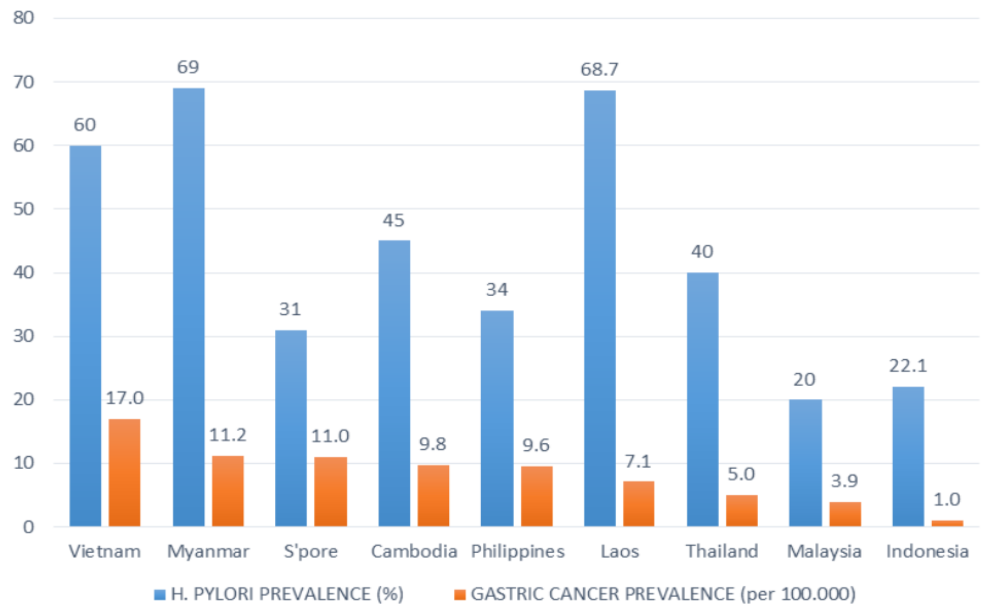


Figure 2.4 ***H. pylori*** **and gastric cancer prevalence in Southeast Asian Countries**
Source: Quach DT et al. Asian Pac J Cancer Prev 2018 (69)

H. pylori is aslo probably a very important cause of several gastrointestinal diseases in Vietnam, including gastric ulcer, dudodenal ucler and possibly gastric cancer. Indeed, the infection rate of *H. pylori* was reported to be 84% and 92.5% in patients with gastric ulcer and dudodenal ulcer, respectively (70). Additionally, a cross-sectional hospital-based study conducted in Ha

Noi also pointed a strong association between *H. pylori* infection and these *H. pylori* related diseases (75).

Gastric cancer was ranked in 2020 as the fourth most common cancer in Vietnam, with 17,906 new cases (76). Unfortunately, the majority of patients were diagnosed when at advanced stages with very poor 5-year survival, causing 14,615 deaths annually (70,76). Gastric cancer in Vietnam is also the highest among Southeast Asian countries . Moreover, approximately 80% of gastric cancer patients in Vietnam were infected with *H. pylori* infection (70), suggesting that *H. pylori* infection may play a vital role in the development of gastric cancer in Vietnam. Given that, the building of programs to prevent and reduce *H. pylori* infection and gastric cancer has to be prioritized in health policies, particularly in young generation.

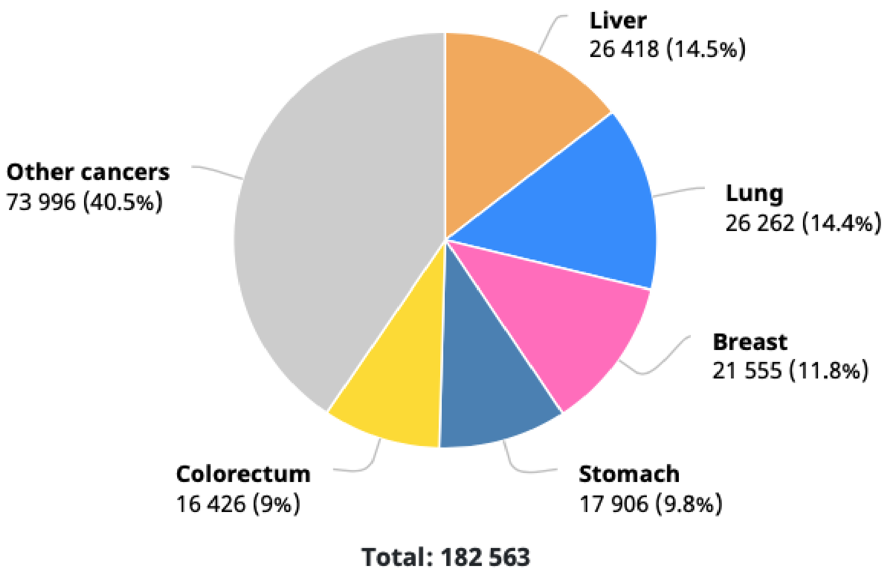


Figure 2.5 Number of new cancer cases in 2020 in Vietnam
Source: GLOBOCAN 2020 (75)

The data on the overall prevalence of *H. pylori* (60%) and the incidence of gastric cancer (17 per 100.000) are reported at the population level (figure 2.4) (70). In children, data on *H. pylori* infection remains scary. Some community-based studies conducted in Vietnam reported a wide range of prevalence rates from 36.7% to 76.8% (72,73,75,77). These studies also reported poor hygiene practices, and crowded living conditions as risk factors for getting *H. pylori* infection. However, most of these studies were conducted in rural or mountainous areas, had a small sample size, and did not address all children ages. Moreover, all previous studies used sera diagnostic methods known to have low accuracy and could not differentiate a life time infection from an active infection. As a result, they may not provide an accurate reflection of the recent situation of *H. pylori* infection in Vietnamese children.

2.2 Ho Chi Minh city

Ho Chi Minh city (HCMC) is the largest city in Vietnam, located in the Southeastern region of Vietnam, covering an area of approximately 2,061 km^2. It shares its borders with several other provinces, including Tay Ninh and Binh Duong provinces to the North, Dong Nai and Ba Ria – Vung Tau provinces to the East, Long An province to the West and the Indochine to the South, with a 15 km coast (78,79).

Figure 2.6 Maps of Ho Chi Minh city and surrounded provinces
Source: https://vpexpress.vn/ban-do-tp-hcm/

2.2.1 Organization and Administrative Structure

Ho Chi Minh City operates under a municipal-level administration, led by the Ho Chi Minh City People's Committee and People's Council. These city-level authorities are responsible for guiding the overall development, policy-making, and governance of the entire city (78). The city is organized into various administrative units, each responsible for governing specific geographic areas and providing essential public services:

- **Districts:** The city is composed of 24 districts, each of which functions as a local administrative unit, including 5 rural districts (Cu Chi, Hoc Mon, Binh Chanh, Nha Be, and Can Gio) and 19 urban districts (Quan-1, Quan-2, Quan-3, Quan-4, Quan-5, Quan-6, Quan-7, Quan-8, Quan-9, Quan-10, Quan-11, Quan-12, Go Vap, Phu Nhuan, Tan Binh, Tan Phu, Binh Thanh, Binh Tan, and Thu Duc). Urban districts are primarily located within the city's central areas and are characterized by higher population densities and more developed infrastructure. Rural districts, on the other hand, often have lower population densities and more agricultural features. Each district is led by a People's Committee and People's Council, responsible for managing local governance and decision-making within its jurisdiction.
- **Wards:** Within each district, there are several wards, which represent the smallest administrative units in the city. Wards are responsible for providing basic public services and addressing local issues within their boundaries. They are managed by Ward People's Committees and People's Councils.

- **Communes:** In certain rural and urban areas, there are communes, which are similar to wards but typically cover a larger geographic area and may have more rural characteristics.

2.2.2 Population Growth

After the national reunion in 1975, the population of Ho Chi Minh City reduced in the time period 1975-1980, mainly due to (1) the return of the "War refugees" to their villages, (2) the evacuation of a portion of the population towards to New Econonomic Zones, (3) the illegal migration abroad (80). However, after 1986 "Đổi Mới", an economic reform, characterized by the economic opening-up and the "socialist-oriented" market, the city's population started to rapidly increase. The population reached almost 5.5 million in 1995, and 10 years later, this number increased to over 7 million (80,81).

In 2019, the official population of HCMC was over 8.9 million people, of which migrants make up about a third . However, it is widely believed that the actual resident population is larger than the official count because of an unquantified "floating popualtion" of illegal inhabitants, and workers. Indeed, a study estimating the actual population of HCMC by counting motorcycles, the main transport in HCMC with 90% of households owning motorcycles, showed that the actual population was three times higher than the official counted (81). Therefore, the actual population of HCMC could be up to 20 million people, making it one of a megacity in the world.

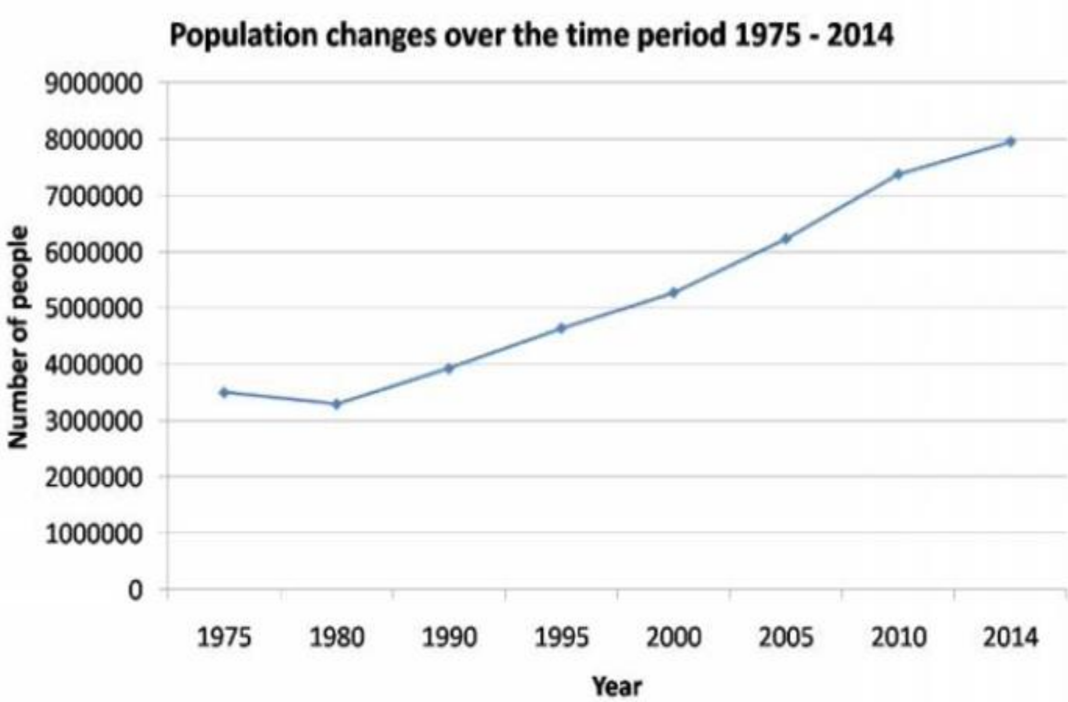


Figure 2.7 The growth of popualtion in Ho Chi Minh city
Source: Statistics Office of Ho Chi Minh city

2.2.3 The economic leadership

The policy of "industrialization and modernization", part of the renovation policy implemented in 1986, played a crucial role in the development of Ho Chi Minh City. Together with the neighboring provinces like Dong Nai, Binh Duong and Ba Ria – Vung Tau, the city forms the Southern Focus Economic Zones of Vietnam. This region, which has been the most dynamic economic development area, has received priority and political support, both local and national, to become the leading economic zone of Vietnam.

Figure 2.8 A view of District 1 from Saigon River
District 1 is the financial and commercial center of the city, where most commerce and trading happen no matter the time of day or night.

The Gross Domestic Product (GDP) growth rate of Ho Chi Minh City demonstrated substantial progress over the years. For instance, in 2004, the city's GDP growth rate reached 9.5%, while the whole country's was 18.4% (82). By the end of 2007, with 2,530 Foreign Direct Investment (FDI) projects worth USD 16.6 billion, the city became the leading destination for foreign investment (83). In the same year, the city's GDP was estimated at approximately USD 2,180 per person, accounting for 20% of the country's GDP. The contribution of Ho Chi Minh City to the annual national budget revenues increased by 30%, accounting for around 20.5% of the total revenues. As of the end of 2012, the city's GDP was estimated to be around USD 3,700 per person (84). By the end of 2014, the city's GDP contributed 9.5% to the country's GDP, with a GDP per capita of USD 5,100. Despite the city accounted for only 9% of the population and 0.6% of the surface area of Vietnam, it contributed to more than 20% and 15% of its GDP and foreign direct investment, respectively, what shows how the city's economy important to Vietnam.

2.2.4 Urbanization and industrialization

Since the implementation of the "Đổi Mới" economic reform, HCMC has undergone rapid urbanization and industrialization. Following the reform in 1986, many people migrated from rural to urban area in search of better job opportunities and higher salaries. From the 1990s to the early 2000s, the development of industries and services further accelerated migration to the city (85). This influx of manpower includes not only rural-to-urban migration but also urban-to-periurban migration.

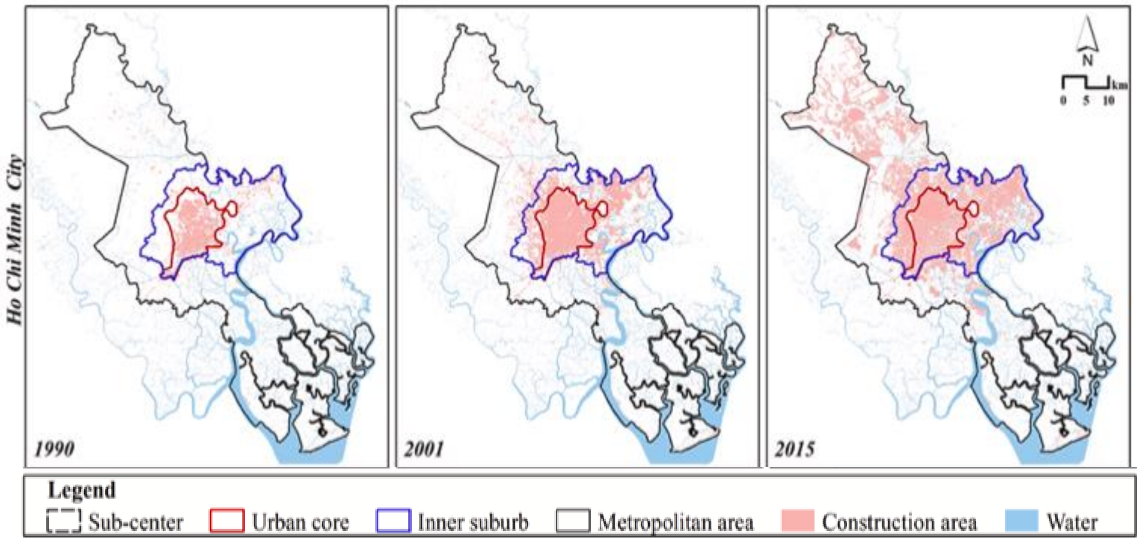


Figure 2.9 Urban development of Ho Chi Minh city
Source: Wenze Yue, Sustainable Cities and Society.
https://www.sciencedirect.com/science/article/pii/S221067072030826X

Figure 2.10 The transformation of Nhieu Loc – Thi Nghe canal
After the implementation of the “Đổi Mới” economic reform in 1986, thousands of temporary huts had gone up along both sides of the canal. Over the years, it become heavily polluted with domestic trash and wastewater from various production facilities. Then in 2002, the city launched a revonation project to upgrade the entire canal with a support of World Bank. By 2015, the renovation project had been completed, transforming it from a dark and smally cannal fully of waste to a clean and fesh canal. The project also helped improve the living conditions of 1.2 million people across Quan-1, Quan-3, Phu Nhuan, Tan Binh, Binh Thanh, and Go Vap district.

The rate of urbanization has averaged 2.15% per year since 2000, leading to an annual increase of approximately 170,000 people, nearly the size of a small disctrict in the city (86). As a result, the urban population of HCMC rose from 4 million in 2010 to nearly 9 million in 2021.

Each district have different urbanization levels, they may be divided into 3 groups:

- The group of rapidly urbanized districts: Quan-1, Quan-3, Quan-4, Quan-5, Quan-6, Quan-10, Quan-11, Phu Nhuan, Quan-8, Binh Thanh, Go Vap, Tan Binh, and Tan Phu district.
- The group of averagely urbanized districts: Quan-7, Quan-12, Binh Tan, and Thu Duc district.
- The group of slowly urbanized districts: Quan-2, Quan-9, Cu Chi, Binh Chanh, Hocmon, Nha Be, and Can Gio district.

Ho Chi Minh city is home to 84 industrial estates, export processing zones (EPZs), and special economic zones (SEZs) with the total area of over 31,000 ha (78,87). The industrialization of HCMC has caused a big substantial change of the employment structure. The number of worker in the agricultural sector has been descreased rapidly, as they have been switching to work in the industrial and service sector. A part of agricultural land has been switched to industrial and residential areas.

However, the rapid urbanization and industrialization have also brought various challenges and probles for HCMC such as rising environmental pollution, inadequate urban infrastructure growth, traffic congestion, bad housing conditions, and lack of schools or hospitals

Figure 2.11 Trafic congestion, flooding, and waste in Ho Chi Minh city

Figure 2.12 Housing conditions in Ho Chi Minh city
(A) A popular house ("tube house" or "rocket house" in HCMC: the houses average 3 meters wide, 10 to 30 meters depth, and 1 to 4 floors. A popular rent room for workers: **(B)** an 10-15 m^2 area for a kitchen, a bedroom, and even a restroom, **(C)** wooden loft used as a bedroom.

Several new urban development areas in Ho Chi Minh City have been formed, serving as an implicit solution to these challenges. One of the most prominent examples is Phu My Hung (PMH), located in District 7, which began its development in the early 1990s. PMH is renowned for its modern and well-thought-out urban planning, making it a highly sought-after destination in the city. The development of PMH includes various residential zones offering a diverse range of housing options. These residential areas encompass high-rise apartments, townhouses, villas, and gated communities, catering to the different preferences and needs of residents. This mix of housing types ensures that PMH provides a balanced and comfortable living environment for its diverse population (88). The establishment of such new urban development areas like Phu My Hung demonstrates the city's commitment to addressing its urbanization and industrialization challenges. These planned and organized urban areas contribute to the improvement of living standards, urban infrastructure, and the overall quality of life for residents. Additionally, these developments often attract local and foreign investments, boosting economic growth and creating new opportunities for businesses and job seekers.

Figure 2.13 **Development of Phu My Hung, a new urban area development in Ho Chi Minh city**

2.2.5 Water Supply

The Saigon Water Corporation (SAWACO), a company owned by the Vietnam Government, is responsible for producing, treating, and distributing clean water to the city's residents and businesses. The city's water supply is sourced from both surface water and groundwater sources. Surface water is obtained from rivers such as Sai Gon River and Dong Nai River, while groundwater is extracted from wells. After extraction, the water undergoes treatment processes to ensure it meets the necessary quality standards for consumption (89).

The city has an extensive water distribution network, comprising approximately 5460 km of pipes, which dilivers treated water to 100% households, businesses, and public facilities throughout the city (90).

The water price in HCMC is fixed by the People Committee with the aim of being affordable for the majority of households. Thus, low prices are preconized with a fixed quota of 4 m^3/month/person at a price of 5300 VND/m^3 (0.4 USD). Despite the government's support, some households still use well water instead of treated water from SAWACO.

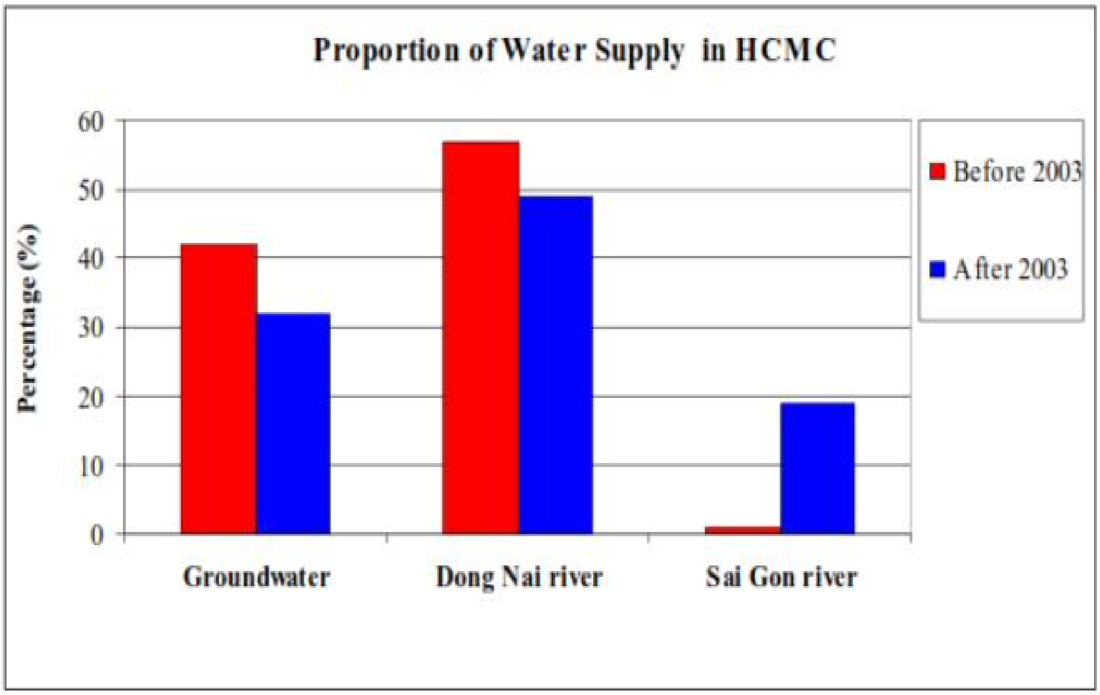


Figure 2.14 Sources of water supply in Ho Chi Minh city
Source: Tran Ngoc et al. Ho Chi Minh City growing with water- related challenges (2015).

Ho Chi Minh City also faces challenges related to water supply, including water scarcity during dry seasons, increasing demand due to urbanization, and potential pollution from industrial activities and agricultural runoff. Addressing these challenges requires careful water resource management and conservation efforts.

2.2.6 Education system

The education system of Ho Chi Minh is structured similarly to the National education system, consisting of four levels: primary (ages 6 – 11 years), lower secondary (ages 12 – 15 years), higher secondary (16 – 18 years), and tertiary education (> 18 years). There are two co-existing education systems in HCMC: public schools represent 96.7% (n = 761) and private schools account for 3.3% (n = 26) (79,81,91).

In HCMC, children are assigned to schools situated in close proximity to their residences, specifically within a ward where their houshold is officially registered. This school assignment approach amins to ensure that children can conveniently access schools without having to travel long distances. The typical school day for primary and secondary school pupils lasts from 7:00 am to 11:00 am, and from 1:00 pm to 5:00 pm, with a one-hour break for lunch and a one-hour break for taking a nap. This add up to a total of 8 hours per day, 5 days a week. Additionally, almost all pupils continue attending private turoring after school to receive supplementary instruction in the subjects they study in the mainstream education (62).

In Vietnam, children are not required to start school until they are 6 years old. However, in major cities such as Ho Chi Minh city, the demand for early years education has led to a large number of private nurseries, kindergartens, and preschools opening across 24 districts of the city. Children usually begin attending these preschools at the age of three, regardless of whether they live in rural or urban area (62).

2.2.7 Healthcare system

In HCMC, there are a total of 394 healtcare facilities providing medical examinations and treatment services. These healthcare facilities are categorized into 92 general hospitals, 42 specialised hospitals, and 260 clinics, based on the type of services they offer (65,92). Hospitals normally provide secondary and tertiary medical services, while clinics focus on primary care, basic treatments and first aid.

Similar to the National healthcare system, the healthcare facilities are also organized into four hierarchical levels: the national, the provincial, the district, and the commune. Eight healthcare facilities are managed and operated at the national level, 41 at the provincial level, 24 at district level, and 321 at the commune level. Approximately, 68% of these healthcare facilities are located in urban area (65).

Since the lower health service levels (district and commune) are not allowed and sometimes not able to perform complex medical care or better treat common health conditions, patients tend to bypass CHSs or district health units to seek services at higher service levels (provincial or central), creating a significant patient overload at the provincial or central levels and under-utilization of services at lower levels. Children face even greater challenges due to the limited number of children’s hospitals. There are only three children's hospitals in HCMC. Two older children hospital, located in two central districts (Quan-1 and Quan-10), and one new children hospital constructed in 2019, located in the rural area (Binh Chanh district). The oldest children's hospital, Children's hopital 1, with a capacity of 1400 beds, is constantly overwhelmed, serving approximately 2000 in-patients at a time. Similar overload situations also happen in the two remaining children's hospitals. The primary reason for this overload is the lack of peadiatric departments and peadiatricians in the district and commune levels. Moreover, there are several policy documents with specific regulations which have allowed cross-district linkage of health insurance payments at the district level since 2016, and future linkage at the provincial level and the national level from 2021 (92). These policy initiatives enable more freedom for patients to choose their own services at the district or provincial or national levels, rather than restricted referral to the district or provincial service within the patient's residential areas. Under this new linkage policy, most patients choose high quality hospitals, especially in children patients. Indeed, the neighbouring provices of HCMC ofen have only one or even no children's hospital in their areas. Consequently, numberous children patients from these provinces seek medical examinations and treatment at the children's hospitals, leading to exacerbate the existing overloaded situtaion in children's hopitals of HCMC.

2.2.8 *Helicobacter pylori* infection in Ho Chi Minh city

The burden of *H. pylori* infection in Ho Chi Minh city

Despite improvements in sanitation, socioeconomic status, and living conditions over the past decades, the prevalence of *H. pylori* infection remains high in Ho Chi Minh city. Recent hospital-based studies reported an infection rate ranging from 57.1% to 65.6% (93,94). These studies also pointed association between *H. pylori* infection and peptic ulcer diseases, active gastritis, atrophy, and intestinal metaplasia. However, data on *H. pylori* infection in children, compared to adults, remain limited. There is no community-based study on *H. pylori* infection in healthy children in HCMC, although it is an overcrowded city (a population density of 12,000 people/km^2) with 1.5 million children.

As the largest city in Vietnam, Ho Chi Minh city faces both developing and developed public health issuses due to rapid economic development and a significant immigration rate in recent decades. Therefore, there is a need of setting up prevention strategies to reduce health burden, particularly in the younger generation. The success of these strategies in HCMC will not only benefit to the city itself, but it will also be a reference for all other places in the country. Unfortunately, *H. pylori* infection has not been a target, and there is no regional data available. Therefore, an accurate estimate of the prevalence of peadiatric *H. pylori* infection is needed to prevent late consequences of *H. pylori* infection in HCMC.

<u>*H. pylori* antibiotics resistance rates in children</u>

In Vietnam, the pediatric treatment recommendation for *H. pylori* is the same as that in the Joint ESPGHAN and NASPGHAN 2016 guidelines (49). The options for antibiotics used to eradicate *H. pylori* in children are limited, including clarithromycin (CLA), levofloxacin (LEV), metronidazole (MET), amoxicillin (AMO), and tetracycline (TET). However, TET is only indicated for children older than 8 years, and LEV is only allowed in adolescents due to potential adverse effects on tooth and cartilage development.

The effectiveness of eradication treatment was influenced by multiple factors, but the primary reason for treatment failure was the development of antibiotic resistance in *H. pylori*. Over the past two decades, there has been an increasing trend in the resistance of *H. pylori* to commonly prescribed antibiotics used in eradication therapy. This rise in resistance can be attributed to various factors, including the over-prescription of antibiotics for other infectious diseases and the widespread use of antibiotics among the population in Vietnam. A recent hospital-based study conducted in Ho Chi Minh city showed concerning levels of resistance to the commonly used antibiotics for *H. pylori* treatment in Vietnamese children. Nearly 80% of children with *H. pylori* infection were resistant to at least one of the commonly used antibiotics, and approximately 70% was resistant to two or more the antibiotics (multidrug resistance).

In particular, the proportion of Clarithromycin (CLR) resistance of *H. pylori* among Vietnamese children was extremely high, ranging from 80.6% to 96.7% (95). In HCMC, the CLA resistance rate was 72.4%, which was 2.5-3.5

times higher than reported rates in American (19%), Europe (24%), and Southeast Asia (29%) (96). This high resistance may be attributed to childhood exposure to CLA through respiratory infection treatment, self-medication, over-the-counter purchase, and inadequate adherence to *H. pylori* treatment regimens utilising CLA as an antibiotic in the first-line therapy. LEV resistance is also a growing concern, with a resistance rate of 67.5% in Vietnamese adults (97). Among children, the resistance rate varies, ranging from 45.1% in the Mekong Delta to 60.2% in HCMC . The high rate of *H. pylori* resistance to LEV in pediatric patients in Vietnam may be attributed to the usage of quinolones in pediatric diarrhea and the inheritance of LEV-resistant strains of *H. pylori* from their parents. The resistence rate of MET in HCMC is alo alarming, with the rate of 38.2%. The AMO resistance of *H. pylori* is one of the most significant concern for clinicians as most eradication regimens include AMO. The resistance rate to AMO detected in children of HCMC was 25.2%, lower than rates reported in Hanoi (88.7%) and Mekong Dealta (71.7%) (95,97). These varying rates of AMO resistance might be due to differences in antimicrobial testing methods, antibiotic consumption, and exposure levels. The considerable levels of resistance pose a big challenge for healthcare system in Vietnam in developing and implementing effective strategies to reduce and treat *H. pylori* in children.

CHAPTER 3: STUDY RATIONALE AND OBJECTIVES

3.1 Rationale of the study

H. pylori infection is widespread, affecting approximately 4.4 billion individuals globally (5). Even in Switzerland, the country with the lowest infection rate (18.9%), over 1.6 million individuals are infected (5), indicating that *H. pylori* infection remains a significant public health issue worldwide. The distribution of *H. pylori* infection varies significantly across regions, countries, and even within the same country (5,9,85). Although its prevalence has been falling in most developed countries, it remains high in developing countries, particularly in South – Eastern Asian regions, where some countries have reported incidence rates as high as 90% (5,9,70). Recent studies pointed out that this difference in trend is likely due to improved sanitation, socioeconomic status, and living conditions in developed countries compared to develping countries (3,5,9). However the precise reasons for this variation are not fully discovered yet, suggesting the need for studies to investigate reasons behind this disparity.

Extensive evidence supports the central role of *H. pylori* in the development of upper gastrointestinal diseases, such as gastritis, gastric ulcer, and duodenal ulcer (2,3). Futhermore, it is recognized as bacterial carcinogen that contributes to the development of gastric cancer later in life . Numerous studies have also reported a strong link between *H. pylori* infection and development of gastric cancer (3,39,40). Additionally, *H. pylori* eradication treatment has been proved to decrease the risk of gastric cancer (40,76). In Vietnam, like other developing countries in South – Eastern Asia, *H. pylori* cancer burden remains high in Vietnam, where it is the third leading cause of cancer deaths, accounting for annual deaths of approximately 15,000 (70,76). The reason for the lingering high gastric cancer incidence in Vietnam is manifold, but a high prevalence of *H. pylori* infection, reportedly as high as 60% (70), appears to be the major contributor. Therefore, reducing *H. pylori* infection rate in Vietnam is crucial for decreasing the burden of gastric cancers in future.

H. pylori is mainly acquired during childhood, and most of infected children do not experience any gastroduodenal symptoms until adulthood (3,7,98). Additionally, several evidences indicated that *H. pylori* can be transmitted from person to person through possible routes, including oral-oral, and fecal-oral (3,22,99). As a result, the bacteria can silently spread from asymptomatic infected individuals to others within family or in communities for a prolonged period, leading to a high burden of *H. pylori* infection in community and posing a significant challenge to the public health system. Therefore, it is crucial to gain a better understanding of transmission modes, possible risk factors or epidemiology of *H. pylori* infection in asymptomatic children to develop effective programs for prevention and reduction of *H. pylori* infection.

Data on the epidemiology of *H.pylori* infection in children in Vietnam are scarce. A study conducted in Ha Noi city, a largest city in the North of Vietnam, reported a sero-prevalence of *H. pylori* infection of 76.8% (74). It was also reported that poor socioeconomic status, improper hygiene practices, and overcrowding living conditions were risk factors for getting *H. pylori* infection. There was no available community-based study on *H. pylori* infection in healthy children in Ho Chi Minh city (HCMC), despite it is the biggest and the most crowded city in the South of Vietnam with a population density of 12,000 p/km^2 (78,81). Its population density is five times higher than Hanoi (2,455 p/km^2), three times higher than Shanghai city (3,800 p/km^2), eighty times higher than the average of Asia (150 p/km^2). All previous epidemiology studies on *H. pylori* infection in children conducted in Vietnam had small sample sizes and used serological tests which are known to have low accuracy in children (73,74,100). In addition, there are big socio-economic and geographic disparities between HCMC and other investigated regions in VietNam. Given that identifying *H. pylori* infection and its risk factors in a large sample of school-aged children population of HCMC by using reliable test are needed to develop preventive strategies in specific settings of HCMC.

Therefore, our study aimed to estimate the spatial distribution of *H. pylori* infection and associated factors in healthy school-aged children in HCMC. The findings of this study will help better comprehend the epidemiology of *H. pylori* infection, and provide a rational basis for intervention strategies.

3.2 Objectives of the study

3.2.1 General objective

The overall objective of this thesis is to enhance the control of *H. pylori* infection by gaining a better understanding of the epidemiology of *H. pylori* infection in school-aged children, identifying specific actions, and strategies for building effective prevention programs.

3.2.2 Specific objectives

Specifically, the research aims were:

- To estimate the feco-prevalence of *H.pylori* infection in school-aged children (6 – 15 years-old) attending primary and secondary public schools in HCMC, Vietnam.
- To identify the geographical distribution pattern of *H.pylori* infection in the region of HCMC.
- To determine the infection-associated factors (socio-demographic factors, behavioral factors, lifestyle factors, environmental factors) in children in HCMC.

CHAPTER 4: METHODS

4.1 Study settings

The "Projet de recherche pour le développement (PRD) – Building strategies for prevention and management of *Helicobacter pylori* infection in children" is a research project including a set of studies in Ho Chi Minh city, Vietnam in a partnetship with the Université catholique de Louvain, and the Université libre de Bruxelles. The PRD project comprises two parallel studies: a community-based study in schools and a hospital-based study in pediatric hospitals in HCMC. Our study, a part of the PRD project, constitutes the community-based study carried out in schools of HCMC.

HCMC is the largest city with a total population of 8.993.082, of whom 1.5 million are school-aged children below 16 years of age. The city has two co-existing education systems: public schools represent 96.7% (n = 761) and private schools account for 3.3 % (n = 26) (61,79,91). Among the 761 public schools, there were 491 primary schools with grade 1^{st} – 5^{th} (6 – 11 years) and 270 secondary schools with grade 6^{th} – 9^{th} (12 – 15 years), representing a total of 1,077,105 pupils. Our study was conducted in public schools of HCMC.

4.2 Population

The target population was school-aged children between 6 and 15 years, of both genders attending public schools across the 24 districts of HCMC.

4.3 Sample size

Because the aim of this study was to estimate the magnitute of *H. pylori* infection among school-aged children in HCMC, the sample size has been calculated based on an expected precision (d) in estimating a proportion (prevalence) of a binary outcome in population with 95% confidence level with a prior prevalence:

$$\frac{\boldsymbol{Z^2_{1-\alpha/2} * p * (1-p)}}{\boldsymbol{d^2}}$$

With. d = 0.075

$Z_{(1-\alpha/2)}$ = 1.96 (for a confidence level of 95%)

p = 0.768 (a priori prevalence of *H. pylori* infection as observed in Hanoi)

A study of Hoang et al. conducted in Hanoi reported a prevalence of *H. pylori* infection of 76.8% (74). Using this prior prevalence, at least 206 children per grade (1^{st} – 9^{th}) were needed to estimate the prevalence with a precision of 7.5%, assuming a loss rate of 20%, and adjusting for a design effect of 1.4 (100). With 9 grades (1^{st} – 9^{th}), the size of 1854 pupils or 9 pupils per class were needed in targeting 216 classes (24 districts*9 grades).

4.4 Sampling

In order to obtain a representative sample of the school-aged children population in HCMC, our plan was to sample all of the 24 districts of HCMC. Children would be taken in the same class in each school grade in every school for ease. To achieve this, a multiple stage sampling method was used as follows:

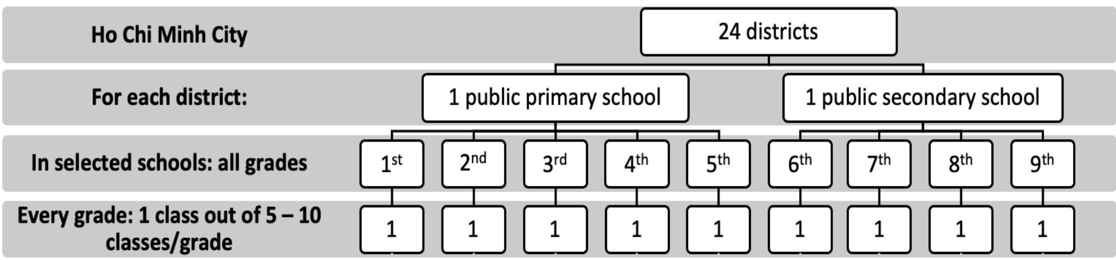
Step 1: All primary and secondary public schools in each of the 24 districts in HCMC were listed.

Step 2: In each of 24 districts, one primary school and the nearest secondary school was randomly selected, corresponding to 48 schools in total.

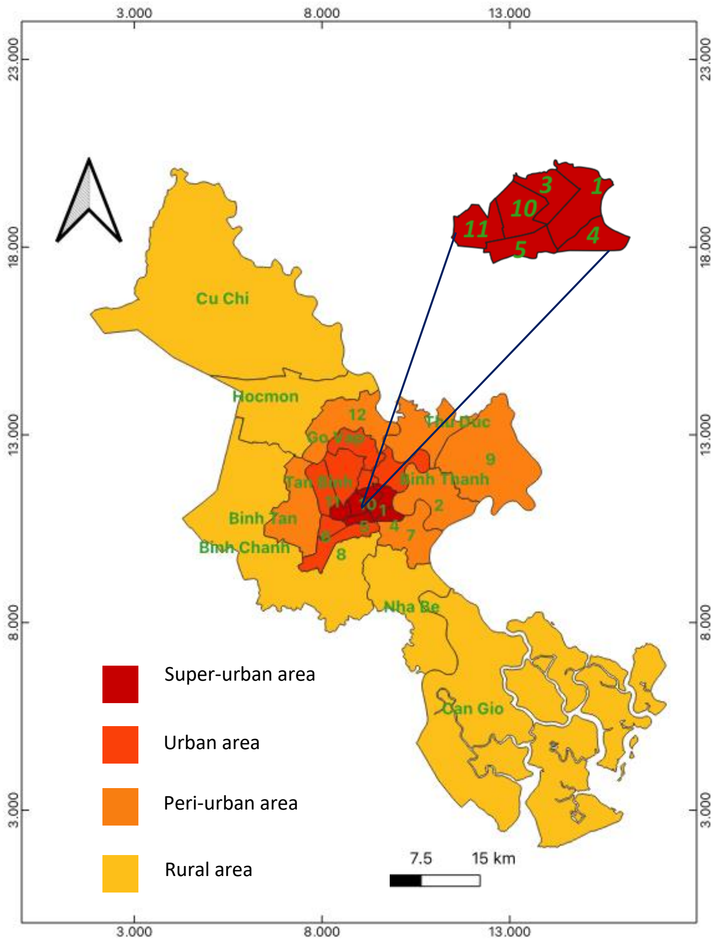
Step 3: A list of all classes in all grades within each these 48 selected schools were created. There would be five grades in a primary school (1^{st} – 5^{th}) and four grades in a secondary school (6^{th} – 9^{th}).

Step 4: A class was randomly selected in each grade, using uniform random sampling.

Step 5: Children in the same class were randomly chosen within the sequential list of inscriptions and were invited to to participate into the study.



The number of school-aged children from each of the 24 districts who participated in the study was also illustrated in figure 4.1. The ratio of the number of children selected in the district (n) and the total number of children in the district (N) was nearly the same for all of the 24 districts. That means we used a multiple-stage and proportional-to-population size sampling to enroll pupils in our study. Eventually, the total number of pupils actually selected and enrolled in this study was 1476.



District name	N	n
District 1	59,883	81
District 2	49,624	67
District 3	38,678	52
District 4	20,675	21
District 5	56,618	76
District 6	52,038	68
District 7	24,732	34
District 8	32,276	45
District 9	82,192	114
District 10	31,376	43
District 11	33,107	45
District 12	30,693	47
Binh Thanh	57,859	79
Go Vap	40,364	53
Phu Nhuan	56,385	78
Tan Binh	60,108	81
Tan Phu	67,842	93
Binh Tan	49,964	69
Thu Duc	24,192	33
Binh Chanh	41,837	57
Cu Chi	56,014	78
Can Gio	18,139	27
Hocmon	59,275	84
Nha Be	33,234	51

Figure 4.1 Mapping of the 24 districts in Ho Chi Minh city and number of 6 – 15 years children selected in each district

N: total number of pupils in the district; n: number of children participating in our study

4.5 Eligibility criteria

Eligibility criteria included health school-aged children of both gender, attending primary (6 – 11 years of age) and secondary (12 – 15 years of age) public schools in HCMC.

Excluded criteria included no documented previous *H. pylori* infection, no past history of gastroscopy or gastrectomy. Those who used antibiotics or bismuth-containing compound within last 28 days or used any proton pump inhibitors during past 14 days were also excluded from the study.

4.6 Data collection

4.6.1 Diagnostic test

The monoclonal enzyme-immunoassay (EIA) stool antigen test, Premier Platium HpSA Plus test (manufactured by Meridian Bioscience, USA) was performed according to the munufacturer's instructions and guidelines to determine *H. pylori* infection status (101).

Parents or children collected a stool sample of 50 grams at home using a provided container with no preservative. They were also instructed on the proper method for collecting the specimen and required to write the time of collection on the container. The samples were then brought to school the next morning and transported to the Micobiology department of the University of Medicine Pham Ngoc Thach for testing. The specimens were stored in 2 – 8^0C for no longer than 2 hours until being tested. The samples were frozen at -80^0C after testing.

The results were interpreted visually and using a spectrophotometer with dual-wavelength (450/630 mm). Optical density (OD, 450/630 mm) values were used to categorize the status of *H. pylori* infection; OD values < 0.100 were classified as negative and OD values ≥ 0.100 were classified as positive, as recommended by manufacturer (101).

4.6.2 Measurements

A standard questionnaire was designed to obtain information regarding to socio-demographic characteristics (age, ethnicity, parent occupation, socioeconomic position), behavioral factors (personal hygiene practice), lifestyle factors (eating habits, sleeping habits, overall health), and environmental factors of the children. The questionnaire was filled by pupils and their parents under the instructions of well-trained interviewers and researchers in the classroom.

4.7 Data entry and analysis

4.7.1 Data entry

Two field data collectors independently entered data into Microsoft Excel sheets for cross-checking and validation purposes. Any discrepancies were resolved by an additional trained staff based on paperback documents. All data were checked for missing values, outliers, and cleaned prior to data analysis.

4.7.2 Data analysis

All analyses were performed using Stata 17.0/IC software for Mac (TX: StataCorp LP).

Characteristics of pupils

The characteristics of pupils were compared using a Person Chi^2 test for categorical variables and a Student's t-test for continuous variables. Trends across ordered categories were tested using the Cochran-Armitage chi-square test.

Geographical distribution of *H. pylori* infection in HCMC

A four-points index for the geographical division of HCMC was built based on population density (PD) and employees density (ED). It was used to demonstrate spatial distribution in prevalence of *H. pylori* infection and to assess a potential link between *H. pylori* infection and the crowded level of HCMC.

The city is administratively divided into 24 districts, comprising 5 rural districts and 19 urban districts. We kept **rural area as the official definition**, included Binh Chanh, Can Gio, Cu Chi, Hocmon, and Nha Be districts. These 5 districts have an estimated population of 1.988.161 inhabitants.

We divided urban districts into three sub-areas:

Peri-urban area: districts with PD below 20,000 p/km^2 and ED below 35,000 p/km^2 were classified as peri-urban areas; it covered Quan-2, Quan-7, Quan-

9, Quan-12, Binh Tan, and Thu Duc districts. These 6 districts have an estimated population of 2.934.441 inhabitants.

Urban area: districts with PD between 20,000 and 35,000 p/km^2 and ED below 35,000 p/km^2 were classified as urban area; it covered 7 districts: Quan-6, Quan-8, Binh Thanh, Go Vap, Phu Nhuan, Tan Binh, and Tan Phu. These 7 districts have an estimated population of 2.958.392 inhabitants.

Super-urban area: districts with a PD above 35,000 p/km^2 or an ED above 35,000 p/km^2 were classified as super-urban area; it corresponded to 6 districts: Quan-1, Quan-3, Quan-4, Quan-5, Quan-10, and Quan-11. These 6 districts have an estimated population of 1.112.088 inhabitants.

Prevalence maps created with QGIS 3.16 for Mac were used to illustrate the spatial distribution *H. pylori* infection across the four-points index of HCMC.

<u>Factors associated with *H. pylori* infection</u>

Associations between factors and feco-positivity for *H. pylori* were assessed using univariate logistic regression and reported as odds ratio with 95% confidence interval. Multivariable logistic regression analysis was performed to assess the independent contribution of each factor to *H.pylori* infection. Only factors associated with *H. pylori* infection with *P*-value < 0.05 in the univariate analysis were considered in the multivariate regression. The statistical significance level was set to 0.05.

HO CHI MINH CITY

Urban area
(Official name)

Rural area
(Official name)

District	PD
Hoc Mon	4,967
Binh Chanh	2,793
Nha Be	2,060
Cu Chi	1,063
Can Gio	102

Super-urban
(PD ≥35,000 p/km^2 **OR** ED ≥35,0000 p/km^2)

Urban
(PD 20,000 – 35,000 p/km^2 **AND** ED <35,0000 p/km^2)

Peri-Urban
(PD <20,000 p/km^2 **AND** ED <35,0000 p/km^2)

District	PD	ED
Quan-4	41,957	15,814
Quan-10	41,074	12,747
Quan-11	40,863	8,598
Quan-3	38,673	29,478
Quan-5	37,219	15,737
Quan-1	18,479	59,613

District	PD	ED
Go Vap	34,304	6,763
Phu Nhuan	33,714	18,741
Quan-6	32,720	6,832
Tan Phu	30,388	8,302
Binh Thanh	24,020	10,514
Quan-8	22,218	2,468
Tan Binh	21,167	9,562

District	PD	ED
Binh Tan	15,074	4,500
Thu Duc	12,399	3,500
Quan-12	11,759	2,871
Quan-7	10,091	5,352
Quan-2	3,621	1,963
Quan-9	3,483	907,0

Figure 4.2 The 4-points index for the geographical division of Ho Chi Minh city
PD: population density (people/km^2); ED: employees density (people/km^2).

4.8 Ethical considerations

Standards of ethics for studies conducted in Vietnam were respected. The study protocol was approved by both the Ethical Review Committee and the Scientific Committee of the University of Medicine Pham Ngoc Thach and the Ethical Review Committee of Université catholique de Louvain – Brussels campus in Belgium.

Written informed consent was obtained from both pupils and their parents. The purpose of the study, possible risks/benefits, and the rights were also explained to both pupils and their parents before obtaining the informed consent. All data were anonymized and used for research purpose only.

CHAPTER 5: RESULTS

5.1 Results 1

Prevalence of *Helicobacter pylori* infection and its geographical distribution among school-aged children in Ho Chi Minh city, Vietnam.

As published:

Che TH, Nguyen TC, Ngo DTT, Nguyen HT, Vo KT, Ngo XM, Truong DQ, Bontems P, Robert A, Nguyen PNV. High Prevalence of *Helicobacter pylori* Infection Among School-Aged Children in Ho Chi Minh City, VietNam. Int J Public Health. 2022 Nov 10;67:1605354.

Abstract

Objectives: There is no study on *Helicobacter pylori* (*H. pylori*) infection in pupils of Ho Chi Minh city (HCMC), the most overcrowded city in Vietnam. Therefore, the aim of this study was to estimate the prevalence of *H. pylori* and its geographical spread among school-aged children.

Methods: A school-based cross-sectional study was conducted among 1476 pupils across 24 districts of HCMC in 2019. Multiple-stage sampling method was used to enroll pupils. We built a four-points index for geographical division based on population density and employees density to evaluate the link between *H. pylori* and crowded level. Stool samples were analyzed by monoclonal enzyme-immunoassay stool antigen-test to assess the infection status. Logistic regression was performed to assess possible factors related to *H. pylori* infection.

Results: The overall prevalence of *H. pylori* was 87.7%. There was a linear increasing trend in the infection rate (P<0.001) across the 4-points index and this trend maintained within both age and gender subgroups (P=0.02).

Conclusion: Prevalence of *H. pylori* was high and it increased with population density or employees density. Therefore, it is crucial to plan and implement the reduction of *H. pylori* infection programs by targeting the highly concentrated population areas of HCMC.

Key words: *Helicobacter pylori*, school-aged children, pupils, Ho Chi Minh city, Vietnam.

Introduction

Helicobacter pylori (*H. pylori*) infection is a common chronic infection, affecting more than 50% of the world's population (5). The prevalence has changed over the last ten years, showing a decrease in developed countries but remaining high in most developing countries (5,6). Like other developing countries in Southeast Asia, the prevalence of *H. pylori* in Vietnam remains high. A recent study in Hanoi in the North of Vietnam showed that the prevalence of the infection was 76.8% (74). There was no such community-based prevalence study on *H. pylori* infection in Ho Chi Minh city (HCMC), despite it is one of the most overcrowded cities globally with a population of 8.993.082 in total and a population density of 12,000 p/km^2. Its population density is five times higher than Hanoi (2,455 p/km^2), three times higher than Shanghai city (3,800 p/km^2), eighty times higher than the average of Asia (150 p/km^2) (78,81). Furthermore, the link between *H. pylori* infection and crowded living conditions has been shown in several studies (102,103). Therefore, assessing the prevalence of *H. pylori* in HCMC is an essential public health issue in HCMC.

H. pylori infection is usually acquired during childhood and tends to persist if untreated (14,98,104). *H. pylori* infection is a risk for duodenal-gastric ulcers, gastric cancer, and its treatment has been proved to decrease cancer risk in individuals with a family history of gastric cancer (3,42). Therefore, reducing *H. pylori* infection rate in the young population is crucial for decreasing the burden of gastric cancers in future.

We therefore conducted a study to estimate the prevalence of *H. pylori* infection and its geographical spread in school-aged children across the 24 districts of HCMC, Vietnam.

Methods

Study design

The present school-based cross-sectional study was conducted across the 24 districts of HCMC. Two education systems co-exist in HCMC; public schools represent 96,7% (n=761) and private schools account for 3,3% (n=26) (78,91). Our study was conducted across the public schools system of HCMC, which consists of 491 primary schools with grade 1^{st} – 5^{th} (6 – 11 years) and 270 secondary schools with grade 6^{th} – 9^{th} (12 – 15 years), representing a total of 1,077,105 pupils .

In order to estimate the prevalence with a precision of 7.5%, a size of 206 pupils is needed for a prior prevalence of 76.8%, as that observed in Hanoi, assuming a loss rate of 20%, and using a cluster design effect of 1.4 (100). With 9 grades, this led to a size of 1854 pupils, or 9 pupils per class if there are 216 classes.

In each of 24 districts, we selected at random one primary school and the closest secondary school. In each of these 48 schools, one class per grade was randomly selected among the 10 to 14 classes within the grade, leading to a total number of 216 classes. In each class, 9 pupils were randomly chosen within the sequential list of inscriptions and were invited to participate into the study.

Eligibility criteria

Eligibility criteria included healthy school-aged children of both sexes, attending primary (6-11 years of age) and secondary (12-15 years of age) public schools in HCMC. Excluded criteria were a history of gastrointestinal endoscopy or surgery, previous *H. pylori* infection, a treatment with antibiotics within the last four weeks, a treatment with a bismuth-containing compound or with a proton pump inhibitor (PPI) within the last two weeks. Pupils diagnosed with an acute or chronic gastrointestinal disorders were also excluded.

A four-points index for a geographical division of HCMC according the crowd

To assess a potential link between *H. pylori* infection and the crowded level of HCMC, we built a four-points index for the geographical division of HCMC based on population density (PD) and employees density (ED) as illustrated on Figure 1.

The city is administratively divided into 24 districts, comprising 5 rural districts and 19 urban districts. Rural area was kept as the official definition, included Binh Chanh, Can Gio, Cu Chi, Hocmon, and Nha Be districts. We divided urban districts into three sub-areas: peri-urban area, urban area, and super-urban area. Urban districts with PD below 20,000 p/km^2 and ED below 35,000 p/km^2 were classified as peri-urban areas; it covered Quan-2, Quan-7, Quan-9, Quan-12, Binh Tan, and Thu Duc districts. Districts with PD between 20,000 and 35,000 p/km^2 and ED below 35,000 p/km^2 were classified as urban area; it covered 7 districts: Quan-6, Quan-8, Binh Thanh, Go Vap, Phu Nhuan, Tan Binh, and Tan Phu. Districts with a PD above 35,000 p/km^2 or an ED above 35,000 p/km^2 were classified as super-urban area; it corresponded to 6 districts: Quan-1, Quan-3, Quan-4, Quan-5, Quan-10, and Quan-11.

Data collection

A standard questionnaire was used to collect relevant data on *H. pylori*-related factors. The questionnaire was filled by pupils and their parents under the instructions of well-trained interviewers and researchers in the classroom. Data was entered into Microsoft Excel sheets by two independent researchers for cross-checking validation. Any discrepancy was resolved by another trained staff based on paperback documents.

Assess *H. pylori* infection status

The positive status of *H. pylori* infection was confirmed by using a monoclonal enzyme-immunoassay (EIA) stool antigen test, Premier Platinum HpSA Plus test (manufactured by Meridian Bioscience, USA). The stool samples were collected and analyzed following the manufacturer's instructions and guidelines (101). Stool specimens were excluded if there was any water or urine in the sample. Results were classified as positive if the cut-off value of optical density (OD) was equal to or greater than 0.100, as recommended by manufacturer.

HO CHI MINH CITY

Urban area
(Official name)

Rural area
(Official name)

District	PD
Hoc Mon	4,967
Binh Chanh	2,793
Nha Be	2,060
Cu Chi	1,063
Can Gio	102

Super-urban
(PD ≥35,000 p/km² **OR** ED ≥35,0000 p/km²)

Urban
(PD 20,000 – 35,000 p/km² **AND** ED <35,0000 p/km²)

Peri-Urban
(PD <20,000 p/km² **AND** ED <35,0000 p/km²)

District	PD	ED
Quan-4	41,957	15,814
Quan-10	41,074	12,747
Quan-11	40,863	8,598
Quan-3	38,673	29,478
Quan-5	37,219	15,737
Quan-1	18,479	59,613

District	PD	ED
Go Vap	34,304	6,763
Phu Nhuan	33,714	18,741
Quan-6	32,720	6,832
Tan Phu	30,388	8,302
Binh Thanh	24,020	10,514
Quan-8	22,218	2,468
Tan Binh	21,167	9,562

District	PD	ED
Binh Tan	15,074	4,500
Thu Duc	12,399	3,500
Quan-12	11,759	2,871
Quan-7	10,091	5,352
Quan-2	3,621	1,963
Quan-9	3,483	907,0

Figure 1 The 4-points index for the geographical division of Ho Chi Minh city
PD: population density (people/km²); ED: employees density (people/km²).

Statistical analysis

Maps of *H. pylori* infection prevalence were drawn using QGIS 3.16 for Mac. Statistical analyses were performed using Stata 17.0/IC software for Mac (TX: StataCorp LP). We report number with percentage for categorical variables and mean ± standard deviation for continuous variables. The demographic characteristics of pupils were compared using a Person Chi^2 test for categorical variables and a Student's t-test for continuous variables. Trends across ordered categories were tested using the Cochran-Armitage chi-square test. Interactions between the crowd index and age or sex were tested using likelihood ratio chi-square test and using Akaike's information criterion. Logistic regression analysis was performed to assess the independent contribution of each factor to *H. pylori* infection. The significance level for all tests was set to 0.05.

Ethical considerations

Written informed consent was obtained from both parents (legal guardians) and pupils, who were also informed that participating in the survey was voluntary. All collected data were stored anonymously and used for research purposes only. The study protocol was approved by the Ethical Review Committee and the Scientific Committee of the University of Medicine Pham Ngoc Thach, and the Ethical Review Committee of Université catholique de Louvain – Brussels campus in Belgium.

Results

A total of 1854 pupils were invited to participate but 20.3% refused. This refusal rate was similar across districts. Sixteen pupils were excluded (4 children who didn't perform the stool test, 5 children were using antibiotics in the last four weeks and 7 children had a missing age or gender). The remaining 1460 pupils were included in the present analysis. Of these, 730 (50%) were boys, and the mean age was 10.1 ± 2.7 years with a range of 6-15 years (Table 1).

Table 1. Characteristics of children in the study

Variables	Total n = 1460
Age (years)	10.1 ± 2.7
6-8	483 (33.1)
9-11	473 (32.4)
≥ 12	504 (34.5)
Gender	
Boy	730 (50)
Girls	730 (50)
Living area	
Super-urban area	317 (21.7)
Urban area	495 (33.9)
Peri-urban area	356 (24.4)
Rural area	292 (20.0)

Values expressed means ± SD or numbers (percentage).

The overall prevalence of *H. pylori* infection was 87.7% (1280/1460, 95% CI: 85.8% - 89.3%). The prevalence was higher in boys (90.0%) compared to girls (85.3%), with a *P*-value of 0.003. Children above the age of 11 (84.1%) were less likely to be infected than yourger age gourps (88.4% in children aged 6 - 8 years and 90.7% in children aged 9 – 11 years) , with *a P*-value of 0.007. The prevalence was significantly higher in boys (90.0%, χ2 test with *P*=0.003), and in children aged 9-11 years (90.7%, χ2 test with *P*=0.007).

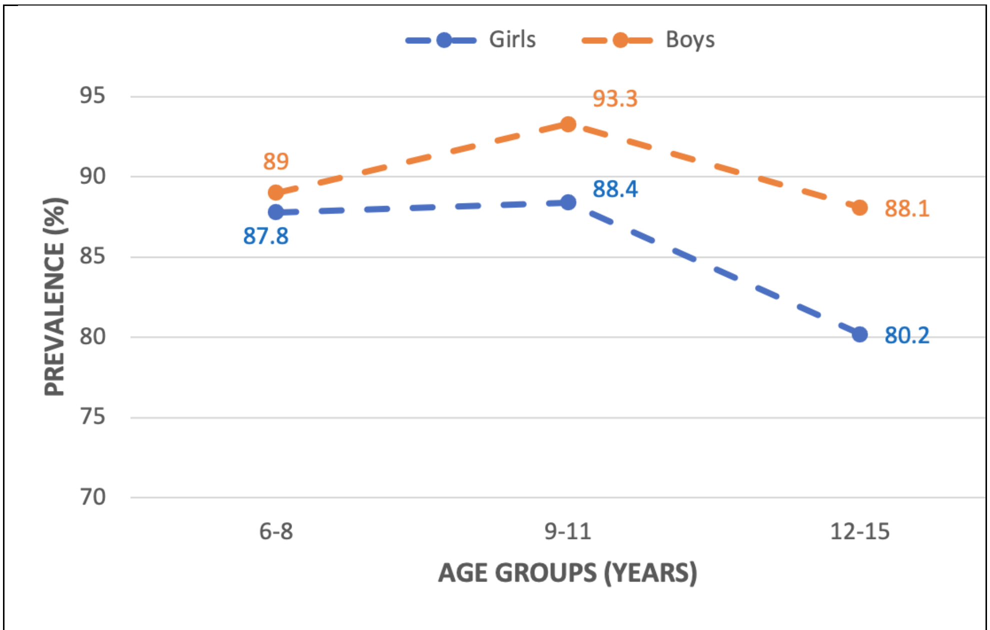


Figure 2. Prevalence of *H.pylori* according to age groups and gender

The prevalence of *H. pylori* infection according to the age groups and gender is reported in Figure 2. In both sexes, the prevalence increased with age, peaked in the 9-11 age group, and then decreased when the child was 12 years or more.

Figure 3 illustrates a linear increasing trend in the prevalence of *H. pylori* infection across the four-points index of crowd in HCMC (80.5% in the rural area, 88.5% in the peri-urban area, 89.3% in the urban area, 90.9% in the super-urban area, Cochran χ2 test with p<0.001).

The increase in *H. pylori* infection with the four points index was the same across age groups (interaction *P*=0.34) and across genders (interaction *P*=0.92). Prevalence of *H. pylori* infection increased as PD or PE increased within both age and gender subgroups (Figure 4).

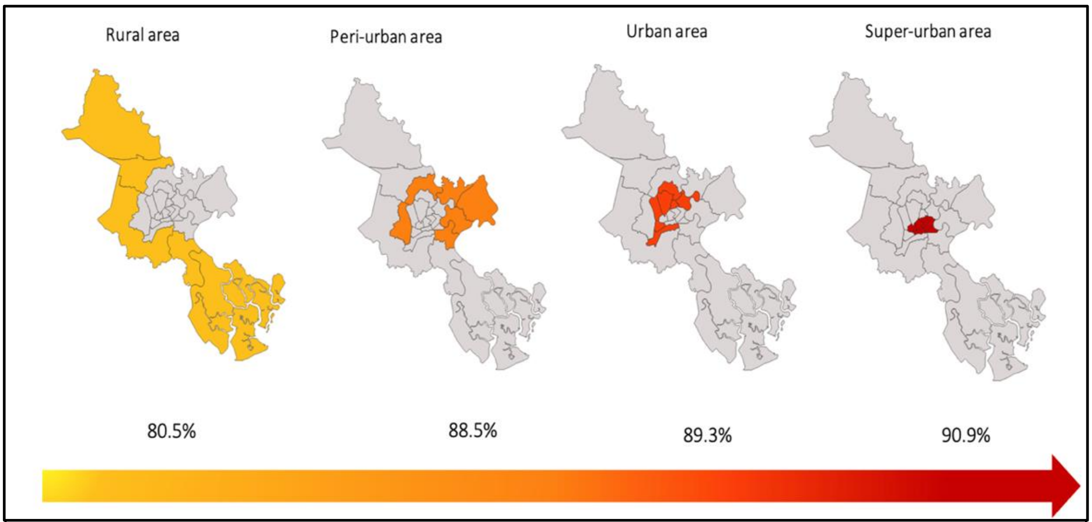


Figure 3. Mapping of the prevalence of *H.pylori* infection in Ho Chi Minh city according to population density (PD) and employees density (ED)

Table 2 shows the results from the logistic regression performed to assess demographic factors associated with *H. pylori* infection. Multiple logistic regression analysis showed that age, gender, and living area were significantly related to *H. pylori* infection. The prevalence of *H. pylori* was significantly increased in children aged 9-11 years [OR = 1.91, 95% Cl: 1.29 –

2.85, p = 0.003] and in boys [OR = 1.5, 95% Cl: 1.08 – 2.06, p = 0.015]. A multivariate analysis also clearly showed that the prevalence of *H. pylori* infection significantly increased with the level of crowded: PD <20,000 p/km^2 and ED <35,000 p/km^2 [OR = 1.85; 95% Cl: 1.19 – 2.88, $p < 0.001$]; PD 20,000 – 35,000 p/km^2 and ED <35,000 p/km^2 [OR = 2.05; 95% Cl: 1.10 – 2.09, $p < 0.001$]; and PD ≥35,000 p/km^2 or ED ≥35,000 p/km^2 [OR = 2.33; 95% Cl: 1.44 – 3.10, $p < 0.001$].

Table 2. Univariable and multivariable analyses for possible factors associated with *H.pylori* infection

			Univariate analysis		Multiple analysis	
	n	% positive	OR (95%Cl)	*p*	OR (95%Cl)	*p*
Age group (years)				0.007		0.003
6-8	483	88.4	1.43 (0.99 – 2.07)		1.61 (1.10 – 2.34)	
9-11	473	90.7	1.83 (1.24 – 2.72)		1.91 (1.29 – 2.85)	
≥ 12	504	84.1	1.00		1.00	
Gender				0.003		0.015
Female	730	85.3	1.00		1.00	
Male	730	90.0	1.55 (1.13 – 2.12)		1.50 (1.08 – 2.06)	
Living area				<0.001		<0.001
Super-urban area	288	90.8	2.40 (1.49 – 3.89)		2.33 (1.44 – 3.10)	
Urban area	283	87.6	2.00 (1.35 – 3.04)		2.05 (1.10 – 2.09)	
Peri-urban area	227	86,3	1.86 (1.21 – 2.89)		1.85 (1.19 – 2.88)	
Rural area	235	80.5	1.00		1.00	

Rural area was defined as official definition. Urban area was divided into three areas: peri-urban area (PD <20,000 p/km^2 and ED <35,000 p/km^2), urban area (PD 20,000 – 35,000 p/km^2 and ED <35,000 p/km^2), and super-urban (PD ≥35,000 p/km^2 or ED ≥35,000 p/km^2).

Discussion

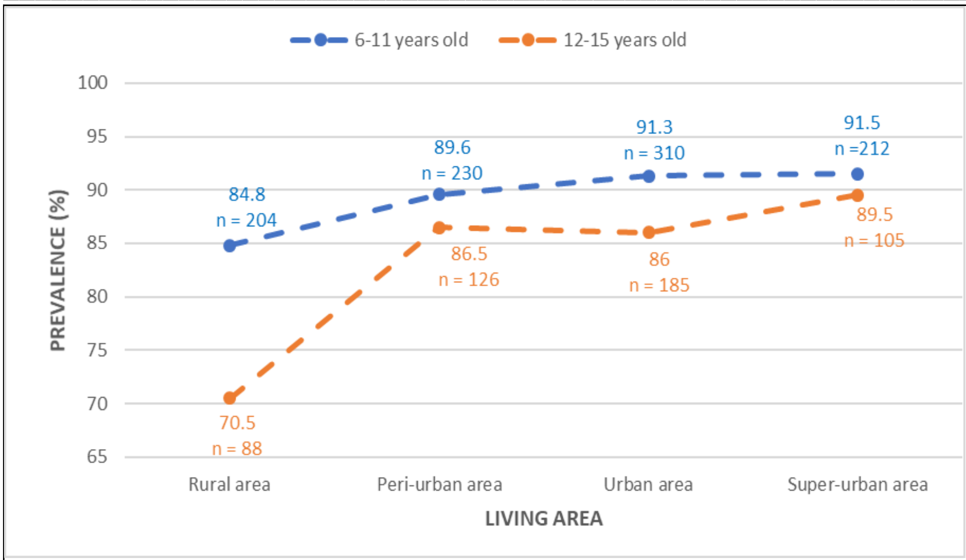
Our study is the first research that reports the current prevalence of *H. pylori* infection among school-aged children in HCMC. The overall prevalence of *H. pylori* in the representative sample of pupils in HCMC was 87,7%. Several studies on the prevalence of *H. pylori* infection in Vietnamese children ranged from 32,1% in the Mekong region to 55.5% in Nghe An (72,73). The prevalence observed in our study was higher compared with those reported in the previous studies, reflecting the increasing trend of *H. pylori* infection over the past decades. However, most of those previous studies were conducted in the countryside or mountainous areas, had a small sample size, and did not address all school-aged children. Moreover, all previous reports used sera diagnostic methods known to have low accuracy and could not differentiate a lifetime infection from an active infection. While in our study, all pupils at all grades in primary and secondary schools in 24 districts of HCMC were recruited. In addition, the Premier Platinum HpSA Plus stool test (Meridian Bioscience, USA) used in our study has proved a reliable tool to detect *H. pylori* infection with an accuracy of 93.4% (105). This accuracy was was calculated based on sensitivity, specificity, and prevalence, following the formula:

$$\text{Accuracy} = (\text{Sensitivity})*(\text{Prevalence}) + (\text{Specificity})*(1 - \text{Prevalence})$$

It corresponds to the proportion of correct classifications, a classical definition of accuracy. Nguyen TVH et al. conducted a validation study of the Premier Platinum HpSA PLUS test (Meridian bioscience, USA) in 232

Vietnamese children and reported a sensitivity of 97% and specificity of 95% (106). Therefore, our results reflect an accurate infection prevalence of school-aged children in HCMC.

Several studies in Japan and Taiwan reported that *H. pylori* infection in children decreased in each age group (107–109). In contrast, two studies from China showed that the infection increased with age (110,111). In our study, the prevalence of *H. pylori* infection increased with age up to 11 years, and then began to decline at 12 years of age in both boys and girls (figure 2). These evidences suggest that the incidence rate of *H. pylori* across age groups varies greatly in different countries and may depend on different socio-economic statuses, living conditions, and lifestyle factors. Furthermore, the prevalence of *H. pylori* in HCMC was already high in the youngest ones, indicating that the acquisition of *H. pylori* occurs in very early childhood. Therefore, the building of programs to prevent and reduce the incidence of *H. pylori* has to be prioritized in public health policies.



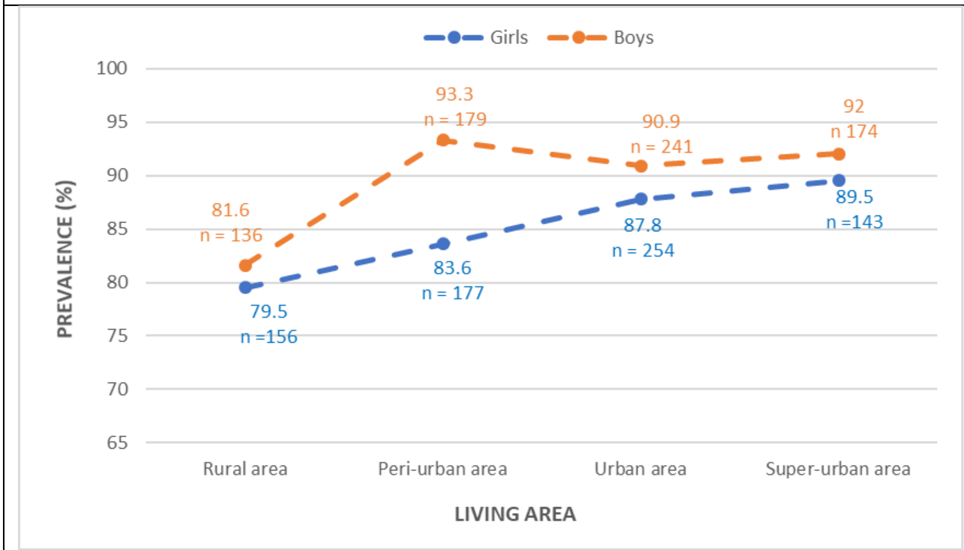


Figure 4. Trend in prevalence of *H.pylori* infection across the crowdy index in HCMC within age and gender subgroups

Rural area was defined as official definition. Urban area was divided into three areas: peri-urban area (PD <20,000 p/km^2 and ED <35,000 p/km^2), urban area (PD 20,000 – 35,000 p/km^2 and ED <35,000 p/km^2), and super-urban (PD ≥35,000 p/km^2 or ED ≥35,000 p/km^2). n: size of age and gender subgroups in each living area.

Although HCMC takes up just 10% of the Southeast (SE) region's land area (2,061.2/23,564.24 km^2), it concentrates up to 50% population (8,993.082/17,828.907 inhabitants) of the SE region with PD of 4,292 p/km^2 (112,113). However, it is widely believed that the population has been seriously underestimated. Indeed, one study was conducted to estimate the actual population of HCMC by counting motorcycles, which is the main transport in HCMC and 90% of household-owned motorcycles, showed that the actual population was higher than three times the officially counted (81). Therefore, the actual PD of HCMC could be up to 12,000 p/km^2, which means HCMC is an overcrowded city. Furthermore, several studies showed that the *H. pylori* infection is related to the crowded level. In order to control for all these qualities, we decided to build a 4-points index for the geographical division of HCMC mainly based on population density to have an insight on the level of crowds across the 24 districts of the city. And this index could aid us in explaining the *H. pylori* infection distribution in HCMC. During the process, we realized that some of the city center districts had relatively low PD compared to others, despite they are the busiest districts, where most offices and buildings are located, and almost all employees come there to work every day. Therefore, using both PD and PE to define the crowdy index woud be more reliable to find the possible link between *H. pylori* infection and the crowd level of HCMC.

By using the 4-points index, our study showed that there was a linear increasing trend in the prevalence of the infection across four areas in HCMC (figure 3), and its trend was also found in both age or genders subgroup (figure 4). A study performed in the North of Vietnam also found that the higher crowded living area was a positive risk factor for *H. pylori* infection (75). Additionally, reports from China , Japan , Jordan , and Nepal also observed that the infection rate significantly increased in people living in crowded areas (108,109,114,115). However, these studies stratified the living area only according to population density whereas our study developed the 4-point index based on both population density and employees density as previously described and explained. That crowdy index can create a more comprehensive understanding of the relation between *H. pylori* infection and the level of populated concentration in HCMC. Therefore, designing the programs to prevent and reduce the *H. pylori* infection in HCMC should focus on the highly concentrated population areas.

The differences in the target population, the diagnostic tests, and the cut-off values of the test cause some difficulties when comparing our results to the findings in other countries. Using the same stool antigen test, a cross-sectional study in Portugal published in 2011 reported that the prevalence of *H. pylori* infection in children (0-15 years old) was 32% (116). The survey conducted in China, among all children aged (0-15 years) published in 2020, reported the prevalence was 32.6% (110). Another cross-section study conducted in Thailand in 2009 reported that the prevalence in children (5-7 years old) was 44.8% (117).

At the early stage of the study, the required sample size was 1854 pupils after applying the appropriate formula, as demonstrated in the study design part. In fact, we had only 1476 pupils were enrolled in the study, which was smaller than the initial number. However, we were assumed that the loss rate of 20% at the beginning of the study development. That means 1483 children were required to enroll in our study. Therefore, our sample size was large enough to reach a precision of 7.5%.

The study has several strengths included this was an all school-ages community-based study that was the first to be conducted in HCMC. The sample size was large with 1854 pupils could represent the sample of pupils of HCMC. Moreover, the stool test used to detect the infection status is highly accurate and also have validated for Vietnamese children with high sensitivity and high specificity (106).

Conclusion

Our study reports an important public health issue of *H. pylori* infection in Ho Chi Minh city. The current feco-prevalence of *H. pylori* among school-aged children remains high and it significantly increased with population density or employees density. We therefore suggest that it is crucial to plan, implement the reduction and prevention of *H. pylori* infection programs by targeting the highly concentrated population areas in HCMC. Further analyses should be focused on the impact of behavioral factors, lifestyle factors and environmental factors of children and their families that might affect the prevalence of *H. pylori* infection.

Acknowledgements

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5.2 Results 2

Factors associated with *Helicobacter pylori* infection among school-aged children in Ho Chi Minh city, Vietnam.

As published:

Che TH, Nguyen TC, Vu VNT, Nguyen HT, Hoang DTP, Ngo XM, Truong DQ, Bontems P, Robert A, Nguyen PNV. Factor Associated with *Helicobacter pylori* Infection Among School-Aged Children From a High Prevalence Area in VietNam. Int J Public Health. 2023 May 11;68:1605908.

Abstract

Objectives: The study aimed to identify prevalence of *H. pylori* infection and associated risk factors among pupils of Ho Chi Minh city (HCMC).

Methods: A total of 1476 pupils aged 6 – 15 years were enrolled in this cross-sectional study using a multiple-stage and proportional-to-population size sampling. Infection status was assessed using stool antigen-test. A questionnaire was used to obtain socio-demographic, behavioral, and environmental factors. Logistic regression was performed to assess possible factors related to the infection.

Results: Of the 1409 children included in the analysis, 49.2% were male and 95.8% were of Kinh ethnicity. About 43.5% of parents completed college or university. The overall prevalence of *H. pylori* was 87.6%. Infrequency of handwashing with soap after toilet, the use of only water to clean after toilet, crowded living areas, larger family size, and younger age were independently contributing to an increased prevalence of *H. pylori*.

Conclusion: *H. pylori* infection is highly prevalent in HCMC, and is associated with poor hygienic practices, crowded living areas, larger family size, and younger age. These findings highlight the importance of fecal-oral route and the attribution of crowded living conditions to the spreading of *H. pylori*. Therefore, preventive programs should be set up with a focus on education of hygiene practices, and oriented to those living in crowded conditions.

Key words: *Helicobacter pylori*, school-aged children, risk factors, Vietnam.

Introduction

Helicobacter pylori (*H. pylori*) was first discovered in 1983 by Warren and Marshall (118). It is well-known as a pathogenic bacteria that causes gastric-related diseases such as gastritis and peptic ulcers (2,3). *H. pylori* is also recognized as bacterial carcinogen that contributes to the development of gastric cancer later in life (39). Numerous studies have also reported a strong relationship between *H. pylori* infection and development of gastric cancer (3,40,42). Furthermore, its role in extra-gastroduodenal disorders including iron deficiency anemia, and idiopathic thrombocytopenic purpura is also well documented in recent reports (3,4). These *H. pylori*-related gastric diseases and other-related disorders together cause over a million deaths each year, making *H. pylori* infection one of the most severe issues to public health worldwide.

H. pylori infection is mainly acquired during childhood and colonizes human gastric mucosa lifelong if not treated (3,98). School-going children are at high risk of infection and many infected pupils do not show any gastroduodenal symptoms up to adulthood (3,7,99). According to a recent systematic review, the worldwide prevalence of *H. pylori* among children was found to be 32.3% (9). It also reported that the incidence rate of *H. pylori* was significantly higher in low-income and middle-income countries than in high-income countries (43.2% vs 21.7%) and in older children than in younger children (41.6% in 13 – 18 years old vs 33.9% in 7 – 12 years old vs 26.0% in 0 – 6 years old). Furthermore, several evidences indicated that *H. pylori* can be transmitted from person to person through possible routes, including oral-oral, and fecal-oral (3,99). Therefore, the bacteria can silently spread from asymptomatic infected individuals to others within family or in communities for an extended period of time, which might lead to high burden of *H. pylori* infection in community with a great impact on the public health system. However, the transmission patterns of *H. pylori* in children, compared to adults, remain scarce. Given that providing further insights on transmission modes and possible risk factors of *H. pylori* infection in asymptomatic children are essential for public health sector to build optimal prevention and reduction *H. pylori* programs.

H. pylori affects over 50% of the global population and its distribution dramatically varies both between and within countries (5). The prevalence of *H. pylori* infection greatly decreased in developed countries, but it is still prevalent in developing countries – particularly in Asia with some countries reporting a prevalence of up to 90% (5). Some recent studies pointed out that this different trend probably reflects the level of urbanization, sanitation, access to clean water, and varied socioeconomic status, but the exact reasons for this variation are not fully discovered yet (3,5). Like other developing countries in South-Eastern Asia, *H. pylori* infection in Vietnam remains high (5,10). A study conducted in Ha Noi city, the largest city in the North of VietNam, showed a sero-prevalence of *H. pylori* infection of 76.8% (75). It was also reported that poor socioeconomic status, improper hygiene practices, and overcrowding living conditions were risk factors for getting *H. pylori* infection. A recent paper reporting on prevalence of *H. pylori* in school-aged children of Ho Chi Minh city (HCMC) showed the prevalence in the range of 80.2% in girls aged 12 – 15 years to 93.3% in boys aged 9-11 years (119). There was no available community-based study focused on the mode of transmission and potential risk factors for acquiring *H. pylori* infection in HCMC, despite it is the biggest city in the South of VietNam. All previous studies on risk factors of *H. pylori* conducted in VietNam had small sample sizes and used serological tests which are known to have low accuracy in children (72–74). In addition, there are big socio-economic and geographic disparities between HCMC and other investigated regions in VietNam. Therefore, identifying the route of transmission and associated risk factors for *H. pylori* infection in a large sample of school-aged children population of HCMC by using reliable tests are needed to develop preventive strategies in specific setting of HCMC.

We therefore conducted a community-based study of a large sample of school-aged children who were assessed for *H. pylori* infection by stool antigen test, to investigate possible risk factors and the route of transmission that may be associated with *H. pylori* infection in school-aged children in HCMC.

Methods

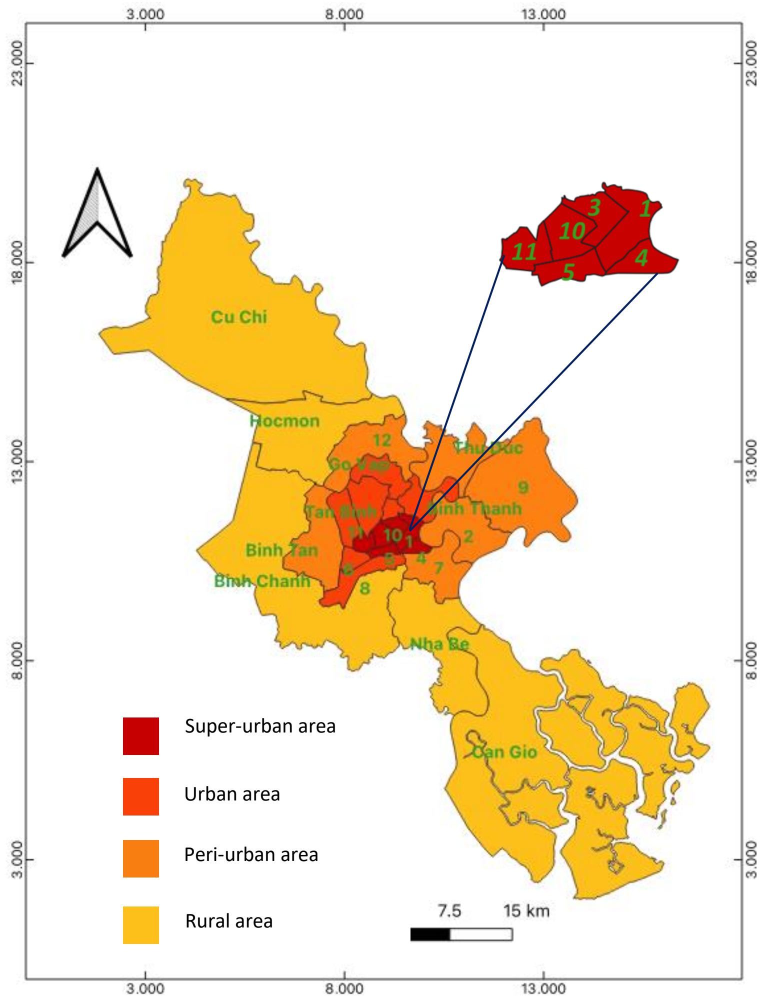
Study design

We carried out a school-based cross-sectional study among public school pupils aged 6 – 15 years across 24 districts of HCMC in 2019. Situated in the Southeast region of VietNam, HCMC is the largest city with a population of 8.993.082 in total, of whom 1.5 million are pupils below 16 years of age

(75,88). Like other parts of VietNam, there are two types of education systems in HCMC: a public system involving most of schools (n=761, 96.7%) and a private system with few schools (n=26, 3.3%) (91). Out of the 761 public schools, there were 491 primary schools with grade 1st – 5th (6 – 11 years) and 270 secondary schools with grade 6th – 9th (12 – 15 years), representing a total of 1,077,105 pupils. Our study was carried out in public schools.

The sample size and the selection procedure have been reported previously (119). Briefly, using the reported prevalence of 76.8% in HaNoi, at least 206 children per grade were required to reach a precision of 7.5% on 95% confidence interval for prevalence in population, assuming a loss rate of 20%, and adjusting for a design effect of 1.4. With 9 grades (1st – 9th), the size of 1854 pupils or 9 pupils per class were needed to enroll in the study because there were 216 classes.

Pupils from public schools across 24 districts of HCMC were enrolled in the study by using a multiple-stage sampling method. In each of the 24 districts, one primary school and the closest secondary school were randomly selected. In each of these 48 schools, one class per grade was randomly chosen, and 9 children in the same class were randomly recruited into the study. The number of school-aged children who participated in the study from each of 24 districts in HCMC was also illustrated in figure 1.



District name	N	n
District 1	59,883	81
District 2	49,624	67
District 3	38,678	52
District 4	20,675	21
District 5	56,618	76
District 6	52,038	68
District 7	24,732	34
District 8	32,276	45
District 9	82,192	114
District 10	31,376	43
District 11	33,107	45
District 12	30,693	47
Binh Thanh	57,859	79
Go Vap	40,364	53
Phu Nhuan	56,385	78
Tan Binh	60,108	81
Tan Phu	67,842	93
Binh Tan	49,964	69
Thu Duc	24,192	33
Binh Chanh	41,837	57
Cu Chi	56,014	78
Can Gio	18,139	27
Hocmon	59,275	84
Nha Be	33,234	51

Figure 1 Mapping of the 24 districts in Ho Chi Minh city and number of 6 – 15 years children selected in each district

N: total number of pupils in the district; n: number of children participating in our study

Eligibility criteria

Asymptomatic healthy school-aged children of both sexes from 6 to 15 years old who studied at primary and secondary public schools in HCMC were invited to participate in the study. The criteria for eligibility included no documented previous *H. pylori* infection, no past history of gastroscopy or gastrectomy. Those who used antibiotics or bismuth-containing compound within last 28 days or used any proton pump inhibitors during past 14 days were also excluded from the study.

Data collection

A standard questionnaire was designed to obtain information regarding to socio-demographic characteristics, behavioral factors, lifestyle factors, and environmental factors of the children. A well-trained interviewer used the questionnaire to survey both pupils and their parents in the classroom at the end of the normal class. Two individual field data collectors independently entered the data obtained from questionnaire into Microsoft Excel. Data entry was validated by two other individual field data collectors and with paper version by an additional trained staff in case of disparities.

Assess *H. pylori* infection status

A monoclonal enzyme-immunoassay (EIA) stool antigen test, Premier Platinum HpSA Plus test (manufactured by Meridian Bioscience, USA) was performed according to the manufacturer's instructions and guidelines to determine *H. pylori* infection status (101). Optical density (OD) values were used to categorize the status of *H. pylori* infection; OD values < 0.100 were classified as negative and OD values ≥ 0.100 were classified as positive, as recommended by manufacturer . Stool specimens with any water or urine were excluded from analysis.

Statistical analysis

Analyses were performed using Stata 17.0/IC software for Mac (TX: StataCorp LP). We report numbers with percentage for categorical variables and mean ± standard deviation for continuous variables. A household wealth index was defined as the first principal component analysis of 28 dummy variables coding for 28 household assets, as proposed by WHO for Demographic and Health Survey (DHS) in VietNam (120). The wealth index was further categorized according to quintiles and reported on an ordinal scale. Associations between factors and feco-positivity were assessed using univariate logistic regression and reported as odds ratio with 95% confidence interval. Multivariable logistic regression analysis was performed to assess the independent contribution of each factor to *H.pylori* infection. Only factors associated with *H. pylori infection* with *P*-value < 0.05 in the univariate analysis were considered in the multivariate regression. The statistical significance level was set to 0.05.

Ethical considerations

Written informed consent was obtained from both pupils and their parents. The purpose of the study, possible risks/benefits, and the rights were also explained to both pupils and their parents before obtaining the informed consent. The study protocol was approved by both the Ethical Review Committee and the Scientific Committee of the University of Medicine Pham Ngoc Thach and the Ethical Review Committee of Université catholique de

Louvain – Brussels campus in Belgium. All data were anonymized and used for research purpose only.

Results

A total of 1476 pupils were enrolled in this study. Out of these pupils, 9 were excluded due to failure to meet inclusion criteria or not performing the stool test. Observations with missing child age, child sex, number of people, number of children in household, parent's education, parent's occupation, types of toilet, and methods to treat water were also excluded from the analysis. Ultimately, the remaining 1409 pupils were included in the final analysis (Figure 2).

Characteristics of study participants

Out of 1409 participants, 693 (49.2%) were boys. The mean age of pupils was 10.1 ± 2.7 years with a range of 6-15 years. Almost participant's ethnicity was Kinh (95.8%), while only 3.3% were Chinese. Nearly half of the parents had high education levels (high school or college/university, 47%). Majority of parents worked in the factory (36.1%) or in the private business (29.2%). Most of families (68.1%) had 4 - 6 members and only one household (0.1%) had two members. The number of children in household was mainly two (61.9%) (Table 1). Among 175 negative children, 102 (58.3%) were boys. Almost these children belonged to Kinh ethnicity (96.6%). Approximately 60% of their parents had a high education level. Most of them always washed their hands with soap after toilet. These negative children were fairly evenly distributed among four areas (Table 1.1b, Annex, Page 177).

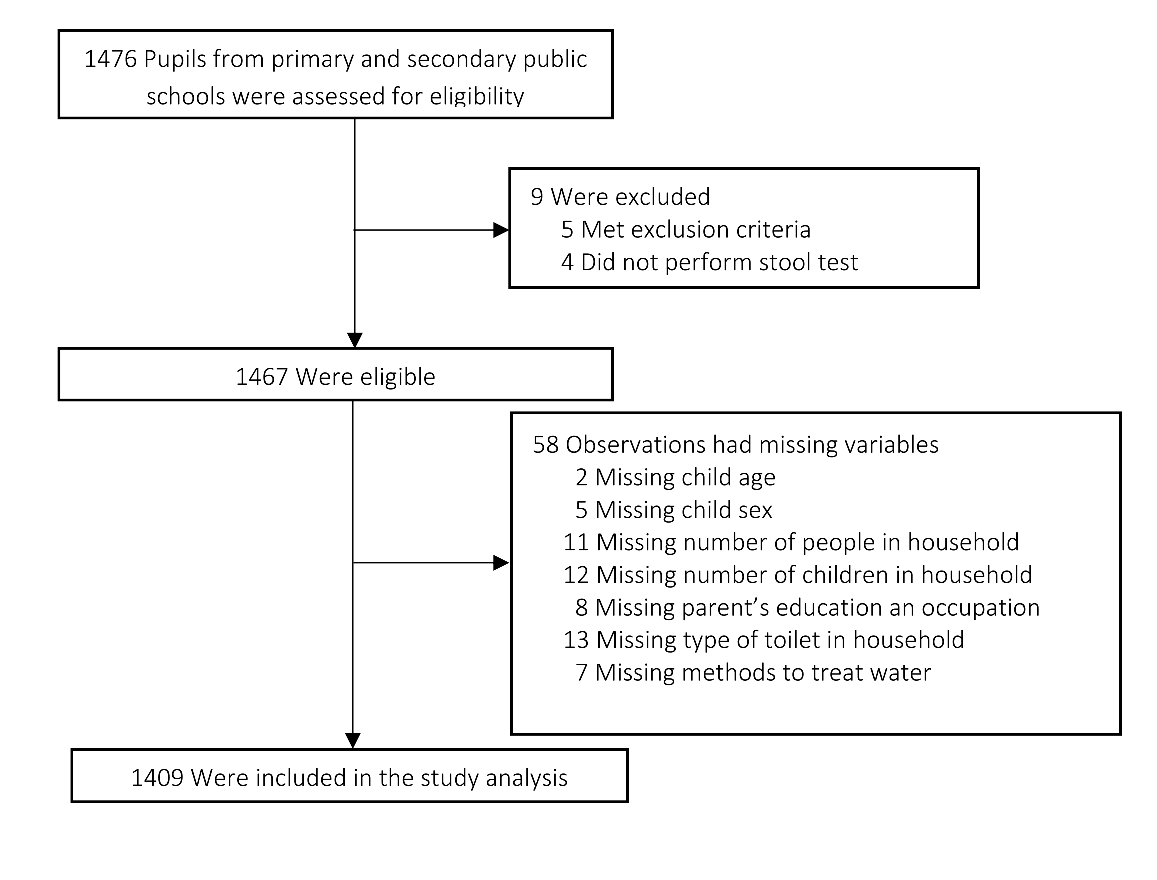


Figure 2. Flowchart of participants

Prevalence of *H. pylori* infection

The overall prevalence of *H. pylori* infection was 87.6% (1,234/1,409). The prevalence was significantly higher in boys (89.5%) compared to girls (85.8%), with an OR of 2.66 (95% Cl: 1.75 – 4.05). Youger age (89.4%) was more likely to be infected than older age (85.3%), with an OR of 1.45 (95% Cl: 1.06 – 1.99). A trend of increasing prevalence was observed across four areas of HCMC, with rates of 80.6% in rural area, 87.6% in peri-urban area, 89.0% in urban area, and 91.6% in super-urban area.

Table 1. General characteristics of children investigated for *H. pylori* infection

Characteristics	Total n (%)
Ethnic group	
Kinh	1350 (95.8)
Chinese	47 (3.3)
Others	12 (0.9)
Educational status of parents	
College/University	161 (11.4)
High school	501 (35.6)
Secondary school	128 (9.08)
Primary school	619 (43.9)
Parent's occupation	
Factory worker	509 (36.1)
Office worker	277 (19.7)
Private business	411 (29.2)
Others	212 (15.1)
Wealth status of household*	
Poorest	263 (18.7)
Poorer	288 (20.4)
Middle	284 (20.1)
Richer	287 (20.4)
Richest	287 (20.4)
Family size	
≤ 2	1 (0.1)
3	145 (10.3)
4 - 6	960 (68.1)
> 6	303 (21.5)
No. of children in household	
1	294 (20.8)
2	872 (61.9)
> 2	243 (17.3)

**Wealth index was defined as the first principal component analysis of 28 household assets, as proposed by WHO for DHS in Vietnam. It was categorized according to quintiles.*

Association between possible risk factors and *H. pylori* infection

Associations between demographic, socio-economic characteristics and *H. pylori* infection in univariate analysis are reported in Table 2. There was a strong inverse correlation between the educational level and *H. pylori* infection (P = 0.01), the prevalence of the infection was the highest (90.3%) for parents with lowest education (primary school). Having parents who

Table 2. Univariate association between *H. pylori* infection and demographic and socioeconomic factors

Factors	n/N	%	OR (95% CI)	*p*
Ethnic group				0.58
Kinh	1181/1350	87.5	1	
Others	53/59	89.8	1.26 (0.54 – 2.98)	
Educational status of parents				0.01
College/University	132/161	81.9	1	
High school	429/501	85.6	1.34 (0.85 – 2.13)	
Secondary school	114/128	89.1	1.73 (0.89 – 3.36)	
Primary school	559/619	90.3	2.05 (1.28 – 3.28)	
Parent's occupation				0.24
Private business	360/411	87.6	1	
Factory worker	450/509	88.4	1.08 (0.72 – 1.61)	
Office worker	247/277	89.2	1.17 (0.72 – 1.89)	
Others	177/212	83.5	0.72 (0.45 – 1.14)	
Wealth status of household*				0.63
Poorest	223/263	84.8	0.80 (0.49 – 1.29)	
Poorer	256/88	88.9	1.15 (0.69 – 1.91)	
Middle	250/284	88.1	1.05 (0.64 – 1.74)	
Richer	254/287	88.5	1.10 (0.67 – 1.82)	
Richest	251/287	87.5	1	

**Wealth index was defined as the first principal component analysis of 28 household assets, as proposed by WHO for DHS in Vietnam. It was categorized according to quintiles.*

completed primary school (OR = 2.05, 95% CI: 1.28 – 3.28) was associated with a higher feco-positive rate. The prevalence in children who had parents working as factory workers (88.4%) or office workers (89.2%) were higher than those who had parents working in private business (87.6%), and the prevalence in children who had parents with other occupations (83.5%) was lower. The prevalence of *H. pylori* infection in children didn't change according to the parent's occupation (P = 0.24). No associations were found with the ethnic group (P = 0.58) or with the wealth status of household (P = 0.63).

Table 3. Univariate association between *H. pylori* infection and water-related factors

Factors	n/N	%	OR (95% CI)	*p*
Mainwater source				0.07
Drilling well	1126/1278	88.1	1.58 (0.98 – 2.55)	
Non-Drilling well	108/131	82.4	1	
Cookwater source				0.06
Drilling well	1063/1204	88.3	1.49 (0.99 – 2.53)	
Non-Drilling well	171/205	83.4	1	
Drinkwater source				0.23
Drilling well	763/863	88.4	1.21 (0.88 – 1.67)	
Non-Drilling well	471/546	86.3	1	
Treatment water				0.49
Boiling water	191/212	90.1	1.43 (0.79 – 2.59)	
Filter	325/367	88.6	1.22 (0.74 – 2.01)	
No treatment	190/220	86.4	1	
Other	528/610	86.6	1.01 (0.65 – 1.59)	

Figure 3 presents relationship between *H. pylori* infection and crowding, lifestyle, and hygiene factors. All crowding-related factors investigated, including family size, number of children, and sharing a bed, were significantly associated with being *H. pylori* positive (all $P < 0.05$). Interestingly, there was an increasing trend in percent feco-positive in both family size subgroups and number of children in household subgroups. The prevalence increased with the number of family members and with the number of children. Pupils who shared a bath towel or a washcloth with other members of household (OR = 1.49, 95% Cl: 1.03 – 2.17) were also significantly more affected with *H. pylori* infection, but no association was found for pupils who used a toothbrush shared with other members in the family (OR = 1.58, 95% Cl: 0.48 – 5.19). Prevalence of *H. pylori* was higher in children who rarely or sometimes washed their hands (91.5%) compared to those who always washed their hands (86.3%) with soap after go to toilet; OR = 1.71 (95% Cl: 1.13 – 2.59). The rate of infection was also higher in individuals who used only water (89.8%) or who used both water and paper (83.9%) compared to those who used only paper (74.2%) to clean after toilet; OR = 1.81 (95% Cl: 0.78 – 4.19) and OR = 3.06 (95% Cl: 1.33 – 7.04), respectively. The remaining hygiene factor (handwashing with soap before having meals) did not show association with *H. pylori* infection ($P = 0.08$). No significant differences were found between children who used or did not use bare hands to take food ($P = 0.36$). There was also no significant association between sharing cups or dishes or sauces or foods with other members in their and *H. pylori* detection (all $P > 0.05$).

Factors	n/N	%	OR (95% CI)
Family size			
≤ 3	108/146	73.9	1
4 - 6	848/960	88.3	2.66 (1.75 – 4.05)
> 6	278/303	91.8	3.91 (2.25 – 6.7)
No. of children in household			
1	244/294	83.0	1
2	773/872	88.7	1.60 (1.11 – 2.31)
> 2	217/243	89.3	1.71 (1.03 – 2.84)
Share a bed with other members of household			
Yes	981/1104	88.9	1.64 (1.15 – 2.33)
No	253/305	82.9	1
Using bare hand to take food			
Frequently or everyday	459/524	87.6	1.00 (0.72 – 1.39)
Nerver of occasionally	775/885	87.5	1
Sharing cups/dishes/sauces/foods			
Yes	847/961	88.1	1.17 (0.84 – 1.63)
No	387/448	86.4	1
Sharing toothbrush			
Yes	33/36	91.7	1.58 (0.48 – 5.19)
No	1201/1373	87.5	1
Sharing bathtowel or washcloth			
Yes	379/419	90.5	1.49 (1.03 – 2.17)
No	855/990	86.4	1
Handwashing with soap before having meals			
Always	515/600	85.8	1
Rarely or sometimes	719/809	88.9	1.31 (0.96 – 1.81)
Type of toilet			
Flushing toilet	1105/1259	87.8	1
Latrine toilet or others	129/150	86.0	0.86 (0.52 – 1.39)
Handwashing with soap after toilet			
Always	911/1056	86.3	1
Rarely or sometimes	323/353	91.5	1.71 (1.13 – 2.59)
Cleaning way after go to toilet			
Water only	837/932	89.8	3.06 (1.33 – 7.04)
Paper only	23/31	74.2	1
Both Water and paper	374/446	83.9	1.81 (0.78 – 4.19)

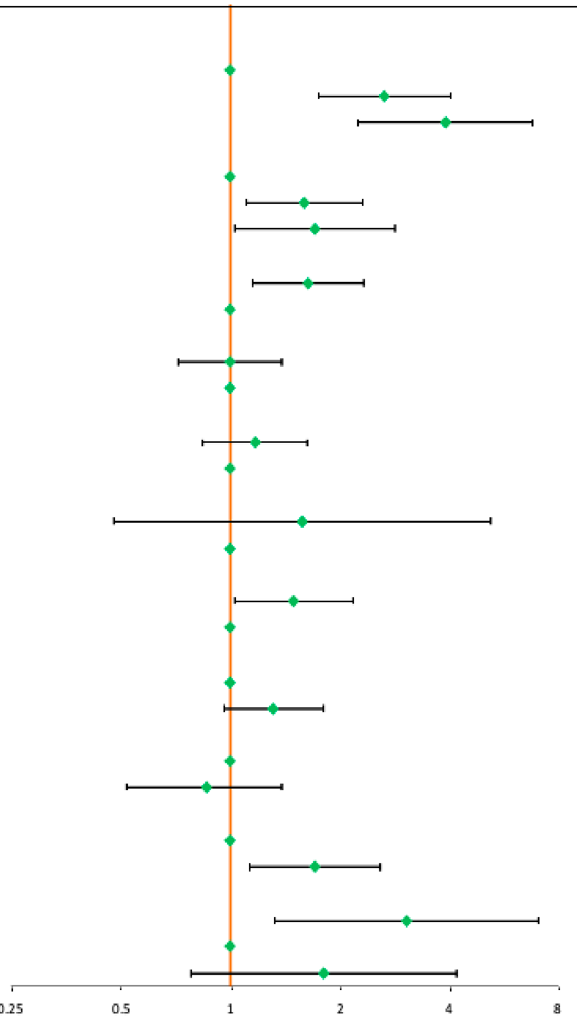


Figure 3. Forest plot of univariate association between *Helicobacter pylori* infection and crowding, lifestyle and hygiene factors. The orange solid vertical line is the reference line. Green dots are the estimated odds ratio. Horizontal black solid lines are 95% confidence intervals

When focusing on the two significant hygiene factors (methods used to wash hands and methods used to clean after toilet), the results showed that there was an increasing trend in prevalence of *H. pylori* infection: prevalence was the lowest in participants who always washed their hands with soap and used only paper to clean after toilet usage (303/372, 81.5%), followed by pupils who always washed their hands with soap but did not use paper to clean after toilet usage (608/684, 88.9%), and it was highest in individuals who rarely or sometimes washed hands with soap whatever using or not using paper to clean after toilet (323/353, 91.5%).

All water-related variables (mainwater source, drinking water source, cooking water source, and methods of water treatment) were not apparently associated with *H. pylori* infection (a $P > 0.05$) (Table 3).

In multivariate logistic regression analysis (Table 4), age, living area, size of family, methods used to wash hands, and ways to clean after toilet were significantly related to *H. pylori* infection. The prevalence of *H. pylori* was significantly higher in pulpils aged 6 – 11 years (OR = 1.66, 95% Cl: 1.18– 2.32, $P = 0.02$) compared to those above 11 years old. It was also significantly increased with the level of crowded: peri-urban area (OR = 1.72, 95% Cl: 1.09 – 2.70, $P < 0.001$); urban area (OR = 2.01, 95% Cl: 1.29 – 3.09, $P < 0.001$); super-urban area (OR = 2.81, 95% Cl: 1.67 – 4.72, $P < 0.001$). Regarding to hygiene-related factors, *H. pylori* infection was significantly higher in pupils who rarely or sometimes handwash with soap after toilet (OR = 1.65, 95% Cl: 1.08 – 2.55, $P = 0.02$), and in children who used only water to clean after going to toilet (OR = 3.13, 95% Cl: 1.28 – 7.64, $P = 0.004$).

Table 4. Association between *Helicobacter pylori* infection and selected factors in a multivariate analysis

Selected factors	n/N	%	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	*P*
Age groups (years)*					0.002
6 - 11	707/791	89.4	1.45 (1.06 – 1.99)	1.66 (1.18 – 2.32)	
≥ 12	527/618	85.3	1	1	
Living area*					< 0.001
Super-urban area	282/308	91.6	2.61 (1.58 – 4.31)	2.81 (1.67 – 4.72)	
Urban area	422/474	89.0	1.96 (1.29 – 2.96)	2.01 (1.29 – 3.09)	
Peri-urban area	310/354	87.6	1.69 (1.09 – 2.62)	1.72 (1.09 – 2.70)	
Rural area	220/273	80.6	1	1	
Family size					< 0.001
≤ 3	108/146	73.9	1	1	
4 - 6	848/960	88.3	2.66 (1.75 – 4.05)	3.04 (1.97 – 4.71)	
> 6	278/303	91.8	3.91 (2.25 – 6.79)	4.09 (2.31 – 7.25)	
Handwashing with soap after toilet					0.02
Always	911/1056	86.3	1	1	
Rarely or sometimes	323/353	91.5	1.71 (1.13 – 2.59)	1.65 (1.08 – 2.55)	
Clean after toilet by using					0.004
Water only	837/932	89.8	3.06 (1.33 – 7.04)	3.13 (1.28 – 7.64)	
Paper only	23/31	74.2	1	1	
Both water and paper	374/446	83.9	2.85 (1.29 – 6.43)	2.02 (0.82 – 5.01)	

**From this community-based study, a recent paper found that age, and living area were associated with H. pylori infection .*

Discussion

Overall, the prevalence of *H. pylori* infection among school-aged children in HCMC was 87.6%. Younger age, living in crowded areas (super-urban area, urban area, peri-urban area), larger family size, infrequency of handwashing with soap after toilet, using only water or both water and paper to clean after toilet were independently associated with an increased prevalence of paediatric *H. pylori* infection.

Table 4. Education level of parents across the four areas

Education level	Area: Super-urban area (n = 308)	Urban area (n = 433)	Peri-urban area (n = 354)	Rural area (n = 273)
	n (%)	n (%)	n (%)	n (%)
College/University (18 - 24 years old)	23 (7.5)	35 (7.4)	46 (13.0)	57 (20.9)
High school (16 - 18 year old)	104 (33.8)	160 (33.8)	117 (33.1)	120 (44.0)
Secondary school (12 - 15 years old)	25 (8.1)	45 (9.5)	32 (9.0)	26 (9.5)
Primary school (6 - 11 years old)	156 (50.6)	234 (49.4)	159 (44.9)	70 (25.6)

In our analysis, the prevalence of *H. pylori* significantly increased when the level of parent education decreased in univariate analysis, but this significant association was not observed in multiple analysis. This was explained by a correlation between level of education and living area. Most of families with higher levels of education resided in rural area, while nearly half of the population with primary school level lived in urban area (Table 4). This trend can be attributed to the rapid urbanization and industrialization of HCMC, which has attracted many people from other provinces and regions to migrate to urban area of HCMC, where many factories, hotels, or restaurants located, to find better job opportunities and higher salaries. Consequently, the urban population of HCMC rose from 4 million in 2010 to nearly 9 million in 2020, which migrants accounting for 70% (78). A part of rural area has also been switched to residential areas, offering more spacious housing with a cleaner environment. As a result, families with higher education levels tend to prefer living in these newly developed areas. This inverse association was also comparable to a study of Nguyet et al. conducted in the North of Vietnam, which indicated low parental education as a risk factor of acquiring *H. pylori* (121). This finding was also consistent with other reports from China, Pakistan, Iran which also pointed that children whose parents had a lower education level were significantly at a higher risk of *H. pylori* infection (18,122,123). In addition, our study found that pupils with parents who completed only primary school had a higher prevalence than those with parents who completed a higher educational degree. A lower level of education can make harder to understand health message. A lower income level leads to live in smaller and more crowdy spaces, and so increased the chances to contract a *H. pylori* infection.

H. pylori infection was not significantly associated with the wealth status of a household, which was inconsistent with previous studies (16,110,124). A possible reason for these conflicting findings might be the different methods used to assess the wealth status. Most previous studies used family monthly income and expenditure, or a combination of multiple indicators such as education level, occupation, and monthly earnings to categorize the wealth index. However, data on income and expenditure were not available in our study. We therefore built the household wealth index based on the ownership of 28 household assets following the DHS guidelines for Vietnam (120). These items were easy to collect and to measure for researchers compared to using monetary indicators. However, the guideline was published several years ago and might be less appropriate at the time that our study was conducted. Therefore, further analyses are needed to discover and verify an appropriate method for evaluating the wealth index of Vietnamese population.

H. pylori could spread from person to person through two main routes: the oral-to-oral route, and the fecal-oral route. The second route was identified to be the predominant mode for *H. pylori* transmission in several studies (22,99). In addition, the pathogen in feces can spread by the fecal-oral route from one person to another's oral cavity through contaminated food, surfaces, or water, which is mainly caused by a lack of adequate sanitation and poor hygiene practices. In our findings, the prevalence of *H. pylori* infection was significantly higher in pupils who infrequently washed their hands with soap after toilet, and it was significantly lower in participants who used only paper to clean after toilet. Interestingly, when carefully assessing the two hygiene factors, results showed that there was an increasing trend in prevalence of *H. pylori* infection: lowest in participants who always washed their hands with soap and used only paper to clean after toilet usage (81.5%), followed by pupils who always washed their hands with soap and did not use paper to clean after toilet usage (88.9%), and highest in individuals who rarely or sometimes washed hands with soap whatever using or not using paper to clean after toilet (91.5%). These evidences support that unhygienic toilet conditions, particularly poor handwashing behavior, increase the possibility of contact with fecal material, which can create an infection vehicle of *H. pylori* and cause the spread of the bacterium both within the family and within the community. In addition, sharing cups or dishes or sauces or foods was not associated with *H. pylori* infection in our findings, suggesting that the oral-oral transmission might not play a main route in spreading *H. pylori* in children in HCMC. Based on these findings, the fecal-oral route appears to be the most likely mode of transmission for *H. pylori* infection among school-aged children in HCMC. Therefore, educating and improving hygiene practices are essential to control the transmission of *H. pylori* and reduce the burden of this infection in the community.

Waterborne infection can be an important route of *H. pylori* infection, particularly in developing countries with a high prevalence of acquiring *H. pylori* (3). Several studies pointed to a positive association between *H. pylori* infection and the consumption of well water (87,113). Other reports also found a strong correlation between *H. pylori* infection and the source of drinking water (110,125). However, water-related variables (source of drinking water or cooking water or methods of water treatment) were not correlated with *H. pylori* prevalence in our study, supporting that the water supply may not act as a reservoir for the transmission of *H. pylori* in the population of HCMC. Indeed, HCMC replaced, developed, and installed new water distribution systems over the past 10 years to ensure treated water for 100% population of the city from 2015. However, some households are still using well water instead of treated water from the government.

Our study is the first to use a random, so representative sample of school-aged children of HCMC to investigate the pathway of *H. pylori* transmission. Our results point the oral-fecal route as an important - but not isolated -

pathway for *H. pylori* spread. Other routes of transmission such as oral-oral are not well documented yet.

Our study has several limitations. Firstly, we relied on the DHS guidelines to build a wealth index based on 28 household assets, but this approach may not be the most suitable way for assessing the wealth status of the family. Several studies showed that a combination of multiple indicators, including education level, occupation, and monetary indicators such as monthly income and expenditure, provides a more comprehensive wealth index compared to solely replying on house assets (16,110,124). However, our study lacked data on monthly income and expenditure, which limited our ability to use those measures to evaluate the wealth status. Secondly, we did not examine the school environmental fators as availability of soap and water at handwashing sites, food-borne factors, sleeping conditions, that could potentially have a significant impact on the prevalence of *H. pylori* infection.

Conclusion

The prevalence of *H. pylori* among school-aged children of HCMC remains high. Younger age, crowded living areas, larger family size, and poor hygienic practices were found to be positively associated with a higher prevalence of *H. pylori* infection among school-aged children of HCMC. These findings highlight the importance of the fecal-oral route and the attribution of crowded living conditions to the spreading of *H. pylori* in HCMC. To decrease the acquisition of *H. pylori* as well as the burden of *H. pylori* among pupils in

HCMC, efficient preventive programs should be set up with a focus on education of hygiene practices, and oriented to those living in crowded conditions. Further analyses are needed to address the impact of school environment on *H. pylori* transmission.

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5.3 Results 3

High antibiotic resistance rates among the five common antibiotics used for *H. pylori* treatment in children in Ho Chi Minh city, Vietnam.

Our project, the “Projet de recherche pour le développement (PRD) – Building strategies for prevention and management of *Helicobacter pylori* infection in children” is a research project, including two different surveys: a community-based study in public schools and hospital-based studies in pediatric hospitals in Ho Chi Minh city, Vietnam. High antibiotic resistance rates were observed among the five common antibiotics used for *H. pylori* treatment in children in Ho Chi Minh city, Vietnam is one of the hospital-based studies carried out between October 2019 and May 2021.

Background: *Helicobacter pylori* (*H. pylori*) is a gram-negative bacterium that can cause several upper gastrointestinal diseases, such as peptic ulcers, gastritis, and even gastric cancer later in life (126). Eradicating *H. pylori* is crucial to prevent such complications (3). However, the rise of antibiotic-resistant *H. pylori* strains poses a significant risk of treatment failure, and potentially leading to higher prevalence of *H. pylori* in the community (127). The limited availability of antibiotics suitable for pediatric use further complicated the treatment process. According to the international guidelines to eradicate *H. pylori* infection, the standard triple therapy (PPI–amoxicillin–clarithromycin or metronidazole) has been the first-line therapy recommended (3,49). Tetracycline is only indicated for children > 8 years of age, and levofloxacin is only allowed in adolescents because of some adverse effects on the development of teeth and cartilage (49). Furthermore, the evidence of antibiotic resistance in each geographic area plays an essential role in choosing empiric regimens. Therefore, a multicenter hospital-based cross-sectional study was conducted by Cam Tu Nguyen to assess the antibiotic resistance patterns of *H. pylori* in Vietnamese children.

Methods: A multicenter cross-sectional study was conducted from October 2019 to May 2021 in two peadiatric hospitals in Ho Chi Minh city (HCMC): the 1400-bed children's hospital 2 located in the urban area (Quan-1) and the 1000-bed city children's hospital located in the rural area (Binh Chanh district). These hospitals provide care services not only for children living in HCMC but also for children residing in neighborhood provinces of HCMC such as Long An, Tay Ninh, Ben Tre, Dong Nai, and Binh Duong. A series of 123 consecutive children aged 4 to 16 years with alarm symptoms (persistent right upper quadrant abdominal pain, dysphagia, odynophagia, persistent vomiting, gastrointestinal blood loss, and weight loss) who underwent upper gastrointestinal endoscopy were recruited. Children who underwent therapeutic endoscopic procedures or children who had a history of upper gastroscopy were excluded. Those who used antibiotics or bismuth-containing compounds within the last 28 days or used any proton pump inhibitors during the past 14 days before endoscopy were also excluded from the series. Five gastric biopsies were taken for *H. pylori* culture (one from the antrum, and one from the corpus), and histopathology (one from the antrum, one from the corpus), as well as rapid urease test (RUT) and PCR assay for ureases gene-ureA (one from antrum). The diagnosis of *H. pylori* infection was confirmed on either positive culture or the combination of histological evidence with at least one other biopsy-based test (RUT or PCR), following ESPGHAN/NASPGHAN criteria (49).

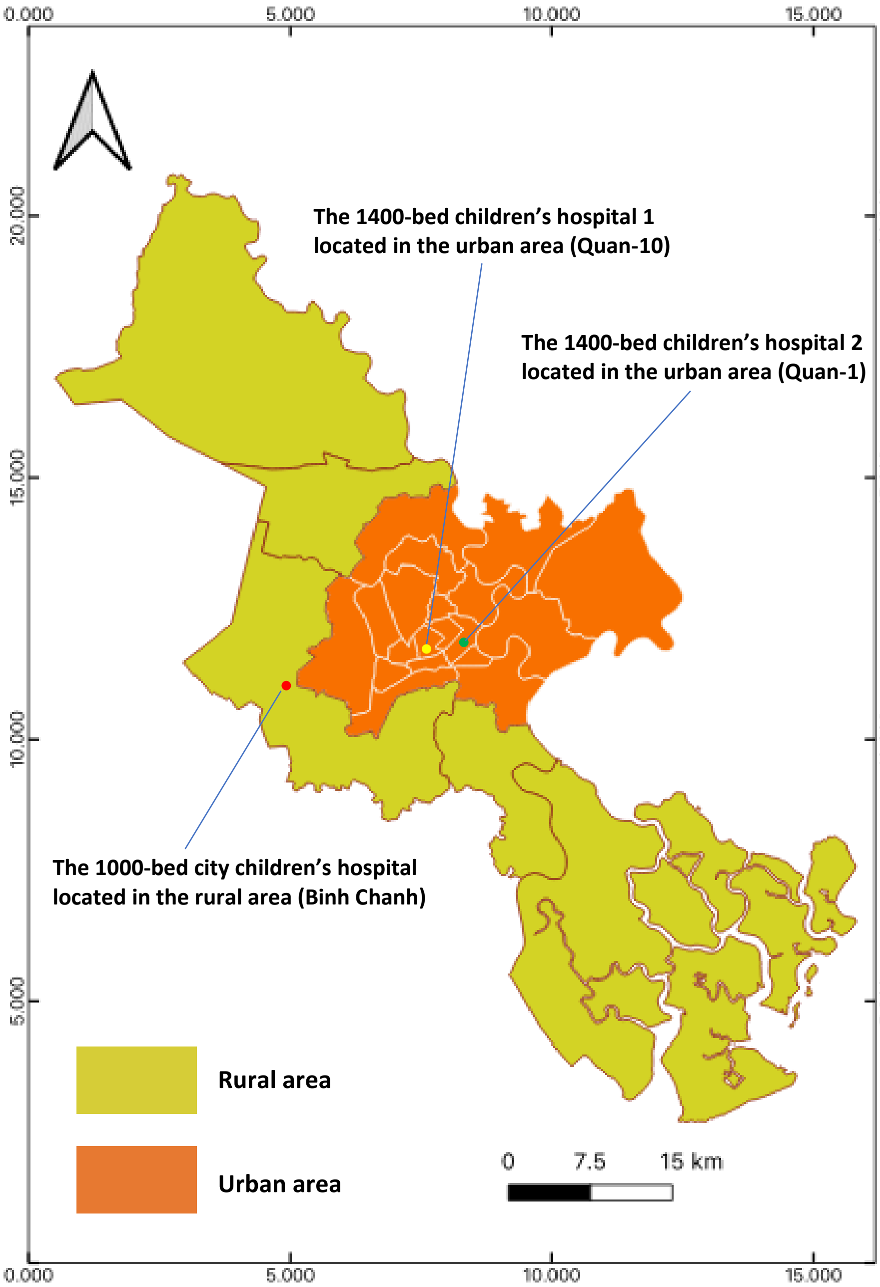

Figure 1. Location of the three children's hopitals of Ho Chi Minh city. The present study was conducted in Children's hospital 2 and City children's

Antibiotic susceptibility to amoxicillin (AMO), clarithromycin (CLA), levofloxacin (LEV), tetracycline (TET), and metronidazole (MET) were determined using E-test (BioMérieux, Belgium). According to the 2019 standards of the European Committee on Antimicrobial Susceptibility (EUCAST) to evaluate susceptibility, the resistance cutoff values were 0.125 µg/mL for amoxicillin, 0.5 µg/mL for clarithromycin, 1 µg/mL for levofloxacin and tetracycline, and 8 µg/mL for metronidazole (128).

Results: The mean age of the 123 pediatric patients was 9.1 ± 2.4 years, ranging from 4.2 to 15.3 years. Most patients were from HCMC (39.9%), followed by South-East provinces (28%), Mekong River Delta (22.9%), and other regions (9.2%). The positive rate for *H. pylori* infection was 80.1% and it was significantly higher in children from HCMC (91.2%) compared to children from other regions: Southeast provinces (75.5%), Mekong Delta River (64,9%). Overall, 79.7% (98/123) of *H. pylori* infected children were resistant to at least one of the five commonly used antibiotics. The resistance rates were particularly high for clarithromycin 72.4%, levofloxacin 60.2%, metronidazole 38.2%, and amoxicillin 25.2%, while tetracycline resistance was relatively low 1.6%. Multi-antibiotic resistance was also prevalent (67.7%) with common patterns including CLA+LEV (20.3%), and CLA+MET+LEV (15.2%).

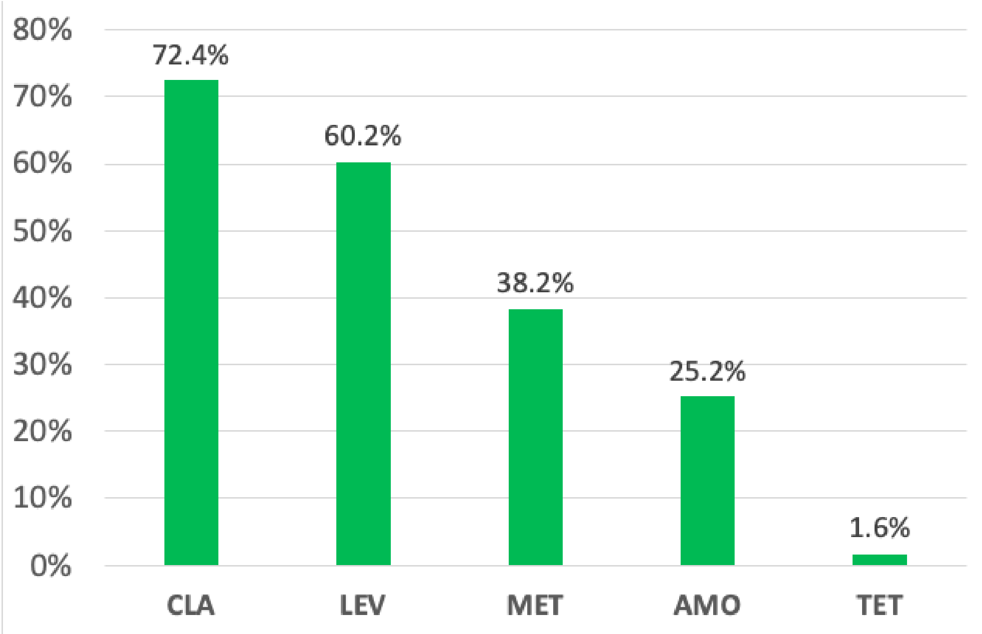

Figure 2. The frequency of *H. pylori* antibiotic resistance in Vietnamese pediatric patients. AMO: amoxicillin, CLA: clarithromycin, LEV: levofloxacin, TET: tetracycline, MET: metronidazole

Conclusion: The prevalence of *H. pylori* infection in symptomatic children was high, especially in children from Ho Chi Minh city. Antibiotic resistance rates of *H. pylori* to CLA, AMO, MET, and LEV were extremely high, whereas TET resistance was relatively low in pediatric patients. These findings suggested that selecting appropriate treatment regimens for *H. pylori* infection in children should be based on antibiotic susceptibility, and considering eradication regimens with TET for children over 8 years old when antibiotic susceptibility testing is unavailable.

CHAPTER 6: DISCUSSION

The present thesis investigates the prevalence of *H. pylori* infection, its geographical distribution, and risk factors among asymptomatic children attending primary schools (6 – 11 years) and secondary schools (12 – 15 years) in Ho Chi Minh city (HCMC).

<u>A high prevalence of *H. pylori* infection among school-aged children in HCMC (87.7%)</u>

Of the cross-sectional study, we could not make a distinction between new infection or reinfection. Furthermore, the stool antigen test used in our study may yield false positive results due to potential cross-reactions with other Helicobacter species, such as Helicobacter heilamanii (129–131). Moreover, the test could also detect coccoid forms of *H. pylori*, which are considered dormant and may lead to false positive results (132–134). Our prevalence was 87.7%, so even 20% of false positive rate, the prevalence remains high.

This high prevalence can be attributed to the increasing resistance of *H. pylori* to the commonly prescribed antibiotics used in eradication therapy. Our recent hospital-based study conducted in HCMC highlighted concerning levels of resistance to the commonly used antibiotics for *H. pylori* treatment in children (135). Nearly 80% of children with *H. pylori* infection were resistant to at least one of the commonly used antibiotics, and approximately 70% was resistant to two or more the antibiotics (multidrug resistance). Specifically, the resistance rates were particularly high for clarithromycin 72.4%, levofloxacin 60.2%, metronidazole 38.2%, and amoxicillin 25.2%, while tetracycline resistance was relatively low 1.6%.

Moreover, the use of tetracycline is only recommended for children above 8 years of age, creating a big challenge for clinicians in selecting effective eradication treatments. This situation not only increases the risk of treatment failure but also potentially contributes to the higher prevalence of *H. pylori* in the community. Therefore, it is crucial for clinicians to base their selection of eradication therapy on antibiotic susceptibility. In cases where antibiotic susceptibility testing in unavailable, considering eradication regimens with tetracycline for children over 8 years old may be a viable option. This apporach aims to improve treatment efficacy and address the growing challenge of antibiotic resistance in *H. pylori* infection among children.

Futhermore, high rates of antimicrobial resistance, not only in case addressed for *H. pylori* infection in children, but also for other common infections in Vietnam, where there is unrestricted access to antibiotics, and a lack of antibiotic stewardship programs**.** In fact, it is estimated that about one-third of antibiotics used annually in Vietnamese adults are taken unnecessarily at the hospital level, and this proportion is likely to be higher at the community-and primary care level (136). These inappropriate use of antibiotics are even worsen in children. A recent study conducted in the children's hospital 1 in HCMC reported that 90.1% and 67.5% of paediatric outpatients were prescribed antibiotics inappropriately for commonly respiratory tract infections, which do not require antibiotic treatment according to treatment guidelines and pathogens detection, respectively (137). There are probably several reasons behind the high rates of antimicrobial resistance in Vietnam, not only in the case of *H. pylori* infection, but also in the other commonly infections. Firstly, Vietnamese health system has four official levels, but there is an unofficial fifth level represented by private pharmacies and drug outlets. Many patients choose these unofficial outlets as their first option for primary care, where antibiotics are often sold without prescriptions, estimating range from 58% to 88% (138,139). Non-prescription antibiotics are frequently brought for children with symptoms of respiratory tract infections (such as cough, runny nose, sore throat), which are usually viral. This might be due to several factors, including convenient opening hours, availability of common medicines, geographical accessibility. However, it is important to note that many pharmacies are run by non-formal pharmacy trained workers, contributing to the high rate of inappropriate use of antibiotics and antimicrobial resistance in Vietnam (68). Secondly, the overuse of antibiotics is like driven by individuals purchasing antibiotics based on self-diagnosis or diagnoses from poorly trained healthcare providers, particularly at the district and commune levels where there is often lack of doctors. Thirdly, there is a common belief among patients that antibiotics are highly valuable and play an important role in symptom management and achieving good health, leading to inappropiate use. Lastly, another contributing factor to the high rates of antimicrobial resistance in Vietnam is the non-adherence to clinical guidelines by physicians (136). This is partly due to the lack of diagnotic tools that can effectively differentiate bacterial and viral infections. Currently, there is a limited capacity for laboratories to provide timely and accurate diagnostic information, particularly for outpatients.

Therefore, addressing these issues will require comprehensive efforts, including improving pharmacy regulations and training, enhancing healthcare provider education on appropriate antibiotic use, promoting public awareness about the appropriate use of antibiotics, and investing in laboratory capacity to support accurate diagnotic and treatment decisions.

The high prevalence of *H. pylori* infection may also possibly due to a high reinfection rate of *H. pylori* (140). Indeed, recent studies reported reinfection rates of *H. pylori* in both infected adults and infected children one year after successful eradication in Vietnam, with rates of 23.5% and 25.2%, respectively (141,142). The rates of reinfection found in Vietnam are higher than the mean annual reinfection rate of *H. pylori* observed in developing countriesn (8.7%) and in developed countries (3.4%). However, they are relatively similar to rates found in population with a high prevalence of *H. pylori* infection, such as Alaska with a reinfection rate of 22%, and Heibei province in China with a reinfection rate of 22.8% (143,144). The severity of reinfection in Vietnam may reduce the benefits of *H. pylori* eradication, such as the healing of ulcers, prevention of progression to gastric atrophy. Moreover, it may increase the likelihood of exposure to *H. pylori* among non-infected persons, thus contributing to a high prevalence of *H. pylori* infection in the community, particularly among children. Because childhood is considered as critical time for acquiring *H. pylori* infection, making children more susceptible to reinfection after successful eradication.

Geographical distribution of *H. pylori* infection among school-aged children in HCMC

By using the 4-points index, our study showed that there was a linear increasing trend in the prevalence of the infection across four areas in HCMC, and this trend was also found in both age or gender subgroups. A study performed in the North of Vietnam also found that the higher crowded living area was a positive risk factor for *H. pylori* infection (75). Additionally, reports from China, Japan, Jordan, and Nepal also observed that the infection rate significantly increased in people living in crowded areas (110,113,114). However, these studies stratified the living area only according to population density whereas our study developed the 4-point index based on both population density and employees density as previously described and explained. That crowdy index can create a more comprehensive understanding of the relationship between *H. pylori* infection and the level of populated concentration in HCMC. Therefore, designing the programs to prevent and reduce the *H. pylori* infection in HCMC should focus on the highly concentrated population areas.

Our study also found that the prevalence is lower than in rural area compared to other areas. Upon closer examination, we observed that districts far from the testing center (the laboratory of the University of Medicine Pham Ngoc Thach, located in an urban district) have also lower prevalence of *H. pylori* infection (Cu Chi 73.7%, Can Gio 77.8%). This lower prevalence could be attributed to a transportation time required for the stool sample from rural area to reach the testing center. A long transportation time might cause a reduction in sensitivity of the stool antigen test and might have induced an underestimate the infection rate among children from rural area. In our study, we conducted research in schools among asymptomatic children in line with the University of Medicine Pham Ngoc Thach, which is why we utilized their laboratory. However, we recognize the importance of addressing this limitation and plan to collaborate with other laboratories in HCMC such as laboratory of City Children's hospital, which is located in rural district, for future projects. This approach aims to reduce transportation-related issues and ensure more comprehensive data collection from different regions, thus providing a more accurate assessment of *H. pylori* infection in various areas of HCMC.

Factors associated with *H. pylori* infection among school-aged children in HCMC

Younger age, crowded living conditions, and poor hygienic practices were associated with an increased prevalence of *H. pylori* infection among children in HCMC

Younger age

In our study, we found a higher prevalence of *H. pylori* infection among younger children (< 12 years) than among older children (≥ 12 years), regardless of sex. Similar findings were reported in studies conducted in Japan and Taiwan, where *H. pylori* infection rates in children decreased in each age group (107–109,145). In contrast, a study conducted in China pointed that children aged > 12 years had a higher prevalence of *H. pylori* infection than children aged < 12 years (146). These evidences indicate that the incidence rate of *H. pylori* across age groups varies greatly in different countries and may depend on different socio-economic statuses, living conditions, and lifestyle factors. However, the higher prevalence of *H. pylori* infection among the younger pupils appears to be consistent aross different regions in Vietnam. Indeed, our result is in line with previous studies conducted in the Highland area and Northern rural area of Vietnam, where younger children had higher prevalence than older children (77,147). A recent study conducted in Hanoi also supported this observation, showing that children aged < 12 years had a higher prevalence of *H. pylori* infection than children aged ≥ 12 years (148). These evidences suggest that there might be a systematic reason behind the higher prevalence of *H. pylori* infection among primary school children (< 12 years) compared to secondary school children (≥ 12 years). This consistent pattern may be related to the size and height of toilet pans in primary and secondary schools in Vietnam. Despite significant differences in height and weight between primary and secondary school pupils, most of public schools in Vietnam have toilet pans of the same size and height for both primary and secondary levels. Consequently, the toilet pans may not be suitable for primary school pupils, leading to increase the possibility of contact with fecal material and the potential spread of the bacterium within school environment. Therefore, further research is needed to investigate school environment, particularly the toilet conditions.

Additionally, it is important to note that the prevalence of *H. pylori* in HCMC was already high in the youngest ones, before 6 years old. Remarkably, our hospital-based study identified cases of newly infected children as young as 4 months of age (135). These findings indicated that *H. pylori* infection can be acquired in as early as 4 months of age or possibly even earlier. This early acquisition of *H. pylori* could contribute to the high prevalence of *H. pylori* infection in HCMC. Therefore, effective measures aimed to preventing and controlling *H. pylori* transmission an early age could have a substantial impact on reducing the prevalence of the infection in the community.

Crowded living conditions

All crowding-related factors investigated, including family size, number of children, and sharing a bed, were significantly associated with being *H. pylori* positive. Interestingly, there was an increasing trend in percent feco-positive in both family size subgroups and number of children in household subgroups. The prevalence increased with the number of family members and with the number of children. These findings are similar to previous reports where crowded living conditions were significantly related to the presence of *H. pylori* infection among children (9,22,99). Furthermore, *H. pylori* is well-documented to cluster in families and in people living in crowded conditions. (3,22) These evidences suggest that crowded living conditions contribute to transmitting *H. pylori* at the household level and intrafamilial transmission maybe a predominant route of spreading *H. pylori* in HCMC.

Poor hygienic practices and the route of transmission

H. pylori could spread from person to person through two main routes: the oral-to-oral route, and the fecal-oral route (3,7,22). The second route was identified to be the predominant mode for *H. pylori* transmission in several studies. In addition, the pathogen in feces can spread by the fecal-oral route from one person to another's oral cavity through contaminated food, surfaces, or water, which is mainly caused by a lack of adequate sanitation and poor hygiene practices. In our findings, the prevalence of *H. pylori* infection was significantly higher in pupils who infrequently washed their hands with soap after toilet, and it was significantly lower in participants who used only paper to clean after toilet. Interestingly, when carefully assessing the two hygiene factors, results showed that there was an increasing trend in prevalence of *H. pylori* infection: lowest in participants who always washed their hands with soap and used only paper to clean after toilet usage (81.5%), followed by pupils who always washed their hands with soap and did not use paper to clean after toilet usage (88.9%), and highest in individuals who rarely or sometimes washed hands with soap whatever using or not using paper to clean after toilet (91.5%). These evidences support that unhygienic toilet conditions, particularly poor handwashing behavior, increase the possibility of contact with fecal material, which can create an infection vehicle of *H. pylori* and cause the spread of the bacterium both within the family and within the community. In addition, sharing cups or dishes or sauces or foods was not associated with *H. pylori* infection in our findings, suggesting that the oral-oral transmission might not play a main route in spreading *H. pylori* in children in HCMC. Based on these findings, the fecal-oral route appears to be the most likely mode of transmission for *H. pylori* infection among school-aged children in HCMC. Therefore, educating and improving hygiene practices are essential to control the transmission of *H. pylori* and reduce the burden of this infection in the community.

Waterborne infection can be an important route of *H. pylori* infection, particularly in developing countries with a high prevalence of acquiring *H. pylori* (7). Several studies pointed a positive association between *H. pylori* infection and the consumption of well water (99,125). Other reports also found a strong correlation between *H. pylori* infection and the source of drinking water (113,149). In our study, while the odds ratio of water-related variables showed an increase but they did not reach significance. However, it is important to note that the sample size in our study was calculated to estimate the prevalence with a precision of 7.5%, and it was not specifically designed to detect factors related to *H. pylori*. As a result, there may have been lack of power to identify factors associated with the infection, particularly water-related factors. Over the past decade, HCMC has made efforts to replace and improve its water distribution systems, aiming to provide treated water to the entire population from 2015 (90). Despite these improvements, some households may still be relying on well water instead of government-treated water. Additionally, it is possible that children may have been exposed to contamination from well water before the implementation of these new water distribution systems. These factors may contribute to the persistence of *H. pylori* infection, even in the context of efforts to enhance water quality and reduce waterborne transmission.

Socioeconomic status

H. pylori infection was not significantly associated with the wealth status of a household, which was inconsistent with previous studies (16,110,124). A possible reason for these conflicting findings might be the different methods used to assess the wealth status. Most previous studies used family monthly income and expenditure, or a combination of multiple indicators such as education level, occupation, and monthly earnings to categorize the wealth index. However, data on income and expenditure were not available in our study. We therefore built the household wealth index based on the ownership of 28 household assets following the Demographic Health Survey (DHS) guidelines for Vietnam (120). These items were easy to collect and to measure for researchers compared to using monetary indicators. However, the guideline was published several years ago and might be less appropriate at the time that our study was conducted. Therefore, further analyses are needed to discover and verify an appropriate method for evaluating the wealth index of Vietnamese population.

Education level

In our study, the prevalence of *H. pylori* significantly increased when the level of parent education decreased in univariate analysis, but this significant association was not observed in multiple analysis. This was explained by a correlation between level of education and living area. Most of families with high level of education resided in rural area, while nearly half of the population with primary school level lived in urban area (Table 4, page 113). This trend can be attributed to the rapid urbanization and industrialization of HCMC, which has attracted many people from other provinces and regions to migrate to urban area of HCMC, where many factories, hotels, or restaurants located, to find better job opportunities and higher salaries. Consequently, the urban population of HCMC rose from 4 million in 2010 to nearly 9 million in 2020, which migrants accounting for 70%. A part of rural area has also been switched to residential areas, offering more spacious housing with a cleaner environment. As a result, sfamilies with higher education levels tend to prefer living in these newly developed areas. The inverse association was also comparable to a study of Nguyet et al. conducted in the North of Vietnam, which indicated low parental education as a risk factor of acquiring *H. pylori* (121). This finding was also consistent with other reports from China, Pakistan, Iran which also pointed that children whose parents had a lower education level were significantly at a higher risk of *H. pylori* infection (18,122,123). In addition, our study found that pupils with parents who completed only primary school had a higher prevalence than those with parents who completed a higher educational degree. A lower level of education can make harder to understand health messages. A lower income level leads to live in smaller and more crowdy spaces, and so increases the chances to contract a *H. pylori* infection.

The test-and-treat strategy for *H. pylori* infection in the high risk group of gastric cancer

The test-and-treat strategy for *H. pylori* infection is not recommended in current guideline of American College of Gastroenterology (3), even in the high-risk group such as those with a family history of gastric cancer. However, a recent randomized trial conducted in South Korea, where a prevalence of *H. pylori* remains high (54%), has evidence findings for using the test-and-treat strategy (42). In this trial, a total of 1676 patients (aged 49 ± 6 years) with confirmed *H. pylori* infection and a first-degree relative with gastric cancer were enrolled. Among these participants, 832 received *H. pylori* eradication therapy at random and 844 were assigned to the reference group. Both groups were flollowed up to 9.2 years to evaluate the occurrence of gastric cancer. The results shown that 10 out of 832 participants (1.2%) in the treatment group developed gastric cancer, while 23 out of 844 (2.7%) developed gastric cancer in the placebo group, leading to a 55% reduction in the risk of developing gastric cancer over about 10-years in patients treated for *H. pylori* eradication (hazard ratio, 0.45; 95% CI: 0.21-0.94).

Similarly to South Korea, the prevalence of *H. pylori* infection and *H. pylori*-related diseases remains high in Vietnam, especially gastric cancer. In 2020, nearly 18,000 new gastric cancer cases were detected, mostly at advanced stages with poor 5 year survival (70). Notably, treatment of asymptomatic *H. pylori* is also not recommended in Vietnam, even for the high-risk group such as patients with a family history of gastric cancer. Considering the similar situation between South Korea and Vietnam, we suggest to conduct a similar clinical trial in Vietnamese population to assess whether test-treat approach for *H. pylori* can reduce the risk of gastric cancer in persons with a family history of gastric cancer.

Sample size and design effect

In our study, we used the prevalence observed in Hanoi of 76.8% to compute a sample size. For a precision of 5%, the sample size would increase from 1854 to 4266. This would be a challenge as the typical class comprises around 40 children, and we had limited money; selecting a precision of 7.5% was more flexible in our situation. We observed a prevalence at 87.7%, higher than the design prevalence of 76.8%, so the precision of our estimated prevalence was higher than that used to calculate the sample size.

In sample size determination (SSD), we also used a design effect of 1.4. This design effect was taken from a study conducted in public schools in HCMC in 2018, which used a sampling method similar to ours, but with full classes of 40 children and an intra-class correlation coefficient (ICC) of 0.01 in a nutritional context (100). In our study, we intended at the beginning to sample full classes also and to keep ICC at 1%. In the pilot phase, it was challenging to keep 40 children per classroom, mainly because of refusals close to 20%, but also because of a high *H. pylori* prevalence. We reduced to 9 the number of children per class in order to cover all districts to know if prevalence was as high in the whole areas of HCMC. For an ICC of 1%, the design effect would be reduced to 1.08 (= 1 + 0.01*8), so we decided not to correct analyses for this design effect.

If a design effect is larger, the standard error is larger than estimated and so, statistical tests are lower, p-values are greater, and confidence intervals are larger. In our study, because of a prevalence close to 100% in many classes (Annex, Tables 2.1a – 2.1d, P. 178 - 179), the variability between classes is expected to be close to 0, or equivalently the observed ICC is expected to be close to 0 as well. The computed ICC was 1% in the study on nutrition in HCMC; it is expected to be lower in *H. pylori* infection. We didn't estimate the intra-class correlation coefficient from our data, but we didn't observe cluster of negative results in classes (Tables 2.1a – 2.1d, P. 178 – 179). It should also be remembered that the SSD was targeting the precision of the estimated prevalence and not factors associated with *H. pylori* infection. Nevertheless, our few significant p-values (see Table 4, P. 112) are highly significant; including a design effect in analyses wouldn't change our conclusion.

CHAPTER 7: CONCLUSIONS AND PERSPECTIVES

H. pylori infection in children is a public health concern in HCMC. Our thesis aimed to highlight the magnitude of *H. pylori* infection among school-aged children in HCMC. Our findings showed that the current feco-prevalence of *H. pylori* among school-aged children remains high, and it significantly increased with population density or employees density. Younger age, crowded living areas, larger family size, and poor hygienic practices were found to be positively associated with a higher prevalence of *H. pylori* infection among school-aged children of HCMC. Our results also showed high rates of antimicrobial resistance among the common antibiotics used for *H. pylori* treatment. These findings highlight the importance of the fecal-oral route, the attribution of crowded living conditions and high rates antibiotic resistance to the high prevalence of *H. pylori* in HCMC. To decrease the acquisition of *H. pylori* as well as the burden of *H. pylori* among pupils in HCMC, efficient preventive programs should be set up with a focus on education for good hygiene practices, not only for those living in crowded conditions but also for all other because the prevalence is high in all places. Further analyses are needed to address the impact of school environment on *H. pylori* transmission. Furthermore, national actions against antibiotics resistance are a priority.

We recommend:

- Public health policies should prioritize the development of programs to prevent and reduce the incidence of *H. pylori* infection, especially among school-aged children. Early childhood interventions are crucial due to the high prevalence observed in youngest age group.

- Planning, implementing the reduction and prevention of *H. pylori* infection programs should be set up with a focus on education about good hygiene practices, and oriented to those living in highly concentrated population areas in HCMC.
- National actions against antibiotics resistance
- National actions on the management, registry of antibiotics intake or prescription
- Strategies should be implemented to raise awareness and provide education of *H. pylori* infection, particularly targeting parents with lower education levels who may face challenges in understanding health messages.
- In the context of a high antibiotics resistance rate, adults are expected to be more infected with *H. pylori* than children. Therefore, the transmission within household would be more likely than a transmission between peers. This hypothesis can be investigated in a study focusing on kindergartens.
- Implementing national programs to improve pharmacy regulations and training, enhance healthcare provider education on appropriate antibiotic use, promote public awareness about the appropriate use of antibiotics, and invest in laboratory capacity to support accurate diagnosis and treatment decisions.

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ANNEXE

Annexe 1: (a) Ethical clearance; (b) Approval note for conducting the study by the Scientific Committee of the University of Medicine Pham Ngoc Thach

ỦY BAN NHÂN DÂN TPHCM
TRƯỜNG ĐẠI HỌC Y KHOA
PHẠM NGỌC THẠCH

Số: 5755 /QĐ-TĐHYKPNT

CỘNG HÒA XÃ HỘI CHỦ NGHĨA VIỆT NAM
Độc lập – Tự do – Hạnh phúc

Thành phố Hồ Chí Minh, ngày 28 tháng 12 năm 2018

QUYẾT ĐỊNH
Về việc chấp thuận các khía cạnh đạo đức trong nghiên cứu

HIỆU TRƯỞNG TRƯỜNG ĐẠI HỌC Y KHOA PHẠM NGỌC THẠCH

- Căn cứ Quyết định số 24/QĐ-TTg ngày 07 tháng 01 năm 2008 của Thủ tướng Chính phủ về việc thành lập Trường Đại học Y khoa Phạm Ngọc Thạch;
- Căn cứ theo quyết định số 4649/QĐ-TĐHYKPNT ngày 16/11/2018 của Hiệu trưởng Trường Đại học Y khoa Phạm Ngọc Thạch về việc thành lập Hội đồng Đạo đức trong nghiên cứu y sinh học Trường Đại học Y khoa Phạm Ngọc Thạch,
- Căn cứ biên bản chấp thuận của HĐĐĐ trong NCYSH ngày 06/12/2018.
- Căn cứ vào quyền hạn và trách nhiệm của Hiệu trưởng Trường Đại Học Y Khoa Phạm Ngọc Thạch;
- Xét đề nghị của HĐĐĐ TNCYSH Trường Đại học Y Khoa Phạm Ngọc Thạch,

QUYẾT ĐỊNH:

Điều 1. Quyết định chấp thuận các khía cạnh đạo đức trong nghiên cứu với tên đề tài: "Tỉ lệ nhiễm Helicobacter pylori và các yếu tố liên quan ở trẻ em tại Thành Phố Hồ Chí Minh, Việt Nam", do TS. BS. Nguyễn Ngọc Vân Phương trưởng Bộ môn Tin Học – Thống Kê Y Học, Trường Đại học Y Khoa Phạm Ngọc Thạch làm chủ nhiệm đề tài, mã số đề tài: CS.2018.05.

Điều 2. Chủ nhiệm đề tài tuân thủ đúng theo hướng dẫn thực hành lâm sàng tốt (GCP) và nội dung đề cương nghiên cứu đã được phê duyệt.

Điều 3. Các Ông, Bà Trưởng Phòng, Ban chức năng và các Ông, Bà có tên trên chịu trách nhiệm thi hành quyết định này./.

Nơi nhận:
- Như điều 3;
- Lưu: VT, NCKH, B. 5

HIỆU TRƯỞNG

TRƯỜNG ĐẠI HỌC Y KHOA PHẠM NGỌC THẠCH

PGS. TS. Ngô Minh Xuân

ỦY BAN NHÂN DÂN TPHCM
TRƯỜNG ĐẠI HỌC Y KHOA PHẠM NGỌC THẠCH

Số: 2684 /QĐ-TĐHYKPNT

CỘNG HÒA XÃ HỘI CHỦ NGHĨA VIỆT NAM
Độc lập – Tự do – Hạnh phúc

Thành phố Hồ Chí Minh, ngày 26 tháng 7 năm 2018

QUYẾT ĐỊNH
Về việc thông qua đề cương nghiên cứu khoa học

HIỆU TRƯỞNG TRƯỜNG ĐẠI HỌC Y KHOA PHẠM NGỌC THẠCH

- Căn cứ Quyết định số 24/QĐ-TTg ngày 07 tháng 01 năm 2008 của Thủ tướng Chính phủ về việc thành lập Trường Đại học Y khoa Phạm Ngọc Thạch;
- Căn cứ Thông tư số 22/2011/TT-BGDĐT, ngày 30/05/2011 của Bộ Giáo dục và Đào tạo về việc ban hành Quy định về hoạt động khoa học và công nghệ trong các cơ sở giáo dục đại học;
- Căn cứ vào Quyết định số 1387/QĐ-TĐHYKPNT ngày 17 tháng 04 năm 2018 của Hiệu trưởng Trường Đại học Y Khoa Phạm Ngọc Thạch về việc thành lập Hội đồng xét duyệt đề tài NCKH cấp cơ sở.
- Căn cứ vào biên bản họp hội đồng khoa học công nghệ xét duyệt đề tài nghiên cứu khoa học cấp cơ sở ngày 24 tháng 04 năm 2018.
- Căn cứ vào quyền hạn và trách nhiệm của Hiệu trưởng Trường Đại Học Y Khoa Phạm Ngọc Thạch;
- Xét đề nghị của phòng Nghiên cứu khoa học,

QUYẾT ĐỊNH:

Điều 1. Quyết định thông qua đề cương nghiên cứu khoa học cấp cơ sở, tên đề tài: "Tỉ lệ nhiễm Helicobacter pylori và các yếu tố liên quan ở trẻ em tại Thành Phố Hồ Chí Minh, Việt Nam", do TS. BS. Nguyễn Ngọc Vân Phương trưởng Bộ môn Tin Học – Thống Kê Y Học, Đại học Y Khoa Phạm Ngọc Thạch làm chủ nhiệm đề tài, mã số đề tài: CS.2018.05.

Điều 2. Chủ nhiệm đề tài có trách nhiệm triển khai và hoàn thành các nội dung nghiên cứu của đề tài theo đúng tiến độ.

Điều 3. Các Ông, Bà Trưởng Phòng, Ban chức năng và các Ông, Bà có tên trên chịu trách nhiệm thi hành quyết định này./.

Nơi nhận:
- Như điều 3;
- Lưu: VT, NCKH, B. 10.

HIỆU TRƯỞNG

TRƯỜNG ĐẠI HỌC Y KHOA PHẠM NGỌC THẠCH

PGS. TS. Ngô Minh Xuân

Annexe 2: Questionnaire

Part 1: Identification

A. Research code* : |_ _ _|-|_ _ _|-|_ _|-|_ _|-|_ _| (Ex : 001-Q01-C1-05-01)

|a a a|-|b b b|-|c c|-|d d|-|e-e|

***Research code :**

aaa: *The number of interviewer (on list A1 : 001-999);*

bbb: *Code of district of the school: Quan-1 to Quan-12 (Q01 - Q12); Binh Thanh (BTH), Binh Tan (QBT), Phu Nhuan (QPN), Go Vap (QGV), Tan Phu (QTP), Thu Duc (QTD), Tan BInh (QTB), Binh Chanh (HBC), Nha Be (HNB), Hoc Mon (HHM), Cu Chi (HCC), Can Gio (HCG);*

cc: *Type of school (C1/C2);*

dd: *School grade (01-09) ;*

ee: *Ordinal number of the pupil in "The list of pupils of the class".*

B. Information of the pupil

Name (first name and surname): ____

Date of birth: ______ Place of birth:(province/city)__________

Sex: 1 [ ] Male 2 [ ] Female

Address: street ___________________ ward _____________ district _________

Father's name: _______________________________________born on _______,

phone: _____________,

Mother's name: _______________________________________born on _______,

phone: _____________,

C. Information of responder:

1. What is the relationship of the responder with this child?

1 [ ] (Biology) Father/Mother 2 [ ] Grandparent

3 [ ] Himself/Herself 4 [ ] Others: (*specify*)______

2. Full Name: ____

Phone: ____________________,

Part 3: The pupil's health

1. What is the order of the child in the family?____/____ (1/1 or 2/3)

2. This child was born on what gestation weeks?____ weeks

3. What is the weight at birth of this child in gram? ______gram

4. Does this child have any malformation?

No [ ] 0 Yes [ ] 1

5. From birth to 24 first months of life, how about the vaccination of this child?

Not finished the national immunization schedule [ ] 0

Finished the national immunization schedule [ ] 1

Unknown [ ] 99

6. Since he/she was born, where has this child spent most of the time in his/her life to live in?

Ho Chi Minh city [ ] 1

Other, please specify ____________ [ ] 2

7. Is there any smoker in your household?

No [ ] 0

Yes, please specify __________ [ ] 1

Unknown [ ] 99

8. Was there anyone in your household diagnosed with *Helicobacter pylori* infection?

No [ ] 0

Yes, please specify ___________ [ ] 1

Unknown [ ] 99

9. Was there anyone in your household diagnosed with gastric cancer?

No [ ] 0

Yes, please specify_____________[ ] 1

Unknown [ ] 99

Part 4: The child's eating habit

1. In the last 6 months, how often did this child do eating by hand?

Every day [ ] 1

Several times/week [ ] 2

Several times/month [ ] 3

Never [ ] 4

2. In the last 6 months, do this child share eating utensils with other family member in a typical meal?

No [ ] 0 Yes [ ] 1

3. Within the last 6 months, did this child share the cup/glass with other family member in a typical meal?

No [ ] 0 Yes [ ] 1

4. Within the last 6 months, did this child share dish/bowl of sauce with other family member in a typical meal?

No [ ] 0 Yes [ ] 1

5. Within the last 6 months, did this child share the same plate with other family member in a typical meal by chopticks?

No [ ] 0 Yes [ ] 1

Part 5: the child's hygiene pratice

1. Does this child wash his/her hand with soap before the meal?

Always [ ] 1

Sometimes [ ] 2

Never [ ] 3

2. Does this child wash his/her hand with soap after going to toilet?

Always [ ] 1

Sometimes [ ] 2

Never [ ] 3

4. Does this child share toothbrush with other family members?

No [ ] 0 Yes [ ] 1

5. Does this child share washcloth with other family members?

No [ ] 0 Yes [ ] 1

6. Does this child share bathtowel with other family members?

No [ ] 0 Yes [ ] 1

7. How does this child do the cleaning after go to toilet?

Water only [ ] 1

Paper only [ ] 2

Both [ ] 3

Others, please specify ___ [ ] 4

Part 6: The child's sleeping habit

1. Does the child have a midday nap at school?

No [ ] 0 Yes [ ] 1

2. Does this child share bed with other family members?

No [ ] 0 Yes [ ] 1

Part 7: The child's family

1. Which ethnic group does this child's father belong to?

[1] Kinh [2] Khơ me [3] Hoa

[4] Chăm [5] Others, please specify _____________

2. What is the education level of this child's father?

College/University	[ ] 1
High school	[ ] 2
Secondary school	[ ] 3
Primary school	[ ] 4
Others, please specify	[ ] 5

3. The occupation of this child's father belongs to?

[ ] 1 Private business

[ ] 2 Factory worker

[ ] 3 Office worker

[ ] 4 Others, please specify ____________________________

4. Which ethnic group does this child's mother belong to?

[1] Kinh [2] Khơ me [3] Hoa

[4] Chăm [5] Others, please specify _____________

5. What is the education level of this child mother?

College/University [ ] 1

High school [ ] 2

Secondary school [ ] 3

Primary school [ ] 4

Others, please specify [ ] 5

6. The occupation of this child's mother belongs to?

[ ] 1 Private business

[ ] 2 Factory worker

[ ] 3 Office worker

[ ] 4 Others, please specify ___________________________

7. How many people currently live in your family?

Adults___

Under 18___

8.Does the child have his own bedroom?

No [ ] 0 Yes [ ] 1

9. What is the kind of toilet that your household members use the most?

[ ] 1 Flushing toilet

[ ] 2 Latrine toilet

[ ] 3 Other, please specify_______________________

10. What is the main water source of your house?

Drilling well [ ] 1

Non-drilling well [ ] 2

Other, please specify__________ [ ] 3

11. What is the drinking water source of your family?

Drilling well [ ] 1

Non-drilling well [ ] 2

Other, please specify__________ [ ] 3

12. What is the cooking water source of your family?

Drilling well [ ] 1

Non-drilling well [ ] 2

Other, please specify__________ [ ] 3

13. Do you treat the water before drinking?

Boiling water [ ] 1

Filter [ ] 2

No treatment [ ] 3

Other, please specify_________ [ ] 4

14. Does you/your family have a povery certificate?

No [ ] 0

A poverty certificate (900.000VND/person/month) [ ] 1

A near-poverty certificate (1.300.000VND/person/month) [ ] 2

15. Do you think you have enough money to support the needs of your family?

1 [ ] No	2 [ ]	3 [ ]	4 [ ]	5 [ ]	6 [ ]	7 [ ]	8 [ ]	9 [ ]	10 [ ] Yes

16. Does your house have any durable good and service below?

No	Household assets	Yes	No
1	Internet		
2	Radio		
3	Tivi		
4	Hi-fi		
5	Camera		
6	Air conditioner		
7	Computer		
8	Laptop		
9	Telephone		
10	Mobiphone		
11	Microwave		
12	Oven		
13	Gas cooker		
14	Infrared cooker		
15	Induction cooker		
16	Fridge		
17	Rice cooker		
18	Pressure cooker		
19	Dish washing machine		
20	Bike		
21	Electric bike		
22	Honda/Scooter		
23	Motorbike		
24	Car		
25	Washing machine		
26	Vacuum cleaner/Hoover		
27	Water heater		
28	Solar system water heater		

Optional questions, voluntary

17. Total income per month of your family?______________________VND

18.Within the last month, how much did your family expend on household?______________________VND

Annexe 3: Tables

Table 1.1b General characteristics of negative children

Variables	Total n (%)
Age groups (years)	
6 - 11	84 (48.0)
>12	91 (52.0)
Gender	
Boys	102 (58.3)
Girls	73 (41.7)
Ethnic group	
Kinh	169 (96.6)
Chinese	6 (3.4)
Others	0 (0)
Educational status of parent	
College/University	29 (16.6)
High school	72 (41.1)
Secondary school	14 (8.0)
Primary school	60 (34.3)
Parent's occupation	
Factory worker	59 (33.7)
Office worker	30 (17.4)
Private business	51 (29.1)
Others	35 (20.0)
Family size	
≤ 2	38 (21.7)
3	112 (64.0)
4 - 6	25 (14.3)
> 6	0 (0)
No. of children in household	
1	50 (28.5)
2	99 (56.6)
> 2	26 (18.9)
Living areas	
Super-urban area	26 (14.9)
Urban area	52 (29.7)
Peri-urban area	44 (25.1)
Rural area	53 (30.3)
Handwashing with soap after toilet	
Always	145 (82.9)
Rarely or sometimes	30 (17.1)
Clean after toilet by using	
Water	95 (54.3)
Paper	8 (4.6)
Both water and paper	72 (41.4)

Table 2.1a *H. pylori* infection according to educational level of parents in each district of rural area

Educational Level	Hocmon		Binh Chanh		Nha Be		Cu Chi		Can Gio	
	n	%	n	%	n	%	n	%	n	%
College/University	10	80.0	19	68.4	3	50.0	21	76.2	5	50.0
High school	38	92.1	23	73.9	16	87.5	24	79.4	9	77.8
Secondary school	7	85.7	6	100	6	83.3	6	50.0	1	100
Primary school	13	84.6	5	80.0	29	89.7	13	69.2	10	70.0

n: number of children participating in the study in the district
%: percentage of positive with *H. pylori*

Table 2.1b *H. pylori* infection according to educational level of parents in each district of peri-urban area

Educational Level	Binh Tan		Thu Duc		Quan-12		Quan-7		Quan-2		Quan-9	
	n	%	n	%	n	%	n	%	n	%	n	%
College/University	6	83.3	2	50.0	3	66.7	1	100	7	100	28	77.8
High school	24	91.7	10	90.0	15	93.3	13	69.2	13	61.5	42	88.1
Secondary school	4	100	2	100	8	87.5	1	100	6	83.3	11	90.9
Primary school	30	100	19	100	19	78.9	18	83.3	40	90.0	33	90.9

n: number of children participating in the study in the district
%: percentage of positive with *H. pylori*

Table 2.1c *H. pylori* infection according to educational level of parents in each district of urban area

Educational Level	Go Vap		Phu Nhuan		Quan-6		Tan Phu		Binh Thanh		Quan-8		Tan Binh	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
College/University			9	100			15	93.3	7	85.7	2	100	2	50.0
High school	8	75.0	31	80.7	26	73.1	36	88.9	30	100	10	80	19	89.5
Secondary school	4	75.0	6	100	6	100	11	90.9	7	85.7	6	100	5	100
Primary school	18	84.2	31	87.1	43	86.1	30	93.3	39	87.2	21	95.2	51	96.1

n: number of children participating in the study in the district
%: percentage of positive with *H. pylori*

Table 2.1d *H. pylori* infection according to educational level of parents in each district of super-urban area

Educational Level	Quan-4		Quan-10		Quan-11		Quan-3		Quan-5		Quan-1	
	n	%	n	%	n	%	n	%	n	%	n	%
College/University	1	100	1	100	3	100	5	80	14	92.9		
High school	6	100	6	83.3	20	90.0	15	86.7	51	88.2	6	100
Secondary school	2	50	4	100	4	90.0	4	75	10	100	5	100
Primary school	6	100	16	93.8	16	93.8	28	89.3	25	92	67	95.5

n: number of children participating in the study in the district
%: percentage of positive with *H. pylori*

Annexe 4: Publications

IJPH | SSPH+

International Journal of Public Health
ORIGINAL ARTICLE
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High Prevalence of *Helicobacter pylori* Infection Among School-Aged Children in Ho Chi Minh City, VietNam

Thai Hoang Che[1,2], *Tu Cam Nguyen*[3], *Dung Thi Thuy Ngo*[4], *Hiep Thanh Nguyen*[5], *Khang Tan Vo*[6], *Xuan Minh Ngo*[7], *Dinh Quang Truong*[8], *Patrick Bontems*[9], *Annie Robert*[2] and *Phuong Ngoc Van Nguyen*[1]*

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Che TH, Nguyen TC, Ngo DTT, Nguyen HT, Vo KT, Ngo XM, Truong DQ, Bontems P, Robert A and Nguyen PNV (2022) High Prevalence of Helicobacter pylori Infection Among School-Aged Children in Ho Chi Minh City, VietNam. Int J Public Health 67:1605354. doi: 10.3389/ijph.2022.1605354

Objectives: There is no study on *Helicobacter pylori* (*H. pylori*) infection in pupils of Ho Chi Minh city (HCMC), the most overcrowded city in Vietnam. Therefore, the aim of this study was to estimate the prevalence of *H. pylori* and its geographical spread among school-aged children.

Methods: A school-based cross-sectional study was conducted among 1854 pupils across 24 districts of HCMC in 2019. Multiple-stage sampling method was used to enroll pupils. We built a four-points index for geographical division based on population density and employees density to evaluate the link between *H. pylori* and crowded level. Stool samples were analyzed by monoclonal enzyme-immunoassay stool antigen-test to assess the infection status. Logistic regression was performed to assess possible factors related to *H. pylori* infection.

Results: The overall prevalence of *H. pylori* was 87.7%. There was a linear increasing trend in the infection rate ($p < 0.001$) across the 4-points index of HCMC and this trend maintained within both age and gender subgroups ($p = 0.02$).

Conclusion: Prevalence of *H. pylori* was high and it increased with population density or employees density. Therefore, it is crucial to plan and implement the reduction of *H. pylori* infection programs by targeting the highly concentrated population areas of HCMC.

Keywords: Vietnam, prevalence, school-aged children, *Helicobacter pylori*, pupils, Ho Chi Minh City

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is a common chronic infection, affecting more than 50% of the world's population [1]. The prevalence has changed over the last 10 years, showing a decrease in developed countries but remaining high in most developing countries [1, 2]. Like other developing countries in Southeast Asia, the prevalence of *H. pylori* in Vietnam remains high. A recent study in

IJPH | SSPH+

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Factors Associated With *Helicobacter Pylori* Infection Among School-Aged Children From a High Prevalence Area in Vietnam

Thai Hoang Che[1,2], *Tu Cam Nguyen*[3], *Vy Ngoc Thao Vu*[1], *Hiep Thanh Nguyen*[4], *Dung Thi Phuong Hoang*[5], *Xuan Minh Ngo*[6], *Dinh Quang Truong*[7], *Patrick Bontems*[8], *Annie Robert*[2] and *Phuong Ngoc Van Nguyen*[1]*

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Objectives: The study aimed to identify prevalence of *H. pylori* infection and associated risk factors among pupils of Ho Chi Minh city (HCMC).

Methods: A total of 1,476 pupils aged 6–15 years were enrolled in this cross-sectional study using multiple-stage sampling method. Infection status was assessed using stool antigen-test. A questionnaire was used to obtain socio-demographic, behavioral, and environmental factors. Logistic regression was performed to assess possible factors related to the infection.

Results: Of the 1,409 children included in the analysis, 49.2% were male and 95.8% were of Kinh ethnicity. About 43.5% of parents completed college or university. The overall prevalence of *H. pylori* was 87.7%. Infrequency of handwashing with soap after toilet, the use of only water to clean after toilet, crowded living areas, larger family size, and younger age were independently contributing to an increased prevalence of *H. pylori*.

Conclusion: *H. pylori* infection is highly prevalent in HCMC, and is associated with poor hygienic practices, crowded living areas, larger family size, and younger age. These findings highlight the importance of fecal-oral route and the attribution of crowded living conditions to the spreading of *H. pylori* in HCMC. Therefore, preventive programs should be set up with a focus on education of hygiene practices, and oriented to those living in crowded conditions.

Keywords: risk factors, *Helicobacter pylori*, Ho Chi Minh City, school-aged children, transmission route

healthcare MDPI

Article

Helicobacter pylori Infection and Peptic Ulcer Disease in Symptomatic Children in Southern Vietnam: A Prospective Multicenter Study

Tu Cam Nguyen [1,2,*,†], Ngoc Le Chau Tang [3], Giao Kim Ngoc Le [4], Vy Thuy Nguyen [5], Khuong Hoang Gia Nguyen [6], Thai Hoang Che [6], Van Thi Tuong Phan [1], Ngoc Minh Nguyen [3], Dinh Quang Truong [7], Xuan Minh Ngo [8], Hiep Thanh Nguyen [9], Annie Robert [10], Patrick Bontems [2,11] and Phuong Ngoc Van Nguyen [6,†]

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Abstract: Background: *Helicobacter pylori* (*H. pylori*) remains a major cause of gastroduodenal diseases. We aimed to evaluate the burden of this infection, particularly peptic ulcer disease in Vietnamese children. Methods: We enrolled consecutive children referred for esophagogastroduodenoscopy at two tertiary children's hospitals in Ho Chi Minh City, from October 2019 to May 2021. Children treated with proton pump inhibitors during the last two weeks or antibiotics for four weeks, and those having a previous or interventional endoscopy were excluded. *H. pylori* infection was diagnosed with either a positive culture or positive histopathology combined with a rapid urease test, or with a polymerase chain reaction of the urease gene. The study was approved by the Ethics Committee and written informed consent/assent was obtained. Results: Among 336 enrolled children aged 4–16 (mean: 9.1 ± 2.4 years; 55.4% girls), *H. pylori* infection was positive in 80%. Peptic ulcers were detected in 65 (19%), increasing with age, and 25% with anemia. *cagA*+ strains were detected at a higher rate in children with ulcers. Conclusions: Prevalence of *H. pylori* and peptic ulcers is high among symptomatic Vietnamese children. It is crucial to have a program for early detection of *H. pylori* to reduce ulcer risk and gastric cancer later.

Keywords: *Helicobacter pylori*; peptic ulcer disease; symptomatic children; virulence factors; biopsy-based tests

1. Introduction

Helicobacter pylori (*H. pylori*) infection remains a significant cause of human gastroduodenal diseases, especially in highly prevalent countries. It is commonly acquired during childhood and can have long-term consequences. *H. pylori* may cause chronic gastritis and gastroduodenal ulcers, affecting both children and adults [1,2]. It can sometimes

Annexe 5: Poster

High prevalence of *Helicobacter pylori* infection among school-aged children in Ho Chi Minh City, Vietnam

Che Hoang Thai[1,2]; Tu Nguyen Cam[3]; Hiep Nguyen Thanh[1]; Khang Tan Vo[1]; Xuan Ngo Minh[1]; Dinh Truong Quang[3]; Patrick Bontems[4]; Annie Robert[2]; Phuong Nguyen Ngoc Van[1].

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INTRODUCTION

Helicobacter pylori (*H.pylori*) infection remains the main risk factor for gastric cancer, that is highly prevalent in Southeast Asia, and gastro-duodenal ulcers.

There are no data on *H.pylori* infection in pupils of Ho Chi Minh City (HCMC), the largest city in the South of Vietnam.

Our study aimed to investigate the prevalence of *H.pylori* infection and its geographical spread among school-aged children across the 24 districts of HCMC, Vietnam.

METHODS

School-based cross-sectional study was conducted aros 24 districts of HCMC in 2019.

Using the previous prevalence of 78.3% observed in Hanoi (the capital of Vietnam) to reach an expected precision of 7.5% on estimating prevalence in population. Assuming a loss rate of 20%, and after adjusting for a design effect of 1.4, at least 206 children per grade (1st – 9th) were required, corresponding to 1854 in total.

In each district, we randomly selected 1 primary school with grade 1st – 5th (6 – 11 years) and 1 secondary school with grade 6th – 9th (12 – 15 years). In each selected school, one class per grade was randomly selected, and 9 children in the same class were randomly selected and invited to participate in the study.

To assess a potential link between *H.pylori* infection and the crowded level of HCMC, we built a 4-points index based on population density and employees density as described in figure 1.

Stool samples were collected and analyzed by monoclonal enzyme-immunoassay stool antigen-test, Premier Platinum HpSA PLUS (Meridian, USA), to assess the infection status.

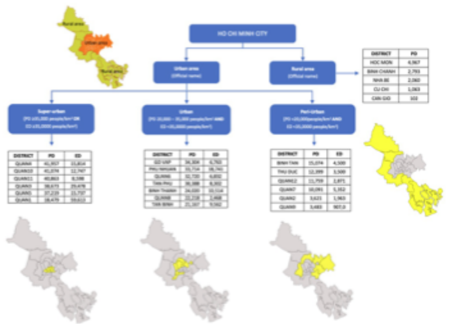

Figure 1. The 4-points index for geographical division of Ho Chi Minh city

Rural area was defined as official definition. Urban area was divided into three areas: peri-urban area (PD <20,000 p/km² and ED <35,000 p/km²), urban area (PD 20,000 – 35,000 p/km² and ED <35,000 p/km²), and super-urban (PD ≥35,000 p/km² or ED ≥35,000 p/km²).

PD: population density (people/ km²); ED: employees density (people/ km²).

Data from completed results of the 2019 Vietnam population and housing census from General statistics office of Vietnam.

RESULTS

The overall prevalence of *H.pylori* in pupils in HCMC was 87.7%.

There was a linear trend in the prevalence of *H.pylori* across four areas of HCMC (80.5% in rural districts, 88.5% in peri-urban districts, 89.3% in urban districts, and 90.9% in super-urban districts, p<0.001).

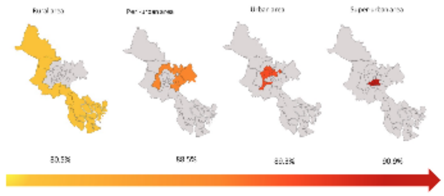

Figure 2. Mapping of the prevalence of *H.pylori* infection in Ho Chi Minh city according to population density (PD) and employees density (ED)

The prevalence was significantly higher in boys (p=0.007) and in children below 11 years old (p=0.03).

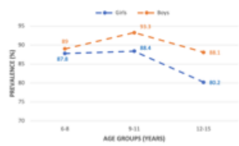

Figure 3. Prevalence of *H.pylori* according to age groups and gender

The increasing trend in *H.pylori* infection across areas was observed in both age groups and also in both genders (p=0.02).

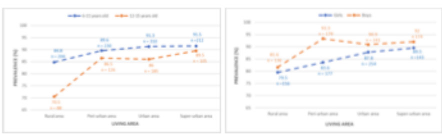

Figure 4. Trend in prevalence of *H.pylori* infection across the crowdy index in HCMC within age and gender subgroups.

n: size of age and gender subgroups in each living area.

CONCLUSIONS

The prevalence of *H.pylori* among school-aged children remains high, even in the youngest ones, suggesting that the infection was acquired in very early childhood, nearby remarkable economic changes of HCMC in the last 10 years. The prevalence increased with population density or employees density. Therefore, It is crucial to plan and implement programs to prevent and reduce *H.pylori* infection by targeting the highly concentrated population areas of HCMC.

We have no conflicts of interest to disclose.
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Che Hoang Thai got a Medical Doctor Degree in 2016 and is currently lecturer at the Pham Ngoc Thach University of Medicine. He is also a pediatrician at Children's hospital 1 in Vietnam. In 2020, Dr. Thai received a grant from the Belgium Government (ARES-CCD) for a Master in Public Health Methodology (MPHM). After this Master Degree, he received a grant for a Ph.D at the UCLouvain in 2021, focusing on *H. pylori* infection in children under the ARES-PRD project "Building strategies for prevention and management of *Helicobacter pylori* infection in children".

L'infection à *Helicobacter pylori* (*H. pylori*) chez les enfants s'est avérée être une menace dans les villes asiatiques, atteignant 77 % à Hanoi ces dernières années. Il n'y avait aucune information de ce type à Hô Chi Minh-Ville (HCMV), malgré le risque de cancer gastrique qu'elle représente. Notre travail visait à évaluer le fardeau de *H. pylori* chez les enfants à HCMV, sa distribution géographique et ses facteurs de risque. Nous avons mené une étude transversale en milieu scolaire à HCMV. Au total, 1 476 enfants de 48 écoles publiques (216 classes) réparties dans 24 districts de HCMV ont participé. L'infection de à *H. pylori* s'est avérée très fréquente (87.7%) et elle augmentait avec la densité de population. Le plus jeune âge, la promiscuité et les mauvaises pratiques d'hygiène étaient associés à une prévalence plus élevée de l'infection par *H. pylori*. Des taux élevés de résistance aux antibiotiques utilisés pour le traitement de *H. pylori* ont également été observés dans des études cliniques. Ces résultats mettent en évidence une transmission fécale-orale. La surpopulation et les taux élevés de résistance aux antibiotiques favorisent l'infection à *H. pylori*. Des politiques nationales contre l'utilisation excessive des antibiotiques sont prioritaires. Des programmes de prévention devraient être mis en place en mettant l'accent sur l'éducation de l'ensemble de la population aux bonnes pratiques d'hygiène.

Helicobacter pylori (H. pylori) infection in children has shown to be a threat in Asian cities, reaching 77% in Hanoi in recent years. Such information was not available in Ho Chi Minh city (HCMC), despite its gastric cancer risk. Our thesis aimed to assess the burden of H. pylori in children in HCMC, its geographical distribution, and risk factors. We conducted a school-based cross-sectional study in HCMC. A total of 1476 children from 48 public schools (216 classes) across 24 districts of HCMC were enrolled. We found that H. pylori infection is highly prevalent (87.7%), and increased with crowded density. Younger age, crowded living conditions, and poor hygienic practices were associated with a higher prevalence of H. pylori infection. High rates of antimicrobial resistance among antibiotics used for H. pylori treatment were also observed in clinical studies. These findings highlight a fecal-oral route for transmission. Crowded living conditions and high rates of antibiotic resistance favor a high H. pylori infection rate. Therefore, national politics against antibiotic resistance are needed. Preventive programs should be set up with a focus on education of the whole population for good hygiene practices.