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Helicobacter pylori in childhood

Aspects of prevalence, diagnosis and treatment

P.E.C. Mourad-Baars

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Helicobacter pylori in childhood

Aspects of prevalence, diagnosis and treatment

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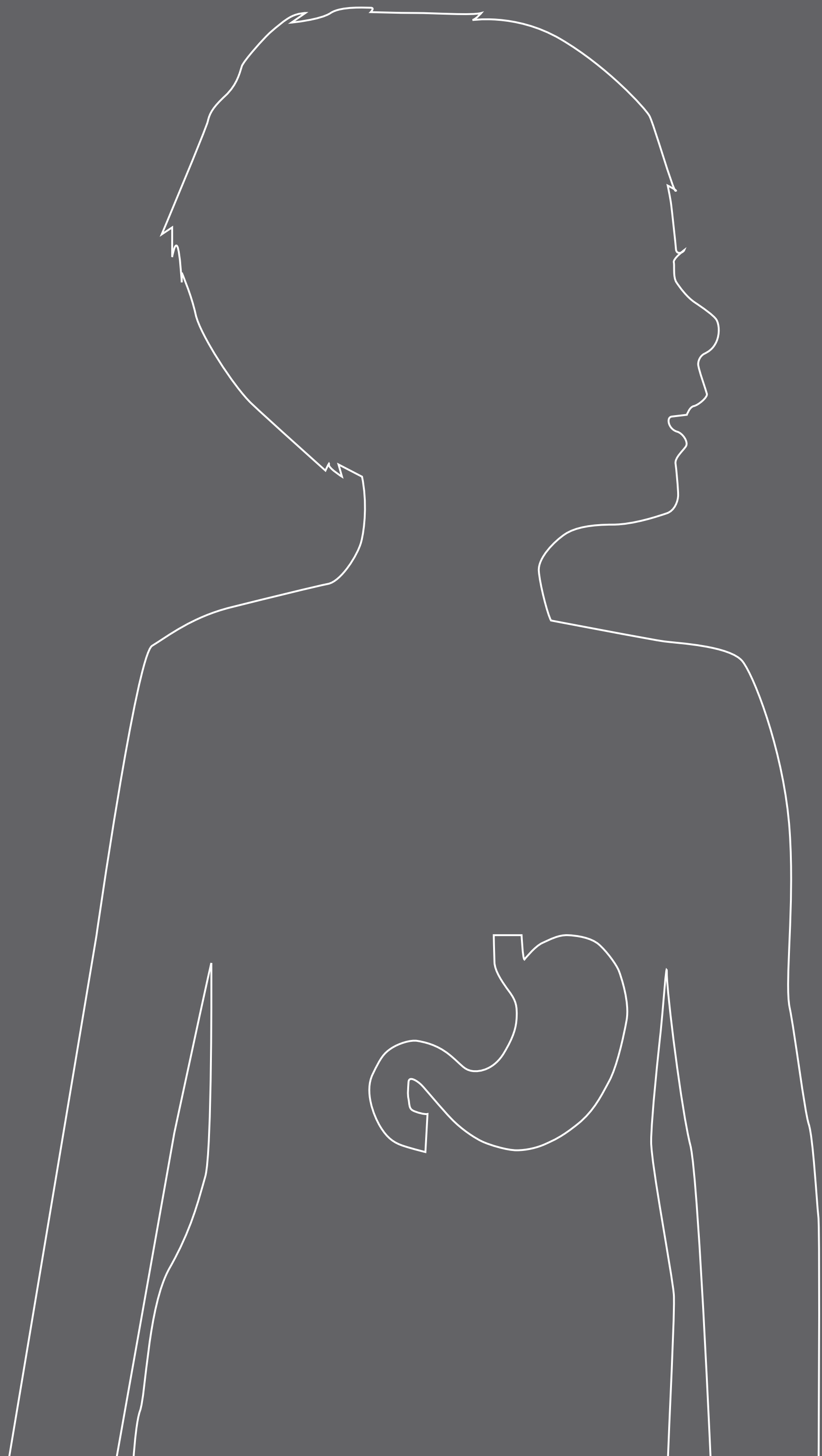

CHAPTER 1
Introduction

1.1 DEFINITION AND HISTORY

Helicobacter pylori (*Hp*) has cohabitated with humans for over 50.000 years, thereby affecting directly more than half of the world's human population. The Gram-negative spiral bacterium with its up to six unipolar sheathed flagella, has the unique ability to colonize the human stomach. Infection with *Hp* has been widespread and therefore geographic variations in the type of *Hp* have successfully been linked to the pathways of early human migrations. Direct evidence of early infections has been found in the form of *Hp*-DNA fragments in a Pre-Columbian mummy dating back to 1350 AD in current Mexico[1].

From 1875 onward, researchers have tried to reveal a positive association between a human infection with microorganisms and the development of peptic ulcers. In line with their hypothesis they already attempted treating ulcers with anti-microbial bismuth-containing compounds. In the 1950s Lykoudis found evidence that peptic ulcer disease and gastritis had in fact an infectious origin and he therefore decided to prescribe antibiotics to thousands of his patients[2].
More than 30 years later two Australian physicians, Barry Marshall (gastroenterologist) and Robin Warren (pathologist), were the first to describe the presence of a Campylobacter-like organism in the stomach of patients suffering from gastritis and peptic ulcers[3,4]. In 1984 Marshall started an attempt to assess Koch's postulates for these diseases in piglets, but the experiment turned out unsuccessful. Eventually, in a bold approach to prove his hypothesis he infected his own stomach with a colony of the bacterium and fell ill himself. He underwent endoscopy and it was revealed that spiral bacteria were present in his antral biopsies and had caused his gastritis. This time, his experiment had succeeded and ever since, *Hp* has been recognized as a human pathogen. Marshall recovered from his gastritis by treatment with amoxicillin and a proton pump inhibitor, and he proved the causal relationship between his disease and *Hp* by fulfilling all four Koch's postulates[5,6].
In recognition of their new discovery, Warren and Marshall were awarded with the Nobel Prize in Physiology or Medicine in 2005, particularly for their role in the observation of "the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease" as well as "further identification of *Helicobacter pylori*". Initially, they named the bacterium "Campylobacter-like organism (CLO)", because of its similarities with the Campylobacter species. Later the name was changed to Campylobacter pyloridis, Campylobacter pylori and, finally, it became known as *Helicobacter pylori* by 1989. Nowadays, *Hp* is recognized as one of the most important pathogens for a wide range of gastrointestinal diseases in the human species. Colonization commonly leads to gastritis and in 10-15% of the cases it progresses to peptic ulcer of the du-

odenum or the stomach, in less than 1% it leads to to mucosa-associated lymphoid tissue (MALT) lymphomas and in 1% to gastric carcinoma[7,8]. However, colonization of the human gastric mucosa does not necessarily result in the development of symptoms and typically more than 70% of infected people remain asymptomatic[7].
The first report on *Hp* in the Netherlands, a letter to the *Lancet* by Langenberg *et al* in 1984, described gastric antral biopsies of 50 outpatients with upper abdominal complaints who were referred for upper gastrointestinal endoscopy[9]. The study unambiguously confirmed a positive association between colonization with *Hp* and gastritis and furthermore noted that *Hp*-associated gastritis may be present in apparently healthy individuals, thereby raising doubts about the clinical significance of *Hp* infections.
In 1986, the first report on the isolation of *Hp* from the stomach of children was published by Hill *et al* [10]. Cadranel and colleagues followed with the first European paper on *Hp* and children. They performed a small prospective study on twenty-five children, eight of whom were found *Hp*-positive. The latter study confirmed the presence of *Hp* in children and established a positive correlation with epigastric pain and chronic gastric inflammation[11].
Currently more than twenty-five *Helicobacter* species have been identified in the gastrointestinal tract of animals and humans, whose infection correlates positively with the occurrence of gastrointestinal and systemic diseases[12].
In figure 1 the rapid development of the scientific field of *Hp*-research, taking off at pioneering work of Marshall and Warren, is illustrated, as well as the relatively small fraction of that work that includes research on children.

1.2 CLINICAL FEATURES OF *HELICOBACTER PYLORI* INFECTION

1.2.1 Transmission and virulence factors

Generally *Hp*-infection is acquired during childhood and if not treated, the infection remains life-long. Spontaneous eradication in childhood appears to occur, but may be attributed to the unreported use of antibiotics to cure respiratory or parasitic diseases.
The principal transmission route of *Hp* has not clearly been defined, nor has its source, but two models prevail: firstly, the vertical route in which parents infect their children, probably by gastro-oral, fecal-oral or oral-oral routes and secondly the horizontal transmission route in which intensive direct contact between infants and children or via for example dentists, endoscopists, or through environmental contamination of food and drinking water[13-21]. For the latter only indirect evidence exists in the presence of *Hp*-DNA in food and water.
Coccoid morphological states of *Hp* have been detected in surface water, but so far

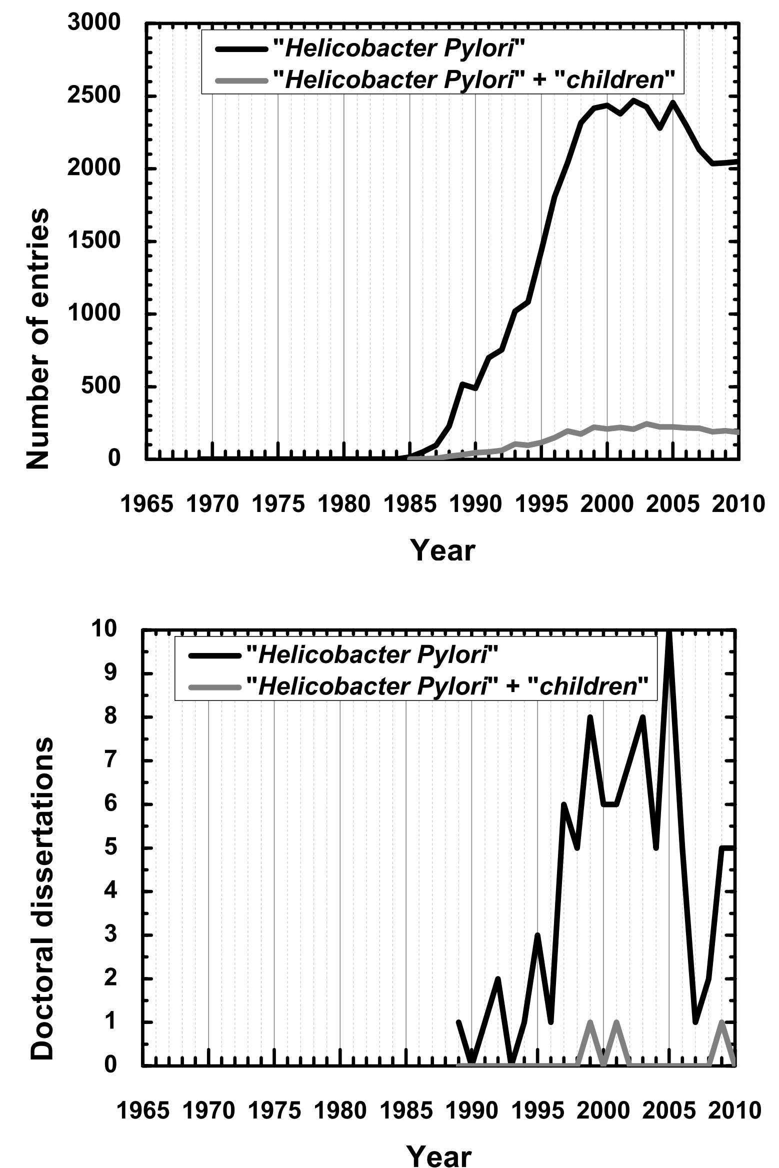

Fig 1: Panel (a) The number of entries in the scientific publications search engine Scopus (www.scopus.com) for the search strings "*Helicobacter pylori*" (black curve) and "*Helicobacter Pylori* children" (grey curve) as a function of the year of publication. Panel (b) The number of doctoral dissertations filed in PubMed (http://www.ncbi.nlm.nih.gov/pmc/) for the search strings "*Helicobacter pylori*" (black data) and "*Helicobacter pylori* AND children" (grey data) as a function of the year of publication.

could not be cultured *in vitro*, raising doubts about their potential for the transmission of the infection. Recognized risk factors for the infection onset are thought to be:
- low socio-economic status and educational level
- living in a family with a high number of siblings (crowding)
- being born to an infected mother
- the use of river or municipal water instead of spring water.

The presence of bacterial virulence factors is one of the possible pathogenic mechanisms of *Hp*-induced gastroduodenal disease; putative virulence factors, especially CagA, VacA, bab A, hom B and OipA, are associated with the gene jhp05632, that encodes for the cell envelope protein glycosyltransferase which appears important for the progression to peptic ulcer disease[22-25].

1.2.2 *Helicobacter pylori* and gastrointestinal symptoms in children

Bacterial virulence, host factors and potentially environmental exposures are considered to be key factors in the type of gastroduodenal inflammation and disease outcome that is associated with *Hp*-infection. It remains unclear as to: (l) why *Hp* causes symptomatic disease in only 20-30% of infected people; (2) how many of the organisms are needed to establish a persistent infection; and (3) who is prone for which disease phenotype. In children, antral predominant gastritis usually develops after colonization. Children may have transient impaired gastric acid secretion (hypochlorhydria), which, as is the case for adults, is thought to add to an increased susceptibility for enteric infections[26,27].

Although dyspepsia in adults is generally regarded to be caused by *Hp*, a specific clinical scenario of *Hp* infection in children is not known. The relation between recurrent abdominal pain (RAP) and *Hp* as described by Apley in 1985[28] has been assessed in many studies, but nevertheless the role of *Hp* in causing such symptoms has remained controversial[29-32]. A literature review over the years 1983-1998 found no specific correlation between RAP and *Hp* infection in children[33]. More recently, a systematic review confirmed the absence of such a correlation. Evidence was found for an association between epigastric pain and *Hp* infection in children except for children in primary care. Other gastro-intestinal symptoms as nausea, vomiting, regurgitation, diarrhea, flatulence or dyspepsia were not associated with *Hp* infection, but randomized control studies on this topic are needed[34].

1.2.3 Peptic ulcer

Duodenal and gastric ulcer disease in childhood are much less common in childhood than for adults and children develop gastric ulcers much less often than duodenal ulcers. Remarkably, the prevalence of non-*Hp*-associated ulcer disease seems to increase lately[35,36]. Longstanding *Hp* infections have been associated with gastric

atrophy and intestinal metaplasia with development of gastric adenocarcinoma in adults. Gastric atrophy and metaplasia have also been described in children[37]. Few cases of gastric MALT-lymphoma in children have been reported, treated only with eradication medication for the *Hp* infection even in immune-compromised pediatric patients. Reports of gastric adenocarcinoma in children are, however, rare[38-44].

1.2.4 *Helicobacter pylori* and extra-intestinal symptoms in children

Idiopathic thrombocytopenic purpura (ITP)

Several reports on recovery of thrombocytopenia after *Hp* eradication in adults and children with chronic ITP have been published[45-48].

In 47 Dutch children with chronic ITP the prevalence of *Hp* infection was 6.4% and all *Hp* positive patients achieved partial or complete remission of their ITP, however the follow-up time was rather short (six months) and unlike in adults the disease is characterized by spontaneous remissions in one-third of the children, particularly in the first year after the diagnosis.

Gastroesophageal Reflux Disease (GERD)

In adults, *Hp* infection might have a protective role against GERD. The role of *Hp* in GERD in children remains controversial and currently insufficient data have been published [49-51].

Short stature

There is insufficient evidence that *Hp* infection is causally related to short stature[52]. Many studies on this item have been conducted in countries where confounders with negative effects on growth in children might play a role: low socio-economic status, helminthic infections, iron deficiency anemia and malnutrition. In those studies height and weight were usually not expressed as standard deviation scores, local reference curves were not available (usually WHO reference curves were used), and parental heights were unknown.

With respect to the possible pathophysiology, laboratory examinations have so far focused on Ghrelin, a strong growth hormone secretagogue, that is predominantly produced by entero-endocrine cells in the stomach. However, so far, most studies have been reported on total Ghrelin concentration, rather than the acylated form or the ratio between acylated and total Ghrelin. It is now generally believed that acylated Ghrelin is the active form of the hormone[27,53-56].

Iron deficiency anemia

There are some plausible explanations for links between iron deficiency anemia

and *Hp* infection. One can imagine that the *Hp* infection can produce gastritis and/or erosions and ulcers with chronic blood loss. Moreover, *Hp* gastritis may have reducing effects on gastric acid secretion and iron absorption. Another mechanism could be utilization of iron by *Hp* for its growth.
Confounders in studies on *Hp* and iron deficiency anemia are low socio-economic state, poor hygienic standards and malnutrition. Only one study described an increasing blood hemoglobin value in *Hp*-positive children after eradication therapy. More well-designed studies in developed as well as developing countries would be useful [57-59].
Recent guidelines recommend that in children with refractory iron-deficiency anemia, testing for *Hp* infection may be regarded after other causes have been ruled out [52].

1.3 DIAGNOSIS

So far, guidelines for the management of *Hp* infection in children recommend endoscopy to exclude other pathological causes for the child's symptoms[52]. The reasoning behind this recommendation is that no specific complex of clinical symptoms and signs has been established for children. Reflux-esophagitis, eosinophilic esophagitis, celiac disease or Crohn's disease could cause symptoms similar to those caused by gastritis or ulcer and can only be ruled out by endoscopy. The current gold standard for the detection of an *Hp* infection is endoscopy with gastric biopsies for histology, a rapid urease test and a culture with susceptibility testing. Cultures of gastric tissues have a specificity of 100%, but a relatively low sensitivity of 38-80%. PCR testing in gastric tissue can detect genes associated with virulence factors and antibiotic resistance. As endoscopy is both invasive and expensive and particularly in children usually requires deep sedation or anesthesia, non-invasive tests have been developed. The ^{13}C-urea breath test (UBT) and the monoclonal stool test have been validated well in children older than 6 years for the detection as well as the eradication control of *Hp*. Unfortunately these non-invasive tests have not sufficiently been validated in younger children, below the age of 6 years[60-62].

1.4 EPIDEMIOLOGY AND PREVALENCE

The prevalence of *Hp* infection varies greatly between developing and developed countries, respectively 90% versus 40% at the age of 40 years, and is declining worldwide over the last decades[63]. The prevalence of *Hp* in children in the Netherlands is 1.2% at the age of 2-4 years and 9% at the age of 7-9 years and most of the infected children are offspring of originally non-Dutch parents[64,65]. In developed countries where the prevalence of *Hp* infection in children is low, the prevalence is increasing with age because of a cohort effect [63,65,66]. In institutions of intellectually disabled persons a relatively high prevalence has been found, including in the Netherlands[15,66-68].
Therefore, despite the decreasing prevalence of *Hp* infection in developed countries such as the Netherlands, *Hp* infections deserve attention and focus should be on those particular population groups, where the prevalence of *Hp* is relatively high.

1.5 RE-INFECTION AND SPONTANEOUS CLEARANCE

Studies in adults in industrialized countries have shown that the rate of *Helicobacter* re-infection following an initial satisfactory eradication response is relatively low (3.4%), while the highest rates of recurrence (8.7%) have been reported in geographical regions with a lower development index and a very high prevalence[69]. Most cases of recurrence take place within twelve months after treatment. Studies using molecular fingerprinting techniques (e.g. polymerase chain reaction) favor the hypothesis that reappearance of a *Hp* strain identical to the pretreatment strain is defined as recrudescence of the *Hp* present in that patient prior to treatment and usually not as a true re-infection and describe lower recurrence rates [69-71]. Niv *et al* described a literature search of 17 studies on *Helicobacter pylori* recurrence in adults in developed countries and in developing countries and found annual recurrence rates of 2.67% and 13.0%, respectively [72]. Studies in children indicate a re-infection rate of 2.3-12.8% depending on the *Hp* prevalence in the country and the follow-up time (see table 1).

Table 1 Re-infection rates in children

Year	Country	*Hp* reinfection (%)	Follow-up Months
1992 [73]	Italy (Turin)	9 0 11	6 12 18
1998 [74]	Japan	2.4	22
1999 [75]	Ireland/Dublin	11.5 (4.3 if >5 years of age)	24+/- 14
2002 [76]	Germany	2.3	15.5
2004 [77]	N-Ireland/ Belfast	2.4	
2004 [78]	Estonia*	6.7	84
2005 [79]	Italy (South)	12.8	18-43
2006 [80]	France	5.4	148 patient years

1.6 GUIDELINES FOR DIAGNOSIS AND TREATMENT

1.6.1 Adults

Updated guidelines on behalf of the European *Helicobacter* Study Group (Maastricht-I11 Consensus Report) for management of *Helicobacter pylori* infection in adults have been published in 2007[81]. One small paragraph in that report provides directives on *Hp* testing in children with recurrent abdominal pain and iron deficiency anemia. In the fourth edition of the Maastricht consensus report (2012) no recommendations on *Hp* in children have been included [82]. The Dutch standard for general practitioners (NHG-standard) lacks recommendations for treating children with *Hp* infection[81,83].

1.6.2 Children

Dutch evidence-based guidelines for *Hp* infection in children do not currently exist. Up to now, directives from Ireland and Canada have been used by the Dutch Society of Pediatrics. Recently NASPGHAN (North American Society Pediatric Gastroenterology, Hepatology and Nutrition) and ESPGHAN (European Society Pediatric Gastroenterology, Hepatology and Nutrition) published joint evidence-based clinical guidelines for the diagnosis and treatment of *Hp* infection in children in North-America and Europe [52,84-87]. In these guidelines it is recommended that the initial diagnosis of *Hp* infection should be based on either histopathology plus a rapid urease test on the biopsy or a positive culture. In contrast, the recommendations for adults suggest a test-and-treat regimen, based on one positive non-invasive diagnostic test under strict conditions. "Test-and-treat" is definitely not recommended in children. First-line eradication therapy usually comprises triple therapy (protonpump-inhibitor + amoxicillin+ clarithromycin (or metronidazole) or bismuth salts + amoxicillin+ metronidazole during 7-10 days, or sequential therapy. The ^{13}C-urea breath test and the monoclonal stool test are reliable non-invasive tests to determine whether *Hp* has been eradicated 4-8 weeks after completion of therapy.

In the Netherlands the publication of evidence based guidelines is scheduled for 2012.

1.7 RESISTANCE TO ANTIBIOTICS

Antibiotic resistance of *Hp* is one of the main reasons for eradication failure in adults as well as in children and there is evidence that the prevalence of resistance is increasing. Generally the prevalence of resistance to macrolides is higher in isolates from children than those from adults as opposed to results for metronidazole[88]. In a French study on 27 treatment regimens a global failure of 25.8% was found and resistance to clarithromycin almost perfectly predicted failure[89]. The resistance rate

to amoxicillin is very low in Europe. More recently alternative therapeutic regimens have been introduced including sequential therapy and quadruple therapy[90-92], to overcome the problems of resistance. So far, promising results have been obtained, although the knowledge on local resistance rates remains key to effective therapy. In most Northern and Western European countries antibiotic resistance does not play a major role, but in Southern-Europe, Africa and Asia high resistance percentages have been found for clarithromycin and metronidazole. The relatively low prevalence of resistant strains in our country (1-5 and 7-33% to clarithromycin and metronidazole, respectively) has led to guidelines on test-and-treat-policy for adults below the age of 45 years (see table 2). However resistance rates are increasing in Dutch adults, and therefore surveillance will remain necessary in order to maintain this regimen for the future.

Table 2 *Hp* resistance to Clarithromycin and Metronidazole in Dutch adults (1993-2003)

Year of publication and reference	Period	Region/city* of the Netherlands	Clarithromycin % (N)	Metronidazole % (N)
1997[93]	1993 1996	Not known	Not tested	7 (245) 32 (509)
1996[94]	1994-1995	North		18 (200)
		South	1 (780)	16 (430)
		Amsterdam*		19 (150)
1999[95]	1997-1998	West	1.6 (123)	24.3 (123)
		North-East	1.5 (65)	21.5 (65)
		South	2.3 (43)	11.6 (43)
2001[96]	1998	Amsterdam*	5 (100)	33 (100)
		Hoogeveen*	1.3 (77)	23.4 (77)
2001[97]	1995-2000	North-East	2.1 (5946)	18.8 (5946)
2003[98]	1995-2000	Zaandam*	4.8 (724)	25.8 (727)
2005[99]	1998-2003	Den Bosch*	3 (959)	14 (960)
2006[100]	1997-2002	Doetinchem*	1 (1123)	14.4 (1125)

1.8 AIM, RESEARCH QUESTIONS AND OUTLINE OF THE THESIS

Introduction of non-invasive diagnostic tests and test-and-treat policy have led to a decreasing prevalence of the infection and its main complications. However, the interest of the scientific society working on *Hp* has mainly highlighted work on adults, as can be seen from Figure 1. There are important reasons for pediatric gastroenterologists to focus on *Hp*:

- the continuous immigration of children from relatively high-prevalence countries (particularly Turkey, Morocco, Somalia and China), as well as the adoption of children from abroad. As the infection is usually acquired early in life, these children may already have been infected in their respective country of origin.
- the increasing resistance rates of the bacterium, which diminishes the eradication rate and which could be the reason for recrudescence of the infection after a negative eradication control.
- the awareness that the first stage of the infection in humans occurs usually during childhood. The discovery of this should have given an impulse on the study of *Hp* in children.

The aim of this dissertation is to present scientific data on the prevalence and treatment of *Hp* infection in children.
The thesis is divided in four parts:
Section A (*chapters 2 and 3*) provides an overview of the literature on *Hp* in pediatrics published between 2005-2006 and 2009-2010 respectively.
Section B (*chapters 4-6*) focuses on the prevalence of *Hp* infection in children and discusses its complications. Chapter 4 contains data on the prevalence of *Hp* infection in young Dutch children within the general population. Chapter 5 describes aspects of one of the severe complications of *Hp* infection in children, ulcer disease, in a European-wide multicenter study. In chapter 6 results of a prevalence study of *Hp* infection in a developing country (Bandung, Indonesia) are described. Here a new monoclonal stool antigen test was used for a group of 150 young Indonesian children.
Section C (*chapter 7*) discusses the main reason for eradication failure, the antibiotic resistance of *Hp*, for both adults and children in the Netherlands.
The results of this thesis are discussed in **Section D**, *chapter 8* and in this chapter also considerations about future scientific research on *Hp* are addressed.

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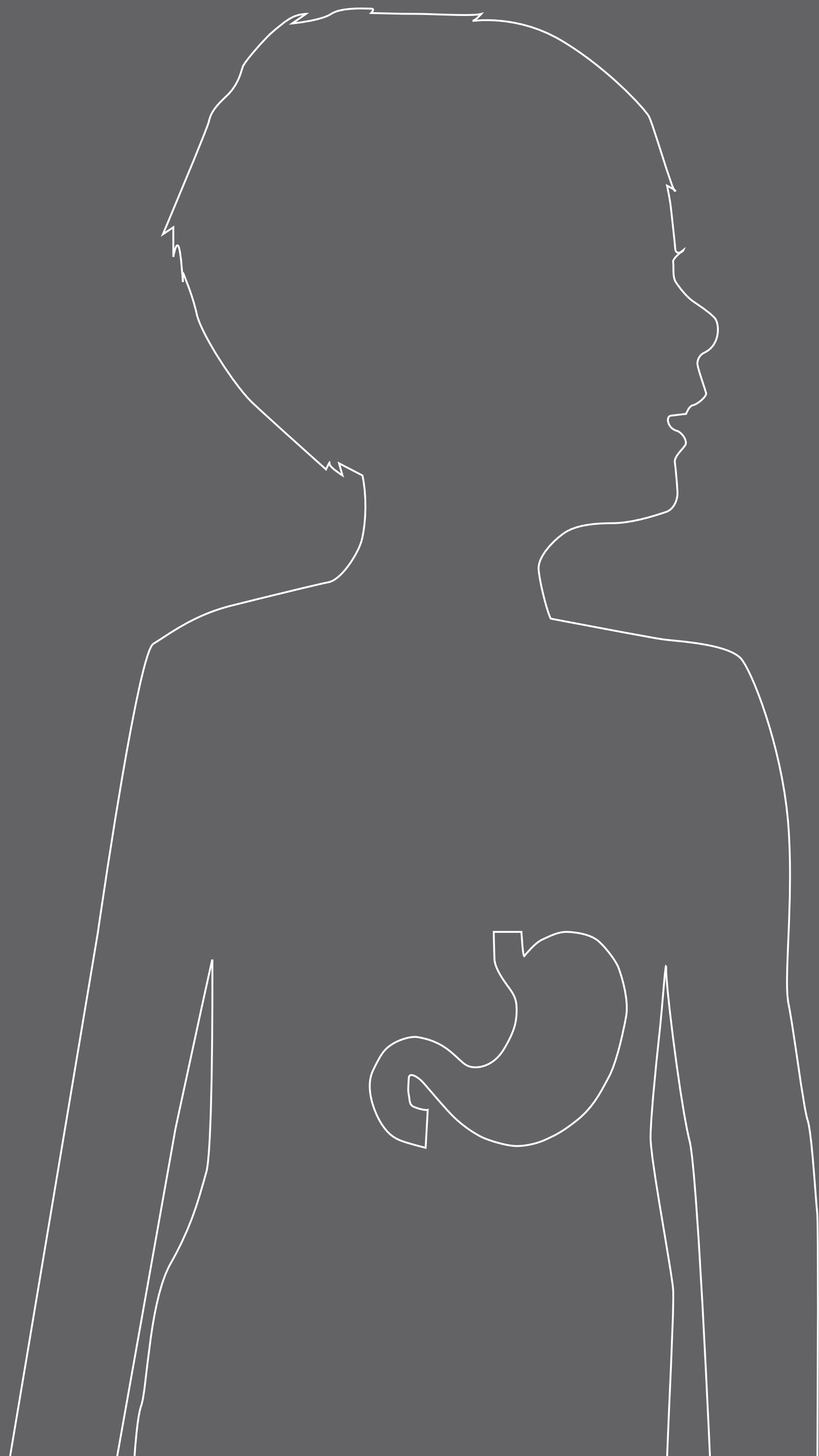
SECTION A

REVIEWS



CHAPTER 2
Helicobacter pylori infection in pediatrics 2005-2006

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ABSTRACT

This review summarizes the literature on *Helicobacter pylori* infection in childhood between April 2005 and March 2006, and includes guidelines of the Canadian *Helicobacter* Study Group Consensus Conference, non-invasive tests, optimum therapy regimens and problems with resistance, and reviews on immune mechanisms in the gastric mucosa that may lead to the development of an effective vaccine.

Keywords: *Helicobacter pylori*; pediatrics; recurrent abdominal pain, stool antigen test.

INTRODUCTION

In 2005 "the Nobel Prize in Medicine or Physiology" was awarded to Marshall and Warren for the discovery in 1982 that most peptic ulcers are caused by an infection with *Helicobacter pylori* [1-3]. It took a further 10 years before literature about *H. pylori* infection in children was published. It is now well accepted that the bacterium is usually acquired during childhood, mainly from the mother via vomitus or fecal oral route [4-9]. Great strides have been made since. In July 2005, the Canadian *Helicobacter* Study Group published an evidence-based Consensus Update on the approach to *H. pylori* infection in children and adolescents, adopted in 2006 by members of the *H. pylori* Pediatric Task Force in the U.S.A [10]. We hope to reach a combined NASPGHAN-ESPGHAN consensus before the World Congress of Pediatric Gastroenterology, Hepatology and Nutrition meeting in Brazil in 2008.

BASIC RESEARCH

Peeters published an excellent overview about the peptide ghrelin, related to motilin which has been suggested to have " saginary" effect in *H. pylori* infection. *H. pylori* infection could influence ghrelin-secreting cells, and impairs the secretion of histamine, pepsin and gastric acid [11]. Eradicating *H. pylori* can lead to a rise in plasma ghrelin, which could promote the development of obesity, increasing the risk of reflux disease and subsequent risks of Barrett's esophagus and esophageal adenocarcinoma. The suggestion that *H. pylori*-eradication may contribute to the obesity epidemic in industrialized countries is an interesting hypothesis to ponder!
Ernst highlighted an article of Velin *et al* about the essential role of mast cells as mediators in experimental *Helicobacter* clearance by vaccination because mast cells-deficient mice could not be protected by immunization; this could be changed by reconstituting them with bone marrow-derived mast cells [12,13].
Ceponis and Jones summarized in the Canadian *H. pylori* Update the current knowledge of specific *H. pylori* factors. *H. pylori* infection can modulate signal transduction pathways in multiple host cell types and specific bacterial factors can activate certain cascades in the mucosa. The bacterium can also lead to opposing effects: anti-apoptotic by inducing NF-kB activity and can suppress interleukin (IL)-4 induced signal transduction [14].

PREVALENCE, INCIDENCE AND TRANSMISSION

The prevalence of *H. pylori* infection is declining in developed countries. Nevertheless, immigrants and indigenous people continue to carry a high burden of

H. pylori infection and disease in their children. In Canada, the immigrant population comprises 200.000 individuals per year. The prevalence in children undergoing gastrointestinal endoscopy from 1990 to 1994 was 26-43%: it has now decreased to about 5%. Jacobson also highlights the study by Miller (2003), in which adopted children have a seroprevalence of 16%, 20% and 49% respectively. He suggests more studies in children with different geographic, socioeconomic and ethnic backgrounds using validated screening tools to see whether eradication is associated with reduced *H. pylori*-related diseases or with significant benefit in adulthood [15].
New data on prevalence come from Asia: Nizami found that early colonization in 148 children showed a decreasing trend with increasing age (80% at 1 month of age, 67% at 9 months). The researchers did not, however, study children over 1 year of age [16]. Singh *et al*. determined prospectively the prevalence of *H. pylori* in 58 children with upper abdominal pain (UAP) and 182 controls. In the UAP-group, the prevalence of *H.pylori* was 53.4%, in the group children without UAP, 28%. The overall prevalence increased with age; 82% of the children with UAP were negative after eradication, with a further 18% after second eradication therapy. All treated children with UAP remained symptom-free for two years. They concluded to a strong link between *H. pylori* infection and UAP [17]. Alborzi *et al* collected stool samples from children from different age groups in Shiraz and found prevalence rates of *H. pylori* of 82, 98, 88, 89 and 57% in age groups of 9 months, and 2, 6, 10, and 15 years, respectively. There was a significant decrease in the 15 year old teenagers [18].
Wong *et al* [19] studied the prevalence of *H. pylori* in symptomatic Chinese children retrospectively from 1997-2004 to assess the impact of an aggressive eradication program. From 159 patients undergoing gastroscopy, 119 had gastritis, 13 had peptic ulcer disease (overall rate of proven *H. pylori* infection 25.6%). They did not find a significant decrease of overall prevalence (33% in 1997, 27.7% in 2004), but reported that increasing age was significantly associated with a higher risk of infection. Their hypothesis was that eradication efforts were unsuccessful, possibly due to Chinese eating habits (cross infection from sharing chopsticks).
Recently, Rowland *et al* published a prospective study of age-specific incidence of *H. pylori* infection in children between 24 and 48 months of age, by using ^{13}C-urea breath test (^{13}C-UBT). They found a positive test in 28 of 327 index children (8.6%) at baseline assessment; during the next 4 years 20 children became infected. As in previous studies, the infection was acquired at a very young age with a declining risk after 5 years of age. Risk factors were having an infected mother, an infected older sibling, and delayed weaning from a feeding bottle [9].

MOTHER TO CHILD TRANSMISSION

The role of infected mothers has been described in 2002 [4] and stated by Konno *et al* [5] in a 5-year follow-up study of 44 children. They collected gastric juice samples for culture and DNA-analysis from 69 *H. pylori*-positive mothers. None of the children acquired *H. pylori* during the first year of life. Five children were tested positive within 5 years by serology and stool antigen test. Strains of the 5 positive children exhibited DNA fingerprinting patterns identical to those of their mothers.
In a follow-up of children up to 36 months old using monoclonal stool test, infected mothers were the main source of *H. pylori* infection of their children, as the mother, being primary carer, has closer contact with the infant than the father in the first year of life [8].

SYMPTOMS

Abdominal pain

The association between chronic abdominal pain and *H. pylori* infection is still hotly debated.
Tindberg *et al* investigated the association between type-specific H.pylori infection and gastrointestinal symptoms in a cross-sectional study of Swedish schoolchildren. Infection was investigated by IgG-antibodies in serum and confirmed by immunoblot and UBT. Abdominal pain was reported by 63% of the children and recurrent abdominal pain (RAP) by 13%; 16% were infected; 73% of these children had CagA antibodies and 59% VacA antibodies. The authors did not find a positive association between *H. pylori*-status and occurrence of abdominal pain: RAP was unrelated to the infection (OR 1.0; 95% CI 0.5-2.1), when adjusted for sex, age and family background. The prevalence of RAP was lower in children with CagA+ and VacA+ infections than among uninfected children [20].
Yang *et al* investigated 1271 children with questionnaires to define RAP or short-term RAP (SRAP) with pain duration from 2 weeks to 3 months. All children with RAP, SRAP or a combination were tested for *H. pylori* by serology. Prevalence rates of RAP and SRAP were 9.8 and 5.5%, respectively. Children with SRAP had a higher anti-*H. pylori* seropositivity rate than those with RAP and controls. One year later 71% of the seropositive children became symptom-free, regardless of persistence of *H. pylori*. Infection was more frequently found in children with SRAP [21].
The subcommittee on chronic abdominal pain reviewed the predictive value of laboratory tests. The authors conclude that the coexistence of abdominal pain and an abnormal test result for *H. pylori* infection does not necessarily indicate a causal relation between the two [22].

Non-ulcer dyspepsia (NUD)

Kalach *et al* [23] investigated 100 children older than 6 years with NUD, by endoscopy for epigastric pain in a prospective double-blind study; 26 children were infected. No differences in age or symptom characteristics between infected and non infected children was found, except for epigastric pain during meals, which was more frequent in non-infected children. They concluded that *H. pylori*-infected NUD-children had no specific symptoms.

At the end of 2005 Talley *et al* published a review on the evaluation of dyspepsia in adults. As is the case in adults, the test-and-treat strategy is not recommended in children with dypepsia: endoscopy is mandatory in this age group [24].

Gastroesophageal reflux disease

The question is whether testing for *H. pylori* is necessary in infants with gastroesophageal reflux disease (GERD). Moayyedi reviewed the literature about the association between GER and *H. pylori* infection. His conclusion is that *H. pylori*-induced hypochlorhydria is frequent in adults, but rare in children and that therefore eradication is unlikely to have an important impact on GERD or protonpump inhibitor (PPI) efficacy in this age group. His advice is that children with GERD, diagnosed clinically or by pH studies, do not need to be tested for *H. pylori*, unless they have endoscopy and have proven infection [25].

As GERD has been suggested as a contributing factor to otitis media, Bitar *et al* investigated the possibility of a role of *H. pylori* in middle ear disease in children, however, all 28 middle ear fluid cultures and polymerase chain reaction (PCR) were negative; also 13 adenoids were negative for *H. pylori* by PCR. Seven of 18 patients had symptoms suggestive of GERD preceding the study, but this had no impact on the results of the study [26].

Gastritis

Leung *et al* emphasized the diagnostic role of macroscopic observation of the gastric mucosa at endoscopy [27]. Ricuarte *et al* studied the prevalence of atrophic gastritis in childhood with this hypothesis. Of 173 children, atrophic mucosa near the antrum-corpus border was present in 16% of the positive children, primarily as pseudo pyloric metaplasia (median age 15 years). As gastric atrophy occurs in infected children living in countries with a high incidence of gastric cancer they recommend that biopsies be taken near the lesser and greater curvature just proximal to the anatomical antrum-corpus border as well [28].

EXTRADIGESTIVE MANIFESTATIONS

In the pediatric age group the following have been reported: anemia, idiopathic thrombocytopenic purpura (ITP), short stature, diarrhea, food allergy and sudden infant death syndrome, some without sufficient evidence [29].

Idiopathic thrombocytopenic purpura

Although the same author in an earlier study in 2003 reported an increased incidence of *H. pylori* in patients with chronic ITP, in a prospective study, Jaing *et al* did not find evidence of an association [30,31]. Sherman and Lin mentioned the potential for molecular mimicry with antiplatelet antibodies, recognizing the CagA protein of *H. pylori* as a possible explanation for an association. The Canadian group did not include ITP as extradigestive manifestation in the recent guidelines [29].

Anemia

The Canadian Consensus Group concluded that there is sufficient evidence available to consider unexplained sideropenic anemia as an extradigestive manifestation and to consider test-and-treat strategy in such cases.

One recent study from India reported reduced hematological response to iron supplementation in asymptomatic children with *H. pylori* compared to children without infection. The mean serum ferritin was similar at admission and improved in both groups of children, but infection had a significant adverse effect on response to iron therapy [32].

Growth

In 2005 two studies have been published about the relationship between growth and *H. pylori* infection: Sood *et al* compared height, weight and body mass index (BMI) of 97 positive children with dyspeptic symptoms to 160 children with dyspepsia without infection. They found no significant difference between mean weight and height SD score in the infected and not infected group [33]. Mera *et al* investigated, whether a newly acquired infection affects height and weight within 16 months by performing UBT and anthropometry every 2-4 months. Authors observed a significant decrease in growth velocity during the first 4 months after infection and the children showed no height catch-up. Infected children had a small decrease in weight, in comparison to non-infected children. Their conclusion is that *H. pylori* infection causes a non-transient negative effect on height and weight in Colombian children [34].

In the Canadian Consensus Report, Sherman and Lin did not find firm evidence for the role of *H. pylori* infection in growth [29].

Other reported manifestations such as food allergy, diarrhea and sudden infant death syndrome were not added to the list of extra intestinal symptoms by the Canadian group.

DIAGNOSTIC TOOLS

None of the non-invasive tests are 100% specific and sensitive. ^{13}C-Urea breath test is reliable for detecting infection in children older than 6 years of age but can give false-positive results in younger children [10]. There was a small number of very young *H. pylori*-positive patients in these studies, making it difficult to validate new diagnostic tests in this age group.
Mégraud *et al* reported in a multinational study on four non-invasive tests that UBT had the best sensitivity in all age groups, followed by serology, stool antigen test and antibody detection in urine. In all tests, except the stool test, better sensitivity was observed with increasing age. The urine office test exhibited a very low sensitivity [35].
The Canadian Consensus group concluded that UBT is currently the best available noninvasive diagnostic test in children and published a list of variables that may influence the results of the test [10,36].
Recently Nugalieva *et al* raised attention to the problem of false-positive UBT and recommended confirmation of a single positive test in low-prevalence populations by using a test that measures a different parameter (UBT confirmed by stool test) [37].
The polyclonal antibody-based stool antigen test (HpSA) is not as reliable as UBT, neither pre-treatment nor after eradication [35], but monoclonal stool antigen tests perform as well as UBT. The Canadian group judged that it was too premature to recommend stool antigen testing as an alternative to UBT, but in settings where the breath test is not available for children, the monoclonal test is an excellent alternative to assess *H. pylori* status pre-and post-treatment [36]. Raguza *et al* evaluated 127 children to compare the accuracy of a modified polyclonal stool antigen test with the gold standard. However, there were no H.pylori positive infants below the age of 2 years in his study. Three patients showed false-positive results and two false-negative results for the HpSA. The sensitivity of the HpSA test was higher in children in the age of 6-18 years (100%) than in 2-6 years (80%). The specificity was respectively 95-100% and 96.4% [38].
Haggarty *et al* also used the polyclonal HpSA test in a study of stool samples from children at two time points, 3 months apart; PCR was performed on all 26 pairs reverting from positive to negative (transient positives), all four persistent positive pairs and 10 randomly selected persistent antigen-negative pairs. In 15 of 26 transient positive stools *H. pylori* was sequenced and identified in 12 and other

Helicobacter spp. were identified in three. They suggested that transient positive stool tests are common and represent *H. pylori* in majority of cases; however some positive stools may represent other *Helicobacter* species [39].
Hauser *et al* compared a multi-step polyclonal versus one-step monoclonal enzyme immunoassay in stool with ^{13}C-UBT in 43 children; 18 children were positive (positive UBT). The polyclonal stool test had a comparatively good sensitivity, but lower specificity compared to UBT. The one-step monoclonal rapid test also had a comparable sensitivity and specificity, when a weakly positive test (visual interpretation) was considered negative [40].
Kalach *et al* tested the rapid monoclonal stool test in 128 children and observed the highest performance of the test in children older than 10 years (sensitivity 100%), in contrast with 75% in those younger than 5 years; in this study he found 11 discordant results of the test compared with gold standard [41].
Antos *et al* evaluated a novel rapid monoclonal one-step immunochromatographic assay for detection of stool antigen and concluded that this quick test shows a good interobserver agreement, but equivocal results in 5% [42].
More studies with quick tests in stools are needed; however, in settings without possibilities for UBT or enzyme immunoassay, the immunochromatographic test could become an alternative to assess *H. pylori* status pre- or post-treatment in children [36].
A study from Thailand evaluated the performance of a rapid office-based serologic test (Assure™ , Genelabs Diagnostics, Singapore) and the immunoblotting for the diagnosis in symptomatic children. The sensitivity of the test was 96%, specificity 94.6% versus immunoblot, 100 and 96,2% respectively, so the test seems to be reliable for the diagnosis of infection in Thai children [43].
The above Assure test was also evaluated in 130 children by Pelerito *et al*. They found a lower sensitivity (75.7%) and a specificity of 95.0%, which increased to 98,6 and 95% when a longer reading time of 45 minutes was considered [44].
The use of serology-based tests could not be advocated by the Canadian Consensus group anymore because of their low accuracy in young children. In a study from Lithuania a seroprevalence of 57% was found; following gold standard it was 79% [45].

Invasive diagnostic methods require endoscopic biopsies and include rapid urease testing, histology and culture. When used in combination, these tests are still considered the “gold standard” for the diagnosis of *H. pylori* in children. The added advantage of this approach is the detection of upper gastrointestinal pathologies including complications of the infection, such as nodular gastritis, peptic ulcer disease, gastric cancer and MALT-lymphoma. Biopsies are necessary for determination of antibiotic resistance and virulence factors [46,47].

TREATMENT

Based on the data in pediatric literature and in adults the Canadian Consensus group decided that the recommended first-line eradication therapy should include a PPI and clarithromycin, combined with either amoxicillin or metronidazole. Higher eradication rates may be achieved by a longer treatment regimen (triple therapy 14 days instead of 1 week) [10].
A randomized clinical trial from Italy showed that a novel 10-day sequential treatment (omeprazole plus amoxicillin for 5 days, followed by omeprazole plus clarithromycin plus tinidazole for another 5 days) achieves a significantly higher eradication rate than standard triple therapy (omeprazole, amoxicillin and metronidazole) for one week [48].
Khurana *et al* summarized data from studies that have examined treatment efficacy, safety, drug resistance and reinfection rates. Treatment efficacy was reduced in the presence of metronidazole and/or clarithromycin resistance. Resistance to metronidazole and/or clarithromycin is common and no therapy has yet been identified as safe and consistently effective to eradicate *H. pylori* infection [49].
Elitsur *et al* assessed the resistance rate against clarithromycin in 16 positive children by the FISH technique in gastric biopsies; the primary resistance rate in this small group was very high (31-38%) [50]; Boyanova *et al* found primary clarithromycin resistance in 12.5% and metronidazole resistance in 15% of Bulgarian children [51]. Recently Koletzko *et al* assessed the bacterial resistance in children from 14 European countries. She reports an overall resistance to clarithromycin of 24%, to metronidazole 25%; and to amoxicillin 0.6%, the last being very low [52].
A Russian group investigated failure of triple therapy; patients were randomized to receive a 2-week course of bismuth, amoxicillin with either nifuratel or furazolidone plus omeprazole. Both schemes produced good cure rates, but nifuratel is preferred because of lower frequency of side-effects. Antibiotics susceptibility tests have not been carried out in this study [53].
The Canadian group discussed whether treatment of *H. pylori* in childhood will alter the two-to sixfold increased risk of developing gastric cancer among infected patients. A population-based test-and-treat policy in children is not justified, except in groups with a high risk of developing gastric cancer (Japanese or those with a strong positive family history) regarding negative cost-benefit analysis [54].

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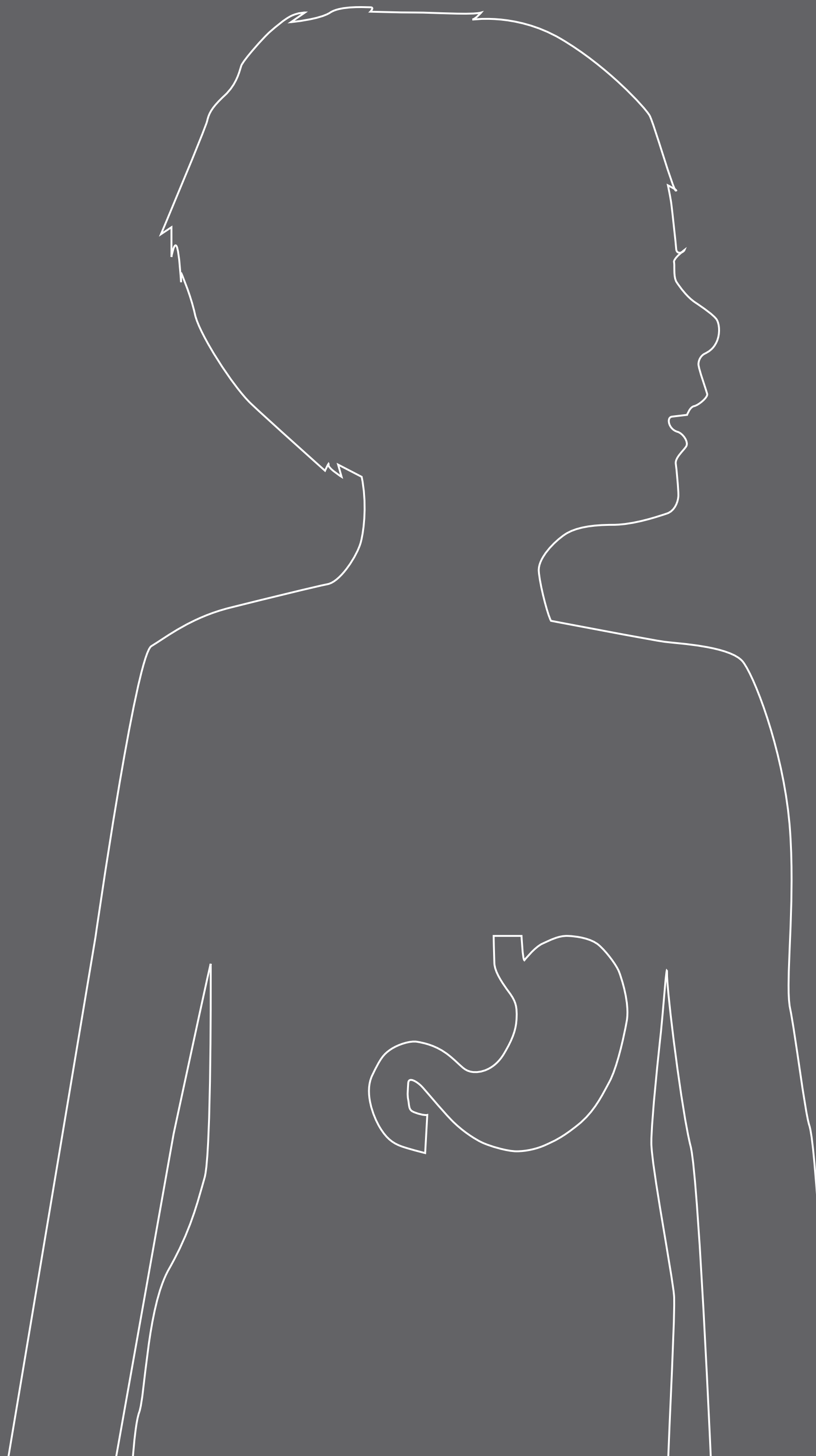

CHAPTER 3

Helicobacter pylori infection and childhood 2009-2010

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ABSTRACT

Pediatric-based *Helicobacter pylori* research continues to contribute significantly to our understanding of both clinical and pathophysiological aspects of this infection. Here, we review the published pediatric *H. pylori* literature from April 2009–March 2010.

Analysis of pediatric *H. pylori* strains continues to suggest that CagA+ and CagPAI competent strains are less prevalent than in adult isolates. Studies from the Middle East report a high *H. pylori* prevalence and intrafamilial transmission. Data continue to show a lack of association between *H. pylori* and recurrent abdominal pain of childhood, gastroesophageal reflux disease, and growth retardation.

Recent probiotic trials have not shown a benefit on *H. pylori* eradication in children, while sequential therapy remains an attractive therapeutic eradication strategy in children, which requires validation in different geographic regions.

Keywords: Review, recurrent abdominal pain, resistance, sequential therapy.

PATHOPHYSIOLOGY

The relationship between apoptosis and *Helicobacter pylori* remains an important aspect of *H. pylori* pathogenesis research. In a Polish cohort of children with symptomatic *H. pylori* infection and gastritis, Fas receptor expression was increased in the CD4+ T cell population in the lamina propria at diagnosis and Fas antigen expression was significantly decreased in both epithelial cells and mucosal lymphocytes following successful eradication[1]. The authors speculate that apoptosis of CD4+ T cells could contribute to bacterial persistence in the mucosa.

Studies of the spectrum of *H. pylori* genetic variability between childhood and adult isolates may help to elucidate age-specific microbial genetic factors involved in pathogenesis. Rick *et al* suggested that in situ hybridization techniques, which reflect in vivo gene transcription, may be superior to testing isolates for CagA in vitro and used this method to confirm the association between gastric mucosal *H. pylori* CagA expression and pediatric gastro-duodenal ulcer disease [2]. While children had a higher prevalence of CagA+ strains compared to adults in one study from China, CagA was not shown to influence their disease phenotype [3]. *H. pylori* strains from symptomatic children in the USA and Greece were more likely to be CagA- and lack a functional CagPAI, although the USA isolates were more likely to retain outer membrane protein (OMP) and adherence gene expression than adult strains, a possible microbial advantage for early life infection and colonization [4,5]. The adherence properties and expression profile of OMP genes of *H. pylori* isolates from 200 symptomatic patients were characterized by Odenbreit *et al* [6]. Apart from AlpA and AlpB, the expression of other OMPs was variable. In vitro interleukin (IL)-8 expression was again shown to be increased by CagA+ strains, while co-expression of OipA, but not OipA alone, further enhanced IL-8 secretion. The presence of the putative virulence factor gene IceA, while common, was not predictive of the extent of inflammation on histology in Slovenian children; CagA and VacA s1 genotypes were associated with more severe gastritis and greater bacterial density [7].

Autophagy, an evolutionary conserved process in eukaryotic cells, is an integral component of our innate immune system and is implicated in the pathogenesis of a number of gastrointestinal diseases [8]. *H. pylori* VacA toxin has recently been shown to induce autophagy in gastric cells in vitro, a potential host defense strategy to limit toxin damage, but auto-phagosome formation may also facilitate bacterial replication and survival [9]. *H. pylori* has also been shown to multiply in autophagosomes in macrophages, suggesting that it may be subverting autophagy for its own benefit [10].

EPIDEMIOLOGY AND TRANSMISSION

The estimated 7.1% prevalence of *H. pylori* infection in asymptomatic children in the Czech Republic is among the lowest reported in Europe [11]. Sykora *et al* found a positive association with increasing age, the number of children in the household (OR 4.26,CI 1.91–9.80), lack of formal education of the father (OR 0.23; CI0.18–0.64), and institutionalization (OR 6.33; CI 2.25–26.50). Their findings are consistent with improving trends in living and housing conditions in recent years and with decreasing family size.

While the prevalence in Western countries and America is decreasing, the high prevalence in Asia remains. Malekzadeh *et al* using stool antigen and serology testing, reported a high prevalence of *H. pylori* infection in 592 Iranian children from Shiraz and 386 children from Rafsanjan (82% and 47%, respectively,) [12]. Iran and Iraq have a high prevalence of CagA$^+$ *H. pylori* [13]. In a study from Pakistan, a seroprevalence of 47% among 1976 children (1–15 years) was reported. The father's educational status, crowding, and increasing age, were the main factors influencing sero-positivity [14].

Understanding the intrafamilial spread of *H. pylori* is an important aspect of transmission research. A study of 100 children with abdominal symptoms (44 *H. pylori*+) found a higher percentage of *H. pylori* infected siblings, mothers, and fathers, tested by urea breath test (UBT), among *H. pylori*+ than *H. pylori*- index cases ($p < .001$, $p < .001$ and $p < .035$, respectively) [15]. Each *H. pylori*+ child had at least one infected family member, implicating the family as the source of *H. pylori* infection in children. Nahar *et al* found evidence of intrafamilial transmission of *H. pylori* by characterizing *H. pylori* in 35 families, including 138 family members, using DNA fingerprinting [16]. Forty-six percent of strains from the mothers shared related genotype with strains from their children. Only 6% of parents shared a related genotype, suggesting mother– child transmission as the most probable transmission route.

In a study from Iran, Amini *et al* described the association between *H. pylori* infection and eating habits (sharing plates, glasses, and spoons) and found a significantly higher prevalence of *H. pylori* infection in families where common dishes were used[17].

Travis *et al* used UBT at 6-month intervals from birth to 24 months to describe possible water-borne transmission of *H. pylori* in a cohort study of 472 children from Mexico and Texas [18]. Their results provide some support for water-borne transmission. On the other hand, Vale and Vitor reviewed water- and food-borne transmission of *H. pylori* and concluded that the principal transmission route remains to be clearly defined [19].

SYMPTOMS

Recurrent Abdominal Pain

The discussion about the association between recurrent abdominal pain (RAP), epigastric pain, unspecified abdominal pain, and *H. pylori* infection in children continues. Thakkar *et al* published a retrospective study on upper digestive endoscopy in 1191 children with abdominal pain; 55 children (5%) were diagnosed with *H. pylori* infection, the second most common diagnosis after reflux esophagitis (23%) [20]. They agreed that earlier studies did not show a causal relation between *H. pylori* infection and abdominal pain in absence of ulcer disease, but conceded that there is a trend to offer eradication therapy once the *H. pylori* infection has been diagnosed. In a meta-analysis, Spee *et al* found no association between RAP and *H. pylori* infection in children and limited evidence for an association between unspecified abdominal pain and *H. pylori* in referred, but not in primary care patients [21]. Although current evidence does not support infection as a significant cause of common symptoms in children, guidelines on *H. pylori* screening in children are contradictory [22,23]. For example, discrepancies exist between the earlier European Pediatric Task Force on *H. pylori* report and the more recent Maastricht III statement, which suggests that although RAP is not an indication for a test-and-treat strategy in children, those with upper gastrointestinal symptoms should be tested after exclusion of other causes of symptoms [23,24].

Peptic Ulcer

H. pylori infection is the most important cause of primary duodenal ulcers in children. A retrospective study of differences between *H. pylori*+ and *H. pylori*- primary ulcers in 43 Chinese children diagnosed >8 years showed that boys vs. girls (91.3 vs. 50%) and older children (12 vs. 10 years) were more likely to have *H. pylori*+ ulcers (53.5%) [25]. In the *H. pylori*- group, ulcer recurrence was more common. In an editorial comment, Oderda *et al* noted the emergence of 'a new disease': *H. pylori*-negative gastric or duodenal ulcer, occurring more frequently in younger children, without gender preference and tending to have a higher recurrence rate [26]. Rick *et al* investigated 51 children, of whom six had gastric ulcers (all *H. pylori*+) and 11 had duodenal ulcers (10 *H. pylori*+), and found *H. pylori* by 16S rDNA and CagA PCR significantly higher in children with ulcer compared with normal children [2].

Gastroesophageal Reflux Disease (GERD)

The role of *H. pylori* in GERD remains controversial, limited by sufficient published data in children. Both a positive and negative association between *H. pylori* and GERD was reported recently [27,28]. Moon *et al* found reflux esophagitis in 13 of 16

H. pylori-positive patients, but in only 38.1% of 404 *H. pylori*-negative children and concluded a positive association. However, the prevalence of *H. pylori* in the study was low, and they did not address CagA status in *H. pylori*-positive patients in the study. On the other hand, researchers in Turkey did not found a positive association between *H. pylori* infection and the severity of esophagitis [28].

DIAGNOSIS

Guarner *et al* published a ten-year review on diagnostic tests in children between 1999 and 2009, concluding that most commercial noninvasive tests now have adequate sensitivity and specificity for detecting the presence of *H. pylori*. They again emphasized that endoscopy with histopathology is the only method that can diagnose and confirm *H. pylori* infection, its lesions and other causes of symptoms; UBT test and monoclonal stool antigen test being good tests for post-treatment control [29].

The same rapid office-based stool test using an immunoassay with monoclonal antibodies was tested in young children in Germany and in France. Prell *et al* compared it to biopsy tests considered as reference in the setting of pre-and post eradication of *H. pylori* and found a sensitivity of 85.5–90.8% and a specificity of 91.0–97.6% [30]. Results of Kalach *et al*. were similar, showing a sensitivity of 87.5% and a specificity of 97.8% [31]. She *et al* confirmed the lack of clinical utility of serology testing in children and adults, including an unacceptably low IgM sensitivity of just 6.8% [32].

EXTRA-INTESTINAL MANIFESTATIONS

Iron Deficiency and Growth

The link between *H. pylori* infection and anemia or sub-optimal growth remains tenuous. Ferrara *et al* presented retrospective data on a heterogeneous group of 102 Italian children aged between 10 and 12 years with iron deficiency anemia, suggesting that children with both *H. pylori* infection (positive stool antigen test) and iron deficiency anemia were more likely to have a reduced height standard deviation score (SDS) in comparison with children with other causes of anemia [33]. However, the data spanning an 8- year period lacked growth velocity assessments, case-matched controls, and details regarding the etiological work-up. A cross-sectional study of children from a low socio-economic background from Mexico found an association between *H. pylori* infection and reduced height compared to uninfected matched controls and suggested that the risk was cumulative per annum above the age of 7 years [34]. In a contrasting study from Turkey, Gulcan *et al* did not find a significant association between anemia and growth retardation; a subgroup analysis did suggest an association between endoscopic mucosal disease and lower height SDS (p = .02) [35]. Chi *et al* did not find an association between *H. pylori* infection and growth failure in their cross-sectional study from Taiwan, albeit of high-school children and based again on height SDS rather than growth velocity [36]. An Australian cross-sectional study of refugee children from Africa also failed to find an association between *H. pylori* infection and subnormal anthropometric measurements [37].

A series of cross-sectional studies from Latin America did not find significant evidence linking *H. pylori* infection and anemia [38]. Children with a positive UBT in Cuba, Argentina, Bolivia, and Venezuela did not have a statistically increased risk of associated anemia in comparison with their UBT negative counterparts. In a study among Arab-Israeli children, a population with a high prevalence of both *H. pylori* infection and anemia, Muhsen *et al* only found a statistically significant association between low ferritin levels and positive *H. pylori* serology in children less than 5 years of age but not among older age groups [39]. Unfortunately, it remains difficult to extrapolate a causal inference from studies of such design.

Idiopathic Thrombocytopenic Purpura (ITP) and Platelet Dysfunction

A multi-center randomized controlled trial of *H. pylori* eradication in children with chronic ITP failed to show an effect of *H. pylori* eradication on platelet recovery [40]. Ferrara *et al* reported a positive effect of *H. pylori* eradication on the outcome of children with chronic ITP with a positive stool antigen test, although their study was not a randomized controlled trial [41]. One translational study described platelet aggregation dysfunction in children with symptomatic *H. pylori* infection, which improved post-eradication [40].

THERAPEUTIC ISSUES

Drug Resistance

Drug resistance is a growing problem in adults as well as in children. Kato and Fujimura studied 61 strains from Japanese children 4–18 years old from 1999–2007 and reported high primary resistance of clarithromycin (36.1%) and metronidazole (14.8%) with consequences for the eradication rate [42]. Double resistance was detected in 6.6% of the strains. In Bulgaria, resistance to clarithromycin and metronidazole was 19% and 16.2%, respectively; multidrug resistance was 1% [43]. Both authors did not find resistance to amoxicillin and recommend susceptibility tests before treatment. Other studies on resistance came from Asia and South America; a low clarithromycin resistance rate was found in Malaysia (2.1%), Taiwan

(10.6%), and Colombia (3.8%), in notable contrast to the high rates of metronidazole resistance in those countries [44–46]. In children from Thailand, clarithromycin resistance was 29.2% [47]. Raymond *et al* determined antimicrobial susceptibility in 530 biopsies between 2004 and 2007 by E-test and molecular methods [48]. Twenty-six percent of strains were resistant to clarithromycin, 61% to metronidazole and 13% to ciprofloxacin in adults; in an earlier study, they reported primary resistance of 22.8% for clarithromycin in children through a one-year period. All authors recommend periodic monitoring of antibiotic susceptibility to tailor treatment and prevent eradication failure.

Sequential Therapy

Pediatric trials of sequential therapy (ST) for *H. pylori* eradication have previously reported a superior efficacy over conventional therapies (CT) [49,50]. Two recent meta-analyses of sequential therapy trials in adults and children suggested a benefit of a sequential therapy eradication regimen over conventional 7- or 10-day eradication regimens. Tong *et al* included 11 randomized controlled trials published up to February 2008 that compared ST to CT, including three pediatric studies [51]. The reported pooled risk ratios for eradication suggested superiority of ST over CT for both 7-day and 10-day regimens (1.23, CI 1.19–1.27 and 1.16, CI 1.1–1.23, respectively). The frequency of adverse effects of therapy was similar between the groups. Gatta *et al* included studies published up to October 2008 in their meta-analysis and again suggested a benefit of ST over CT, with an odds ratio for eradication of 1.98 (95% CI: 0.96–4.07) in the pediatric trials [52]. While publication bias is an unlikely explanation of the findings, a number of over-riding concerns remain concerning the use of ST based on these analyses to date. The quality of the studies included was variable, and almost all were conducted in Italy. In addition, the number of patients in the individual trials has been relatively small and compliance concerns regarding a regimen that involves changing medications at the mid-point persist. Whether the medications in the ST regimen would be as effective if given 'conventionally' rather than sequentially is also unclear. These questions remain to be answered by well-designed, multi-center, high-quality studies in different geographic regions before ST is adopted as the new 'first line' eradication regimen for children.

Probiotic Therapy

A lack of benefit of probiotic administration on *H. pylori* eradication in children was reported in two studies this year. In a randomized, double-blind placebo-controlled trial, Szajewska *et al* randomized children receiving 7 days of triple eradication therapy to either supplementation with 10^9 colony-forming units of Lactobacillus GG (n = 44) or placebo (n = 39) [53]. Subjects were recruited over a 40-month period, and complete data were only available in 34 of 44 children in the probiotic group and 32 of 39 children in the placebo group. No statistically significant benefit of probiotic supplementation over placebo was evident in terms of either eradication (69% versus 68%) or side effects. There was a no significant trend toward less regimen-associated diarrhea in probiotic treated children (6% versus 20%), although the study may have been underpowered to detect such differences with significance. In a study using functional food to deliver probiotics (cheese containing Lactobacillus gasseri OLL2716), Boonyaritichaikij *et al* studied the effects of probiotic supplementation in two groups of asymptomatic kindergarten children in Thailand – with or without *H. pylori* as determined by stool antigen testing (n = 132 and 308, respectively) [54]. The eradication arm of the study was single-blinded and nonrandomized, whereas the prevention arm was randomized and stratified for age and gender. Compliance was evaluated by the children's teachers. No statistically significant difference was detected between placebo and probiotic treatments in either the eradication or prevention arm of the study.

RE-INFECTION AND SPONTANEOUS BACTERIAL CLEARANCE

The extent of spontaneous clearance of *H. pylori* infection in childhood remains unclear. The Pasitos cohort study was established in 1998 to prospectively study *H. pylori* infection in Hispanic children [55]. A recent follow-up report from this study examined the effect of incidental antibiotic exposure on subsequent *H. pylori* clearance, based on ^{13}C-UBT changes and parental documentation of medication exposure [56]. Medication dose and duration were not recorded. A remarkable 78% of 218 children with a previously positive UBT subsequently tested negative, especially those between ages 1–3. Of the 205 children with complete medication exposure data, 36% received at least one antibiotic course following the initial positive UBT, while 68% had a subsequent negative UBT. Notwithstanding the number of significant limitations of this study, incidental antibiotic exposure in this study cohort seemed to account for a relatively limited proportion of 'spontaneous clearance' of *H. pylori* infection.

VACCINATION

A recent editorial questioned the benefit of eliminating *H. pylori*, as only 10–15% of hosts develop ulcerations and only 1% gastric adenocarcinoma. Vaccination can not yet be recommended, as our understanding of the bacterium is too

preliminary to make complete eradication a feasible option [57]. Several studies have suggested the merits of prophylactic immunization [12,58]. Rupnow *et al* quantified the cost-effectiveness of a prophylactic vaccine in the USA, using variables including costs of vaccine, vaccine administration, gastric cancer treatment, efficacy, quality adjustment caused by gastric cancer, and discount rate for periods of 10–75 years. They concluded that with a time horizon beyond 40 years, the use of such a vaccine could be cost-effective in the USA, especially if administered to infants or newborns. However, the problem is that the efficacy is unknown. This strategy would be different in less developed countries, where rates of *H. pylori* prevalence remain high. If prevention of ulcer disease is included in the calculation, vaccination may also have some shorter term cost-benefits [58]. In Australia, Hickey *et al* reported that transcutaneous immunization (TCI) with a lipid-based formulation against *H. pylori* infection in mice partially protected them against challenge with live *H. pylori*; this was not associated with development of gastric inflammation [59]. Successful vaccination strategies in mice have not proven effective in human subjects. However, TCI may be effective as a route for inducing protection against *H. pylori* colonization and warrants further study.

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SECTION B

PREVALENCE

CHAPTER 4

Low prevalence of *Helicobacter pylori* infection in young children in the Netherlands

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ABSTRACT

Aim of the study

To investigate the seroprevalence of *Helicobacter pylori* infection in young children from the general population in the Netherlands.

Methods

Determination of IgG antibodies against *H. pylori*, using an enzyme-linked immunosorbent assay technique (cut-off 0.32 Absorption index (AI), in serum from 1258 children who were 2-4 years of age. The serum was obtained from a serum bank of 6127 children who attended the community child healthcare cnters in the Dutch province of Zuid-Holland.

Results

In general, we found a seroprevalence of 1.2% of *H. pylori* infection, with a significant difference between the children with parents who were both Dutch (0.5%) and the children with at least one non-Dutch parent (2.6%) (p<0.001).

Conclusions

The prevalence of *H. pylori* infection in young infants in the general population in the Netherlands is low. Children with at least one non-Dutch parent form a risk group, however, for *Helicobacter pylori* infection in the Netherlands.

Keywords: *Helicobacter pylori*, epidemiology, infants, children, seroprevalence, the Netherlands.

INTRODUCTION

Helicobacter pylori (*Hp*) infection is acquired early in life within families [1, 3] and is recognized as a causative agent of gastritis, ulcer disease, gastric carcinoma and mucosa-associated lymphoid tissue-lymphoma [2]. In industrialized countries the prevalence of the infection is much lower than in developing countries and the rate of infection is related to factors such as ethnic origin. The Netherlands form a multicultural community: in 1997 1,063,987 inhabitants belonged to ethnical minorities; 279,958 of which originated from Turkey, 232,991 from Morocco and 287,219 from Suriname [4]. Data from Dutch adults show a prevalence of *H. pylori* infection of 33% to 50% [5, 6]. In Turkish immigrants with reflux-esophagitis the prevalence of *Helicobacter pylori* infection is as high as 61% (vs. 33% in Dutch adults with reflux-esophagitis) [7].

As there are no data on the frequency of *H. pylori* infection in young infants in the Netherlands, the objective of this study was to investigate its prevalence in this group of children.

METHODS

We determined the titers of IgG antibodies against *H. pylori* (*H. pylori*-IgG) in serum from 1258 children who were 2-4 years of age born in the Netherlands. The sera came from a serum bank built up during a screening study on celiac disease in 1998. This study pertained to 6127 children from the general population, who attended the community child healthcare centers in the Dutch province of Zuid-Holland[8]. The healthcare centers are attended regularly by 98% of all children born in this area. The parents gave informed consent to store the sera of their children at -20° C to be used anonymously for research purposes. We selected the sera from all the 427 children with at least one non-Dutch parent and from the 31 children who, after the screening, were diagnosed with celiac disease. In addition, we also analysed 800 randomly chosen sera from the 5669 children of whom both parents were Dutch (computerized selection, SPSS-10 Bijleveld Press, Utrecht, the Netherlands) (Table 1). Specific IgG antibodies against *H. pylori* were measured in serum using a validated in-house enzyme-linked immunosorbent assay (ELISA) technique[9,10]. In brief: a mixture of six pooled *H. pylori* strains was sonicated and adjusted to a protein concentration of 3 mg/ml. Each well of a microtiter plate (Dynatech Laboratories, M129A Chantilly, Virginia, USA) was coated overnight with 100 µl antigen solution (1 µl suspension/ml) and washed three times with phosphate-buffered saline (pH 7.5) containing 0.05% Tween 20. IgG antibodies were measured in serum diluted 1:200, by an ELISA technique using peroxidase-labeled conjugates specific for human IgG.

Table 1. Frequency of positivity of IgG antibodies against *H. pylori* (Hp-IgG) in 1258 Dutch children.

Children		N	Positive Hp-IgG * N (%)
Both parents of Dutch origin		800	4 (0,5)
At least one non-Dutch parent		427	11* (2,6)
Countries of origin	Suriname	48	1 (2)
	Morocco	34	2 (6)
	Turkey	31	3 (6)
	Germany	17	1 (6)
	Ghana	4	1 (25)
	Somalia	3	2 (66)
	India	2	1 (50)
	Other countries	288	0 (0)
Celiac disease		31	0 (0)
Total		1258	15 (1,2)

(* = p<0,001)

Table 2. Frequency of *H. pylori* infection in pediatric populations in the Netherlands

Author	Age (yrs)	n	Population	Serum test	Year of investigation	Frequency (%)
Van de Meer *et al* 1992	11[b] 7[b]	82 [a] 39	Hospital	ELISA	1989	8,5 [a] 5,1
Roosendaal *et al* 1997	6-8 12-15	154 160	General: viral infection	ELISA	1993	9 11
Schipper *et al* 2000	8,2[b] 6,5 [b]	279 [a] 903	Hospital	Pyloriset EIAG	1998-99	9,7 5,6
Actual study	2-4	1258	General: health-care centers	ELISA	1998	1,2

ELISA enzyme-linked immunosorbent assay; EIAG enzyme immuno assay for IgG
a Children with recurrent abdominal pain.
b Mean age

The absorbency index was calculated from the mean of two readings of the optical density of the serum, corrected for a uniform standard positive serum used in all assays. Sera with an absorbency index higher than 0.32 are considered positive for IgG antibodies against *Helicobacter pylori* [9]. The sensitivity of the ELISA is 98.5% with a specificity of 92% for *Hp* infection [10].
The study was approved by the medical ethical committee of the Leiden University Medical Center.
Statistical analysis was based on the two-sample t-test for proportions and on the $\chi 2$ –square test. For the comparison of prevalence rates with observed proportions from earlier studies, the one-sample t-test for proportions was used, using the previously observed proportions as null hypothesis.

RESULTS

We found anti-*H. pylori*-IgG titers higher than 0.32 AI in 15 children, indicating *H. pylori* infection in 1.2% of the children aged 2-4 (Table 1). This frequency is lower than the one previously found among Dutch children (Table 2) [12-13]. None of the children with celiac disease had increased anti-*H. pylori*-IgG titers in serum.
A significant difference was seen in the frequencies of *H. pylori* infection in the children with two Dutch parents (0.5%) and in those with at least one non-Dutch parent (2.6%; p<0.001 Table 1). The non-Dutch parents of all the children with *H. pylori* infection were, one case excepted, not European and in six cases belonged to the most common ethnical minorities in the Netherlands: that is, Surinamese, Moroccan and Turkish. From the entire group of non-Dutch parents, 58 came from Africa, 75 from Asia, 161 from Europe, 10 from North-America, 85 from South-America and 38 from the Middle East.

DISCUSSION

To our knowledge this is the first study on the prevalence of *H. pylori* infection in young children in the Netherlands. We found a frequency of *H. pylori* infection lower than the one found in the Netherlands before (Table 2). A possible reason for this may be the young age of the children in our study-group, as it is well-known that the frequency of *H. pylori* infection increases with age[11]. Another possible reason for the low frequency of *H. pylori* infection in our group may be the good health status of the children, as they were attending the healthcare centers, which are preventive and not curative institutions in the Netherlands. The studies on *H. pylori* infection previously performed in the Netherlands concerned older children with health complaints who attended the hospital because of abdominal pain[12, 13],

surgery[12] or suspected viral infections[14]. In addition, as shown by Roosendaal *et al* [14], *H. pylori* infection rates in Dutch children have continuously declined over the last decades, demonstrating a persistent birth cohort effect. This decline will result in a very low prevalence of *H. pylori* infection in the Dutch population during the next few years. One question is whether our population is representative for the general Dutch population of 2-4 years of age. Possibly, this is not the case, since we selected the sera from all the children with non-Dutch parents and from all the children with celiac disease. On the other hand, the total study group is big enough to be considered representative for our region. Moreover, the results of our study (1.2% *H. pylori* infection) may represent an underestimation of the true prevalence of *H. pylori* infection, because we used the determination of serum antibodies against *H. pylori* to identify the infection. Although our technique was a home made ELISA with a well-known high sensitivity and specificity for *H. pylori* infection in adults, the sensitivity of serological tests may be lower in children under the age of six [11]. On the other hand, our home made Elisa contains the extract of 6 *H. pylori* strains [10], which improves the sensitivity and specificity and to cover infrequent strains of *H. pylori*. In addition, our home made ELISA has already been used in two other studies in children [15, 16], showing that the increase in sensitivity with age is not significant. A recent collaborative European study using the kit Pyloritest EIA-G III (Orion Diagnostics, Espoo, Finland), also with a cut-off of 0.32 AI, has shown that serology can have excellent performance among children[17], but the authors observed a trend in better performance with increasing age or with lowering the cut-off. If we assume a lower cut-off of positivity for our young children (usually 30% lower than the cut-off for adults for other ELISA tests), in our group there were only 39 children with a cut-off higher than 0.20 AI, giving a frequency of positivity of 3%, which still is very low.

The golden standard to assess *H. pylori* infection in children remains upper gastrointestinal endoscopy with biopsies[2, 23], but this invasive method is not acceptable for epidemiological studies. Nowadays, stool and breath tests can be used in epidemiological studies on *H. pylori* infection [11, 18], but till now these tests have not been validated in young children as those in our study. The availability of a serum bank from a large population of young healthy Dutch children offered us a unique opportunity to perform this study, even if we assume a certain degree of false negative results from serology in children younger than 5 years[19]. In addition, serology is a well-accepted non-invasive test to perform epidemiological studies[20] and it allowed us to compare the results in our population with those found in the other Dutch pediatric studies, which also used serological techniques (Table 2).

We found no positive anti-*H. pylori*-IgG among the sera from the children with celiac disease identified by the mass screening study [8], which is in agreement with the results of an Italian study of 81 children with celiac disease [21].

We found that the children with at least one non-Dutch parent had a significantly higher prevalence of *H. pylori* infection (2.6%) than the children with two Dutch parents (0.5%), ($p<0.001$). Interestingly, only 1 of these 11 children had European non-Dutch parents (Germany, Table 1). Children with parents from Ghana, Somalia and India were relatively frequently infected by *H. pylori*. The number of children in this category, however, is small (n = 9) and the results should be interpreted with caution. The frequency of *H. pylori* infection found among the children with parents from the three largest ethnical minorities in the Netherlands (i.e. Surinamese, Moroccan and Turkish) was 5.3%. This is significantly higher in comparison with the frequencies in children from Dutch parents (0.5%) ($p<0.001$) and in children from non-Dutch parents in general (2.6%) ($p<0.05$). No information is available about the prevalence of *H. pylori* infection in Morocco, Somalia, Suriname and Ghana, but it is assumed to be high. The seroprevalence of *H. pylori* in 346 children from eastern Turkey was 44% with a corresponding one of 85% in their mothers and 76% in the fathers[22].

In conclusion, we have found that the frequency of *H. pylori* infection among young children in the Netherlands in general is low, but that it is significantly higher among Dutch children from the ethnical minorities. In developed countries the prevalence of *Hp* infection is rapidly decreasing mainly due to better socio-economic conditions, but in developing countries the incidence of infection still is very high. Our results indicate that immigration to Europe from countries with high rates of *H. pylori* infection induces the existence of a group of children with high risk for *H. pylori* infection. Pediatricians should be aware of this fact, as *H. pylori*-pathology may be particularly frequent among these children who will benefit from early diagnosis and treatment.

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CHAPTER 5

Frequency and risk factors of gastric and duodenal ulcers or erosions in children: a prospective 1-month European multicenter study

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ABSTRACT

There are no solid figures of the frequency of ulcer disease during childhood in Europe. We assessed its frequency and analyzed known risk factors.

Patients and methods

Ulcers, erosions, indications, and risk factors were recorded in all children undergoing an upper gastrointestinal endoscopy in a prospective study carried out during one month simultaneously in 19 centers of 14 European countries.

Results

Ulcers and/or erosions were observed in 56 out of 694 children. Children with ulcers/erosions were significantly older than those without lesions (10.3±5.5 vs. 8.1± 5.7 years, P = 0.002). *Helicobacter pylori* infection was present in 15 of 56 children (27%); NSAIDs were used in eight, steroids in five, immune-suppressive drugs in five, antibiotics in six, antacids in one, H2-blockers in six and proton pump inhibitors in eight children (more than one risk factor was detected in 32 of 56 children). No risk factors were observed in 24 of 56 children (43%). The main indications for endoscopy were epigastric or abdominal pain (24%) and suspicion of gastroesophageal reflux disease (15%). Similarly, epigastric tenderness, hematemesis, melena, and weight stagnation were significantly associated with ulcers/erosions, whereas sex, *H. pylori* infection, socioeconomic status and lifestyle factors were equally distributed.

Conclusion

Although limited by the short-time duration and the heterogeneity of the patients included throughout the 19 centers, our study shows a frequency of 8.1% of ulcers and/or erosions in children, occurring mainly in the second decade of life. *H. pylori* infection and gastrotoxic medications were less frequently implicated than expected.

Keywords: child, erosion, ulcer, *Helicobacter pylori*

INTRODUCTION

Not only peptic ulcers, but also erosions, reported in 10–20% of symptomatic children infected with *Helicobacter pylori* undergoing upper endoscopy [1–6] remain less frequent than in adults. However, these data originate from monocentric studies enrolling a small series of children. In one large prospective European multicenter study, aimed at assessing antibiotic resistance of *H. pylori* strains, including more than 1400 symptomatic infected children, gastric, or duodenal ulcers were found during endoscopy in 3.5% of children below 6 years of age, in 4.6% children aged 6–11 years, but in 10.4% of those older than 12 years [7].
Ulcers or erosions also occur in *H. pylori* negative children, especially those receiving NSAIDs [8]. Other known causes of peptic lesions are stress, Crohn's disease, and various exogenous agents [1–4].
There is a lack of solid data about the frequency of ulcer disease in children and the frequency of *H. pylori* and other etiologic risk factors in children with peptic ulcer disease. The European Paediatric Task Force for *H. pylori* decided to conduct a descriptive prospective study in 14 European countries to evaluate in a population of children referred for upper gastrointestinal (GI) endoscopy, not only the frequency of gastric or duodenal ulcers, but also erosions in relation to *H. pylori* infection and various other risk factors.

PATIENTS AND METHODS

Patients

The study was carried out for one month (January - February 2007) in 19 centers in 14 European countries. In each center, all the children aged below 18 years undergoing an upper gastrointestinal endoscopy were enrolled. Parental informed consent and child ascent was obtained as requested by local ethical policy in the different European countries for the collection of clinical information in an anonymous form including the following items: presence of gastric or duodenal ulcers and/or erosions, age, sex, known chronic disease, and indication for endoscopy. In addition, in children with ulcers and/or erosions, macroscopic and microscopic findings were collected including the *H. pylori* status and further information on potential risk factors such as medications during the last 4 weeks, ethnic background, and education level of the parents. Lifestyle habits including smoking and alcohol consumption were also collected. The Ethics Committee of Queen Fabiola Children's University Hospital, Brussels, Belgium approved the protocol for anonymous data collection and analysis.
H. pylori status was determined in patients exhibiting gastric and or duodenal

ulcers but also erosions according to various diagnostic methods as already defined by our conference consensus [9]: histology according to the updated Sydney classification [10], culture, rapid urease test, ^{13}C urea breath test, and stool antigen. *H. pylori* infection was confirmed when culture or at least two diagnostic tests were positive. The *H. pylori* status was considered as not valid, that is, false negative result, when children received antibiotics, proton pump inhibitors (PPI's), H2 -blockers or antacids within 4 weeks before endoscopy.

To limit interindividual interpretation of endoscopic findings between observers in the different centers, a clear description and definition of ulcers and erosions was adopted by consensus according to the minimal standard terminology for digestive endoscopy: ulcer was defined as a deep defect in the mucosa with an inflamed edge; erosion was defined as a small (<3mm) superficial defect in the mucosa, of white or yellow color [11]. Endoscopic bleeding signs were noted according to the Forrest *et al* [12] classification into six classes: IA arterial spurting bleeding, IB arterial oozing bleeding, IIA visible vessel, IIB sentinel clot, IIC hematin covered flat spot, and no stigmata of hemorrhage.

Ulcers and/or erosions were also subclassified as secondary, when children showed *H. pylori* infection, received either NSAIDs or steroids during the four weeks preceding endoscopy, or presented with a known chronic disease, that is, inflammatory bowel syndrome (IBD), polyarthritis, and other rheumatic diseases. Primary lesions were those without an identified etiology.

Statistical Analysis

Calculation of mean, median, standard deviation, and range of all quantitative parameters, descriptive analysis of repartition for qualitative parameters, performance of the $\chi 2$ test or Fisher's exact test when appropriate and the monovariate analysis were done using the Stat-View System (Abacus, California, USA), whereas the multivariate analysis was done using the STATA 7.0 System (Stata Inc., California, USA). All tests performed were two-tailed, with P value less than 0.05 considered significant.

Frequency of gastric and/or duodenal ulcer/erosion was analyzed in the study cohort, and their clinical signs and additional descriptive data. Frequencies of risk factors in the group of children with gastric and/or duodenal ulcer/ erosion compared with those without lesions were precise with 95% confidence interval. The risk factors considered are sex, age as a variable divided into two age groups (<10 and >10 years), indications of endoscopy, and known chronic disease. Risk factors were first analyzed using monovariate analysis, then logistic regression analyses using a backward stepwise procedure to adjust for every variable. Only variables exhibiting a *P* value less than 0.2 by the monovariate analysis were analyzed by

Table 1 Distribution of patients with gastroduodenal ulcers and/or erosions according to age with their risk factors in the investigator centers*

	Patients without ulcers and/or erosions n (%)	Patients with ulcers and/or erosions n (%)	Total	Age (years) Mean (SD)	Number of patients Age > 10 years n (%)	OR	95% CI	*P value*
Belgium	46 (78.0)	13 (22.0)	59	7.69 (5.8)	22 (37.2)	3.9	2,0-7.7	0.00004
Italy	25 (80.7)	6 (19.4)	31	7.85 (5.2)	14 (45.1)	3.1	1.3-7.7	0.01
Turkey	30 (83.3)	6 (16.7)	36	6.17 (3.5)	5 (13.8)	2.6	1.0-6.3	0.05
France-Bordeaux	25 (86.2)	4 (13.8)	29	7.13 (4.9)	10 (34.4)	2.1	0.7-5.8	NS
Poland-Wroclaw	77 (89.5)	9 (10.5)	86	11.1 (4.3)	52 (60.4)	1.5	0.7-3.0	NS
Sweden	28 (90.3)	3 (9.7)	31	10.4 (5.4)	18 (58,0)	1.4	0.4-4.4	NS
Poland-Warsaw	90 (91.8)	8 (8.2)	98	10.11 (5.4)	54 (55.1)	1.1	0.5-2.3	NS
Spain-centre 1	54 (93.1)	4 (6.9)	58	9.51 (5.8)	33 (56.8)	0.9	0.3-2.5	NS
Spain-centre 2	31 (96.9)	1 (3.1)	32	9.03 (6.3)	15 (46.8)	0.5	0.1-2.8	NS
France-Lille-centre 1	65 (98.5)	1 (1.5)	66	3.5 (4.4)	11 (16.6)	0.2	0.1-1.2	0.04
Hungary	19 (99.5)	1 (0.5)	20	7.26 (5.1)	8 (40,0)	0.9	0.2-4.6	NS
France-Lille-centre 2	51	0	51	3.4 (4.3)	6 (11.7)	0	0	0
Germany	26	0	26	9.15 (5.3)	11 (42.3)	0	0	0
Czech Republic	26	0	26	12.08 (5.2)	19 (73,0)	0	0	0
Greece	24	0	24	9.09 (4.6)	12 (50,0)	0	0	0
Netherlands	14	0	14	7.99 (6.2)	5 (35.7)	0	0	0
United Kingdom	7	0	7	10.92 (5.7)	5 (71.4)	0	0	0
Total	638	56 (8.1)	694					

CI : confidence interval ; NS : not significant ; OR : Odds ratio ; SD : standard deviation

*The age was significantly different between the different investigator centers, $p < 0.0001$ according to the multivariate analysis of variance analysis.

the multivariate logistic model. Results were expressed as odds ratios (ORs) with 95% confidence intervals. Statistical significance was set up at the P value less than 0.005, and all *P* values were two-tailed.

RESULTS

Frequency and description of ulcers and/or erosions

A total of 694 children were enrolled, 349 female and 345 male, median age 8.2 years (range 1 month–18 years), unevenly distributed between the different centers.
Ulcers and/or erosions were observed in 56 of 694 children (8.1%), with a wide variation (0–22%) in the 19 European centers (Table 1). In each and all centers, children with ulcers and/or erosions were significantly older than those without lesions (10.26±5.5 vs. 8.11± 5.7 years, P=0.002).
Endoscopic lesions were reported as gastric ulcers in seventeen and duodenal ulcers in seven, gastric erosions in 31, and duodenal erosions in 15 children. Most of the gastric lesions were located in the antrum (44/48) and the majority of the duodenal lesions were located in the bulb (15/22). Duodenal erosions in nine, duodenal erosions and ulcer in two, duodenal erosions and gastric ulcers in one, duodenal ulcers in five, gastric erosions in 21, gastric and duodenal erosions in two, gastric and duodenal erosions and gastric ulcers in one, gastric erosions and ulcers in seven, and finally gastric ulcers in eight. Endoscopic bleeding signs were present in nine of 56 children (16%). No age difference was observed between children with gastric compared with those with duodenal lesions. Among the 56 children with peptic ulcers and/or erosions, 24 lesions (11 ulcers and 13 erosions) were classified as primary and 32 (13 ulcers and 19 erosions) as secondary (Table 2).
H. pylori infection was present in 15 of 56 children (27%). These *H. pylori* infected children showed gastric erosions in five, duodenal erosions in three, combined gastric and duodenal erosions in one, combined gastric erosions and ulcers in four, gastric ulcers in one, and duodenal ulcers were found in one. No significant relationship could be found between *H. pylori* infection and the type of peptic lesions. *H. pylori* status was considered as not valid in six of 24 (25%) and 12 of 32 (37.5%) patients of the primary and secondary lesions (Table 2).

Gastro–duodenal ulcers versus erosions

The risk factors in children with ulcers were compared with those with erosions and did not show any statistically significant difference: age more than 10 years 14 of 24 (58.3%) versus 19 of 32 (59.4%), female sex ratio 13 of 24 (54.1%) versus 15 of 32 (46.8%), *H. pylori* infection six of 24 (25%) versus six of 32 (18.7%), previous intake of medication 13 of 24 (54.1%) versus 16 of 32 (50%) recurrent abdominal pain and

Table 2 Primary and secondary ulcers and/or erosions characteristics

	Primary Lesions (n=24)		Secondary Lesions (n=32)	
	Ulcers +/- erosions n=11	Erosions only n=13	Ulcers +/- erosions n=13	Erosions only n=19
***H. pylori* status**				
Positive	0	0	6	9
Negative	11	10	7	10
Not tested	0	3	0	0
***H. pylori* status**				
Valid	9	9	9	11
Not valid*	2	4	4	8
Known chronic diseases				
IBD	0	0	3	4
Polyarthritis or other rheumatic diseases	0	0	0	2
Chronic neurologic disease	1	2	0	1
Chronic lung disease	0	0	1	0
Chronic liver disease	0	1	0	2
Food allergy	0	0	0	1
Other allergic disease (except food allergy)	0	0	0	1
Oncology (during chemotherapy)	1	0	0	0
Celiac disease	1	1	0	0
Others	2	0	2	0
Absence of known chronic disease	6	9	7	8
Drug consumptions				
None	5	7	6	9
Yes	6	6	7	10
NSAIDs	0	0	5	3
PPIs	0	3	0	5
H2 blockers	2	1	2	1
Antacids	0	0	1	0
Antibiotics	0	0	3	3
Steroids	0	0	1	4
Immuno-suppressive drugs	0	0	1	4

* *H. pylori* status not valid, i.e. due to the use of either antibiotics, or PPIs or H2 blockers during the 4 weeks preceding endoscopy
IBD: inflammatory bowel disease; PPIs: protonpump inhibitors; NSAIDs: non-steroidal anti-inflammatory drugs

Table 3 Known chronic diseases in all enrolled patients

Known chronic diseases	Patients without ulcers and/or erosions n (%)	Patients with ulcers and/or erosions n (%)	Total n	OR	95% CI	*P* value
Chronic neurologic disease	34 (5.3)	4 (7.1)	38	1.5	0.5 - 4.2	NS
Chronic cardiac disease	4 (0.6)	0	4	1.3	0.1 - 23.3	NS
Chronic lung disease	22 (3.4)	1 (1.7)	23	0.7	0.1 - 3.9	NS
Chronic liver disease	31 (4.8)	2 (3.5)	33	0.9	0.2 - 3.3	NS
Chronic renal disease	4 (0.6)	0	4	1.3	0.1 - 23.3	NS
Food allergy (FA)	12 (1.8)	1 (1.7)	13	1.3	0.2 - 7.6	NS
Other allergic disease (except FA)	18 (2.8)	1 (1.7)	19	0.9	0.2 - 5.0	NS
IBD	40 (6.3)	7 (12.5)	47	2.2	1.0 - 5.2	0.05
Celiac disease	37 (5.7)	2 (3.5)	39	0.7	0.2 - 7.8	NS
Polyarthitis or other rheumatic diseases	5 (0.7)	2 (3.5)	7	5.3	1.2 - 24.1	0.04
Clotting disorders	4 (0.6)	0	4	1.3	0.1 - 23.3	NS
Prematurity	8 (1.2)	0	8	0.7	0.1 - 11.6	NS
Post-Transplant	4 (0.6)	1 (1.7)	5	3.8	0.6 - 24.8	NS
Oncology (during chemotherapy)	4 (0.6)	1 (1.7)	5	3.8	0.6 - 24.8	NS
Others	68 (10.6)	4 (7.1)	72	0.7	0.3 - 1.9	NS
Absence of known chronic disease	343 (53.7)	30 (53.57)	373	1.0	0.6 - 1.7	NS
Total	638	56	694			

CI : confidence interval ; IBD : inflammatory bowel disease ;
NS: not significant ; OR : Odds ratio

epigastric pain as main symptoms 17 of 24 (70.8%) versus 16 of 32 (50%), known chronic diseases 10 of 24 (41.6%) versus 18 of 32 (56.3%). In three children with gastro–duodenal erosions *H. pylori* status was not assessed.

Complementary data

The median age of the 56 children presenting with ulcers or erosions was 11.38 years (range 0.16–17.8) with a sex ratio of 1. Known chronic diseases were reported in 321 of 694 children (Table 3), which were significantly higher in patients with ulcers and/or erosions as compared with those without lesions. Seven of 32 children presented with IBD and two of 32 with polyarthritis, or other rheumatic diseases (Tables 2 and 3). Main indications for endoscopy were epigastric or abdominal pain and suspicion

of gastroesophageal reflux disease (Table 4). Epigastric pain, hematemesis, melena, and weight stagnation were significantly found as the main indication of endoscopy in patients with ulcers and/or erosions as compared with those who lacked lesions (Table 4).

H. pylori infection was detected in only 15 children (27%) using different diagnostic methods: culture in 11, histology in 11, rapid urease test in 4, ^{13}C urea breath test in three, and stool antigen test in two. In three children, histology failed to detect *H. pylori*, whereas culture was positive. *H. pylori* infection may, however, be underestimated as *H. pylori* status was considered as not valid in six of 24 (25%) and 12 of 32 (37.5%) patients with primary and secondary lesions and in three children *H. pylori* status was not assessed (Table 2). In three children (5.3%), *H. pylori* was eradicated earlier.

Other complementary data concerned previous *H. pylori* eradication in three children (5.3%). Thirty children were born in Western Europe (53.5%), 24 in Eastern Europe (43%), and two in Africa (3.5%); 20 mothers were born in Western Europe (35.7%), 22 in Eastern Europe (39.2%), 10 in Africa (17.8%), two in the Middle East, one in Asia, and one in North America.

The educational level of mothers and fathers, according to the number of schooling years, was unevenly distributed: less than 9 years for thirteen mothers and nine fathers, respectively, 9–11 years for 6 mothers and 13 fathers, more than 12 years for 23 mothers and 16 fathers, and university degree for six mothers and ten fathers; the educational level was not known in eight couples of parents.

A median of four members of the family were living in the house (range 2 -9), and 36 children (64%) slept in a personal bedroom.

A history of peptic ulcer in a first-degree family member was reported in six children (10.7%). A history of use of medication within four weeks before endoscopy was reported in 29 children (51.7%); some children were taking more than one drug: NSAIDs in eight (14.3%) where five of 13 and three of 19 in the secondary ulcers and/or erosions and erosions only groups, respectively, antacids in one (1.7%), H2-blockers in six (10.7%) where three of 24 (two ulcers and/or erosions, one erosions only) and three of 32 (two ulcers and/or erosions, one erosions only) in the primary and secondary lesions groups, respectively, PPIs in eight (14.3%) where three of 24 (three erosions only) in the primary and five of 32 (five erosions only) in the secondary lesions groups, antibiotics in six (10.7%) where three of 13 (three ulcers and/or erosions) and three of 19 (three erosions only) in the secondary lesions groups, steroids in five (8.9%) where one of 13 (one ulcer and/or erosion) and four of 19 (four erosions only) in the secondary lesions groups, and immuno-suppressive drugs in five (8.9%) where one of 13 (one ulcer and/or erosion) and four of 19 (four erosions only) in the secondary lesions groups (Table 2). In addition, a history of

Table 4 Indications of endoscopy in all enrolled patients

Indications of endoscopy	Patients without ulcers and/ or erosions n (%)	Patients with ulcers and/or erosions n (%)	*Total* n	OR	95% CI	*P* value
Suspected gastroesophageal reflux disease	87 (13.6)	3 (5.3)	90	0.4	0.14-1.25	NS
Established gastroesophageal reflux disease	66 (10.3)	1 (1.7)	67	0.2	0.1-1.2	0.05
Abdominal distress/pain	92 (14.4)	11 (19.6)	103	1.5	0.7-2.9	NS
Epigastric pain	51 (7.9)	9 (16.1)	60	2.3	1.1-4.8	0.03
Pain awaking child at night	23 (3.6)	4 (7.1)	27	2.2	0.8-6.4	NS
Heartburn	5 (0.7)	0	5	1.0	0.1-18.7	NS
Nausea	2 (0.3)	0	2	2.6	0.1-47.5	NS
Vomiting	16 (2.5)	0	16	0.3	0.1-5.6	NS
Halitosis (foul smelling breath)	1 (0.01)	0	1	3.8	0.2-92.7	NS
Hematemesis	8 (1.2)	5 (8.9)	13	7.9	2.6-24.1	0.00005
Melena	9 (1.3)	3 (5.3)	12	4.3	1.2-15.3	0.03
Weight stagnation	32 (5.0)	7 (12.5)	39	2.8	1.2-6.6	0.02
Chronic diarrhea	20 (3.1)	1 (1.7)	21	0.8	0.2-4.4	NS
Anemia	7 (1.1)	0	7	0.8	0.1-13.3	NS
Iron deficiency	2 (0.3)	0	2	2.3	0.1-47.5	NS
Anorexia	5 (0.7)	0	5	1.0	0.1-18.7	NS
Malaise	11 (1.7)	1 (1.7)	12	1.5	0.3-8.3	NS
G-Tube	22 (3.4)	1 (1.7)	23	0.7	0.1-4.0	NS
Foreign body	4 (0.6)	0	4	1.3	0.1-23.3	NS
Suspected caustic ingestion	9 (1.4)	1 (1.7)	10	0.9	0.2-5.3	NS
Work-up for celiac disease	60 (9.4)	0	60	0.1	0-1.3	0.02
Work-up for IBD	34 (5.3)	6 (10.7)	40	2.3	0.9-5.5	NS
Work-up for portal hypertension	34 (5.3)	2 (3.5)	36	0.8	0.2-3.0	NS
Other	38 (5.9)	1 (1.7)	39	0.4	0.1-2.2	NS
Total	638	56	694			

CI: confidence interval; IBD: inflammatory bowel disease; NS: not significant; OR: Odds ratio

Table 5 Multivariate analysis of children with gastroduodenal ulcers and/or erosions

	Adjusted OR[a]	95% CI	*P* value*
Results of the multivariate analysis were adjusted according to the investigator centers Age (vs <10 yrs)			
> 10 yrs	1.6	0,7-3,6	0,28
Indication of endoscopy (vs. all other indications)[b]			
Suspected or established gastroesophageal reflux	0.4	0.1-1.2	0.08
Epigastric pain	2.9	1.0-8.2	0.05
Pain awaking child at night	4.4	1.2-16.7	0.03
Hematemesis	12.7	3.0-53.2	0.001
Melena	6.6	1.4-31.9	0.02
Weight stagnation	3.6	1.3-10.2	0.015
Known chronic diseases (vs. all other relevant)			
IBD	2.1	0.8-5.7	0.13
Polyarthritis or other rheumatic diseases	6.7	0.5-84.3	0.14

CI: confidence index; IBD: inflammatory bowel disease; OD: Odds ratio

[a] Odds ratio adjusted on all the variables of the model

* *P* value obtained by a multivariate logistic model

[b] only variables exhibited a *P* value <0.20 by the monovariate analysis were analyzed by multivariate logistic model

alcohol consumption was reported in seven (12.5%) and tobacco smoking in three children (5.4%).

Risk factors

Ulcers and/or erosions were more frequently reported in three centers located in Belgium, Italy and Turkey (Table 1). In all enrolled children, the analysis of gastro–duodenal ulcers and/or erosions showed the same risk factor results when analyzed separately and as conjointly that epigastric pain, hematemesis, melena, and weight stagnation were reported as the most significant clinical symptoms considered as risk factors for erosions or ulcers (Table 4). Known chronic diseases such as IBD, polyarthritis, or other rheumatic diseases were also considered significant risk factors (Table 3).

Children aged above 10 years represented another significant risk factor. Adjust-

ment of the multivariate analysis according to the centers showed that the same risk factors were as significant and that pain-awaking at night also reached a level of significance (Table 5).

Finally, no significant relationship could be shown between *H. pylori* infection and the type of peptic lesions. As *H. pylori* status was not systematically determined in children without ulcers and/or erosions, thus an OR could not be determined for children with or without ulcers/erosions.

DISCUSSION

In this prospective European study, the frequency of ulcers or erosions in children referred for upper endoscopy reached the unexpected high figure of 8.1%. This frequency is possibly underestimated because of the use of gastro-protective drugs including H2 -blockers and PPIs preceding the endoscopic procedure.

An important variation of this frequency was observed among the different centers participating in the study (0–22%). This can be because of several factors in the different countries: referral habits, use of gastro-protective drugs before endoscopy, interference of adult gastroenterologists taking care of adolescents, and possible unknown geographical influences.

Lesions of ulcers or erosions were observed mainly around the second decade of life, and children older than 10 years were significantly more at risk for ulcers and erosions alike. Epigastric tenderness, pain-awaking at night, hematemesis, melena, and weight stagnation were significant risk factors for lesions of ulcers or erosions alike.

The fact that *H. pylori* infection was observed in only 15 of 56 (27%) children in different European centers is in agreement with the decreasing prevalence of *H. pylori* infection in adults observed in Europe [13]. The relationship between chronic peptic ulcer disease and *H. pylori* is widely documented in adults, especially in relation with duodenal ulcers. A higher rate of *H. pylori* infection was also described[3] in children with duodenal ulcers compared with those with gastric ulcers (62 vs. 20%, $P<0.001$). However, in other pediatric papers, *H. pylori* infection was detected in only three of 11 (27%) children with duodenal ulcer [5]. Similarly, in an older publication, it was reported in six of 11 children with duodenal ulcers (55%) versus two of four with gastric ulcers (50%) [14]. In this study, we did not find in any of the different European countries a significant relationship between *H. pylori* infection, present in 15 of 56 children (27%) and the location of the gastric or duodenal ulcers/erosions. Furthermore, the fact that *H. pylori* status was considered as unreliable because of the use of either PPIs, H2 - blockers or antibiotics in the period preceding endoscopy in six of 24 and 12 of 32 children with primary and secondary lesions, may also influence the low frequency of *H. pylori* infection observed in our series (Table 2). A recent review of studies published during the last 10 years including a total of 16 080 patients, showed that *H. pylori* was found in 81% of duodenal ulcers, but a lower frequency of 77% was reported when only the last 5 years were considered[15]. Associations with *H. pylori*-negative duodenal ulcer were mainly because of false negative results of diagnostic methods and the use of NSAIDs [15]. This important puzzling observation needs a confirmation by a study on a wider scale taking into consideration a longer period of time extending necessarily over several seasons. Even more important is the correlation to the prevalence of *H. pylori* infection in each specific geographic area as stressed by a recent Israeli paper [6] describing a high incidence of ulcers in their Israeli Arab or Russian immigrant children and also as observed, in this study, in three European centers of Belgium, Italy, and Turkey (Table 1).

A much lower frequency of ulcers and erosions (2%) is reported in earlier papers [3, 5] without reference to age, although with a higher frequency of *H. pylori* associated ulcers and the same trend is reported in the PEDS-CORI database [16]. In contrast, ulcers were detected in 80 of 1180 *H. pylori*-positive children (6.8%) in a prospective European multicenter study [7].

Our results confirm data published earlier stressing the fact that the age of the children is an important risk factor: OR of 3.1 in children older than 11 years [7] and OR of 2.1 in this study in children older than 10 years. However, we did not observe the age difference between children with gastric ulcer reported [3] to be younger than those with duodenal ulcer (6.5 vs. 10.5 years).

Several investigators have suggested that night-time pain associated with nocturnal awakening, fasting pain relieved by food intake, pain associated with meals or postprandial bitter taste, heartburn and epigastric pain are clinical signs that help to distinguish ulcer-positive from ulcer-negative children, although positive for *H. pylori* infection [2]. However, in contrast, these clinical symptoms are not found to be specific by Roma *et al* [3], as no significant difference was found with regard to clinical symptoms between children with and without ulcer. In this study, the main indications for endoscopy were epigastric or abdominal pain (24%) and suspicion of gastroesophageal reflux disease (15%). Hematemesis, melena, epigastric tenderness, and weight stagnation can be considered as significant risk factors for erosions or ulcers alike.

Together with *H. pylori* infection, the use of NSAIDs is considered as an important risk factor not only for gastrointestinal mucosal injuries in adults, but also in children [17]. In adults, both *H. pylori* infection and the use of NSAIDs independently and significantly increase the risk of peptic ulcer and upper gastrointestinal bleeding (UGIB) [18]. In children, few studies address the relationship between NSAIDs intake, *H. pylori* infection, and other risk factors inducing mucosal injuries and UGIB.

In a retrospective review of the medical records of 112 Taiwanese children who

presented with UGIB, *H. pylori* infection was present and closely related to primary duodenal ulcers in 55% of patients without any other underlying disease, contrasting with the lower figure of only 17% in children with several underlying diseases[19]. UGIB is frequently related to drug ingestion with almost 50% of cases showing gastric erosions secondary to drug intake [20].

In a recent study based on data of the French Pharmacovigilance system [21] analyzing all serious upper gastrointestinal complications such as gastritis, duodenitis and esophageal lesions, gastric or duodenal ulcers were reported in 61 children aged 11 months - 15 years during treatment with niflumic acid, ibuprofen, and tiaprofenic acid, associated with NSAIDs in children. This important study concludes that NSAIDs used in children for fever or moderate pain are associated with a risk of serious UGIB complications that increase with duration, dose, and association with a salicylate.

In our study, medication intake during the last 4 weeks was reported in 29 out of 56 children with erosions or ulcers (51.7%), but NSAIDs consumption in only eight children (14.3%) and steroids in another five (8.9%). The frequency of tobacco (three cases, 5.4%) and alcohol (seven cases, 12.5%) consumption is low in the population of children with ulcers and/or erosions and does not allow drawing firm conclusions about their influence on the pathogenesis of ulcers.

In this study, we found that the frequency of primary gastric and duodenal ulcers and/or erosions was slightly lower than those of secondary lesions (24/56 and 32/56), lower than the figures of 19 of 36 primary peptic ulcers and 17 of 36 secondary lesions published by Drumm *et al.* [22] 20 years ago.

Finally, because the results of this study show, in children, a higher frequency than expected of ulcers or erosions, the evaluation of the multiple risk factors for the detection or prevention of ulcers and/or erosions needs a prospective case–control study over several seasons taking into account the specific prevalence of *H. pylori* infection in each different geographical area. These lesions should be systematically screened in symptomatic children with epigastric pain, hematemesis, melena, or weight stagnation to adapt adequately the subsequent treatment.

CONCLUSION

In this prospective European multicenter study a frequency of 8.1% of ulcers and/or erosions was observed in children mainly around their second decade of life with a high variability among different centers. Epigastric tenderness, pain awakening the child at night, hematemesis, melena and weight stagnation can be considered as significant risk factors for ulcers or erosions indifferently. The unexpectedly low frequency of *H. pylori* infection and NSAID-use in these patients questions its etiologic role causing ulcers and/or erosions.

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CHAPTER 6

Low prevalence of *Helicobacter pylori* infection in Indonesian young children: a longitudinal community-based cohort study

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ABSTRACT

Background:
In Indonesian adults and preschool children the reported prevalence of *Helicobacter pylori* (*Hp*) infection is considerable high, with rates between 49% and 54%. Data on the prevalence in younger children is scarce. We studied the prevalence of *Hp* in young children across the socio-economic classes of Bandung, Indonesia.

Methods:
Subjects were 150 healthy infants aged 3-24 months, living in the rural area of the Kiaracondong subdistrict. The socio-economic status (SES) was assessed according to the salary of the father. Stool samples were collected in September 2003 and September 2005 and tested for *Hp* using a monoclonal enzyme immunoassay (IDEIA™HP STAR, Dakocytomation, Denmark).

Results:
At first occasion, 4 out of 150 stool samples were *Hp* positive, giving an overall prevalence of 2.7%. All positive samples derived from the two youngest age groups (3-9 months), indicating a prevalence of 8% in that specific subgroup. The youngest infected child was 3 months old. Three of the 4 positive tested children belonged to the lowest SES, while 1 belonged to the medium status. Two years later, all 112 samples available for follow-up tested negative, whereas *Hp* specific treatment had not been provided.

Conclusion:
The prevalence of *Hp* infection in the age group 3-9 months is considerably high (8%) and indicates a very early acquisition of the infection. Spontaneous clearance is possible, since a follow-up analysis 2 years later revealed negative results for 3 patients tested originally positive.

Keywords: *Helicobacter pylori*; children; stool test; monoclonal enzyme immunoassay; Indonesia.

INTRODUCTION

Helicobacter pylori (*Hp*) is known to be responsible for chronic gastritis, predisposes to gastric and duodenal ulcers, and has been recognized as a type 1 gastric carcinogen in humans by the International Agency for Research on Cancer since 1994[1]. In spite of this, only a small part of the infected persons develop gastric cancer, so additional factors as virulence genes of the bacterium and life style of the host may be involved in progression toward cancer[2,3].
Infection with *Hp* is ubiquitous with prevalences of 40-50% in adults of developed countries and 80-90% in developing countries[4]. In general initial infection occurs during childhood[5], while chronic disease predominantly emerges at adolescent or adult age. In developing countries, the acquisition of the infection appears to occur earlier in life than in developed countries and in the latter a smaller percentage of children are infected. The infection prevalence increases with age and is usually associated with low socio-economic status (SES), crowding conditions, and poor hygiene[6-8]. Humans are the main source of *Hp* infection. The routes of transmission are unclear, although the presence of *Hp* in saliva, dental plaque, and stool seems compatible with both oro-oral and faecal-oral inter-human transmission[9].
Indonesia and Japan reportedly have a similar prevalence of *Hp* infection in adults, but the incidence of gastric cancer in Yogyakarta and Semarang (Indonesia) is 2% and 1%, respectively, of that in Japan. Tokudome *et al*. observed a *Hp* seroprevalence of only 2% both in man and woman in a study in 171 persons of the general population in Semarang, Indonesia, significantly lower than the 62% and 57% they observed for Japan and suggested that the rarity of gastric cancer in Semarang may be attributable to the relatively low prevalence of *Hp* infection[10,11]. On the contrary, Abdullah *et al*. observed in dyspeptic patients in Jakarta (Indonesia) and Japan similar percentages of *Hp* infections, but the Japanese patients had a significantly higher grade of gastritis and prevalence of mucosal atrophy and intestinal metaplasia, both precursors of gastric carcinoma[12]. A decreasing incidence of *Hp* has been shown in Jakarta from 1998 - 2005 with a stable incidence of intestinal metaplasia[13]. There are recommendations to vaccinate people in developing countries with high *Hp* prevalence to prevent gastric carcinoma, but before such a vaccine would be recommended, prevalence rates of *Hp* infection in different areas in those countries are needed[14].
Only few data exist on the prevalence of *Hp* infection in Indonesian children, and are either part of prevalence studies on adults that include some older children[15], or comprise exclusively older children[16] with exception of two conference abstracts[17,18] (table 1). Furthermore, most of the studies were based on determination of non-standardized antibody tests against *Hp*, lacking the sensitivity and specificity

desirable for young children. The aim of our study was to conduct a prevalence study of *Hp* infection in young children living in the crowded rural district of Bandung, Indonesia. We also took the opportunity to investigate the same population two years later to recognize changes in prevalence rates of *Hp* infections.

Table 1. Reported frequency of *Hp* infection in Indonesia

Year and Reference	City/ Region	Age (years)	Number tested	Prevalence (%)	Test method
2000[17]	Mataram	3-7	unknown	49.4	Serology
2000[18]	Surabaya	10-75	unknown	31.2	Serology
2006[16]	Jakarta	< 14	51	53	Serology
2005[15]	Jakarta	16-74	63	9.5	Stool
2005[11]	Yogyakarta	Adults	91	4(f), 5(m)	Serology and UBT
2005[10]	Semarang	Adults	171	2(m), 4(f)	Serology
This study (2003-2005)	Bandung	0.3 - 4	150	2.7	Stool

F: female; M: male; UBT: Urea Breath Test

METHODS

Study design and population

A longitudinal community-based cohort study was conducted in a peri-urban area of Bandung (Kiaracondong subdistrict), on the island of Java, Indonesia. Stool samples were collected in September 2003 and September 2005.

In September 2003, 150 healthy children aged less than 2 years of age were enrolled for the study. The children were randomly selected from the district population across the socio-economic status (SES). The parents of the participating children gave informed consent before sampling. The ethics committee of the local Padjadaran State University (Bandung) approved the design and concept of the study.

Sample collection and stool tests

Fresh stool samples were collected at the children's homes, by local health nurses. The parents (mostly the mothers) were interviewed about their SES and the health status of their children.

The children were divided into 6 age categories of three months each (table 2). Stool samples were collected and stored in cooled boxes at -4°C immediately and transported within 3 hours of collection to the laboratory of the Hasan Sadikin

Table 2. Prevalence of *Helicobacter pylori (Hp)* in 150 young Indonesian children using stool antigen detection with monoclonal *Hp*SA EIA.

Age (months)	n	*Hp* positive n	*Hp* positive %
3-6	25	3	2
6-9	25	1	0.7
9-12	25	0	0
12-15	25	0	0
15-18	25	0	0
18-24	25	0	0
Total	150 (m:82; f:68)	4 (m:2; f:2)	2.7

SES*	n	*Hp* positive n	*Hp* positive %
Low	32	3	2
Medium	113	1	0.7
High	5	0	0

*Socioeconomic status Indonesian Government Criteria: salary of the father: < 500.000 Rupiah: low; 500.000-1.000.000 Rupiah: Medium; > 1.000.000 Rupiah: high.

General Hospital for processing. The samples were stored at –70°C before being shipped in dry ice-cooled boxes to the Netherlands. Within one week after arrival at the Laboratory of Medical Microbiology, Leiden University Medical Centre, all 150 samples were tested blindly for *Hp* antigens using a monoclonal enzyme immunoassay (IDEIA™HP STAR, Dakocytomation, Denmark) in one run following the instructions of the manufacturer.

Twenty-four months later, new stool samples of the same children were collected by the same health care nurses. At this occasion a questionnaire was filled out by the parents, assisted by the trained health nurse. This questionnaire provided us data about living conditions, family size, and the education level of the parents and the history of abdominal complaints, gastro intestinal bleeding or carcinoma within the family as well as health complaints and medication (antibiotics) of the index child. Samples were transported similarly as described above and were again tested by the same monoclonal stool-test, blinded, in one run. Samples having readings ≥ 0.190 units were considered positive and samples ≤ 0.190 negative.

RESULTS

In total 150 infants (82 male and 68 female) aged 3-24 months were enrolled (table 2). The SES was low for 32 (21.4%), medium for 113 (75.3%) and high for 5 (3.3%) of the children.
Four stool samples tested *Hp*-positive at the first sampling (2 females), resulting in an overall *Hp*-prevalence of 2.7%. All positive samples originated from the groups aged 3-6 and 6-9 months, leading to a prevalence of 8% for this subgroup. The youngest infected child was aged 3 months and 4 days. Three of the positive children belonged to the lowest SES and 1 to the medium SES.
Two years after the first sampling, the *Hp* status was followed-up. Unfortunately, due to a big fire in the subdistrict, 27 children originally tested negative had moved elsewhere and attempts to trace their actual addresses remained unsuccessfully. In addition, one *Hp* positive tested child died from pneumonia and 10 *Hp* negative tested children could not be traced, so in total 112 children were available for the follow-up study. All samples in the follow-up were *Hp*-negative, including 3 of the children who were previously tested positive. Those 3 children had never been treated with antibiotics. None of their parents reported abdominal pain, ulcer or gastric carcinoma, nor underwent abdominal surgery.

DISCUSSION

In this longitudinal community-based cohort study we found a prevalence of 2.7% *Hp* infection in healthy Indonesian children of a very young age (<2 years), tested by an EIA in stools using an *Hp* specific monoclonal antibody. This result agrees with the previous reported prevalence of *Hp* infection in that age in some other developing countries, but it is not as high as in other countries in South-East Asia such as Bangladesh, Pakistan and Malaysian Borneo (table 3). Interestingly, none of the 150 children tested positive at the follow-up after 2 years, including 3 children who tested *Hp* positive at the first sampling. One possibility to explain our findings is that the stool test used lacks sensitivity and specificity. Gold standard for detection of *Hp* infection in children is upper endoscopy with biopsies for pathology, urease-test and culture[19]. However, these invasive tests are not suitable for epidemiologic studies in healthy children. The ^{13}C-Urea Breath Test (UBT) is the most appropriate non invasive diagnostic tool to diagnose *Hp* infection and to confirm therapeutic successes of eradication. A disadvantage of this UBT is the need of relatively expensive analytical equipment.
Serologic tests are unreliable in young children and have revealed disappointing results with respect to the diagnostics of acute *Hp*-infection, since the antibody

Table 3. Prevalence of *Hp* infection in children in different countries in South and East-Asia

Year and reference	Country	Age years	Number tested	Prevalence (%)	Test method
1996[27]	Korea	1-4	52	13	Serology
1996[28] 1999[29]	Bangladesh	0.1-0.25 0.8-1.3 6-9	36 (follow-up)	61 33 84	UBT
2009[35]	Bangladesh	0.3-4	68 (follow-up)	0 9 57 60	Serology (EIA) Serology (IB) UBT Stool-PCR
	Bangladesh	2	238	60 49	Serology Stool-antigen
1997[36]	Singapore	<5	unknown	3	Serology
1999[30]	Taiwan	3 4 5 6	112 356 658 232	4.5 4.4 9.4 11.7	Serology (ELISA and Latex-agglutination)
1999[31]	Malaysia	0,5-5	119 92 50	Mal: 5.9 Chin:7.6 Indians:10	Serology
2001[32]	Malaysia (West)	10-19	30 50 16	Mal:10 Chin:40.0 Indians: 37.5	Serology
2004[22]	Malaysian Borneo	<2 2,1-4	21 17	34 35	Stool (Premier Platinum HpSA)
2005[33]	Pakistan	0.1 0.2 0.3 0.5 0.8	61 42 121 64 30	80 79 76 58 67	^{13}C-UBT
2005[23]	Japan	0-12 months 5 year	51 44 (follow-up)	0 11	Serology and stool (HpSA)
2006[34]	Vietnam	<3 3-6 years	217 140	22.6 32.9	Serology

PCR: Polymerase chain reaction; EIA: enzyme immunoassay;
UBT: Urea Breath test; IB: immunoblot

reaction remains positive for months after successful eradication therapy[20]. Therefore, a specific *Hp* antigen test in stool samples is the preferred non-invasive test to use for epidemiological surveys. We applied a monoclonal stool antigen test in Indonesian children, that has previously been validated in children in Europe with a high sensitivity and specificity (98 and 99% respectively), albeit less sensitivity and specificity for younger children[21]. The performance of this test is significantly better than the polyclonal *Hp*SA test [9,22,23]. Our stool test has also been validated in Egyptian children and demonstrated a good sensitivity (94%), but less specificity among children less than 6 years[24]. In contrast Megraud *et al* demonstrated that UBT, stool antigen and antibody detection in serum and urine in children, showed a trend for improved sensitivity with age except for the stool test in comparison with biopsy-based tests[25]. The low specificity of the test applied in our study, prompted us to use a second assay (*Hp*SA, Meridian, Bioscience, Europe) on the 4 positive tested samples. All positive tested samples remained positive. The Canadian Consensus Group on *Hp* has judged that, in settings where the breath test is not available for children, the monoclonal test forms an excellent alternative to assess the status of *Hp* [21,26].
Table 3 summarizes results of the available studies of *Hp* infection in children in different countries in South and East-Asia. Most of the studies used serology or stool tests using polyclonal antibodies [15,22,23,27-35,36]. Only one study compared serology to monoclonal stool test in children of Bangladesh and showed a prevalence of 49% at the age of 24 months[35]. In previous studies from Mataram, Indonesia, the prevalence of *Hp* among kindergarten children (3-7 years) using passive hemagglutination (PHA) was 49.9%[17].
In most of the studies the prevalence of *Hp* infection increases with age, but in our study we did not observe this phenomenon. In developing countries with high *Hp* prevalence most of the children become *Hp*-infected at a very young age. The absence of *Hp* infection for 9-24 months old children in our study could be due to the small sample size, which is unlikely, especially given the 0% estimate 2 years later, the small number of children from low SES included in the older groups or both.
Low *Hp*-prevalence populations have been described earlier in Java[13] and in a multiracial population in Malaysia[11,32,37-39] as well as in Africa[40]. Boey and Goh already described in Malaysia the phenomenon of a different *Hp* prevalence between ethnic groups in one area for both adults as well as children, the so-called "racial cohort phenomenon": the prevalence of 6% in "Malay"children < 5 years was lower than that in "Chinese and Indian" children (7.6 and 10% respectively) living in the same area[31,32]. Possibly such a phenomenon could be responsible for the low prevalence in our population: the closeness of the community in the Kiaracondong district of Bandung may explain the low prevalence of *Hp* in our study.

The unexpected negative results in the second sampling could be associated with spontaneous clearance of the infection, as it has been previously shown in 42% of infected Egyptian children aged 6-17 months after a follow-up of 6 months[41]. This phenomenon has been also observed in 77% of a prospective cohort of Mexican children at 24 months of age. Interestingly 19% of the children were infected again later[42]. Transient *Hp* infection has also been reported in Japanese children followed-up from birth till 24 months, even if this could also have been the result of false positive initial tests (*Hp*SA)[43]. Another possibility is that *Hp* infection disappeared because of antibiotic therapy for other infectious disorders. However, the parents of the children in our cohort, who cleared the *Hp* infection, did not report any use of antibiotics during the follow-up period in their questionnaires.
Most of the children from our study group were breastfed for more than a year. It has been suggested that breastfeeding may play a role in preventing the acquisition of *Hp* infection during the first year of life due to passive immunity by anti-*Hp*-antibodies in the milk[44]. However, contra dictionary results have also been published reporting a positive correlation between breastfeeding and *Hp* infection[45], and no correlation both in Brazil[46] and in Germany[47]. However these studies have the limitation that they were conducted years after the period of breastfeeding[48,49], or were based on self-reported history. In addition less-sensitive serology tests were used in most of these studies [23,34,45-47].

Strengths and limitations of our study

Our study is the first study of *Hp* prevalence in young Indonesian children, determined by the monoclonal stool test. Although the test has not been validated in Indonesian children, it performs well in children of the same age group from Bangladesh. Moreover, retesting positive stool samples with a polyclonal stool test gave same results.

CONCLUSION

The results of our study demonstrate that the prevalence of *Hp* infection, detected by a monoclonal stool test, is 2.7% in healthy children aged 3-24 months living in a crowded subdistrict in Bandung, Indonesia. In the group aged 3-9 months the prevalence is 8%. This indicates that very young children already acquire *Hp*, despite the local practice of prolonged breastfeeding. Interestingly (spontaneous) clearance of *Hp* infection was observed. Our study supports the recommendation to start very early with infection preventive measures, such as vaccination if available in future. More extensive data on the *Hp* prevalence in the various (sub)populations and age groups living in the area are needed to justify such invasive measurements.

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SECTION C

DIAGNOSIS

CHAPTER 7

Antibiotic resistance of *Helicobacter pylori* in the Netherlands

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ABSTRACT

Guidelines on treatment of Helicobacter pylori (*Hp*) infections in adults and children recommend triple therapy (amoxicillin, clarithromycin or metronidazole and a proton pump inhibitor). The increasing antimicrobial resistance of *Hp* is one of the main reasons for eradication failure. Failure of first eradication treatment has shown to diminish future eradication success and therefore it is clinically relevant to get information on local resistance of *Hp*. In the Netherlands data on the resistance of *Hp* to clarithromycin and metronidazole are available up to 2003. No recent data on *Hp* resistance are known from adults or from children. We investigated the resistance prevalence of *Hp* to clarithromycin and metronidazole in 1080 Dutch adults and 72 children from 2000 to 2009. *Hp* resistance to clarithromycin was 8.5-9.4% and 6.5-7.2% in adults and children, respectively, while resistance to metronidazole was detected in 20.7-22.9% and 10.4-11.7%, respectively. Resistance to both clarithromycin and metronidazole was found in 2.8% of the adults and it was absent in children. Resistance rates were low compared to previous data of adults in the Netherlands, while resistance rates in children were low compared to other European countries. We conclude that a test-and-treat regimen is justified for adults as well as for children in the Netherlands.

Keywords: Helicobacter pylori, resistance, clarithromycin, metronidazole, children, adults

INTRODUCTION

Hp is acquired mainly in childhood and is one of the most important pathogens for a wide spectrum of human gastrointestinal diseases, including acute and chronic gastritis, peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma (MALT) and gastric malignancy[1]. After detection, the bacterium should be eradicated, as spontaneous clearance of the infection is rare.

Consensus guidelines on treatment of *Hp* in adults as well as in children recommend 7-10 day triple therapy, i.e. amoxicillin, clarithromycin or metronidazole and a proton pump inhibitor (PPI) in areas with clarithromycin resistance prevalences less than 15-20% and other regimens if the resistance rate is higher [2,3]. With this regimen eradication rates vary from 60-90%. However, the success rate of standard triple therapy for *Hp* eradication is decreasing worldwide and is a point of concern. Reasons for therapeutic failure include lack of compliance to therapy and an increasing antimicrobial resistance of *Hp* to clarithromycin and/or metronidazole [4-12].

Resistance of *Hp* to clarithromycin mainly results from point mutations occurring in the 23S rRNA gene, and resistance to metronidazole is associated with alterations of the nitroreductase-encoding genes rdxA and frxA as well as an increase of the TolC effluxpump [13,14]. *Hp* resistance to clarithromycin and metronidazole is thought to be caused by the extending use of clarithromycin for respiratory infections (especially in children) and the use of metronidazole for parasitic infections. Prescription of alternative regimens containing tetracyclines or bismuth is not allowed in children in many countries. Currently sequential therapy is a topic of research in adults as well as in children to improve the eradication rate [15-18].

The gold standard for assessment of *Hp* infection is upper endoscopy plus mucosal biopsies of the antrum and corpus of the stomach, hereby allowing getting material for the urease test, histology and culture to determine the in vitro susceptibility of the bacteria before any treatment. Determining the in vitro susceptibility before starting treatment will increase the eradication rate after first treatment and seems to be cost effective for clarithromycin-resistant *Hp* [19]. The additional advantage of endoscopy is that it can detect complications of *Hp* infection such as ulcer and carcinoma and that it is able to rule out other upper gastrointestinal disorders such as celiac disease, esophagitis and Crohn's disease. However, endoscopy is an invasive and expensive procedure and requires the use of sedation or anesthesia in children.

Dutch guidelines for adults recommend test-and-treat, which is safe and as effective as prompt endoscopy in absence of alarm symptoms in persons less than 45 years

of age [20]. In the Netherlands there is also a tendency to test-and-treat *Hp* infection in children, even if the current recommendation is to perform a culture of stomach biopsies taken during endoscopy. According to this approach, non-invasive tests include antibody-based stool tests (with a sensitivity of 80-98%), the urea breath test and serology. However, serology does not distinguish between an active and a past infection [21].
In the Netherlands, data on resistance of *Hp* isolated from adults are only known between 1993 and 2003. Resistance to clarithromycin varied from 1 to 5%, while resistance to metronidazole was 7-31% [9,22-28]. Since 2006 no further data have been published and resistance of *Hp* in children has never been investigated. The aim of this study was to analyze the prevalence of *Hp* resistance in adults and children in comparison to reported data on European individuals, in order to estimate whether the test-and-treat approach is justified in the Netherlands.

MATERIAL AND METHODS

Design of the study

We conducted a single center, retrospective database study at Leiden University Medical Center (LUMC), the Netherlands, from January 2000 to December 2009 to analyze the resistance to clarithromycin and metronidazole of *Hp* positive cultures of biopsies from the gastric antrum and/or corpus of adults and children.

Patients

The endoscopy unit of the LUMC is a reference center for family doctors as well as medical specialists, with a regional and national function. All consecutive patients that were referred for upper gastrointestinal endoscopy and had positive biopsies for *Hp*, were included. The data of all *Hp*-isolates were divided into two groups: 0-17.99 years of age (children) and ≥18 years of age (adults). If later biopsies and positive cultures from a patient were obtained after an interval of ≥3 months, the results were analyzed separately, and used to estimate the development of resistance under appropriate treatment. In adults, information about the medical history was limited due to the fact that most of them had been sent for endoscopy by the family doctor with an incomplete history of abdominal complaints. For most patients it was unknown whether or not they had undergone non-invasive testing before, and whether or not they had been treated before.
The children attended the outpatient department of our hospital or a regional hospital before endoscopy. None of the children had been treated for *Hp* before the first endoscopy. Children with a migrational background (at least one non-Dutch parent, or adopted from abroad) were analyzed separately.

Culture and susceptibility testing

Cultures for *Hp* were carried out at the laboratory of Medical Microbiology of the LUMC. Biopsies from gastric antrum and corpus were sent to the laboratory as soon as possible in NaCl 0.9% and inoculated on a blood plate (BioMérieux, France) and on a specific plate for *Hp*, Pyloria agar (PYL-plate, Bio-Mérieux, France). Plates were checked after 3-5 and 7 days of incubation under microaerophilic circumstances. *Hp* positivity was determined with a Gram stain and a positive oxidase, katalase and urease test. Minimal inhibitory concentrations (MIC's) were determined by the epsilometer test (E-test) (AB Biodisk, Solna, Sweden). Strains were considered clarithromycin sensitive if MIC ≤0.25 and resistant if MIC > 0.25 mg/L and metronidazole sensitive if MIC ≤ 8mg/L, intermediate if >8 and ≤ 16 mg/L and resistant if > 16 mg/L, according to the Eucast-criteria (www.eucast.org).

Table 1A. Demographic data and results

Parameters	Adults (N=1080)	Children (N=72)
Male (N) Mean age, yr (range)	510 55.8 (18.7-90.3)	42 11.5 (2.9-17.8)
Migrational background (%)	unknown	69
Number of Hp isolates (N)	1137	77
Resistance to Cla % #		
R	8.5	6.5 △
S	81.2	83.1
NT*	10.4	10.4
Resistance to Cla, % of tested strains	9.4	7.2
Resistance to MNZ, % #		
R	20.7	10.4 △ △
I	0.4	3.9
S	69.2	74.0
NT*	9.7	11.7
Resistance to MNZ, % of tested strains	22.9	11.7
Double Resistance** %	2.8	0

R = resistant; S = sensitive; I = intermediate. Cla = Clarithromycin. MNZ = Metronidazole.
NT* = Not tested due to viability problems of the strains
**Double resistance to Clarithromycin and Metronidazole
percentage of isolates
△ non-Dutch N= 3, △ △ non-Dutch N= 6

Table 1B. Changing resistances in second and third occasion biopsies

	Adults N	Children N
Patients with two cultures	41	2
Cla S→ Cla R	4	0
MNZ S→ MNZ R	4	0
No change	33	2
Patients with three cultures	6	2
Cla S→Cla R	1	0
MNZ S→MNZ R	2	0
No change	3	2
Patients with four cultures	1	0
No change	1	

R = resistant
S = sensitive
Cla = Clarithromycin.
MNZ = Metronidazole

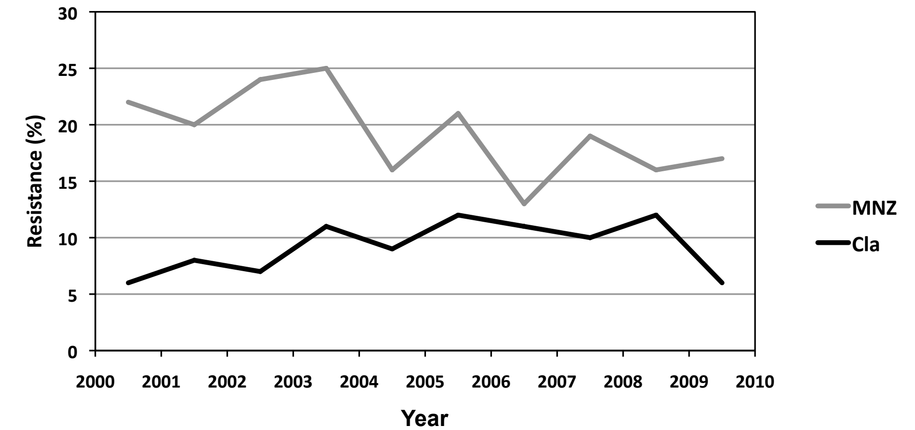

Fig 1. *Hp* resistance to Clarithromycin and Metronidazole in 2000-2009 in the Netherlands

RESULTS

From 2000 to 2009 all *Hp*-positive patients who underwent endoscopy, were included: 72 children and 1080 adults. Of these 1152 patients, 1214 cultures were positive for *Hp*. Susceptibility of clarithromycin and metronidazole could be determined in 1088 (90%) and 1095 (90%) cultures, respectively.
Demographic data and results of resistance to antibiotics are summarized in Table 1A and Table 1B. *Hp*-resistance rate against clarithromycin was 8.5-9.4% in adults and 6.5-7.2% in children. Resistance to metronidazole was observed in 20.7-22.9% of adults and 10.4-11.7% of children. Resistance to both clarithromycin and metronidazole in adults was 2.8%, equally divided among genders, and there was no time trend (2.9% in 2000-2004, 2.7% in 2005-2009). Double resistance to clarithromycin and metronidazole was absent in children. The *Hp* resistance rate against any antibiotic in the whole decade was 29.2% in adults, and 16.9% in children.
Sixty-nine percent of the children with *Hp* positive cultures in the study had a migrational background. Three out of 5 clarithromycin resistant strains of the children and 6 of 8 metronidazole resistant strains were detected in offspring of a non-Dutch mother. All clarithromycin resistant strains in children were detected in the period after 2004, while metronidazole resistant strains were divided equally over the whole period. The development of resistance to clarithromycin and metronidazole over time reveals that the resistance to clarithromycin slightly increased in the Netherlands (with the exception of the year 2009) and that resistance to metronidazole slightly decreased (figure 1).

DISCUSSION

We have shown that the *Hp* resistance to clarithromycin in the Netherlands has increased from less than 5% [22,24,26-28] to 8.5-9.4% in adults. This rate is comparable to the resistance rates reported in the European multicenter study in 1998 [9] and in studies from Finland, UK and Belgium in the same period [29-31]. However, this resistance rate is low compared to increasing resistance rates to clarithromycin in adults in other European countries [32-36], where prevalences have been reported of 17-26% (supporting information Table 2). In case of secondary resistance rate, the resistance rate can be up to 68%, as was shown in a study from France [34]. We speculate that the most likely explanation of the low resistance rates of *Hp* to clarithromycin in the Netherlands is the relative reluctance of prescribing antibiotics by physicians in the Netherlands [37]. The prescription of clarithromycin, the most commonly used macrolide in primary care, has stabilized since 2003 (www.swab/nethmap.nl).
Hp resistance to metronidazole in this study was 20.7-22.9%, while in the period before 2003 rates in Dutch adults varied from 7 to 33%. This, again, is comparable to data from Sweden (16.2%) [38] and much lower than the resistance rates in other European countries (27-61%) [29-36,39] and the resistance rates reported in the European multicentre study in adults (33.1%) [9] (Supporting information Table 2). Metronidazole resistance of *Hp* varies geographically, being higher in developing countries, where this class of antibiotics is frequently used to treat parasitic infections.
Double resistance to clarithromycin and metronidazole was detected in only 2.8% of the strains of adults and remained stable over time. The low resistance rates to clarithromycin and metronidazole as well as the stable and low double resistance rate are remarkable, because since implementation of the stool antigen test in 2000, the test-and-treat regimen has been introduced gradually in the Netherlands without determining the susceptibility of *Hp* before treatment. With such approach, one would have expected higher secondary resistance rates, since the endoscopic samples were probably more often from patients who failed first line therapy. Resistance of *Hp* to amoxicillin in Europe and USA is very low and stable, and clinically negligible. In 1998, Glupczynski reported 0% primary resistance to amoxicillin all over Europe with an exception for Italy (8.2%) and Copenhagen (4%) [9]. We did not determine the susceptibility to amoxicillin in all *Hp* positive strains systematically during the last decade.

This study is the first to report data on the antimicrobial resistance of *Hp* in children living in the Netherlands. The prevalence of *Hp* in young children is low (1.2%-9%) and most infected children are offspring of at least one non-Dutch parent [40,41]. In our study 69% of the *Hp*-positive children had a migrational background. We determined resistance rates of 6.5-7.2% to clarithromycin and 10.4-11.7% to metronidazole. Seventy-five percent of the metronidazole resistant strains and sixty percent of the clarithromycin resistant strains were detected in offspring of a non-Dutch mother.
The resistance rates of *Hp* strains of children in our study are much lower than the resistance rates of 24% and 25% to clarithromycin and metronidazole, respectively, that were detected in an European study [42], and also lower than rates reported from various European countries [36,43-50](Supporting information Table 3). In the European study, 41% of the children with resistant strains were offspring of non-European mothers, while double resistance to both clarithromycin and metronidazole was 6.9%. Several studies have suggested that resistance to clarithromycin is generally higher in children than in adults, probably due to the previous use of macrolides in respiratory infections [9,30,35]. However, we detected lower clarithromycin resistance rates in children (6.5%) compared to adults (8.5%).

A limitation of the study is the single center design, due to unavailability of data on resistance rates in the past ten years and differences in testing methods and standardization in other centres. Therefore, geographical variation in resistance rates could not be analyzed. However, previous publications on Dutch adults have reported only a slightly different resistance pattern in diverse areas of our country [9,22,24], although the resistance rate may be higher in an area of the country with a higher percentage of immigration. Another limitation is the retrospective nature of our study, conducted on the basis of medical files. Since general practitioners in the Netherlands directly refer adult patients for endoscopy, primary or secondary resistance could not be distinguished. In general, secondary resistance is far higher than primary resistance, and is one of the main reasons for eradication failures [33,35]. Although it is likely that some of the *Hp*-positive patients in our study have used antibiotics before, and in spite of the gradual introduction of the test-and-treat-regimen in adults in 2007 [2], the resistance rates have remained low.

The resistance rate for clarithromycin in Dutch adults below 15-20% supports the recommendation [2] to routinely perform a test-and-treat regimen. However, we believe that also in the coming years surveillance of regional *Hp* resistance is needed, and that this should be well communicated to the clinicians. Previous studies have shown that most physicians are insufficiently acquainted with regional resistance data [51-53]. Up-to-date information on resistance rates is needed to timely modify treatment regimens. Hopefully, future development of non-invasive susceptibility tests for clarithromycin in stool samples will facilitate this.
Our data on resistance rates in children suggest that also in this age group a test-and-treat approach can be used. However, we suggest that the clinician should

inquire if the child has received clarithromycin in the previous 3 months, and if so, exchange this by metronidazole. In case of eradication failure after the first clarithromycin-based triple therapy, a second triple therapy with metronidazole instead of clarithromycin could be prescribed. A second eradication failure should lead to referral of the patient for endoscopy with biopsies for susceptibility testing.

CONCLUSION

The resistance of *Hp* to clarithromycin, although still low compared to resistance in other European countries, is slowly increasing in Dutch adults, while the resistance to metronidazole has been stable. The resistance of *Hp* to clarithromycin and metronidazole in Dutch children is low compared to European data and lower than in adults. Double resistance to both clarithromycin and metronidazole is low in adults and absent in children. Both for adults and children, a test-and-treat approach can be used, but continuing surveillance of antibiotic resistance remains necessary.

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Tables 2 and 3
Supporting information

Table 2 Reported prevalence of *H.pylori* resistance to clarithromycin (CLA) and metronidazole (MNZ) in adults in Europe

Year and reference	Country	Period	No of ptts	CLA res %	MNZ res %	AMX res or CLA + MNZ
2004[1] 2011 [2]	Finland	2000-2002 2000-2008	292 505	2 8	38 40	Not done AMX 0
2006 [3]	Sweden		333	1.5	16.2	0
2007 [4]	Italy	2004-2006	255	16.9	29.4	
2007 [5]	UK (Gwynned) UK (Essex)	2000-2005 2000-2005	664 646	8.3 12.7	28.6 36.3	Cla +MNZ 4.4 Cla + MNZ 8.4
2009 [6]	Poland	1997-1998 2007-2008	66 76	9.1 18.4	36.4 44.7	Amoxy 0
2010 [7]	France	2004-2007	530	26 P* 19 S** 68	61.1	Amoxy 0
2010 [8]	Ireland	2007-2008	222	13.2 P* 9.3 S* 32.4	31.5	Cla+MNZ 8.6
2008 [9] 2010 [10]	Bulgaria	1996-1999 2007-2009	120 428	9.8 18	27.5 27.3	
2005 [11] 2011 [12]	Belgium	2002 1990-2009	436 7903	3 P* 5.2 S** 8,5	31 P 26.1 S 49	Amoxy 0 Amoxy 0
2001 [13]	European multicenter study	1998	1274	9,9	33.1	0.8

P*: primary resistance S**: secondary resistance

Table 3 Reported antibiotic resistance of *H.pylori* in children in Europe (last decade)

Year of publication and reference	Year (period)	No of patients	Country	Resistance		
				CLA %	MNZ %	AMX %
2001 [14]	1989-1995 1995-2000	551	Belgium	6-16 16.6	18 18	0 0
2001 [15]	1998-2000	98	Poland	23.5	unknown	unknown
2002 [16] 2008 [9] 2010 [10]	2000-2001 2005-2007 2007-2009	115 75 73	Bulgaria	12.4 Naive 18.7 Naive 27.4 Naive	15.8 Naive 16 Naive 16.4 Naive	0
2000 [17] 2005 [18] 2011 [19]	1998-1999 1999-2003 2000-2009	58 109 1115	Portugal	44.8 39.4 34.7	19.0 16.5 13.9	0 0 0
2001 [20] 2009 [21]	1991-1995 2002-2006	246 101	Spain	3.5 54.6	19.9 35.7	0 0
2003 [22] 2010 [23] 2011 [24]	1997-2000 2002-2008 2002-2009	117 153 74	Austria Vienna Vienna Graz	20.3 34 22	16 23 22	0 0
2006 [25]	2000-2003	58	Germany	9	16	unknown
2008 [26]	2002-2006	157	Italy	42	12	unknown
2007 [27]	1994-2005	377	France	22.8	36.7	0
2006 [28]	1999-2002	1233	Europe* (Multicenter)	24	25	0.6

* no Dutch children included

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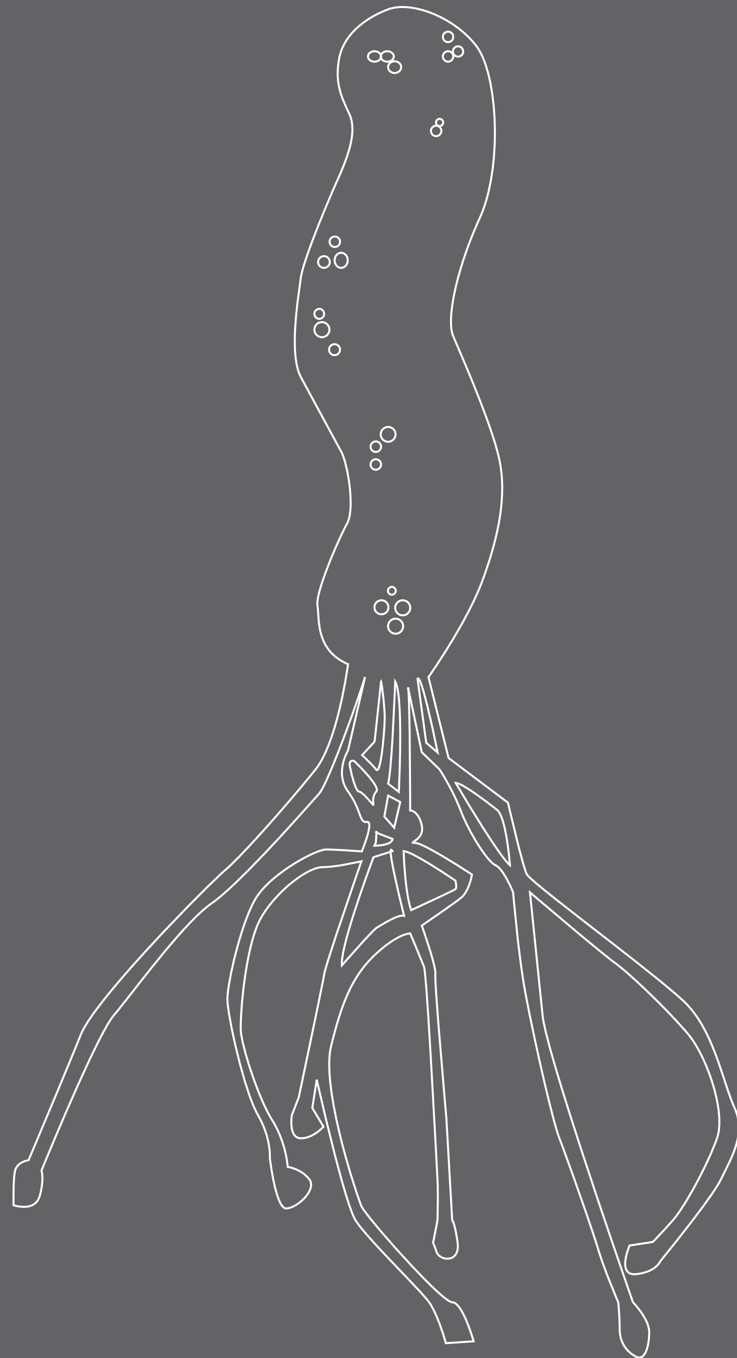
SECTION D

GENERAL DISCUSSION



CHAPTER 8
General discussion, conclusions and topics for further research

ABSTRACT

This chapter discusses the key findings of our research on the prevalence, diagnosis and treatment of *Helicobacter pylori* (*Hp*) infection in children, including some technical aspects on *Hp*-related clinical issues. The final part concerns some aspects of prevention of *Hp* infection and future directions of *Hp*-related research.

8.1 PREVALENCE

Prevalence of *Hp* infection

The prevalence of *Hp* infection varies from 11-70% in Europe to more than 90% in adults in Africa and Asia. This variability is highly related to geography, ethnicity, age and socioeconomic status. The prevalence of *Hp* infection has decreased considerably in the last decades in developed countries [1-4]. Roosendaal *et al* predicted a decrease of the prevalence in Dutch adults between 1940 and 2040 from 50% to less than 20% , based on a decrease of prevalence from 20 to 10% in the 6-15 years age group over 15 years (1978-1993) in the Netherlands [5].
We found a relatively low prevalence in young children (2-4 years of age) in the general Dutch population (1.2%), and a significantly higher prevalence among Dutch children of the same age belonging to ethnical minorities (2.6%) (Chapter 4). A recent study showed that *Hp* prevalence in Dutch children (7-9 years of age) has stabilized between 1993 and 2005 at a level of 9% [6]. This stabilization can be explained in two ways. First, it may be that no further decrease can be expected by improving living conditions and decreasing family size because these have already reached the maximum level attainable. Alternatively, a potential decrease by improvements of these factors may be neutralized, at least partly, by increased immigration of children from high prevalence countries. As a result of this stabilization, colonization with *Hp* is expected to remain common in the coming decades [6]. Notably, more than 90% of the children, investigated in this study, were of Dutch descent, so that the study group does not reflect the current population, of which 21% is reported to be of non-Dutch descent (www.cbs.nl). So, the authors may have underestimated the prevalence of *Hp* among schoolchildren of the general population in the Netherlands.
During the last five years, the number of people from high prevalence countries in Eastern Europe (Poland, Romania, Bulgaria) and Asia and the Middle East (China, Afghanistan, Iran, Iraq), who immigrated into the Netherlands has tripled (www.cbs.nl), which may have a significant influence on the *Hp* prevalence. Since the infection is usually acquired at a young age, it is likely that these immigrants and their offspring have already been infected in the country of origin, and form a risk group for *Hp* infection and its pathology.
After discovering that a group of adopted children that emigrated from Indonesia to the Netherlands tested *Hp*-positive, we decided to investigate the prevalence of *Hp* infection in their country of birth. We expected to find a high prevalence of *Hp* in a crowded district of Bandung (Chapter 6). Interestingly, the prevalence in the young Indonesian children was relatively high (8% for the youngest participants).

However, this could not be confirmed in a follow-up two years later when the prevalence was 0%. Given previous data on the prevalence of *Hp* in Indonesia [7], we speculate that the prevalence of *Hp* infection may differ between various areas in a country. We therefore suggest that before deciding on eradication campaigns in high prevalence countries, the local variation of prevalence should first be monitored.

Apart from the group of children of non-Dutch parents living in the Netherlands (recently immigrated or adopted from abroad), another risk group for *Hp*-infection consists of institutionalized children. The prevalence of *Hp* in residential children with developmental disabilities is reportedly higher than in the general population [8-11]. In the Netherlands the prevalence of *Hp* has been described by Bohmer *et al* in institutionalized persons (N=338, 11-89 year, median age 51 yr), of whom a small number of children [12]. The *Hp* prevalence in these adults was significantly higher (83%) than the prevalence of *Hp* in adults of the general Dutch population (50%).

A third group we have identified to be at risk for severe *Hp* infection are children with immunodeficiencies / X-linked agammaglobulinemia (XLA), because of their proneness to recurrent gastrointestinal infections. Although *Hp* infections in immunocompetent children are limited to superficial infections of the stomach, bacteremia with *Helicobacter* (species) has been found in patients with immunodeficiencies [13,14]. Diagnosis can be delayed by the fact that recognition in standard automatic blood cultures is problematic, since *Hp* germs multiply slowly. Therefore extended culturing after one week of incubation is often applied, if no bacterial growth is observed in the first week. We published a case report and literature review on this topic [15].

In the 10 years database of *Hp*-strains from adults and children at the Leiden University Medical Center, the prevalence of *Hp*-resistance to antibiotics was low, despite dramatically increasing percentages of drug resistance to clarithromycin, metronidazole and amoxicillin reported elsewhere [16-21]. Possibly, the restricted prescription of antimicrobial drugs and the lack of over-the-counter availability of antibiotics in the Netherlands may have contributed to the low prevalence of drug resistance. Intensive surveillance of the resistance patterns will remain important, given the expected changes in the composition of the population in the Netherlands.

With respect to the virulence of *Hp* strains, special attention should be given to children originating from high prevalence countries, for example as a result of adoption or immigration. This group is at increased risk of developing gastroduodenal lesions as they may have been infected with the more virulent strains (CagA positive), that are dominant in these countries. These strains cause more pathology, and at an earlier age [22,23]. In contrast, in the Dutch population CagA positive strains are currently vanishing. Den Hoed *et al* detected CagA-seropositivity in only 8.2% of the *Hp*-positive Dutch children 6. An earlier Finnish cohort study determined a decline in prevalence of CagA antibodies from 43% to 8% among subjects 14-44 years old over a 21 year period, suggesting that CagA positive strains are disappearing more rapidly than CagA negative strains [24].

Prevalence of gastric and duodenal ulcers

In the prospective multicenter study on the frequency and risk factors of gastric and duodenal ulcers or erosions in European children undergoing an upper endoscopy, we found a frequency of 8.1%, occurring mainly in adolescence. However, *Hp* infection was only detected in 27% of the children with ulcer or erosion (Chapter 5). In an earlier multicenter study on antibiotic resistance of *Hp* in Europe a frequency of 10.4% of ulcers was found in children above the age of 12 years [25]. More ulcers and/or erosions (16.7 to 22%) were found in research centers in Belgium, Italy and Turkey. In all these countries the clinical presentation was similar: clinical symptoms such as epigastric pain, hematemesis, melena and weight stagnation, as well as known chronic diseases such as inflammatory bowel disease (IBD) and rheumatic diseases. If aged above 10 years of age, nocturnal pain was also significantly associated with ulcers [26].

Oderda *et al* reported on *Hp* negative ulcers [27] and highlighted that in countries with a relatively low *Hp* prevalence most ulcers are not associated with *Hp*-infection. So, pediatricians should be aware that gastric and duodenal ulcers in European children, surprisingly, occur more often in *Hp*-negative than in *Hp*-positive children. Therefore, despite the decreasing prevalence of *Hp* in Europe, the frequency of ulcers and/or erosions may still increase due to the increasing prevalence of chronic diseases such as inflammatory bowel diseases (IBD) and rheumatic diseases, as well as the increasing use of drugs, that can cause ulcers as a side-effect (ulcerogenic drugs) in children. The development of gastro-duodenal ulcers is multifactorial, so that many other factors than *Hp*, such as genetic susceptibility, can lead to the development of ulcers.

As mentioned above, the risk of developing peptic ulcer disease (PUD) is higher in pediatric and adult patients that are CagA seropositive or carry CagA positive status. However, not all CagA positive patients develop ulcers, depending on the in situ expressing of CagA. A positive correlation was established between acute and chronic gastritis, bacterial density and CagA-expression. Moreover, Figura *et al* showed that most patients with non-ulcer-dyspepsia harbor both CagA positive and –negative strains [28-31]. For practical reasons, in our study the CagA status of *Hp* or other virulence factors of the bacterium in positive patients with ulcer and/or erosions could not be investigated.

8.2 DIAGNOSIS

In the last decade, much progression has been made in the development of non-invasive diagnostic tools. However, none of these tests are 100% specific and sensitive (Chapter 2) and most tests have been poorly validated in infants below the age of 2 years in low prevalence populations. At the present time the monoclonal stool test is most frequently used, and has an adequate sensitivity and specificity (97-98% and 95-100%, respectively) for the detection of *Hp* [32]. Therefore easily accessible testing of *Hp* has become feasible. The ^{13}C-urea breath test requires special devices and equipment for children, but provides a non-invasive test with an optimal performance, even better for post-treatment control. Serology testing is only useful in epidemiological studies, since this does not discriminate between a past or ongoing infection. The availability of these tests has aroused discussion about the necessity to perform endoscopy in suspected cases versus a test-and-treatment regimen.

Endoscopy versus test-and-treat: implications for the differential diagnosis

Recently, an evidence-based guideline was published for diagnosis and treatment of *Hp* infection in children in Europe and North-America. This guideline recommends endoscopy (including rapid urease testing, histology and/or culture) in all suspected cases, including those with a positive non-invasive test [33]. When used in combination, these tests are still considered "the gold standard" for the diagnosis of *Hp* in children. The main reason for recommending endoscopy is that the symptoms of *Hp* infections are little specific, so that other causes for the symptoms of the child need to be ruled out (e.g. celiac disease, Crohn's disease and gastro esophageal reflux). The added advantage of endoscopy is that other pathologies of the upper gastrointestinal tract can be found, as well as complications of the infection.
However, there are also arguments against endoscopy. First, it is an invasive method that cannot be performed in children without anesthesia or deep sedation. Second, the diagnosis of celiac disease has become easier by recent sensitive antibody-tests, and does no longer require taking duodenal biopsies in selected cases [34]. Third, in children the diagnosis of Crohn's disease is not only based on abdominal complaints, but also on the presence of peri anal alterations (skin tags, fistulas, abcess), extra-intestinal manifestations as mouth ulcers and growth retardation, and a positive family history for inflammatory bowel disease. Fourth, a child suspected for suffering from gastroesophageal reflux, is usually first empirically treated with antacids, proton pump inhibitors or life style intervention before endoscopy is considered.

In our opinion, the decision to perform endoscopy before treatment of *Hp* or not, is dependent on the information obtained at the medical history, physical examination and laboratory investigations. The medical history, given by the child and his/her parents is crucial and should include at least detailed information on the symptoms, family history of gastric ulcers or carcinoma, and country of birth. A thorough examination of the patient, accompanied by laboratory results, is required to assess the likelihood of the various disorders in the differential diagnosis.

Endoscopy versus test-and-treat: implications for the therapeutic strategy

As mentioned above, the recent guideline for children recommends susceptibility monitoring[33] before treatment, implying that endoscopy should always be performed. Theoretically, the obvious advantage is that if the susceptibility for antibiotics is known, the appropriate antibiotics can be administered. However, in our country this would mean that one should have to perform an endoscopy in all cases, as non-invasive susceptibility tests with sufficient diagnostic accuracy are not yet available. While the specificity of PCR in stool is high, the sensitivity is still too low for practical use [35-37]. Susceptibility testing is more important in countries, where high resistance rates of *Hp* play a role, than in our country.
The question is if this general guideline is also applicable for the Netherlands, thus whether a test-and-treat regimen in Dutch children is justified instead of endoscopy and culture with susceptibility testing. This decision should be primarily based on the prevalence of resistance to antibiotics, particularly to clarithromycin. Therefore, it is important to collect information on the patients' previous use of antibiotics, particularly regarding clarithromycin. Current guidelines for adults suggest susceptibility testing only if the resistance rate of *Hp* to clarithromycin is higher than 15-20% [38]. Our study shows that the resistance to clarithromycin in the children was only 6.5% and the resistance to metronidazole 10.4%. As a result of the low resistance rate in children in the Netherlands, we believe that an *Hp* test-and-treat regimen without susceptibility testing is justified as first-line therapy, on the condition that the local and national antibiotic susceptibility of *Hp* are being monitored regularly. Currently, the only way to monitor *Hp* resistance is to analyze databases from centers that perform *Hp* cultures from gastric biopsies.

In conclusion, it seems justified to follow the directive for the adult test-and treat regimen policy, with some exceptions, for example if there are reasons to exclude pathology, or in acute situations (such as hematemesis or melena). In case of one or more risk factors (use of ulcerogenic drugs, family history of ulcers or gastric carcinoma), performing an endoscopy may be necessary to exclude other pathology

and to provide an appropriate treatment and follow-up. In case of eradication failure, susceptibility testing of *Hp* to clarithromycin would be desirable, before a second eradication therapy is applied. Since a non-invasive way of resistance testing (for example by molecular methods in feces) is still not available, in such cases endoscopy is advised.

8.3 TREATMENT

Considering that resistance percentages of *Hp* in the Netherlands are low, the most applied triple therapy (amoxicillin, clarithromycin and a proton pump inhibitor (PPI)) is generally effective in eradicating *Hp*. In case of previous use of clarithromycin, one could consider substituting clarithromycin for metronidazole. If triple therapy does not succeed in the first round, a regimen of amoxicillin, metronidazole and PPI is recommended to cover secondary resistance to clarithromycin. Clarithromycin resistant strains form the main cause of treatment failure, particularly in children [39]. To a lesser extent, this is due to metronidazole resistant strains. In case of a second eradication failure, an alternative regimen (higher doses of the drugs [21], more drugs [40], other drugs (such as bismuth [41] or quinolones [42]), or longer duration of therapy can be considered. A sequential therapeutic scheme, combining a PPI and amoxicillin for the initial five days to reduce the bacterial load and to destroy the bacterial cell wall, followed by a PPI and clarithromycin and metronidazole for the remaining five days, could increase the eradication rate. However, evidence for sequential therapy to be more effective than triple therapy is lacking [43-45]. The downside of these alternative regimens is that they may diminish patient compliance and may increase side effects. As the eradication rate strongly decreases after the first therapy, a well-tailored therapy regimen for children is important.
Recent Italian studies suggest that *Hp* positive immigrants probably should be managed differently from Italian patients [46,47], and possibly this is relevant as well for children in the Netherlands. For example it is unclear if in children of non-Dutch parents efficacy of eradication therapy is lower than in children of Dutch origin, because of worse compliance or by higher antibiotic resistance (Chapter 7).

8.4 PREVENTION

In analogy to the prevention of other infectious diseases, vaccination against *Hp* has been considered. De Vries *et al* calculated that vaccination could possibly be a cost-effective intervention if the prevalence would be higher than 20% [48]. However, these authors exclusively assessed effects 20 years or more after vaccination (such as ulcers and gastric cancer), while pediatricians deal with somatic consequences

(growth, anemia) and cognitive function impairment in children with *Hp* infection [49-51] over a shorter time interval. These effects are particularly worrying in developing countries with a relatively high prevalence.
At present, no *Hp* vaccine has been registered yet, but possible target groups and alternative strategies are being defined. Partly for this reason we assessed the prevalence in young children in the Netherlands in 2006. Due to the low prevalence of *Hp* in the Netherlands, we believe that general preventive measurements, such as vaccination, are not indicated for all children.
In recent years, interest in vaccines has declined, because of publications on adverse effects of *Hp* eradication in adults in developed countries, such as increase of gastroesophageal reflux disease and Barrett's esophagus. Furthermore, it has been suggested that *Hp* eradication may contribute to the obesity epidemic and the increasing prevalence of asthma and allergy. Meanwhile, considerable progression has been made in eradication success rates [52] in developed countries and data on a decline of the prevalence of gastric cancer do also not speak for a vaccination campaign. In high-prevalence countries, however, mass vaccination against *Hp* may be an attractive and practical strategy to eliminate *Hp*-related disease. In some of these countries public health authorities have initiated programs for screening and eradicating of the bacterium.
Thus far, attempts to develop a good (preventive as well as curative) vaccine against *Hp* have faltered for perceptual, practical and financial reasons. Vaccine candidates, delivery routes and adjuvants for preventive and therapeutic strategies were investigated in experimentally infected mice with significant reductions in *Hp* colonization, but only few of these studies achieved complete *Hp* eradication [53]. In humans, few clinical vaccination studies have been performed and the results have been disappointing [52,54]. Moreover, the genetic diversity of *Hp* (sometimes within a single patient) forms a complicating factor. A few years ago, however, a phase 1 study with clinical testing of a vaccine was published with promising results. All healthy non-infected volunteers responded to one or two of the three recombinant antigens (VacA, CagA and NAP) and in 86% of cases the vaccine mounted IgG antibody responses to all of these. Booster immunization after 2 years elicited a strong antibody response to the three antigens in all subjects. The safety of this intramuscular vaccine was satisfactory [55]. However, no further publications on this topic in *Hp* infected persons have been published by the same research group.

8.5 CONCLUSIONS OF THIS THESIS

In this dissertation we present the results of our research on *Helicobacter pylori* infections in childhood, focusing on the prevalence, diagnosis and treatment of the

infection. Our studies were conducted in the Netherlands, Europe and Indonesia. We discuss diagnostic tests, therapeutic regimens, resistance and preventive measurements. We highlight clinical and pathophysiological aspects of the infection and describe which particular strains are prevalent and how transmission occurs. Presently, there are no established correlations between a *Helicobacter pylori* infection and recurrent abdominal pain, gastroesophageal reflux disease or growth retardation. We present data on the prevalence of *Helicobacter pylori* in young infants in the Netherlands and observe that children with at least one non-Dutch parent form a risk group. We assess risk factors in a Europe-wide study on gastroduodenal erosions and ulcers in childhood. In our study, *Helicobacter pylori* infection and gastrotoxic medications were relatively little implicated as etiology of that pathology. The prevalence of *Helicobacter pylori* infection in Indonesian young children is relatively high and points at an early acquisition of the infection. Finally, the resistance of *Helicobacter pylori* to clarithromycin and metronidazole was assessed for adults and children in the Netherlands. Low resistance rates were found, but the resistance in adults is increasing. We conclude that a test-and-treat regimen is justified for the Netherlands.

8.6 TOPICS FOR FUTURE RESEARCH

1. In the Netherlands the prevalence of *Hp* infection in children is low, so screening and treating is probably not useful. Exceptions should be made for identified risk groups. So far, in the Netherlands research in institutionalized individuals has only been carried out in studies on adults, in which some children have been included. We believe that the prevalence of *Hp* should be determined among children in institutions for disabled persons, as they live closely together and possibly part of their abdominal and/or nutritional complaints are caused by *Hp* infection. A gain in the quality of life in those children may be obtained by eradicating *Hp*.
2. Multicenter studies in children with primary or secondary immunodeficiencies are desirable. It is presently unknown what the prevalence is of *Hp* in these children. They often need intensive care nursing and ulcerogenic medication. Scientific studies should be initiated to investigate whether test-and-treat regimen can prevent ulcers and bacteremia in the therapeutic pathway of these children. Within this group of patients particular attention should be drawn to children of non-Dutch parents from high-prevalence countries.
3. In most Dutch children suspected for *Hp* infection a test-and-treat-regimen is justified. Exceptions are children with acute presentations of the disease, such as hematemesis or melena or children with eradication failure. A prospective study on the effects of such strategy should be performed.

4. Regular local surveillance of antibiotic resistance of *Hp* in adults and children is necessary for optimal treatment and prevention of eradication failure and secondary antibiotic resistance.
5. In future Dutch guidelines on treatment of children with *Hp* infection, the choice of antibiotics should be tailored, depending of the development of resistance of *Hp* to clarithromycin and metronidazole in the Netherlands and the resistance rate in the country of birth of the infected children and their parents.
6. The development of a sensitive and specific non-invasive susceptibility test for *Hp* in stool samples would be a significant improvement of the management of children with suspected *Hp* infection before first triple therapy as well as after failure of eradication.
7. Pediatric-based *Hp* research is necessary for our understanding of both clinical and pathophysiological aspects of the infection. Since the prevalence of *Hp* in children of the general Dutch population is low, those studies should ideally be multicenter-based.

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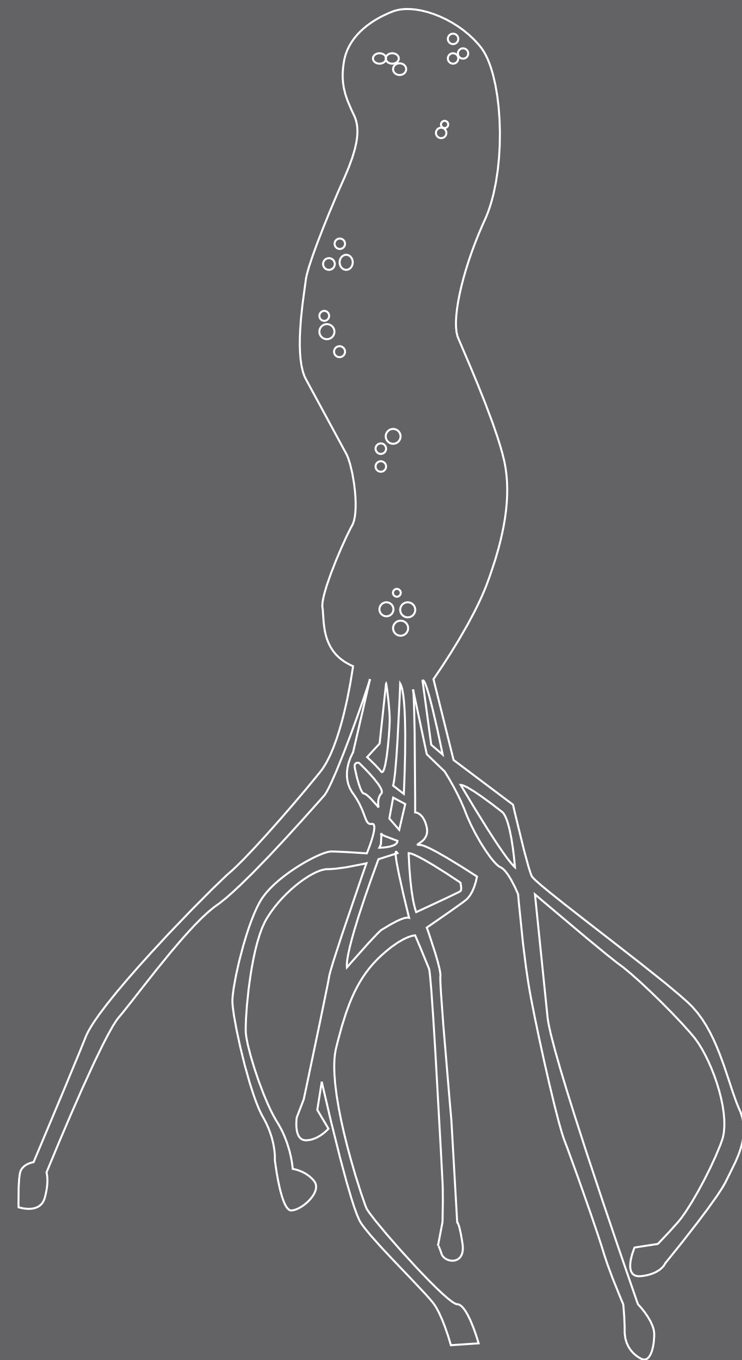


CHAPTER 9
Summary
Samenvatting

SUMMARY

In this thesis the prevalence, diagnosis and treatment of *Helicobacter pylori* (*Hp*) infection in children are discussed.

Chapter 1 is a general introduction to the subject: it describes the history, clinical features such as transmission and virulence factors, gastrointestinal and extra-intestinal symptoms, as well as the diagnosis, epidemiology, reinfection and spontaneous clearance of the infection. Some aspects of current guidelines for diagnosis and treatment are discussed as well as the increasing prevalence of resistance to antibiotics.

Section A: Reviews

In **Chapter 2** the developments on *Helicobacter pylori* in children are discussed for the time frame April 2005 until March 2006. The Canadian Helicobacter Study Group Consensus Conference published their evidence-based evaluation of the approach to *Helicobacter pylori* infection in children and adolescents that was later adopted by the Pediatric Task Force in the USA. Various diagnostic tools for *Helicobacter pylori* infection in children were compared, such as the urea breath test, stool tests, monoclonal and polyclonal tests, as well as newly introduced rapid tests, urine test and serology. Increasing resistance of *Hp* in children led to the introduction of novel therapeutic schemes, for example sequential therapy (omeprazole plus amoxicillin, followed by omeprazole plus clarithromycin and tinidazole) instead of conventional triple therapy. At the time, extra-digestive manifestations of *Hp* in children were not yet documented sufficiently. During the period of the review the Nobel Prize was awarded for the discovery of *Helicobacter pylori*, and its role in gastritis and peptic ulcer disease and this led to a renewed interest in the field.

Chapter 3 provides a review of the advances in pediatric *Helicobacter pylori* infections from April 2009 - March 2010. Studies on genetic variability between adult and childhood *Hp*-isolates did not establish a correlation between CagA, VacA and the histopathology in children. There was no association of *Hp* and some extra-intestinal manifestations as recurrent abdominal pain, gastroesophageal reflux disease and growth retardation. However, the guidelines on *Hp* screening in children with those symptoms were contradictory.
The emergence of *Hp*-negative duodenal and gastric ulcers in children was noted and led to the European multicenter pilot study on ulcers/and erosions, described in chapter 5. The number of publications on drug resistance had increased enor-

mously up to 2010 and this increase has continued since. We concluded that the results of studies on sequential therapy were too variable to adopt this new first-line eradication regimen for children. No benefits were found for probiotic administration on *Hp* eradication in children. Vaccination studies were performed dealing with the way of administration of the vaccine, the efficacy and the cost-benefit and the translation of studies from mice to humans.

Section B: Prevalence

Chapter 4. In view of the generally early acquisition of *Hp* infection, we studied the prevalence of *Hp* infection in young children living in the Netherlands. Prevalences were very low (1.2%), with a significant difference between the children with parents who are both Dutch (0.5%) and children with at least one non-Dutch parent (2.6%). The frequency of *Hp* in children with parents from the three largest immigrants groups in the Netherlands (originating from Suriname, Morocco and Turkey) was 5.3%. Therefore, children belonging to the aforementioned groups are at a relatively high risk and would benefit most from early diagnosis and treatment.

Chapter 5 describes the results of a prospective European multicenter study on the frequency and risks of gastric and duodenal ulcers or erosions in children. The study showed a frequency of 8.1% of ulcers and/or erosions, mainly appearing during teenage years. It appeared that an *Hp* infection was implicated less frequently as a cause of this pathology than expected.

Chapter 6 reports a follow-up study on the prevalence of *Helicobacter pylori* infection in young children living in Bandung, Indonesia. The prevalence of *Hp* in the age-group 3-9 months was 8%, indicative of a very early acquisition of the infection. However, two years later all children tested negative. This chapter also discusses the limitations of the diagnostic test, the role of breastfeeding in prevention and (spontaneous) clearance of the infection.

Section C: Diagnosis

Chapter 7 deals with *Hp* resistance related issues. The antimicrobial resistance of *Hp* was determined in a 10-year's retrospective database study at Leiden University Medical Center, the Netherlands. Resistance to clarithromycin was detected in 8.5-9.4% of the *Hp* isolates of adults and in 6.5-7.2% of the children, respectively. Resistance to metronidazole was detected in 20.7-22.9% of the adults and 10.4-11.7% of the children, respectively. These resistance rates are low in comparison to rates

in other countries and are compatible with a test-and-treat regimen for both adults and children in the Netherlands.

Chapter 8 comprises the general discussion and conclusions of this thesis. It also provides an outlook for future research on *Hp* infection in children.

SAMENVATTING

Dit proefschrift beschrijft het onderzoek, dat ik als kinderarts-maag-, darm- en leverziekten heb gedaan over de besmetting met en behandeling van de *Helicobacter pylori* infectie bij kinderen. Doel van het onderzoek was om de kennis van de infectie met deze bacterie en de daarmee verband houdende onderwerpen als de prevalentie, het stellen van de diagnose en de behandeling te vergroten. Wij voorzagen dat de kinderarts een belangrijke rol speelt bij deze relatief nieuw ontdekte infectieziekte, aangezien deze in de jeugd wordt opgedaan, zonder behandeling levenslang aanwezig blijft en tot ernstige ziekte kan leiden.
In de praktijk leidt dit tot de vraag of het zinvol is om de besmetting te voorkomen (bijvoorbeeld door vaccinatie) of ongedaan te maken (door te screenen en te behandelen). Richtlijnen hierover zijn niet eensluidend en niet zomaar te vertalen naar de Nederlandse situatie. Om hier zorgvuldig mee om te gaan hebben we **Sectie A** van dit proefschrift, "**Wetenschappelijke vorderingen ten aanzien van *Helicobacter pylori***", gewijd aan de internationale ontwikkelingen betreffende de infectie bij kinderen in de tijdsintervallen 2005-2006 en 2009-2010. Een belangrijk aspect in de literatuur van beide intervallen was de ontluikende resistentievorming van de bacterie tegen antibiotica.
Hoewel *Helicobacter pylori* wereldwijd voorkomt en meer dan 50% van de bevolking ermee besmet is, zijn er grote lokale verschillen: in Nederland komt de infectie relatief weinig voor. Immigratie vanuit landen met een hogere infectiegraad, zoals Turkije, Marokko, Suriname en Somalië of adoptie heeft echter gevolgen (gehad) voor de besmettingsgraad in Nederland. Aangezien de infectie meestal op kinderleeftijd plaatsvindt en door één van de ouders wordt overgedragen, hebben wij deze specifieke groepen kinderen apart onderzocht.
In **Sectie B** van dit proefschrift, "**De *Helicobacter pylori* infectie in verschillende omgevingen**" bespreken we onze bevindingen over de infectie bij kinderen in Nederland, in Europa en in een relatief arm district in Indonesië. We hebben niet alleen voor het eerst vastgesteld, dat besmetting ook in Nederland al op zeer jonge leeftijd optreedt, maar ook dat de besmetting vaak pas in de puberteit gerelateerd kan worden aan ernstige ziektebeelden. Met ons langetermijn-onderzoek in Indonesië kon het fenomeen, dat spontaan klaren van de infectie mogelijk is, in kaart worden gebracht. In een unieke Europese studie is het mogelijk gebleken, om de infectie in diverse landen te bestuderen en een relatie te leggen met ernstige verschijnselen op de kinderleeftijd.
Naar aanleiding van de in sectie A geconstateerde toenemende resistentievorming van de bacterie elders in de wereld, hebben we het in **Sectie C** "**Diagnostiek van *Helicobacter pylori* infectie**" beschreven onderzoek verricht. We onderzochten het

resistentiepatroon van in Leiden gekweekte *Helicobacter pylori*- stammen tegen de twee antibiotica, die het meest voor de behandeling van de infectie gebruikt worden. Met de resultaten van dit onderzoek konden we een behandelingsstrategie vaststellen voor *Helicobacter*-positieve kinderen in Nederland.
Het proefschrift wordt afgesloten met **Sectie D** "**Algemene Discussie**". Dit gedeelte is onder meer bedoeld om onze bevindingen te relateren aan de praktijk van behandeling van kinderen met een *Helicobacter pylori* infectie.

Hieronder treft u een korte samenvatting aan per hoofdstuk.

Hoofdstuk 1 is een algemene inleiding tot het onderwerp: het beschrijft de geschiedenis van *Helicobacter pylori*, de klinische kenmerken zoals transmissie en virulentiefactoren, en de symptomen die de bacterie kan geven op het gebied van het maag-darmkanaal en daarbuiten. Ook het stellen van de diagnose en diverse aspecten van epidemiologie, reïnfectie en het spontaan klaren van de infectie worden besproken. Bovendien worden aanbevelingen uit recente richtlijnen voor de diagnose en behandeling belicht, evenals het probleem van de toenemende resistentieontwikkeling voor antibiotica.

Sectie A: Wetenschappelijke vorderingen ten aanzien van *Helicobacter pylori*

In **Hoofdstuk 2** worden de ontwikkelingen besproken zoals die in de literatuur beschreven worden tussen april 2005 en april 2006. In die periode publiceerde de Canadese *Helicobacter pylori* werkgroep richtlijnen voor een *Helicobacter pylori* infectie bij kinderen en adolescenten. Deze richtlijnen werden later in de Verenigde Staten overgenomen door de "Werkgroep voor kinderen met een *Helicobacter*-infectie". Verschillende diagnostische methoden werden vergeleken: in uitademingslucht, ontlasting, urine en bloed. Resistentieontwikkeling tegen antibiotica leidde tot nieuwe behandelingsregimes zoals sequentietherapie (bestaande uit een maagzuurremmer met één antibioticum gedurende enkele dagen, gevolgd door een maagzuurremmer met twee andere antibiotica in de dagen daarna) in plaats van de traditionele drievoudige therapie (een kuur van 7-10 dagen met een maagzuurremmer en daarbij twee dezelfde antibiotica gedurende de hele kuur). Onderzoek liet nog niet voldoende aanwijzingen zien voor verschijnselen buiten het maag-darmkanaal bij kinderen, die veroorzaakt zouden kunnen worden door de infectie. In het jaar van de beschreven vorderingen werd de Nobelprijs uitgereikt aan Marshall en Warren voor de ontdekking in 1983 van *Helicobacter pylori* en de rol die de bacterie speelt bij maagontsteking en ulcus-ziekte. Hun eervolle erkenning leidde tot een hernieuwde wetenschappelijke belangstelling voor infectie met deze bacterie.

Hoofdstuk 3 beschouwt de wetenschappelijke voortgang over *Helicobacter pylori* infecties bij kinderen tussen april 2009 en april 2010. In die tijdsperiode verschenen er studies over een mogelijk verband tussen virulentiefactoren zoals CagA en VacA en de microscopische bevindingen van het maagslijmvlies bij kinderen, zoals dat ook bestaat bij volwassenen; bij kinderen werd dit verband niet gevonden. De literatuur liet geen associatie zien tussen de infectie en recidiverende buikpijn, gastro-oesofageale refluxziekte en achterblijvende groei. Richtlijnen over screening op *Helicobacter* infectie van kinderen met deze symptomen waren echter niet eensluidend. Een in dat jaar verschenen publicatie over *Helicobacter*-negatieve maag- en dunnedarmzweren bij kinderen deed de leden van de Pediatric Task Force van de Europese *Helicobacter pylori* Studie Groep ertoe besluiten om de studie, zoals beschreven in hoofdstuk 5 van dit proefschrift, te starten. Het toenemend probleem van resistentie van de bacterie tegen antibiotica vond zijn weerslag in het grote aantal publicaties hierover, een trend die tot op heden voortduurt. Een gunstig effect van probiotica bij de behandeling van *Helicobacter pylori* bij kinderen kon nog niet worden aangetoond. Er verschenen diverse vaccinatiestudies, die zich vooral richtten op de wijze van toediening, de efficiëntie en de kosten-batenanalyse van een eventueel vaccin en het extrapoleren van de onderzoeksresultaten aan muizen naar mensen.

Sectie B: De *Helicobacter pylori* infectie in verschillende omgevingen

Hoofdstuk 4 (*Nederland*). Aangezien een infectie met *Helicobacter pylori* meestal wordt verworven op kinderleeftijd, bestudeerden we de prevalentie bij jonge kinderen in Nederland. Deze was heel laag (1.2%) met een duidelijk verschil tussen kinderen van twee Nederlandse ouders (0.5%) en kinderen met minstens één niet-Nederlandse ouder (2.6%). De prevalentie bij kinderen met ouders uit de drie grootste immigrantengroepen in Nederland (Surinaams, Marokkaans en Turks) was 5.3%. Daarom hebben kinderen, behorend tot één van die groepen een relatief hoog risico op een *Helicobacter* infectie en zouden zij het meest kunnen profiteren van vroege opsporing en behandeling van de infectie.

In **hoofdstuk 5 (*Europa*)** worden de resultaten beschreven van een Europese studie, waaraan 19 onderzoeks- en behandelcentra deelnamen. De prevalentie van en risicofactoren voor maag- en dunnedarmzweren en erosies bij kinderen werd onderzocht. Wij vonden een prevalentie van 8.1%, en deze zweren en erosies bleken voornamelijk te ontstaan gedurende de puberteit. Tot onze verbazing waren een *Helicobacter pylori* infectie en het gebruik van bepaalde pijnstillers (NSAID), beide beschouwd als een belangrijke risicofactor voor maagslijmvliesschade bij zowel volwassenen als kinderen, minder frequent oorzaak van de zweren en erosies dan verwacht.

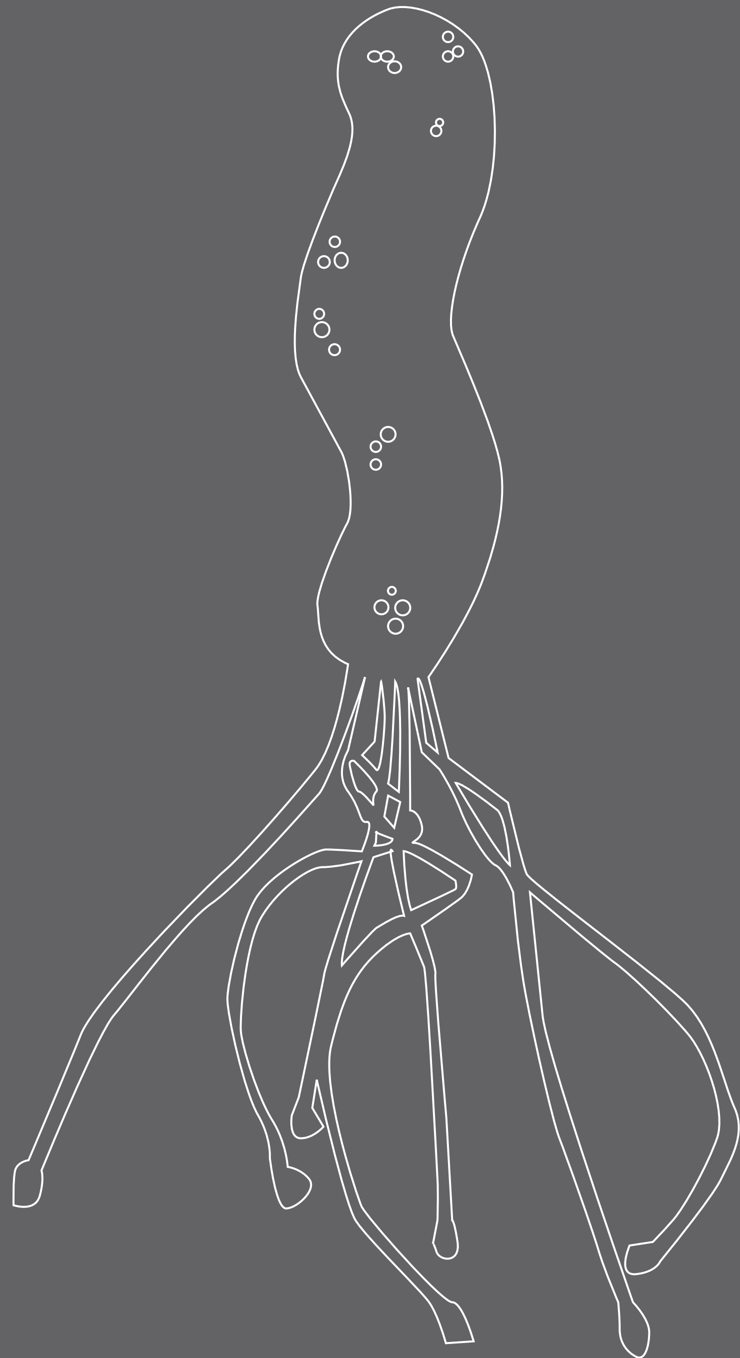
Hoofdstuk 6 (*Indonesië*) beschrijft een follow-up onderzoek over de prevalentie van *Helicobacter pylori* infectie bij jonge kinderen in een district van Bandung op Java, Indonesië, gebruikmakend van een diagnostische test in de ontlasting. Wij vonden een prevalentie van 8% bij kinderen van 3-9 maanden oud, een bevestiging dat kinderen in dit gebied op zeer jonge leeftijd de infectie oplopen. Bij hernieuwd testen van dezelfde kinderen twee jaar later, bleek echter geen van de kinderen meer positief in de ontlastingstest. In dit hoofdstuk bespreken wij dit klaren van *Helicobacter pylori* infecties. Daarnaast richten wij ons op de beperkingen van de diagnostische test en de rol van borstvoeding ten aanzien van preventie van de infectie.

Sectie C: Diagnostiek van *Helicobacter pylori* infectie

Hoofdstuk 7 omschrijft het resistentiepatroon van *Helicobacter pylori* tegen antibiotica. De resistentiegegevens van *Helicobacter pylori* werden geanalyseerd in een database van de afdeling Medische Microbiologie van het Leids Universitair Medisch Centrum (LUMC), over een verzamelperiode van 10 jaar. Resistentie tegen het antibioticum clarithromycine werd in 8,5-9,4% van volwassen- en in 6,5-7,2% van kinderkweken aangetroffen. De resistentie tegen het antibioticum metronidazol was respectievelijk 20,7-22,9% in kweken van volwassenen en 10,4-11,7% in die van kinderen. Deze getallen zijn laag in vergelijking met bevindingen uit andere landen en rechtvaardigen een beleid in Nederland, waarbij de behandeling mag worden ingezet zonder resistentiebepaling vooraf, voor zowel volwassenen als voor kinderen.

Sectie D: Algemene beschouwing

Tot slot worden in **Hoofdstuk 8** de hoofdresultaten van dit proefschrift in een breder perspectief geplaatst en worden suggesties gedaan voor vervolgonderzoek. Daarnaast biedt dit hoofdstuk een open aanbeveling aan de Nederlandse kinderartsen en huisartsen omtrent de behandeling van *Helicobacter pylori* infecties bij kinderen.



Appendices

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LIST OF ABBREVIATIONS

CagA	Cytotoxin-associated gene A
Cag PAI	Cag-Pathogenicity Island
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
EHPSG	European Helicobacter pylori Study Group
FISH	Fluorescence in situ hybridization
GER	Gastroesophageal reflux
Hp	Helicobacter pylori
HpSa	Helicobacter pylori Stool antigen
MALT	Mucosa-associated lymphoid tissue lymphoma
NAP	Neutrophil activating protein
NHG	Nederlands Huisartsen Genootschap
NASPGHAN	North-American Society for Paediatric Gastroenterology, Hepatology and Nutrition
NSAID	Non-steroid anti-inflammatory drugs
PCR	Polymerase Chain Reaction
PPI	Proton Pump Inhibitor
UBT	Urea Breath test
VacA	Vacuolating cytotoxin gene A

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CURRICULUM VITAE

The author of this thesis was born on the 6th of May 1946 in Amsterdam, the Netherlands. She attended secondary school at the "*Fons Vitae Lyceum*" in Amsterdam, where she passed her exam Gymnasium β. From 1964 till 1969, she studied medicine at the University of Amsterdam; afterwards, she moved to Nijmegen University, where she conducted her clinical training and specialized as a pediatrician under supervision of Prof.dr. E. Schretlen and Prof.dr. G.B.A. Stoelinga. Then, she moved to Leiden to start as a fellow Neonatology at Leiden University Medical Center (LUMC) under supervision of Prof.dr. J.H. Ruys and later moved her focus towards pediatric gastroenterology.

From November 1983 up to March 2008 she was a staff member at the department of Pediatrics of the St.Elisabeth hospital (presently Rijnland hospital) in Leiderdorp. During that period she served on the Supervisory Board of the Medical Daycare "Margriet". From 1994, she joined her later thesis advisors, Prof.dr. J.M. Wit and dr. M.L. Mearin in a part-time position at the Department of Pediatrics of the LUMC. There, she developed her focus on pediatric gastroenterology.

From 2000 onwards, she has been a registered pediatric gastroenterologist in the Netherlands. In addition to her duties as a general pediatrician, pediatric gastroenterologist and a teacher, she got involved in scientific research on pediatric gastroenterology. From 2008 she chose to work exclusively at the LUMC, thus enabling her to carry out her clinical research on *Helicobacter pylori* infections in children. She spent a sabbatical leave on a visit to Bandung, on the island of Java, Indonesia, in order to investigate the prevalence of the *Helicobacter pylori* infection in children in collaboration with local pediatricians. Eventually, her research activities led to the work described in this dissertation. Since 2005 she is an active member of the Pediatric Task Force of the European *Helicobacter pylori* Study Group.

Nel Mourad-Baars has been married to Abdelmassih Mourad for more than 35 years. They are blessed with three children, Daniël, Selma and Maurice and all three have preceded her with PhD theses in Geography (2008), Medicine (2010) and Chemistry (2009), respectively.