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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.

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.

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. 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.

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 pyloriidis, 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. 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. 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. 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. 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.

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.

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. 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...
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 jh05632, that encodes for the cell envelope protein glycosyltransferase which appears important for the progression to peptic ulcer disease33-35.

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: (i) why Hp causes symptomatic disease in only 20-30% of infected people; (ii) how many of the organisms are needed to establish a persistent infection; and (iii) who is prone for which disease phenotype. In children, antropical 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 infections36,37.

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 198538 has been assessed in many studies, but nevertheless the role of Hp in causing such symptoms has remained controversial39-41. A literature review over the years 1983-1998 found no specific correlation between RAP and Hp infection in children39. 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 needed42.

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 lately43-46. 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 children47. 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, rare48-50.

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 published49-51. 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 published49-51.

Short stature

There is insufficient evidence that Hp infection is causally related to short stature42. 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 hormone52-54.

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 status, 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. 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.

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. 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 13C-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.

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. The prevalence of Hp infection is low, the prevalence is increasing with age because of a cohort effect. In institutions of intellectually disabled persons a relatively high prevalence has been found, including in the Netherlands. 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. 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. 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 2.6% and 13.0%, respectively. 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>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Hp reinfection (%)</th>
<th>Follow-up Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1992</td>
<td>Italy (Turin)</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>1998</td>
<td>Japan</td>
<td>2.4</td>
<td>22</td>
</tr>
<tr>
<td>1999</td>
<td>Ireland/Dublin</td>
<td>11.5 (4.3 if &lt;5 years of age)</td>
<td>24±/± 14</td>
</tr>
<tr>
<td></td>
<td>Germany</td>
<td>2.3</td>
<td>15.5</td>
</tr>
<tr>
<td>2004</td>
<td>N-Ireland/ Belfast</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>Estonia</td>
<td>6.7</td>
<td>84</td>
</tr>
<tr>
<td>2005</td>
<td>Italy (South)</td>
<td>12.8</td>
<td>18±/± 43</td>
</tr>
<tr>
<td>2006</td>
<td>France</td>
<td>5.4</td>
<td>148 patient years</td>
</tr>
</tbody>
</table>
1.6 GUIDELINES FOR DIAGNOSIS AND TREATMENT

1.6.1 Adults
Updated guidelines on behalf of the European Helicobacter Study Group (Maastricht-I1 Consensus Report) for management of Helicobacter pylori infection in adults have been published in 200780. 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 included81. The Dutch standard for general practitioners (NHG-standard) lacks recommendations for treating children with Hp infection82,83.

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 Europe84-86. 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 13C-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 metronidazole87. In a French study on 27 treatment regimens a global failure of 25.8% was found and resistance to clarithromycin almost perfectly predicted failure88. The resistance rate to amoxicillin is very low in Europe. More recently alternative therapeutic regimens have been introduced including sequential therapy and quadruple therapy89-92, 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)

<table>
<thead>
<tr>
<th>Year of publication and reference</th>
<th>Period</th>
<th>Region/city* of the Netherlands</th>
<th>Clarithromycin % (N)</th>
<th>Metronidazole % (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997**</td>
<td>1993-1996</td>
<td>Not known</td>
<td>Not tested</td>
<td>7 (245)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>South</td>
<td>16 (436)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amsterdam*</td>
<td>19 (150)</td>
<td></td>
</tr>
<tr>
<td>1999**</td>
<td>1997-1998</td>
<td>West</td>
<td>24.3 (123)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>North-East</td>
<td>21.5 (65)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>South</td>
<td>11.6 (43)</td>
<td></td>
</tr>
<tr>
<td>2001**</td>
<td>1998</td>
<td>Amsterdam*</td>
<td>33 (100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hoogeveen*</td>
<td>23.4 (77)</td>
<td></td>
</tr>
<tr>
<td>2001**</td>
<td>1995-2000</td>
<td>North-East</td>
<td>18.8 (5946)</td>
<td></td>
</tr>
<tr>
<td>2003**</td>
<td>1995-2000</td>
<td>Zaandam*</td>
<td>25.8 (727)</td>
<td></td>
</tr>
<tr>
<td>2005**</td>
<td>1998-2003</td>
<td>Den Bosch*</td>
<td>14 (960)</td>
<td></td>
</tr>
<tr>
<td>2006**</td>
<td>1997-2002</td>
<td>Driel*</td>
<td>14.4 (1125)</td>
<td></td>
</tr>
</tbody>
</table>
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.

REFERENCES


Chapter 1


