Helicobacter pylori infection among children in Sweden

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CONTENTS

LIST OF ORIGINAL PAPERS

The thesis will be based on the following papers, which will be referred to by their Roman numerals.

I. Granström M, Tindberg Y, Blennow M.
Seroepidemiology of *Helicobacter pylori* infection in a cohort of children monitored from 6 months to 11 years of age.
Journal of Clinical Microbiology 1997;35:468-70.

II. Tindberg Y, Blennow M, Granström M.
Clinical symptoms and social factors in a cohort of children spontaneously clearing *Helicobacter pylori* infection.
Acta Paediatrica 1999;88:631-5.

III. Tindberg Y, Bengtsson C, Granath F, Blennow M, Nyrén O, Granström M.
Determinants for *Helicobacter pylori* infection in Swedish school children: Lack of evidence of child-to-child transmission outside the family.
Gastroenterology, in press 2001.

IV. Tindberg Y, Nyrén O, Blennow M, Granström M.
Helicobacter pylori infection and gastrointestinal or abdominal symptoms in Swedish school children.
Submitted for publication.

V. Tindberg Y, Bengtsson C, Bergström M, Granström M.
The accuracy of serologic diagnosis of *Helicobacter pylori* infection in school-aged children of mixed ethnicity.
Helicobacter, in press 2001.

ABBREVIATIONS

cagA	Cytotoxin associated gene A
C. jejuni	*Campylobacter jejuni*
CDC	Center for Disease Control
DU	Duodenal ulceration
EBV	Epstein-Barr virus
ELISA	Enzyme Linked Immuno-sorbent Assay
GERD	Gastroesophageal reflux disease
GI	Gastrointestinal
H. pylori	*Helicobacter pylori*
IARC	International Agency for Research on Cancer
IR	Incidence rate
MALT	Mucosa-associated lymphoid tissue
mL	millilitre
NIH	National Institute of Health
NPV	Negative predictive value
NUD	Nonulcer dyspepsia
PCR	Polymerase chain reaction
PI	Performance Index
PPI	Proton pump inhibitor
PPV	Positive predictive value
RAP	Recurrent abdominal pain
Sens.	Sensitivity
SES	Socio-economic status
SIDS	Sudden infant death syndrome
Spec.	Specificity
UBT	Urea breath test
vacA	Vacuolating cytotoxin A gene

INTRODUCTION

Helicobacter pylori history

Spiral shaped or curved bacilli were found in animal and human gastric mucosa already 100 years ago (Salomon 1896, Krienitz 1906), but until the 1980'ies no clinical significance was attached to the presence of these organisms. In 1983, Warren and Marshall, detected the organisms and associated the presence of these spiral-curved bacteria on the antral mucosa with gastritis in adults (Warren and Marshall 1983). Several subsequent reports supported a relationship between the organism and gastroduodenal disease, notably gastritis and duodenal ulcer disease (Marshall et al. 1984, Jones et al. 1984, Kasper et al. 1984, Blaser et al. 1987).

Initially termed Campylobacter-like, the organism was later assigned the name *Campylobacter pyloridis*, because of its principal localisation in the lower, pyloric parts of the stomach. The name was later changed to *Helicobacter pylori,* indicating its spiral shape, when taxonomic features of the bacterium showed that it was a genus of its own (Goodwin et al. 1989).

As a result of the accelerating research on both microbiological aspects and the pathogenic role of this newly discovered bacteria, the role of *H. pylori* in duodenal and gastric ulcer disease was established and reported in a consensus statement in 1994 (NIH Consensus 1994). Also, theories of an etiological link between *H. pylori* and gastric cancer and mucosa-associated lymphoid tissue (MALT)-lymphomas prompted a number of epidemiological studies. These reports were reviewed by the International Agency for Research on Cancer (IARC), who concluded that *H. pylori* is carcinogenic to humans and thus classified *H. pylori* as the first bacterial Group 1 carcinogen (IARC Monograph 1994).

The microorganism

In vivo, H. pylori is a spiral- or rod-shaped bacterium only a few micrometers long and actively motile. The bacteria have up to six sheeted flagella projecting from either pole and being of great importance for its mobility. *H. pylori* is Gram-negative, urease-, catalase- and oxidase- positive. The characteristic high urease activity is used by the bacteria to convert urea to neutralising ammonia when colonising the gastric mucosa (Scott et al. 1998).

Furthermore, the bacterium is microaerophilic, thus growing out to visible glittering colonies at *in vitro* culture after 3-5 days in a moist, microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2) at 37°C.

In comparison with other bacteria, *H. pylori* has a relatively small genome, i.e. 1700 kilo basepairs. This is believed to result in a limited metabolic repertoire and few regularly networks (Tomb et al. 1997), which is consistent with its requirement for special conditions of culture and its restricted niche in the human gastric mucosa. Like other bacteria *H. pylori* has "housekeeping genes" for survival and growth and also species-specific virulence genes that are required for a successful *in vivo* establishment.

Bacterial virulence and host factors

Even though *H. pylori* infection in humans has been convincingly linked to the development of gastrointestinal (GI) diseases, only a small percentage of colonised individuals will express clinical manifestations. The virulence of the infecting strain is likely to be a major determinant for developing the diseases, although the process is a complex interaction between host and bacteria.

A proposed determinant for the outcome of colonisation is the ability of the bacterium to attach to the gastric epithelium. *In vitro* studies have indicated that *H. pylori* strains express a variety of adhesins that recognise a number of epithelial receptors (Dunn et al 1997). One of these receptors is the antigen of the Lewis blood group system that is not only synthesised by erythrocytes and blood vessel endothelium but by secretorial cells of the stomach (Sakamoto et al. 1989). Borén et al. (1993) demonstrated that the blood group antigen Lewis b on the gastric epithelial cells acted as an *H. pylori* receptors *in vitro*, and it has further been shown that the Lewis b receptor recognises the product of the *H. pylori babA* gene (Ilver et al. 1998).

Bacterial strains producing vacuolating cytotoxin activity (VacA) have been more commonly isolated from people with peptic ulcers than without, and infections with strains possessing *cagA* (cytotoxin associated gene A) is more commonly found in people with peptic ulceration and gastric adenocarcinoma than in controls. While the secreted protein (VacA) has been shown to be toxic to human cells in tissue culture and inducing epithelial cell damage in mice, the function of the CagA protein is unknown.

The *cagA* gene, however, is a marker of the *cag* pathogenicity island (PAI), which includes genes necessary for the enhanced inflammation induced by pathogenic strains. Based on the presence of these markers, infecting strains are often referred to as being a Type 1 (*cagA*+ and *vacA*+) or a Type 2 (*cagA*- and *vacA*-) strain (Xiang et al. 1995).

Both the presence of CagA and its correlation to gastroduodenal disease vary between populations from different geographic areas (Perez-

Perez et al. 1997). In Western countries a positive correlation has been observed between CagA and peptic ulcers (Crabtree et al. 1991) as well as gastric cancer (Blaser et al. 1995, Parsonnet et al. 1997, Ekström 2000). As a contrast, a number of studies from Asia have not been able to confirm this due to an almost 100% prevalence of CagA expression in several of the populations that have been observed (Miehlke et al. 1993, Mitchell et al. 1996, Pan et al. 1997).

The lack of an association between *cagA* and upper GI disease in some populations may reflect that the expression of CagA does not necessarily correlate with the presence of *cag* PAI (Jenks et al. 1998). Also, differences in *cagA* genotype may result in differences in virulence among *cagA*+ strains (Yamaoka et al. 1998). The location and density of *cagA*+ strains may also be of importance for the development of DU (Hamlet et al. 1999), and the *cagA* has been shown to exhibit substantial sequence heterogeneity among strains from different populations (van Doorn et al. 1999).

A recent work focusing on genetic susceptibility for gastric cancer reported that interleukin-1 gene polymorphism, suspected of enhancing production of the pro-inflammatory cytokine interleukin-1β, was associated with an increased risk of both hypochlorhydria induced by *H. pylori* and gastric cancer (El-Omar et al. 2000).

The host and microbial factors that determine the outcome of the relationship are being actively explored and further research is needed before the clinical outcome of the infection can be more precisely predicted. Also, the reason why so many but not everyone is infected in *H. pylori* endemic areas might be further explored on a genetic level.

Evidence of causality

Classical evidence of a causative role of *H. pylori* in the development of gastritis was demonstrated by Marshall et al. in a successful attempt to fulfil Koch's postulates (Table 1). Marshall himself, being a healthy volunteer, drank a pure culture of *H. pylori* and did within a few days develop symptoms of dyspepsia as well as an acute gastritis, confirmed by endoscopy (Marshall et al. 1985).

Table 1. Koch's postulates (according to the original wordings by Löffler, 1884)

1.	The organism must be shown to be constantly present in characteristic form and arrangement in the diseased tissue.
2.	The organism, which from its behaviour appears to be responsible for the disease, must be isolated and grown in pure culture.
3.	The pure culture must be shown to induce the disease experimentally.

Furthermore, infection with *H. pylori* does not occur at random, but with a strong association to inflammation of the gastric mucosa, termed chronic superficial gastritis (Warren et al. 1983, Blaser 1990). Also, compelling evidence occurred when *H. pylori* induced gastritis was treated with antibiotics, resulting in disappearance of *H. pylori*, normalisation of the inflammatory process and restitution of the gastric mucosa (Rauws et al. 1988).

H. pylori infection seems to be the most significant risk factor for duodenal ulceration (DU). The main arguments for a causal relationship between *H. pylori* and DU are that the infection precedes the disease and that the disease disappears after treatment of the infection. While ulcers are known to relapse in many cases after symptomatic treatment, eradication of *H. pylori* has been shown to almost always prevent ulcer recurrence (Marshall et al. 1988, Graham et al. 1992).

H. pylori associated gastritis may, over decades, develop into atrophic gastritis, a precursor for gastric cancer (Correa et al. 1976). The geographic correlation between *H. pylori* prevalence and gastric cancer incidence and mortality, and the coinciding decline of the two in some parts of the world, support a causal pathway between infection, subsequent inflammation, and gastric cancer (Forman et al. 1993a, IARC Monograph 1994,). Knowledge of the causative mechanisms is however lacking. The regression of MALT lymphoma after *H. pylori* eradication (Wotherspoon et al. 1993) and a recent observation that high-dose antibiotics in connection with hipreplacement surgery reduced gastric cancer risk during follow-up, provide indirect evidence that antibiotics may prevent gastric cancer (Akre et al. 2000).

THE DISEASES

Gastritis

The majority of individuals infected with *H. pylori* develop an inflammatory condition, with infiltration of polymorphonuclear lymphocytes in the antrum and the fundus of the stomach. The association between chronic superficial gastritis (Marshall et al. 1984, Blaser 1990) and GI symptoms is uncertain. It will sometimes, however, develop into chronic atrophic gastritis with loss of epithelial glands over a period of decades (Karnes 1991).

Also in children, *H. pylori* infection is associated with the development of chronic gastritis (Drumm et al. 1987a and 1987b). A French investigation based on upper endoscopies revealed progressive inflammatory changes also among asymptomatic children (Ganga-Zandzou et al. 1999). A common finding at gastroscopies in *H. pylori* infected children is a typical pattern of antral nodular hyperplasia (De Giacomo et al. 1990, Ashorn et al. 1994).

A recent large-scale endoscopy-based investigation of 396 Greek children with recurrent abdominal pain (RAP) reported no significant differences in frequency and character of symptoms between *H. pylori* positive and negative groups (Roma et al. 1999). Histologically confirmed gastritis was the most prominent finding in *H. pylori* positive (98%) as compared to negative (19%) children ($p<0.001$).

Ulcer disease

A peptic ulcer is defined as a defect in the gastric mucosa of at least 0.5-cm in diameter and penetrating through the muscularis mucosa, with or without bleeding and perforation (Kuipers et al. 1995). The discovery of *H. pylori* infection has dramatically changed the understanding of the pathogenesis of ulcer disease and also its treatment. *H. pylori* infection is diagnosed in 95% of all DU patients worldwide (Borody et al. 1991, Kuipers et al. 1995). The remaining DUs, especially in elderly, are due to NSAID use and other rare causes like Zollinger-Ellison syndrome and Crohn's disease (Borody et al. 1991).

The corresponding prevalence of infection in gastric ulcer patients has been more complicated to establish since the association between gastric ulcers and atrophic changes of the mucosa might have resulted in false-negative results from biopsy based diagnosis. Serological confirmation of *H. pylori* infection has, however, verified an *H. pylori* seroprevalence of 60-100% in gastric ulcers (Kuipers et al. 1995).

A study of paediatric patients presenting with unspecified RAP showed that peptic ulcers were more common in *H. pylori* positive as compared to negative children (5% *versus* 1%, p=0.05) (Roma et al. 1999). The numbers were too small to allow for a separate risk analysis for duodenal and gastric ulcers in children, respectively.

Seroepidemiological evidence from different populations indicates that the lifetime risk for peptic ulcer disease among *H. pylori* infected individuals is 6-20%, which is at least 3-4 times higher than for uninfected individuals (Kuipers et al. 1995, Feldman et al. 1998).

Nonulcer dyspepsia

The possible role for *H. pylori* in nonulcer dyspepsia in adults, which is the most common gastroenterological diagnosis in Sweden, accounting for 2% of all outpatient consultations (Nyrén 1987), has been controversial. The topic has also received much interest from physicians and not the least from the pharmaceutical industry. Two recent randomised double-blind placebo-controlled studies in adults with nonulcer dyspepsia did not find that *H. pylori* eradication relieves symptoms (Blum et al. 1998, Talley et al. 1999). A third well-designed trial, however, reported a benefit of eradication treatment in a small subgroup of patients (McColl et al. 1998). Ad hoc analyses in subgroups must, however, be interpreted cautiously.

Gastric cancer

Although >50% of the population worldwide is infected with *H. pylori*, and gastric cancer is the second most common cancer in the world (Parkin et al. 1999), only a fraction of all *H. pylori* infected (1-2%) is likely to develop gastric carcinoma (Kuipers 1998). Still, the majority of epidemiological work has sustained the role of *H. pylori* in the pathogenesis of gastric cancer through the years, although the relationship seems to be complex also involving environmental and host genetic factors.

The classification of *H. pylori* as a human carcinogen by the IARC (1994) was mainly based on the first prospective studies relating the later development of gastric carcinoma with serological evidence of *H. pylori* antibodies in earlier stored sera. The British and American studies showed odds ratios (ORs) for gastric cancer ranging from 2.8-6.0 in *H. pylori* infected as compared to uninfected controls (Forman et al. 1991, Parsonnet et al. 1991, Nomura et al. 1991).

The association has been confirmed in a number of case-control studies with establishment of *H. pylori* exposure status at time of sampling (cancer diagnosis among the cases) (Eslick et al. 1999). One of these, also controlling for potential background factors, confirmed the positive association between *H. pylori* and non-cardia tumours with an OR of 3.1 (Hansson et al. 1993).

A problem with the case-control studies, however, is the induction and latent periods, i.e. the period of time from causal action to disease presentation, which for cancers is likely to be decades. At the time of the exposure assessment, the atrophic changes preceding the carcinomas could have led to clearance of the infection and thus a negative serological result, resulting in an underestimation of the true risk. A recent study, which tried to control for the latter, found that 71% of non-cardia adenocarcinoma was attributable to *H. pylori* (Ekström 2000).

In contrast to the above mentioned positive association between the infection and non-cardia cancer of the stomach, *H. pylori* infection does not seem to be associated with cancers of the cardia. Some authors have even found an inverse relation between the two (Hansen et al. 1999), especially for CagA-positive strains (Chow et al. 1998).

Gastroesophageal reflux (GERD)

Even though GERD is primarily a motility disorder, other patophysiological disturbances seem to play a role in its pathogenesis. Whether *H. pylori* may be one of the players remains controversial and conflicting results have been reported. Indications that the curing of *H. pylori* infection in DU patients may provoke reflux esophagitis have been reported (Labenz et al. 1997). Although not finding any significant difference in the prevalence of *H. pylori* carriage in patients with GERD and its sequel compared with the controls, Vicari et al. (1998) found that patients carrying CagA-positive strains might be at a decreased risk of complications of GERD.

A 2-year follow-up, based on 276 DU patients randomised to either eradication therapy or long-term omeprazole treatment, found that both regimens were equally effective in controlling dyspeptic symptoms and GERD in patients with a healed DU (Bytzer et al. 2000). Furthermore, the healing of ulcers was not found to increase the risk of GERD.

From a paediatric point of view, the endoscopy-based study of Greek children presenting with RAP reported that esophagitis was more common in the *H. pylori* negative (48%) as compared to the positive (27%) group ($p<0.001$) (Roma et al. 1999). A similar finding has been reported also from a smaller study of Finnish children (Ashorn et al. 1993).

Extragastric manifestations

A number of associations between *H. pylori* and extragastric disorders have been suggested in the literature, although conclusive results are not available for many of them. For instance, some early reports indicated an association between *H. pylori* infection and cardiovascular diseases (Mendall et al. 1994, Patel et al. 1995), although later studies have rather suggested that these results might have been confounded by other factors (Whincup et al. 1996, Rathbone et al. 1996).

Iron deficiency has been reported to be associated with *H. pylori* infection (Milman et al. 1998) and the reversal of iron deficiency anaemia after *H. pylori* eradication has been observed in adults and children (Marignani et al. 1997, Choe et al. 1999 and 2000). The observations could be due to subclinical bleedings from the GI tract but are also likely to reflect the genetic findings indicating that *H. pylori* scavenge iron, presumably from its host (Tomb et al. 1997).

In some paediatric studies, an association between *H. pylori* infection and short stature has been suggested (Patel et al. 1994, Goodman et al. 1997, Perri et al. 1997). These observations would, however, need to be confirmed in future prospective studies also taking, for instance, genetic and socio-economic/nutritional factors into account.

Among children in developing parts of the world, a positive association has been noted for *H. pylori* and *Vibrio cholerae* (Clemens et al. 1995, Shahinian et al. 2000). The higher prevalence of *H. pylori* in children with chronic diarrhoea as observed in Gambia (Sullivan et al. 1990) might, however, have been a result of a higher susceptibility for *H. pylori* in these malnourished children. In contrast, a population-based study of German pre-school children found an inverse association between *H. pylori* and diarrhoea (Rothenbacher et al. 2000b), even though the reported prevalence of diarrhoeal illness in 43% of the children during the last 3 months might be questioned.

A recent report, attracting attention in the media and thus being of interest for paediatricians, concluded that *H. pylori* were associated with sudden-infant-death syndrome (SIDS) (Kerr et al. 2000). The publication has, however, been thoroughly criticised for methodological problems including the possibility of severe bias (Rowland et al. 2001). Also, a subsequent study did not confirm the results (Elitsur et al. 2000). Thus, there is no conclusive evidence of a causative association between *H. pylori* and SIDS.

PAEDIATRIC ABDOMINAL PAIN

Paediatric gastroenterology differs in a number of ways from gastroenterology in adults. The spectrum of diseases is different but also the clinical expression may differ for the same diagnoses in children as compared to adults (Table 2, after Lindquist 1995). Paediatric gastrointestinal (GI) diseases will not only affect somatic functions but also psychological maturation and development. Thus, the effects observed will be different for infants, toddlers, pre-school and school children as well as for adolescents.

Table 2. Gastrointestinal disorders in the paediatric patient.

Examples of manifestations
1. Infectious diseases
2. Functional gastrointestinal symptoms
3. Malabsorbation
4. Gastrointestinal intolerance and allergies
5. Feeding or nutritional disturbances
6. Inflammatory bowel diseases
7. Liver- and pancreatic diseases
8. Others, e.g. motility disorders, metabolic and endocrine disorders

The quantitatively most dominating category is the GI infectious diseases. The second most common group, according to the few reports from the industrialised part of the world, is likely to be the functional disorders.

Functional symptoms apply to symptoms arising without organic causes identifiable by investigations. Recurrent abdominal pain, which has been described in 10-20% of school children (Apley 1957 and 1975, Bury 1987, Alfvén 1993, O'Donohoe et al. 1996), is likely to be the most common functional GI disorder in childhood.

The clinical expression in children of the functional disorders with symptoms from the GI tract varies according to age. "Failure to thrive" is a symptom seen in infants and toddlers with manifestations similar to malabsorbation. Constipation, abdominal pain and irritable bowel will be more common in older children (Table 3, after Lindquist 1995).

Table 3. Functional gastrointestinal symptoms in children of different ages.

Symptom	*Age (years)*
Failure to thrive	0-2
Constipation	2-8
Abdominal pain	5-14
Irritable bowel syndrome	6-18

Recurrent abdominal pain

In a publication from 1957, Apley suggested a definition of recurrent abdominal pain (RAP), i.e. abdominal pain severe enough to interfere with the child's normal activities and occurring at least three times over a three-month-period. The definition has remained generally accepted throughout the years.

It is now nearly half a century ago since Apley made the large investigation of abdominal complaints in 1,000 English school children drawn from working class and middle class families in a thriving town. Apley found RAP in 11% of the school children, predominantly in girls as compared to boy (12.3% *versus* 9.5%). While boys had a stable prevalence of RAP, the girls showed a maximum peak of incidence at 9 years of age declining thereafter. Similar findings have been reported by other investigators (Bury 1987, Lundby et al. 1990, Alfvén 1993, O'Donohoe et al. 1996).

RAP is a common complaint in school children, often troublesome to the patient, the parents and the physician. The symptom is seen also in pre-school children and adolescents, but these groups have not been studied as extensively. A questionnaire to 602 Swedish adolescents (89% response rate) revealed monthly abdominal pain in >1/3 of the participants, with even higher numbers for tiredness and headache (Larsson et al. 1991).

The importance of psychosomatic reactions in RAP has been stressed. This has included the increased risk for RAP if living in flats as compared to garden cities (Lundby et al. 1990), in instable as compared to more stable sociodemographic areas (Alfvén 1993) and also if having relatives with organic or psychosomatic disorders (Apley et al. 1957). Apley even discussed the possibility of functional disorders developing into organic disorders later in life. According to Apley, only 5% of the children presenting with RAP were found to have an organic disease explaining their symptoms (Apley et al. 1957). A similar prevalence of organic disease in 4% of children with RAP has been reported from Australia (Bury 1987).

H. pylori infection and RAP – hospital-based studies

Some hope to find an explanation for a larger number of the paediatric RAP patients was raised with the discovery of *H. pylori*. The finding of an association between the infection and gastritis (Drumm et al. 1987a and 1987b) also in children encouraged investigators to search for a role of the infection also in RAP. An improvement of symptoms as well as of the gastritis after eradication of *H. pylori* infection (Drumm et al. 1988, Glassman et al. 1989, De Giacomo et al. 1990) were noted in the first reports.

Several studies in a clinical setting have evaluated the possibility of a relationship between *H. pylori* infection and the non-specific symptoms of RAP. Hospital-based studies of children undergoing upper endoscopies due to RAP have, however, yielded divergent results.

An important disadvantage of the hospital-based case-control studies is, however, the possibility of selection bias, when the controls may be selected due to conditions that were treatment at the hospital. Another problem might be confounding by background factors.

Chong et al. (1995) found *H. pylori* infection in 17% of 218 children with RAP as compared to 10% in 238 controls and concluded that *H. pylori* might be a cause of RAP in childhood, even though no adjust-ments for possible confouning factors were made. Other groups could not find an association between the two (Ashorn et al. 1993, Gormally et al. 1995, Blecker et al. 1996b). As an example Wewer et al. (1998) found *H. pylori* in 21% of 438 children with RAP as compared to 10% in 93 *H. pylori* negative controls (p=0.3). One study even found a negative association between *H. pylori* and RAP (Hardikar et al. 1996).

Macarthur et al. (1999) designed a study of RAP cases and controls without any GI disease attending their local Canadian primary care paediatrician. The group found a low rate of *H. pylori* infection (<5%) for both cases and controls and concluded that the low rate of *H. pylori* infection could not be a major cause of RAP.

A recent large-scale endoscopy-based investigation of 396 Greek children with RAP reported no significant differences in prevalence and character of symptoms between *H. pylori* positive and negative groups (Roma et al. 1999).

H. pylori infection and RAP - community-based studies

The only community-based investigation, so far, of *H. pylori* infection and RAP in 640 school children in London found no association between the infection and RAP (O'Donohoe et al. 1996). A community-based study of pre-school children in Germany, although not specifically inquiring about RAP, found no association between the infection and a symptom-score of abdominal complaints in the investigated age group (Bode et al. 1998b).

A meta-analysis of studies on the role of *H. pylori* in different symptoms (Macarthur 1995) and a consensus report on *H. pylori* infection in children (Drumm et al. 2000) both concluded that up to now there is no evidence of an association between the infection and RAP in children.

Management

Recurrent abdominal pain

A suggested approach for investigations in children presenting with RAP has been a detailed history and a thorough physical examination, followed by a laboratory investigation directed by the clinical findings. This could include a complete blood count (or blood haemoglobin), erythrocyte sedimentation rate, urinary analyses and stool tests for occult blood or ova and parasites (Lindberg 1994, Frazer et al. 1996). Further investigations, such as breath tests for lactose malabsorbation and IgA class reticulin antibody tests for celiac disease should be guided by specific suspicions based on history and symptoms.

In most cases, where a physiological component has been excluded or identified and treated, ongoing support and consistent medical follow-up of RAP has been believed to promote the child's functioning (Bury 1987, Frazer et al. 1996).

H. pylori infection in children

In the recent consensus statement on *H. pylori* infection in children (Drumm et al. 2000) it was agreed that children with non-specific abdominal pain (or RAP) should not undergo non-invasive or endoscopy-based tests only to seek evidence of *H. pylori* infection. It was concluded that there is no identified clinical picture indicating a need to screen for *H. pylori* in children. However, further studies are needed to try to determine whether a subgroup of infected children has symptoms due to the infection.

*W*ith an increased acceptance of upper endoscopies in children, possibly as a result of the *H. pylori* era, the importance of organic pathology in the RAP syndrome has been highlighted (Ashorn et al. 1993, Roma et al. 1999). In the mentioned consensus statement it was thus concluded that upper gastrointestinal endoscopy with biopsies is the preferred method of investigation in children with upper digestive symptoms suggestive of organic disease, such as ulcer disease or esophagitis (Figure 1) (Drumm et al. 2000). Other causes of organic disease (i.e. lactose maldigestion, celiac disease, constipation, liver and biliary system disease) should be excluded with non-invasive methods before the endoscopy is performed.

Paediatric H. pylori treatment

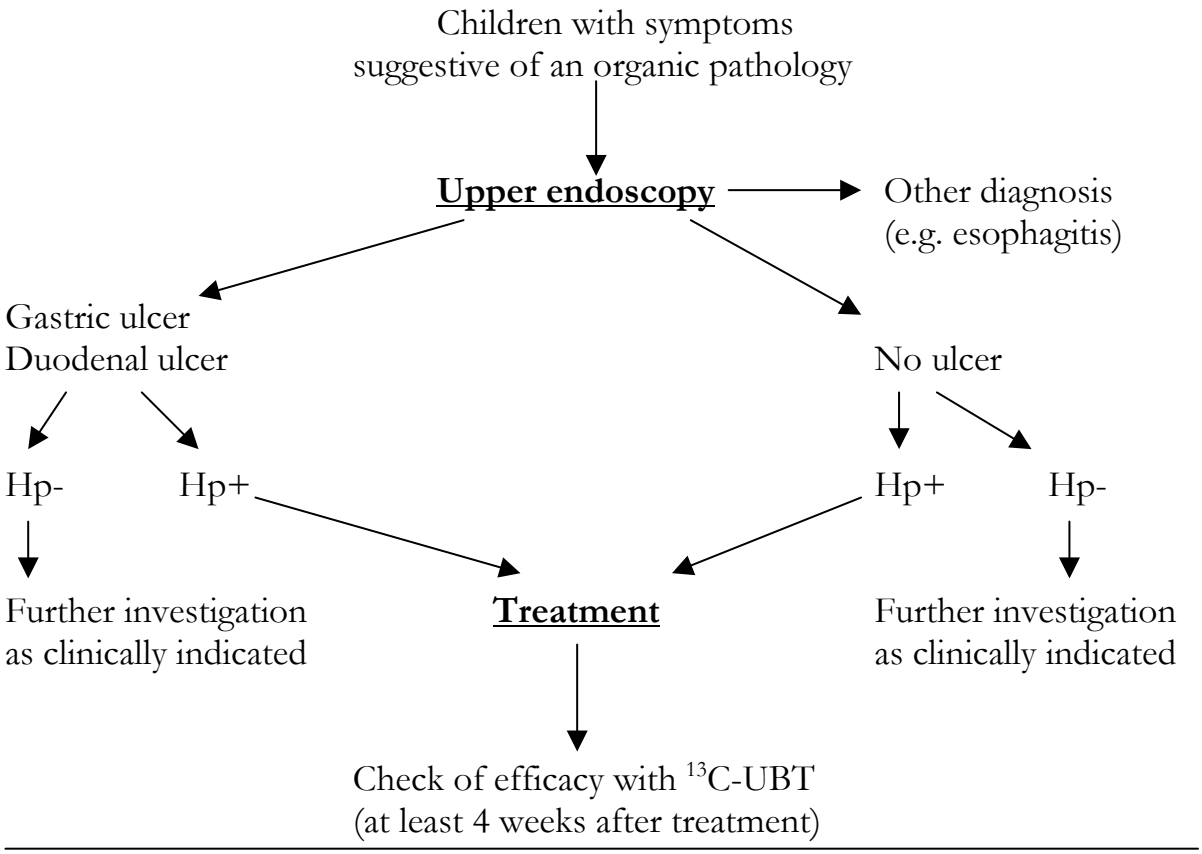
According to the consensus from 2000, it was suggested that if *H. pylori* was identified at endoscopy treatment should be offered to the patient. If an ulcer was found at endoscopy or if the child had a history of a previous ulcer, treatment should definitely be given. However, in the absence of an ulcer, the patient and the parents should be fully informed about the

treatment and that the eradication of the bacteria might not lead to a relief of the symptoms. Thus, an option to refuse the treatment should be given (Drumm et al. 2000).

Treatment of *H. pylori* infection seems to require, in most cases, a triple combination of drugs also in children. So far, in Sweden, no such regimens have been registered with the indication to eradicate *H. pylori* infection in children. Also, no double-blind randomised and placebo controlled trials of different eradication regimens has been reported for children and adolescents. Thus, the regimens used in the paediatric clinic are the same as for the adult patients, i.e. in Sweden a combination of amoxicillin, metronidazole or clarithromycin and a proton pump inhibitor (PPI) (Casswall et al. 1998, Tirén et al. 1999).

It has also been proposed that any attempt to eradicate *H. pylori* infections in children should be monitored by a reliable non-invasive test, at present ^{13}C-UBT (Drumm et al. 2000). A reservation was made in the consensus statement, as the UBT is not fully evaluated in younger children (<5 years).

Figure 1. Consensus statement on management of children with upper GI symptoms, where other explanatory causes have been excluded.



EPIDEMIOLOGY

Prevalence and incidence rates

Although *H. pylori* infection is endemic and despite more than 15 years of research, the mode and route of transmission remain elusive. Studies of the epidemiology of the infection have shown that the prevalence increases with age, is higher in developing compared to developed countries and inversely related to socio-economic conditions (Mégraud et al. 1989, Forman et al. 1993b).

Cohort phenomenon

In epidemiology the word *cohort* is often used to define a group of people who share a common experience or condition. For instance, the birth cohort share the same period or year of birth while a cohort of smokers has the habit of smoking in common. To separate the effect of age from birth cohort one needs not only data on persons of the same age from different cohorts but also data on persons of different ages from the same cohort.

This was achieved in two studies presented in 1993, one from South Yorkshire, UK (Banatvala et al. 1993) and one from Australia (Cullen et al. 1993). In both studies, longitudinally collected sera (from 1969-1989) were tested for *H. pylori* and revealed indications of a cohort phenomenon. The authors concluded that most infections would have been acquired in childhood and that few new infections had occurred during adulthood, also supported by the results of Kuipers et al. (1993) and Parsonnet et al. (1992). Thus, the increasing prevalence with age in developed countries is likely to be explained by a fall in the rate of early acquisition in successive birth cohorts.

Prevalence and incidence as indicators of H. pylori acquisition

Prevalence refers to the number of infected persons present in the population at a defined point in time. Incidence on the other hand, represents the number of new infections acquired during a given period in a specified population. Since, in most cases, the onset of *H. pylori* infection is not possible to identify clinically, longitudinal studies of uninfected persons are required for accurate measurement of acquisition. The same principle can be used for measuring spontaneous elimination of infection.

Studies on seroprevalence in children, as well as in adults, show marked variation between and within countries, notwithstanding the possible bias introduced when the majority of studies have non-randomly selected

subjects from the community population. For instance, prevalence data from patients undergoing endoscopy might be liable to bias by indications for endoscopy. In seroepidmiological studies, on the other hand, bias could be introduced if invited volunteers perceive that they would benefit from participation.

In developing countries the prevalence of *H. pylori* infection often reaches 50% already among 5-year-olds, whereas in developed countries the prevalence is low (<10%) (Table 4 and 5).

Most of the differences in the prevalence of adult infection are likely to be explained by differences in childhood infection rates (Table 6 and 7). A large seroepidemiological study with randomly selected subjects from southern China clearly showed this (Mitchell et al. 1992). The higher prevalence noted in urban as compared to rural areas was demonstrated to be determined by the prevalence reached in each community by the age of 5 years. After the age of 5, the subsequent acquisition of infection was estimated to approximately 1% per year, which is also comparable to the findings of others (Velhuyzen van Zanten et al 1994).

Incidence of H. pylori – developing countries

When discussing acquisition *versus* loss of infection, the term conversion reflects the change in infection status from negative to positive in a given test. Accordingly, reversion means the change from positive to negative.

Few longitudinal studies, accurately measuring *H. pylori* acquisition and loss of infection, are available. The studies performed among children point at early childhood as the critical period for acquisition with, however, different incidences in developed as compared to less developed parts of the world.

One of the first prospective studies, performed in Peru, used repeated ^{13}C-UBT in 56 children from 6 to 30 months of age, and reported a prevalence of 74% at entry (Klein et al. 1994). This high prevalence was not found at any other age during follow-up. Instead, frequent elimination and reinfection in these young children from a developing country was for the first time observed.

High acquisition rates early in life have been observed also in other less developed parts of the world. A seroepidemiological study of Ethiopian children found an incidence of 31- 24 per 100 person-years, in a follow-up from 2 to 6 years of age (Lindkvist et al. 1999a). Rates almost as high were reported for an Estonian birth cohort followed to the age of 4 years (Lindkvist, 1999b) and for Turkish 1-4-year-olds residing in Germany (Rothenbacher et al. 2000).

Table 4. *H. pylori* prevalence among asymptomatic children in Europe and the United States

Population	*Author*	*Comment*	*Age, yrs*	*No.*	*% Hp+*
Belgium	Lanciers et al. 1996 [a]	Belgian	6 [b]	44	6
		Non-Caucasian[c]	6 [b]	28	20
Finland	Ashorn et al 1996 [d]		2	195	5
	Ashorn et al 1995 [d]		12	70	6
Germany	Rothenbacher et al. 1999 [d]	German	6	475	5
		Turkish	6	72	44
Italy	Dominici et al. 1999		12-19	186	30
Spain	Cilla et al. 1997	Middle-class	1-9	203	11
			10-19	101	30
Turkey	Us et al. 1998		1-4	58	16
			5-9	62	31
			10-14	57	47
			15-19	53	58
UK	Patel et al. 1994 [c]		11	720	11
	O'Donohoe et al. 1996	Mixed ethnicities	5-14	640	17
USA	Staat et al. 1996 [d]	European-American	6-9	*	12
			10-14	*	18
		African-American	6-9	*	30
			10-14	*	43

[a] Pre-surgery blood samples
[b] Mean age
[c] African and Mediterranean children
[d] Community-based sample
*Age distribution not given

Table 5. *H. pylori* prevalence among asymptomatic children in other parts of the world

Population	*Author*	*Comment*	*Age (yrs)*	*No.*	*% Hp +*
Bangladesh	Clemens et al. 1996		2-4	82	46
			5-9	238	62
Chile	Hopkins et al. 1993		3-9	670	29
Colombia	Goodman et al 2000 [a]		2	77	53
			8	92	80
Ethiopia	Lindkvist et al. 1998		2-4	248	48
Gambia	Sullivan et al. 1990		1.5-3.3	136	27
			3.3-5	135	46
Korea	Malaty et al. 1996[a]		1-4	52	13
			5-9	81	9
			10-14	64	25
			15-19	55	45
Mexico	Torres et al. 1998 [a]		1-4	527	24
			5-9	1809	42
			10-14	1854	55
Nepal	Kawasaki et al. 1998		4-9	36	18[b]
			10-19	175	44[b]
Nigeria	Holcombe et al. 1993		<10	100	69
			10-19	43	91
South Africa	Pelser et al. 1997	Black and coloured	<2	104	14
			2-5	103	48
			5-10	104	67
South. China	Mitchell et al. 1992 [a]	Urban community	<5	*	31
			5-10	*	40 [b]
		Rural community	<5	*	15
			5-10	*	21[b]

[a] Community-based sample
[b] Approximated from graph
* Age distribution not given

Table 6. *H. pylori* prevalence among asymptomatic adults in Europe and the United States

Population	*Author*	*Year of birth*[#]	*Age (yrs)*	*No.*	*% Hp +*
Belgium	Blecker et al. 1993 [a]		20-29	213	12
			30-40	276	22
Denmark	Rosenstock et al. 1996 [b]	1952	30	905	13
		1932	50	909	28
Germany	Rothenbacher et al. 1999	1955-75 [c]	25-45	825	25
		1955-75 [d]	25-45	106	86
Italy	Dominici et al. 1999	1963-72	26-35	641	51
		1943-52	46-55	634	72
Spain	Cilla et al. 1997	1962-71	20-29	251	47
		1942-51	40-49	165	78
		1922-31	60-69	124	75
Sweden	Bergenzaun et al. 1996	1962-71	20-29	68	7
		1942-51	40-49	71	31
		1922-31	60-69	36	47
Turkey	Us et al. 1998	1967-76	20-29	67	63
		1947-56	40-49	75	81
		1927-36	>60	80	65
USA	Graham et al. 1991 [e]	[f]	20-29	39	12
		[f]	40-49	38	38
		[f]	60-69	65	50
		[g]	20-29	38	42
		[g]	40-49	34	75
		[g]	60-69	54	90

[#]Approximated from information in the methods
[a] Antenatal care patients
[b] Community-based study
[c] German mothers
[d] Turkish mothers
[e] Houston, community group members
[f] European-American origin
[g] African-American origin

Table 7. *H. pylori* prevalence among asymptomatic adults in other parts of the world

Population	*Author*	*Year of birth*[#]	*Age (yrs)*	*No.*	*% Hp +*
Chile	Hopkins et al. 1993		>25	268	69
China	Mitchell et al. 1992 [a]	1962-71[c]	21-30	*	65 [b]
		1942-51 [c]	41-50	*	78 [b]
		1962-71 [d]	21-30	*	41 [b]
		1942-51 [d]	41-50	*	58 [b]
Japan	Kumagai et al. 1998	1959-66	20-27	26	65
		1935-42	44-51	80	89
		1919-26	60-67	102	89
Korea	Malaty et al. 1996a	1963-72	20-29	23	57
		1943-52	40-49	40	80
Mexico	Torres et al. 1998 [a]	1958-67	20-29	1944	76
		1938-47	40-49	954	84
		1918-27	60-69	506	89
Nepal	Kawasaki et al. 1998	1967-76 [c]	20-29	212	58 [b]
		1947-56 [c]	40-49	89	81 [b]
		1927-36 [c]	60-69	50	79 [b]

[#]Approximated from information in the methods
[a] Community-based study
[b]Approximated from graph
[c] Urban area
[d] Rural area
*Age distribution not given

H. pylori incidence in developed countries

The incidence rate for *H. pylori* infection is generally lower among children in more developed parts of the world. A serological follow-up of Finnish infants noted an incidence of 1.5 per 100 person-years between 0 and 1 year and 3.7 per 100 person-years between 1 and 2 years of age (Ashorn et al. 1996). A corresponding incidence of 1.6 per 100 person-years was observed for the period between 3 and 12 years (Ashorn et al. 1995). Only one seroreversion occurred among the older children. The latter, however, was opposed in a Finnish longitudinal study of 337 children, where no seroreversion was noted among the 17 identified seropositive children monitored to 20 years of age (Rehnberg-Laiho et al. 1998).

A similar low incidence was reported from Japan in an 8-year follow-up of 112 children >6 years of age (Kumagai et al. 1998). In addition to a seroconversion rate of 1.1 per 100 person-years a corresponding seroreversion rate of 1.8 per 100 person-years was observed, and the overall prevalence among children was found to decline from 32 % to 17% between 1986-94. In New Zealand a seroconversion rate of 0.1 per 100 person-years was seen between 11 and 21 years of age and a corresponding seroreversion rate of 0.4 per 100 person-years resulted in a fall of the overall prevalence of infection to 4.1% at age 21 (Fawcett et al. 1998).

A longitudinal study of a birth cohort from the 1960'ies in a bi-racial community in the US, followed from 7-9-years of age to young adulthood found an incidence of 1.9 per 100 person-years and a corresponding seroreversion rate of 1.8 per 100 person-years (Malaty et al. 1999). However, a four-fold higher seroconversion rate in blacks as compared to whites and a significantly higher seroreversion rate in the white youngsters resulted in an overall prevalence of 65% and 20%, respectively, for blacks and whites at 19-21 years of age.

As a complement to the few available longitudinal studies, a number of cross-sectional studies have been used to estimate the incidence of infection at different ages (Mitchell et al. 1992, Lindkvist et al. 1996, Kawasaki et al. 1998, Redlinger et al. 1999). Some of these should, however, be interpreted with caution since differences in point estimates of infection in different age groups, in one and the same population, might be generated from small and non-representative subgroups.

Among adults in developed parts of the world an incidence of 0.3-0.5 per 100 person-years, with a slightly higher rate of seroreversions, has been reported (Parsonnet et al. 1992, Cullen et al. 1993, Kuipers et al. 1993, Veldhuyzen van Zanten et al. 1994). The estimated acquisition rate for adults in developing parts of the world, however, is somewhat higher (Parsonnet 1995).

Maternal antibodies and age at H. pylori acquisition

The available data on prevalence and incidence of *H. pylori* infection clearly point at early life, i.e. before 5 years of age, as the most important period for acquiring the infection.

A few prospective longitudinal studies of infants have contributed data on passive mother-to-child transfer of anti-*H. pylori* IgG antibodies and the time for the first seroconversions or acquisitions of infection as measured by UBT (Blecker et al. 1994 and 1996b, Gold et al. 1997). Blecker and Gold both observed full concordance between seropositive maternal sera and

seropositive cord-blood. Ashorn et al. (1996), however not having access to maternal sera, reported what was likely to be maternal anti-*H. pylori* IgG antibodies in 11% of newborn term Finnish infants. Detectable maternal antibodies disappeared in the vast majority of the children by the age of 6 months. The relatively few verified acquisitions of infection during the first year of life did not occur until the maternal antibodies had disappeared. Also, the majority of infants contracting the infection had seronegative mothers.

As a curiosity it might be mentioned, that the youngest child, from whom *H. pylori* has been isolated, was a 6-day-old full-term infant presenting with symptoms of vomiting, poor weight gain and frequent crying (Raymond et al. 1995).

Protection by breastfeeding

Breastfeeding has been suggested to protect against early acquisition of *H. pylori* infection. In Gambia, where infants normally are breastfed until the age of 2 years, a group of 12 infants were followed prospectively in parallel with measuring IgA levels in the mothers' breast milk (Thomas et al. 1993). The infants of the mothers with the highest IgA antibody levels in the milk were found to have the latest onset of infection.

An *in vitro* study of adhesion mechanisms of the *H. pylori* organism to the human gastric mucosa showed that human κ-casein, in contrast to κ-casein purified from bovine milk, effectively inhibited the adhesion of the organisms to the mucosa (Strömqvist et al. 1995). The finding may be relevant for the studies of breastfeeding and the possible protection from *H. pylori* infection early in life.

The authors of the prospective study from Finland (Ashorn et al. 1996), finding only initially cord-blood negative children seroconverting before the age of 2 years, speculated on the possibly protecting role of breastfeeding by seropositive mothers. This was based on the relatively long breastfeeding observed for these Finnish infants, i.e. 7 months. The shorter breastfeeding among Belgian infants was, however, thought to exclude a major protective role by such mechanisms in the Belgian setting (Blecker et al. 1994 and 1996a).

Spontaneous elimination of infection

For some time it was believed that, after the establishment of *H. pylori* infection, the organism would persist in the stomach for life. "Spontaneous clearance" of the infection was first noted in the elderly and might be explained by gastric atrophy, i.e. loss of the natural niche of the organism.

Later, clearance of the infection, or rather "transient infections", have been noted predominately in children (Ashorn et al. 1995, Kumagai et al. 1998, Malaty et al. 1999). One explanation might be a lower expression of *H. pylori* binding receptors in the gastric mucosa of children and adole-scents. This was indicated in as study by Celik et al. (1998), which noted a lower rate of Lewis b adhesins in *H. pylori* isolates from 32 children and adolescents. Treatment with antibiotics has also been suggested to play a role in the loss of early infections (Rothenbacher et al. 1999).

From an epidemiological research perspective, the evidence of a selfresolv-ing form of *H. pylori* infection makes it necessary to distinguish between factors associated with the chronicity of the infection and those associated with its acquisition. With the general decrease in infection rates, as seen in the developed parts of the world, spontaneous clearance might also be observed even more often in the future.

Reinfection rates

Reinfection rates after treatment of *H. pylori* infection have also been used to estimate the risk of infection at different ages. While reinfection was rare in Irish children >5 years of age (Rowland et al. 1999), a high rate of reinfections have been reported for children and adults in other parts of the world (Oderda et al. 1992, Ramirez-Ramos et al. 1997, Gurel et al. 1999).

However, the difficulties in separating true reinfections from recrudescence should not be overlooked. This was extensively investigated in a study by Bell and Powell (1996), who found that reinfection varied markedly with the effectiveness of the treatment protocol, and also with the time interval used to define successful treatment.

Transmission

Mode and route of transmission

Despite the large volume of research on *H. pylori*, many of the important questions regarding mode and route of transmission remain unanswered. The fastidious nature of the *H. pylori* organism, which makes isolation and culture difficult, the lack of accurate serotyping systems and the inability to identify acute infection clinically, has delayed a deeper understanding of the transmission mechanisms. Also, the lack of adequate techniques for detecting the bacteria in material other than the gastric tissue has hindered identifying portals of entry and exit as well as the identification or ruling out of environmental reservoirs.

Risk factor association

Several epidemiological studies have examined risk factors for *H. pylori* infection, with lower socio-economic conditions being the most consistently identified. However, social classifications by occupation, level of education or earning are merely markers for groups of people sharing certain characteristics or practices and not a specific cause of infection.

Studies of adults have revealed a stronger association between *H. pylori* infection and childhood living conditions than for current living conditions (Mendall et al. 1992, Webb et al. 1994, Malaty et al. 1994a and 1998), thus supporting acquisition early in life. The risk of introduced recall bias when adults and elderly were asked about living conditions before the age of 5 years should not be ignored. However, studies performed among children of today have confirmed the finding of an inverse association between socio-economic conditions and *H. pylori* infection (Fiedorek et al. 1993, Malaty et al. 1996a, Staat et al. 1996).

Person-to-person spread or a common source of infection

Evidence supporting both person-to-person spread and a common source of infection has been presented, but the respective importance of the two has not yet been possible to establish. Also, the questions of which routes are the most important, the direct route *via* oral-oral, gastro-oral or faecal-oral transmission or the indirect route *via* vectors including water, food and animals remain unanswered. The suggested routes of transmission may also be of varying importance in different settings.

Person-to-person transmission of *H. pylori* has been suggested in a number of studies pointing at domestic overcrowding early in life as an important risk factor for infection (Mendall et al. 1992, Malaty et al. 1994a). A common exposure to infection could, however, not be excluded. Two studies from the UK (Whitaker et al. 1993, Webb et al. 1994) identified childhood crowding, increasing number of siblings and bedsharing as possible risk factors for transmission of the organism. Although statistical analyses could not separate the relative importance of the three, the findings indicated transmission via close personal contact early in life, also supported by results of Fall et al. (1997).

Studies among children of today have confirmed the importance of domestic crowding (Patel et al. 1994, Goodman et al. 1996, Malaty et al. 1996b, Staat et al. 1996, McCallion et al. 1996, Rothenbacher et al. 1998a). Also, the increased risk for *H. pylori* infection noted among institutionalised children and adults point at the possibility of transmission either between individuals or by a common source (Vincent et al. 1994, Lambert et al. 1995, Lewindon et al. 1997).

A higher *H. pylori* prevalence in urban as compared to rural areas has been reported from developed (Whitaker et al. 1993, Rosenstock et al. 1996) and less developed parts of the world (Mitchell et al. 1992, Goodman et al. 1996, Lindkvist et al. 1998, Kawasaki et al. 1998). The relative crowding in households in expanding towns as compared to rural areas, with consequences for closer interpersonal contacts as well as for the general level of sanitation, might explain these findings.

Even though childhood living conditions seem to be important for *H. pylori* acquisition, factors like crowding, number of siblings and bed-sharing have not been able to explain all infections. Mixing of children at nurseries, day-care centres and schools, have been suggested to contribute some infections early in life (Webb et al. 1994, Mendall et al. 1995, Cave 1997). Studies with the aim to disentangle these effects have, however, not been published so far.

A study of twins indicated that not only environmental but also genetic factors influence the acquisition of *H. pylori* infection (Malaty et al. 1994b). Further genetic studies may address the importance of distinct genetic factors such as the cell surface antigens.

Intrafamilial transmission

The finding of clusters of *H. pylori* infections within families (Mitchell et al. 1987, Drumm et al. 1990) has been confirmed in several epidemiological studies (Malaty et al. 1991, Oderda et al. 1991, Dominici et al. 1999). However, the concordance of infection status among family members is consistent with either person-to-person transmission or shared exposure to environmental reservoirs. Also, the question on the direction of transmission between individuals remains to be resolved.

Evidence of mother-to-child transmission was presented in two studies based on UBT, one from China (Ma et al. 1998) and one from Germany (Rothenbacher et al. 1999), where the infection rate was highest in offspring's of infected mothers.

An association between increasing numbers of siblings and *H. pylori* infection for older birth cohorts was mentioned above (Whitaker et al. 1993, Webb et al. 1994). Recent investigations of pre-school children in Germany could not find an association between increasing numbers of siblings and *H. pylori* prevalence (Rothenbacher et al. 1998a and 2000a).

At odds with the above-mentioned German study, a study from Colombia showed that the number of older infected siblings had a particularly strong effect on infection status in the child (Goodman et al. 2000). The authors thus concluded that transmission from older to younger siblings might be a major pathway for *H. pylori* infection in populations

where large families are common. However, the possibility of parent-to-child transmission in the Colombian setting cannot be ignored since 61% of firstborn children were infected, as were 63% of children with no other siblings. Also, the overall prevalence of infection was >90% in this particular setting (Goodman et al. 1996), making risk factor detection very difficult.

Epidemiological findings have been presented in favour of transmission of *H. pylori* also between spouses, although the possibility remains that the partners would have been exposed to a common exogenous source of infection. Malaty et al. (1991) found a higher rate of infection among spouses of infected otherwise healthy index cases when age, race, family size and income was taken into account. Schutze et al. (1995) demonstrated that reinfection, after successful eradication therapy, could occur by person-to-person transmission between spouses. A large seroepidemiological investigation of 277 couples attending an infertility clinic showed, however, that neither *H. pylori* infection in the spouse nor duration of sexual cohabitation was associated with an increased risk of infection when age and nationality was taken into account (Perez-Perez et al. 1991).

Molecular typing

The infecting strains of *H. pylori* are genetically diverse. The identified long-term colonisation of individuals with multiple, polyclonal *H. pylori* strains (Taylor et al. 1995, Salama DM et al. 1995) as well as the recombination of bacterial genes *in vivo* (Atherton et al. 1995) makes studies of transmission patterns even more complicated. However, molecular evidence of intrafamilial person-to-person transmission has been presented (Vincent et al. 1994, Raymond et al. 1998) and so has evidence favouring a common source of infection.

One study using molecular typing, i.e. DNA fingerprinting, of isolated bacteria from the gastric mucosa identified clonal variants of *H. pylori* from three generations of family members in an ulcer family (Nwokolo et al. 1992). In another study of a peptic ulcer family, cluster analysis after DNA fingerprinting revealed a close relationship between the heterogeneous isolates (van der Ende et al. 1996). A study of the 8 parents of 4 infected children found identical strains in one, similar strains in one and different strains in two families (Bamford et al. 1993). All groups concluded that their results indicated an environmental source of infection, however, also allowing intrafamilial cross infection with *H. pylori.*

Oral-oral

In theory, person-to-person transmission of *H. pylori* infection could occur by an oral-oral, a faecal-oral or a gastro-oral route, or all. The oral-oral route

of transmission has been suggested to be a major route of transmission in the developed part of the world, in a number of studies. Furthermore, the oral cavity has been proposed a reservoir for the bacteria.

The latter has been based on the culturing of *H. pylori* from saliva (Ferguson et al. 1993) and dental plaque (Krajden et al. 1989) and the identification of *H. pylori* DNA in the oral cavity by polymerase chain reaction (PCR) (Li et al. 1996). However, only a subset of *H. pylori* infected individuals in these studies tested positive from the oral cavity in the various tests and negative studies have been published (Bernander et al. 1993). Differences in results may be due to the population examined, sampling methods or diagnostic techniques. Also the complexity of the oral flora makes the isolation of *H. pylori* from the oral cavity complicated.

Some epidemiological results favour spread of *H. pylori* infection by an oral source. For instance, premastication of food was more common in Burkino Faso families were both mother and child were *H. pylori* infected than in families were only the mother was infected (Albeneque et al. 1990). A study of Chinese immigrants in Australia found, after adjustments for socio-economic conditions, an association between the use of chopsticks and an increased risk of *H. pylori* infection (Chow et al. 1995).

Epidemiological evidence against the oral-oral route would be that the prevalence of infection does not increase among teenagers, as is the case for Epstein-Barr virus (EBV) with its age-specific peak in young adults, possibly as a result of kissing. One study determined the prevalence of the two infecting agents in the same population and concluded that *H. pylori* and EBV were not likely to be spread by the same route (Luzza et al. 1998).

Faecal-oral

H. pylori has been cultured from faeces (Thomas et al. 1992, Kelly et al. 1994) and found in faeces by PCR (Mapstone et al. 1993). In addition, data concerning water as a possible source of infection has been based on the hypothesis of faecal contamination of the water.

In Peru, children whose homes had external water, as compared to an internal water source, were 3 times as likely to be infected (Klein et al. 1991). In addition, children from high-income families, whose homes were supplied with municipal water, were 12 times more likely to be infected than were those from high-income families whose water supply came from community wells.

The water itself showed proof of presence of *H. pylori* by means of PCR (Hultén et al. 1996), even though this finding have failed to be convincingly reproduced and uncertainty remains as to whether the identified *Helicobacter* DNA was representative for viable infectious

microorganisms. *H. pylori* have so far not been cultured from water. Still, speculations are made as whether the converted coccoid form of the bacteria (Bode et al. 1993) is of significance for the transmission of the infection.

For other child populations in less developed parts of the world an association between absence of running or municipal water and *H. pylori* infection has been reported (Goodman et al. 1996, Lindkvist et al. 1999). A study from China, on the other hand, found a similar infection prevalence in rural residents who drank river water compared to those who drank well water, although most participants practised the custom of boiling drinking water and storing it in a flask (Mitchell et al. 1992)

The indications of foodborne transmission via unboiled fruit and vegetable, as noted in Chile and the Colombian Andes (Hopkins et al. 1993, Goodman et al. 1996), have also favoured water as an exogenous source of infection. However, over 130 Swedish travellers who had recently visited developing countries and most of whom had reported at least one episode of diarrhoea did not become *H. pylori* infected (Lindkvist et al. 1995). This would suggest that neither water nor food was a significant source of infection.

The isolation of viable *H. mustelae* from ferrets in an animal model with induced hypochlorhydria (Fox et al. 1993) also supported the theory of a faecal-oral route. As hypochlorhydria occurs in acute *H. pylori* infection in humans, children have been suggested to shed the bacteria in their faeces during the acute phase of infection thus being ready to contaminate other children, or adults, in the society. More recently, *H. pylori* was also isolated by culture and PCR from the stools of adults with induced diarrhoea (Parsonnet et al. 1999), supporting the hypothesis of *H. pylori* transmission during episodes of gastrointestinal tract infection.

Studies within a given population comparing the prevalence of *H. pylori* and Hepatitis A virus, which is spread predominantly by the faecal-oral-route, has failed to provide evidence of a common route of transmission for the two (Hazell et al. 1994, Furuta et al. 1995, Redlinger et al. 1999).

Gastro-oral

Iatrogenic gastro-oral transmissions of *H. pylori* via contaminated endoscopes and pH probes have been documented (Langenberg et al. 1990). Already before the *Helicobacter* era an epidemic of hypochlorhydric gastritis after experiments involving gastric intubation was described (Ramsey et al. 1979) suggestive of iatrogenic spread of an, at that time, unknown pathogen.

A higher prevalence of infection was also reported for gastro-enterologists (52%) as compared to age-matched blood-donor controls (21%) (Mitchell et al. 1989). The finding was thought to reflect patient-to-doctor spread of *H. pylori* possibly due to doctors not using gloves at examination and handling of instruments. The infection rate among the endoscopy nurses, however, did not differ significantly from that among the controls.

The gastro-oral route, where vomitus would act as the medium of transmission, has been suggested a route of transmission among children, who are more likely to vomit, especially during episodes of gastroenteritis (Axon 1995). The theory was supported by two recent studies isolating *H. pylori* by means of culture and PCR in vomitus from children presenting with acute gastroenteritis (Leung et al. 1999) and also from adults with induced vomiting and diarrhoea (Parsonnet et al. 1999). The latter group postulated that the declining incidence of gastroenteritis that occurs as countries make the transition from developing to developed may contribute to the observed decline in *H. pylori* infection in industrialised nations.

Non-human reservoirs as vectors

The only consistent sources of *H. pylori* are the gastric mucosa of the human and some non-human primates. *H. pylori* organisms have been isolated from domestic cats, with strains antigenically related to strains in humans (Handt et al. 1994). Epidemiological data from developed parts of the world has, however, not been able to identify any major importance of any house animal as a vector in *H. pylori* transmission (Graham et al. 1991, Fiedorek et al. 1993, Staat et al. 1996, Bode et al. 1998a). As a matter of fact, an association between higher social class and pet-ownership could possibly explain a noted lower prevalence of infection in pet owners in these studies.

Vector transmission has also been proposed in a study of house flies (*Musca domistica*) showing how the flies, in a laboratory setting, could harbour viable *H. pylori* on their bodies and in their GI tract for later dissemination in their excreta (Grubel et al. 1997). The route was suggested to be of particular importance in warm and developing countries, where sanitary and domestic facilities are poor. The easy access for flies to untreated sewage and their ability to carry *H. pylori* to either food or human mucosal surfaces was suggested as an explanation of the high prevalence of infection in less developed parts of the world.

However, since recent genome analysis has shown that *H. pylori* bacteria do not have the ability to survive outside the host (Tomb et al. 1997), it seems reasonable to interpret data on environmental and animal reservoirs with caution.

DIAGNOSIS

Invasive or non-invasive diagnostic methods

The invasive tests for *H. pylori* infections are those requiring gastric biopsy samples; culture, histology, rapid urease assays and polymerase chain reaction (PCR) which by amplifying segments of DNA from *H. pylori* can detect very small numbers of bacteria. Since these methods require upper endoscopy they are generally not considered suitable for paediatric epidemiological investigations, for ethical reasons and for the cost.

The non-invasive methods, serology and urea breath test (UBT), both being indirect methods, have the advantages of sampling the whole stomach and duodenum and, thus, avoiding the pitfalls of sampling error and variable efficacy that might be a result of culture and histology.

Culture

Culturing of bacterial colonies from gastric biopsies is regarded as a definitive proof of *H. pylori* infection. However, the ability to culture the organism, and thus the sensitivity of the test, may vary between laboratories since the method is most technically demanding. The organisms are sensitive to temperature and medium already during transportation and the culture itself requires special conditions and 3-5 days of incubation. The main advantages are the excellent specificity and the opportunity to test for susceptibility to antibiotics in patient management (Mégraud et al. 1996). Also, the genotype of the isolate with regard to CagA and VacA status can be determined, although this investigation is limited to research centres.

Histology

H. pylori are one of the microorganisms that can be recognised by histology. Also, the histopathologist is uniquely placed both to identify infection and to assess the disease process associated with it, i.e. gastritis and in adults premalignant or neoplastic changes. Whereas the sensitivity of histology is generally high, also this method may be affected by observer related factors and topographical changes in the stomach may introduce sampling errors.

Rapid Urease Test

This test uses the high concentration of pre-formed urease enzyme activity in *H. pylori* infected gastric biopsy samples. This will bring about a pH change when placed in a urea containing medium. The sensitivity of the test depends on the number of bacteria present in the sample, which may have consequences for its use when evaluating treatment failures etc. The specificity is very good when the test is read at one hour, but declines with

the length of the incubation. Thus, at 24 hours false positive results may be obtained by other urease producing bacteria (Moayyedi et al. 1998).

^{13}C-Urea Breath Test

The UBT is based on the principle that an ingested solution of isotope-labelled urea will be rapidly hydrolysed by the expressed urease of *H. pylori*. The released $^{13}CO_2$ is absorbed across the mucus layer of the gastric mucosa, and hence, via the systemic circulation, excreted in the expired air. The test gives a direct measure of a current gastric infection (Logan et al. 1998), also reflecting the bacterial load and grade of histological gastritis (Debongnie et al. 1991, Logan et al. 1991).

The UBT is a very sensitive and specific method, thus providing a "gold standard" against which the accuracy of other diagnostic tests can be validated (Hawtin 1999). Occasionally, oral urease producing bacteria may cause false positive results (Hamlet et al. 1995). Also, antibiotics, bismuth salts and PPI, which reduce the extent of *H. pylori* infection, may introduce false negative results. One disadvantage is the high initial cost for the test, caused by the need for expensive mass spectrometric equipment.

UBT is recommended for monitoring the effect of eradication treatments, and it is a suitable test for epidemiological investigations, particularly in children. Reservations have, however, been made since the test is less reliable in children <5 years (Kindermann et al. 2000) and it is not yet evaluated among the youngest (<2 years) (Drumm et al. 2000). However, recent ^{13}C-UBT studies in children have shown that high accuracy can be obtained, provided that the test is validated in a paediatric population (Rowland et al. 1997, Kalach et al. 1998, Cadranel et al. 1998, Corvaglia et al. 1999, Bazzoli et al. 2000).

Serology – by ELISA

Serology is the easiest way to identify *H. pylori* infection in individuals not undergoing gastroscopy, being based on the principle of detecting circulating antibodies against *H. pylori*. The enzyme-linked immunosorbent assay (ELISA) is the most commonly used method with its simple and non-expensive features making it suitable also for large-scale screening (Cutler et al. 1998, Laheij et al. 1998).

A positive test does not necessarily indicate a current infection but, like the UBT, the test is not affected by sampling errors. An initially lower specificity of the method was due to false positive results due to non-specific cross-reaction with other organisms, such as *Campylobacter* sp. (Perez-Perez et al. 1986, Johansen et al. 1995). Many different types of antigen preparations can be used in the ELISA and the upper limit of

normal values, i.e. the cut-off level, needs to be evaluated for each assay system and in the population being investigated.

It has been suggested that the cut-off value needs to be corrected for children since they produce lower antibody responses than adults (Andersen et al. 1994). Serological studies in infancy have their own limitations, not only due to delayed and low antibody production, but also to the occurrence of maternal antibodies up to the age of 3-7 months (Blecker et al. 1994, Ashorn et al. 1996, Gold et al. 1997). A lower specificity for some serological tests has been reported for children and could possibly be ex-plained by the focal nature of the infection, when compared with histology or culture (Raymond et al. 1996), or the transient infections seen at this age as also discussed by Sunnerstam et al. (1999).

Serology – by Immunoblot

Immunoblot is more costly and time consuming than ELISA, but has the advantage of allowing the analysis of antibody response to specific bacterial proteins. Furthermore, it has been demonstarted to have a high validity also in paediatric patients (Raymond et al. 2000). In the future, knowledge of CagA and VacA status, as well as yet undiscovered proteins being markers of specific *H. pylori* virulence, may be relevant for treatment and prevention of the complications of the infection.

Other methods

Other tests include the detection of anti-*H. pylori* IgG antibodies in saliva (Simor et al. 1996) and urine (Alemohammad et al. 1993). Similarly to the UBT, measurement of ^{14}C-urea in the urine has been reported to reflect *H. pylori* status (Pathak et al. 1994). However, these methods have not been completely standardised and have not conferred significant advantages over previously mentioned methods.

Recently, an enzyme linked immunoassay for detection of *H. pylori* antigen in faeces was developed. Studies in adults (Makristathis et al. 1998, Vaira et al. 1999) and children (Ni et al. 2000, Oderda et al. 2000) have indicated validity of the test when compared with invasive methods in symptomatic patients. These first promising results indicate that the test might be an alternative for epidemiological studies as well as in the clinic if UBT is not available, as suggested by the Maastricht II Consensus (2000).

An adjusted cut-off value for children might be needed also for the stool antigen test (Oderda et al. 2000). Also, a population-based study among children yielded a lower sensitivity of the test than was obtained in the clinic (Rothenbacher et al. 2000a). The latter could be due to differences in reference methods but implicate a need of further validation in the general population.

AIMS OF THE INVESTIGATIONS

To identify the prevalence and incidence and the age of acquisition of childhood *H. pylori* infection

To identify risk factors for *H. pylori* infection among children in Sweden e.g.

- family background
- close child-to-child contacts outside the family
- the role of antibiotic treatments during childhood

To elucidate the possible role of *H. pylori* infection, and of specific infecting strains, on abdominal pain and gastrointestinal symptoms in school children

To validate current non-invasive diagnostic methods for *H. pylori* infection as for paediatric epidemiological purposes

SUBJECTS AND METHODS

Settings, subjects and study designs

Papers I-II

In order to investigate prevalence, incidence rates and risk indicators for *H. pylori* infection among children in Sweden, we used stored sera and data originally collected for a pertussis vaccine trial. The recruitment of the children had been made at the local child health care centres, enrolling all children at birth and having a >99% attendance during the first year of life for offered routine check-ups and vaccinations.

All (754) infants born between March 21, 1984 and May 14, 1984 and registered at 93 child health care centres in the southern part of Stockholm were identified. Of these, 435 (58%) children were excluded for reasons such as declining participation, no answer, contraindications, expected relocations out of the study area and communication difficulties. The remaining cohort of 319 children entered the vaccination trial (Blennow et al. 1988, 1989 and 1990). At our 11-12-year follow-up, 305/319 (94%) children were still identifiable and contacted (Tindberg et al. 1999).

Thus, paper I and II were conducted as a seroepidemiological retrospective cohort study monitoring children from the age of 6 months to 11 years. The studies were based on stored sera drawn at 6 months (n=319), 8 months (n=303), 10 months (n=303), 18 months (n=258), 2 years (n=242 and n=233 for sera drawn with a 2-week-interval), 4 years (n=201) and for the present follow-up at 11-12 years of age (n=201+7).

Questionnaires were completed before the blood sampling at ages 6 months (n=295), 2 years (n=236), 4 years (n=203) and 11-12 years (n=263). At the 11-year-contact, the parents of the children were asked to answer some additional questions on abdominal pain and if we were allowed to use the stored sera from their child to detect antibodies against *H. pylori*. Due to the original requirements for language skills, needed for the informed consent and also for answering the questionnaires during follow-up, the study children had a high proportion of Swedish-born parents (92%).

To improve the participation rate at 11 years of age, families could choose between visiting our study nurse at their local health care centre or to be visited at home. Children could also choose between capillary and venous blood sampling. For children who had moved out of Stockholm between 4 and 11 years of age, material and instructions for blood sampling at their nearest health care centre was mailed after we had received the questionnaire.

For paper I, prevalence and incidence rates were estimated from all available samples from 201 children bled at 11 years of age, all samples from 55 children monitored to 4 years of age and all samples from 38 children monitored to 2 years of age, i.e. 294 children and 1,857 sera.

Paper II was based on questionnaires and serial sera from the same 294/305 children. Data were analysed as a case-control study investigating 1) the association between *H. pylori* infection, at any time during childhood, and background variables in early life and 2) the possible role of *H. pylori* infection in the development of abdominal complaints in childhood.

Papers III-IV

To investigate determinants for an established *H. pylori* infection among 10-12-year old children in Stockholm, Sweden (paper III) and the association between *H. pylori* infection and abdominal or gastrointestinal (GI) symptoms (paper IV), we invited pupils at 11 schools in the town of Stockholm and nearby southern suburbs. All 11 invited schools accepted participation thus contributing 36 classes and 858 eligible pupils.

Like in most European countries of today, the Swedish population is a mix of people with different ethnic backgrounds. In 1998, 0.97 millions (11%) of the Swedish population were born abroad. Another 0.78 millions (9%) were born in Sweden but had at least one parent born abroad, giving an immigrant background in 20%. In Stockholm County, with a population of 1.78 million, 30% had an immigrant background. This proportion was 36% at 10-12 years (Official Statistics of Sweden, 1999).

Practically all 10-12-year-olds in Stockholm County attend public schools, serving the local population. For these cross-sectional school-based case-control studies, information on social background variables among 4th to 6th graders (i.e. 10-12-year-olds) was obtained from the local School Health Care authorities in late 1997. A non-random selection of schools was made to ensure a maximum range of exposure of *H. pylori* infection, i.e. geographic origin and socio-economic conditions.

Being aware of the problem to motivate 10-12-year-old children to volunteer for research and not the least for a blood sample, a number of efforts were made to reduce non-participation:

1) Information meetings/lectures were given in each of the 36 classes during January 1998. The pupils met the physician and learnt about abdominal anatomy and microbiology (including *H. pylori*, which was adopted by the children as the "*Helicopter bacteria*"). The children were also informed about some basic principles of research, such as the im-

portance of participation irrespective of outcome (in this case abdominal pain).

2) At the information meeting, every child was given a standardised questionnaire to answer at home together with his/her parents. In order to increase the participation rate, the questionnaire was translated to the most common foreign languages, thus being available in Swedish, Turkish, Spanish, Arab, Persian, Serbo-Croatian, English and Somali. The translated questionnaires allowed for all children and parents to respond in a language in which they felt comfortable.
3) One pupil in each class was responsible for the collection of returned and sealed questionnaires, which were kept in a box in the classroom. The questionnaires were collected before the blood sampling, performed between February and April 1998.
4) For the blood sampling itself, classmates were invited to give their blood samples on the same day (for children not being at school on this day a second opportunity was offered in April). Children were welcomed to the study nurse and the physician at the school health care at each school together with their best friend or in a few cases (n=2) their teacher or a parent. Girls were bled before the boys in each class, and each child could choose between capillary and venous blood sampling. With this approach there was no need for local anaesthetics.

Overall, 756 (88%) children answered the questionnaire while 695 (81%) children participated also with a blood sample.

Paper V

The setting was the same as for paper III and IV. The validation of non-invasive diagnostic methods used in the present studies was, however, based on identified *H. pylori* seropositive and borderline cases (n=133) and negative controls (n=37) from the same school classes. Children were offered a ^{13}C-UBT (September-December 1998) serving as reference method. Previously identified seropositive and borderline cases also donated a new blood sample on the same day as performing the UBT.

Ethics

The ethical review board of Huddinge University Hospital approved all the studies, and all children and their parents gave informed consent before participation.

Laboratory methods (identification of *H. pylori* cases)

Serology (paper I-V)
All sera were screened by an in-house ELISA to detect IgG antibodies against *H. pylori*. A sonicated *H. pylori* antigen, based on culture of seven clinical isolates and NCTC 11638 strain, was used to coat 96-well microplate at a concentration of 5 μg/ml. Sera were diluted 1:1000, first 1:100 in phosphate buffered saline (PBS) only and then 1:10 in PBS containing 70 μg/ml of sonicated *Campylobacter jejuni* antigen (five clinical isolates) to remove cross-reacting antibodies. Alkaline phosphate-conjugated anti-human IgG (Euro-Diagnostica, Malmö, Sweden) was used to detect bound antibodies (Sörberg et al. 1997).

In this semi-quantitative ELISA, the patient serum dilution had been chosen to give a high sensitivity and quantitative values up to an OD of 1.5. OD values above 1.5 gave underestimates of true antibody concentrations. The cut-off value for this ELISA of an OD value of 0.36 at A_{405} had been established in Swedish adults, 83 being *H. pylori* culture-positive and 45 being negative by microscopy, culture and rapid urease test, giving a sensitivity of 100% (83/83) and a specificity of 96% (43/45) (Befrits et al. 1993).

For paper I and II, seropositivity was based on the cut-off OD value of 0.36, as established in adults, motivated by the fact that repeated freeze-thawing of sera could have induced some none-specific background antibodies even at the high serumdilution used.

For paper III and IV seropositivity was based on an adjusted cut-off OD value of 0.22, established in the present population of school children of mixed ethnic background. Furthermore, immunoblot and ^{13}C-UBT were used to confirm the serologic ELISA results. In practice, an infected case was both ELISA positive with an OD value ≥0.22 and either Helico Blot 2.0 or ^{13}C-UBT positive.

In paper V all ELISA values between the OD values of 0.20 and 0.36 were tested against the reference method (^{13}C-UBT) as to establish an optimal cut-off, i.e. the least percentage of falsely classified results.

Immunoblot (paper III-V)
All sera with an ELISA OD value of ≥0.1 were tested by immunoblot, using Helico Blot 2.0 (Genelabs, Singapore) for detection of IgG antibodies against *H. pylori* specific antigens. The kit consists of Western blot membrane strips made with a surface antigen-enriched preparation of *H. pylori* including CagA (116 kDa), VacA (89 kDa) and the urease subunit (30 kDa).

All buffers and reagents were supplied with the kit and used according to the manufacturer's instructions. Positive and negative controls were included with each batch of strips. As recommended by the manufacturer, a serum sample was considered to have tested positive by Western blot analysis if it had reacted with any of the bands 116 kDa, 89 kDa or 35 kDa or with any two bands from among 30-, 26.5- or 19.5-kDA antigens. Sera not fulfilling the criteria were classified as negative.

For paper IV, and based on available immunoblot results, each infected child was classified as having a Type 1 (CagA and VacA positive) or a Type 2 (CagA and VacA negative) strain infection (Xiang et al. 1995).

^{13}C-Urea Breath Test (paper III-V)

The test was performed after a fasting period for >4 hours, without a prior test meal. Triple basal samples of expiratory air were collected in screw-capped glass tubes. The children drank a solution of 100-mg ^{13}C-urea in 25 ml of tap water followed by 25 ml of water to rinse the mouth. Physical activity, food and drinks were avoided during the test. A second triple breath sample was obtained 30 minutes after ingestion of the urea. Breath samples were refrigerated until analysed using isotope ratio mass spectrometry (Oksanen et al. 1997).

UBT results were calculated as the difference in relative enrichment of $^{13}CO_2$ between baseline and 30 minutes samples. Correction for body weight, and thus the increased metabolism and CO_2 production in children, resulted in results expressed as [(%/mmol) x kg], with a grey zone for values between 0.10 and 0.20 [(%/mmol) x kg]. The established correction corresponds to a cut-off at the 3.5 δ per mil over baseline level.

This breath test had been validated in 91 adult patients with dyspepsia, showing a sensitivity, specificity, positive (PPV) and negative predictive value (NPV) of 92%, 95%, 96% and 91%, respectively, with histology and rapid urease test as reference methods (Oksanen et al. 1997). The sensitivity and specificity of 100% for the test was also established in 40 children of varying ethnic background and 5-16 years of age, undergoing upper endoscopy due to abdominal disorders and with *H. pylori* status confirmed by histology, culture and urease test on biopsies (M Bergström, unpublished data). The cut-off for the UBT established among those children was used in the present validation.

For paper IV, the UBT results allowed us to classify infected children as exhibiting low-moderate (0.2-1.55 [%/mmol] x kg) or high UBT levels (>1.55 [%/mmol] x kg). The cut-point corresponded to that of the upper quartile of the infected group.

Measurement of other variables

Exposure and outcome (paper II)

Possible risk factors for *H. pylori* infection in the present investigation were: length of breastfeeding (answered at the age of 6 months), family size and type of day-care attendance (2 and 4 years), size of family and numbers of rooms of the house (11 years), history of atopic disease in the child (4 years), number of courses of antibiotics during childhood (11 years), history of ulcer in the parents (11 years) and country of birth of parents. All information on exposure was given blind to the outcome, i.e. *H. pylori* status.

When investigating the possible role of *H. pylori* infection on abdominal pain, infection status served as independent variable while abdominal pain was the dependent variable. At age 11, the parents of each child answered the following questions on the occurrence of 1) unspecified abdominal pain in the child during childhood, 2) unspecified abdominal pain during the preceding 6 months and 3) recurrent abdominal pain (RAP) during the preceding 6 months. The same nurse asked the third question before collecting the blood samples. When asking about RAP, the definition sug-gested by Apley (1957) was used, i.e. "at least three episodes of abdominal pain over a three-month-period of such severity that it has affected the normal activities of the child".

Information on abdominal symptoms was given blind to *H. pylori* status, since all answers were turned in before the blood sampling was performed. An exception was made for 7 children recalled at age 12 in 1996, as they were found to have been seropositive earlier in childhood but not bled at the age of 11 years. However, *H. pylori* status at the age 11-12 years was unknown also for these children at the time of answering the question on RAP.

Measure of exposure (paper III)

Socio-economic status (SES) was classified according to the Swedish Socio-economic Classification (Swedish socio-economic classification, 1995) for parents separately and thereafter transformed into a household index. The latter was reflecting either the highest SES of the two parents, the SES of the only parent working or the SES of a single parent (Swedish socio-economic classification, 1995). Individual and household indexes were divided into two categories: 1. Blue-collar workers, unqualified self-employees and unemployed, and 2. White-collar employees.

Family size was classified as 2-3, 4-5 or ≥6 persons of the household and day-care attendance as having attended a day-care centre (15-20 children per group), some other form of day-care or no day-care outside the home. Use of antibiotics during childhood was classified as none, 1-5, 6-10 or ≥11 courses during childhood.

Due to the large number of countries of birth represented (44 for children, 54 for mothers and 64 for fathers) family background was classified into three groups. For this grouping different exposure opportunities were hypothesised based on the estimated overall *H. pylori* prevalence in the parent's country of origin: low (origin in Scandinavia, Western Europe or the US), medium (Eastern Europe, South America or Asia) or high (Middle East or Africa) exposure opportunity to *H. pylori*. The age of the child at arrival in Sweden was classified as being born here, arriving before the age of 4 or arriving later.

Identification of cases with GI symptoms (paper IV)

All questions regarding symptoms were limited to the preceding six-month-period. The questionnaire inquired about occurrence and frequency of abdominal pain (never, occasionally, ≥ once a month, ≥ once a week, daily), intensity of the abdominal pain (mild, moderate or severe) and if it had interrupted daily activities or occurred at night. Questions were also asked about the occurrence and frequency of other GI symptoms; i.e. acid regurgitation, constipation and diarrhoea (defined as any episode of loose stools).

Apley's criteria for RAP were used. Children reporting abdominal pain, regardless of severity, more than once a week constituted another studied subcategory, which partly overlapped the RAP category. The other GI symptoms were classified according to their frequency.

Information on possible confounders for *H. pylori* infection and GI symptoms, such as family size, parents' occupations and ethnic background, was the same as in paper IV.

Statistical methods

Paper I

The prevalence numerator included all prevalent cases of identified *H. pylori* seropositive children at a given point in time. The prevalence denominator was the total number of study children bled at the same time.

The incidence rate numerator included all incident cases of *H. pylori* seropositivity for the given time period. The incidence rate denominator (person-years) was computed as the average of the population at risk at the start (total number of subjects minus the ones already infected) and at the end of the period (total number of subjects minus the ones remaining infected) times the length of the period.

Paper II

The associations between explanatory variables and *H. pylori* infection were tested in univariate analyses using the Mantel-Haenszel method (Epi Info 6, CDC Atlanta, GA). Prevalence odds ratios (OR), which is explained in Figure 2, and 95% confidence intervals (CI) were used as measures of association.

The 95 % confidence interval is constructed in such a way that the probability is 95% that the interval would contain the "true" value.Thus, the CI reflects the uncertainty due to small numbers (random errors) and does not account for systematic errors (such as confounding, selection bias and misclassification). According to tradition, Fisher's exact test was used to test for statistical significance and two-tailed *p* values were given.

Figure 2. Calculation of the odds ratio.

	Exposed	Unexposed
Cases	A	B
Non-cases	C	D

The odds for exposed = A/C
The odds for unexposed = B/D
The odds ratio (OR) = (A x D) / (B x C)

When estimating any association between *H. pylori* infection and abdominal complaints, adjustments were made for gender, since gender was found to be a risk factor among the unexposed and possibly associated with the exposure in the study base, thus fulfilling the criteria for a confounding factor.

Paper III

The association of each variable with *H. pylori* infection was tested in univariate analyses using the Mantel-Haenszel test (Stokes et al. 1995). The effects of gender, age, household SES, family size, age at arrival in Sweden, day-care attendance, use of antibiotics and *H. pylori* prevalence among class mates were calculated with the Mantel-Haenszel test after stratification by family origin. The impact of maternal *versus* paternal geographic origin, and also SES, on *H. pylori* status in the child were estimated in multivariate logistic regression analyses using the SAS GENMOD procedure (Stokes et al. 1995). Two factor interactions between explanatory variables were tested using likelihood ratio tests.

In the final multivariate model *a priori* decided possible risk factors and confounders were included (Clayton et al. 1998). Children with both parents born in low *H. pylori* prevalence areas were excluded since they carried no information on factors modifying *H. pylori* infection prevalence.

Paper IV

To assess the association of abdominal pain and other GI symptoms and their potential determinants (here being *H. pylori* infection or strain type-specific infection and socio-economic conditions) analyses were performed using logistic regression models.

In the full model investigating the effect of *H. pylori*, adjustments were made for gender, age, SES, family size and immigrant background. Two factor interactions between explanatory variables were tested using likelihood ratio tests. All analyses were carried out using the SAS software package and logistic regression analyses performed by using the GENMOD procedure.

Paper V

In our validation of non-invasive diagnostic methods for *H. pylori* infection, sensitivity, specificity, positive predictive value (PPV), negative predictive (NPV) value and accuracy (also called performance index=PI) were calculated according to the formulas in Table 8.

Table 8. Definitions of sensitivity, specificity, PPV, NPV and PI

Parameters and definitions
Sensitivity = no. of true positives in test/total true positives x 100
Specificity = no. of true negatives in test/total true negatives x 100
PPV = no. of true positives in test/all positives in test x 100
NPV = no. of true negatives in test/all negatives in test x 100
PI = no. of true positive and true negative in test/all tested samples x 100

RESULTS

Prevalence and incidence rates

Paper I

Overall, 40 (13.6%) of 294 Swedish-born children had serological evidence of *H. pylori* infection at any time between 6 months and 11 years of age. Three infants were seropositive already at 6 but not at 8 months of age, probably reflecting waning maternal antibodies in these children. The first two seroconversions were noted between 8 and 10 months. The highest prevalence of 10.1% was noted at 2 years of age, followed by an overall decline in seroprevalence (Table 9), presumably indicating clearance of infections. By the age of 11-12 years, 6/40 initially seropositive children remained seropositive, while 32 had become seronegative, one declined blood sampling and one was deceased.

Table 9. Serological evidence of *H. pylori* infection Swedish children*

Age at sampling	No. of children	Prevalence, %	No. of new cases
6 months	247	1.2	
8 months	266	0	0
10 months	254	0.8	2
18 months	239	5.4	12
2 years	237	10.1	15
4 years	185	7.5	10
10.8 years	201	3.0	1♣

* 92% of the parents were also born in Sweden

♣Age at infection unknown since previous samples were lost

Based on the overall observed 40 cases, an incidence rate (IR) of 1.7 infections per 100 child-years was calculated for the entire 10.5-year follow-up. Already during the first year of life, an IR of 4.6 infections per 100 child-years was observed between 8 and 10 months. The highest IR of 13.3 infections per 100 child-years was calculated for the period between 18 and 24 months, declining to 2.4 for the next 2-year-period. No further new cases were confirmed for the remaining 7 years.

Paper III

In total 112 (16%) of the 695 participating children (whereof 589 children were born in a low prevalence area, i.e. Scandinavia, Western Europe or the US) had serological evidence of *H. pylori* infection. However, a marked difference in prevalence was observed for children with parents of different geographic origin as shown in Table 10.

Table 10 *(paper III).*
H. pylori prevalence by parental origin among 695 Swedish 10-12-year-olds

Factor	*H. pylori* infected n/total	%	Adj OR* (95% CI)
Place of birth of the mother			
Low prevalence area[1]	11/481	2	1.0
Medium prevalence area[2]	25/83	30	13.8 (4.1-46.2)
High prevalence area[3]	76/119	64	25.2 (6.6-96.5)
Place of birth of the father			
Low prevalence area[1]	13/451	3	1.0
Medium prevalence area[2]	22/92	24	1.4 (0.4-4.6)
High prevalence area[3]	77/139	55	2.7 (0.7-10.0)

* GENMOD procedure, adjusted for gender, household SES, family size and place of birth of the other parent
[1] Scandinavia, Western Europe or the US
[2] Eastern Europe, South America or Asia
[3] Middle East or Africa

Risk factors for infection

Age and gender (paper II and III)

Within the narrow age range (10-12 years) in paper III, the gender and age of the child was unrelated to *H. pylori* prevalence when parental origin, SES of the household and family size were taken into account.

In the longitudinal study, a large variation in seroprevalence was noted among different ages in the children, with a fall in prevalence after the age of 2 years, indicating clearance of infection. Also, girls seemed to be at higher risk of infection in the crude analyses (Table 12).

Exposure opportunity at home (paper III)

The noted large difference in infection prevalence among Swedish school children according to parental origin confirmed our hypothesis that the prevalence of *H. pylori* infection among parents would be an important determinant for exposure opportunity within the family.

When just looking at children born in Scandinavia, the odds ratios (OR) for infection, adjusted for gender, SES and family size, were 5.6 (95% CI 1.8-17.3) and 39.1 (95% CI 16.7-91.3) if at least one parent was born in medium or high prevalence areas, respectively, relative to the risk of infection when both parents were born in low prevalence areas.

Maternal origin was more important for the risk of *H. pylori* infection than was the origin of the father, as shown in Table 10. This was confirmed in a model restricted to 69 children with parents who were

discordant with regard to the country of birth. Having a mother born in a medium or a high prevalence area as compared to having a father with such a background was associated with an OR of 71 (95% CI 3.4-1,000) after adjustment for gender of the child, household SES and family size.

Age at arrival in Sweden (paper III)

The time spent by the child in the country of origin seemed to matter. Children coming from medium prevalence areas had an OR of 9.0 (95% CI 2.8-28.4) if they were 4 years or older at arrival in Sweden, compared to children with the same background but who arrived before the age of 4, or who were born in Sweden. Adjustments were made for gender, household SES and family size. The corresponding data for children with an origin in high prevalence areas was less clear due to small numbers in some groups.

Socio-economic status (paper III)

A confirmation of lower SES as a risk factor for infection was established only among children with appreciable exposure opportunity at home, i.e. children with at least one parent from a medium or high prevalence area (Table 11). For children of blue-collar workers as compared to white-collar employees the risk of infection was 12 and 3 times higher for children with an origin in medium or high prevalence areas, respectively. The low infection prevalence among children with both parents originating from low prevalence areas precluded a meaningful analysis.

Table 11 *(paper III).*
Risk factors for *H. pylori* infection in school children by area of family origin

	Medium prevalence area [1]			High prevalence area [2]		
	H. pylori infected		Adjusted odds ratio[#]	*H. pylori* infected		Adjusted odds ratio[#]
Factor	n/total	%	(95% CI)	n/total	%	(95% CI)
Household SES						
White-collar worker	1/29	3	1.0*	15/41	37	1.0*
Blue-collar worker[♣]	22/72	31	12.4 (2.5-62.0)	62/101	61	2.9 (1.2-6.9)
Family size						
2-3 persons	8/35	23	1.0[φ]	7/26	27	1.0[φ]
4-5 persons	12/53	23	0.9 (0.3-2.7)	40/73	55	3.0 (1.1-7.9)
≥6 persons	4/9	44	2.9 (0.4-19.5)	29/38	76	10.0 (2.9-34)

[1] Eastern Europe, South America or Asia [2] Africa or the Middle East
[#] Mantel-Haenszel χ^2-test, adj. for: * sex and family size [φ] sex and household SES.
[♣] Also including unqualified self-employed and unemployed

The mother's occupation was a stronger determinant for infection in the child than was the occupation of the father. Among 601 study children with information on both maternal and paternal occupation, the risk for *H. pylori* infection was 2.6 times higher (95% CI 1.2-5.6) if the mother was a blue-collar worker, unqualified self-employed or unemployed than if she was a white-collar employee. The analysis was adjusted for gender and geographic origin of the child, family size and SES of the father. The corresponding data for paternal occupation was 1.2 (95% CI 0.6-2.5).

Family size (paper II and III)

Likewise, increasing family size was a statistically significant determinant for infection in some children, i.e. having at least one parent from a high prevalence area (Table 11). A similar, but non-significant trend, with an increased risk for infection was seen also for children in the largest families (≥6 persons) with an origin in medium prevalence areas (paper III).

For 10-12-year-old children with both parents from low prevalence areas the *H. pylori* prevalence was too low to allow for detection of any association with family size (paper II and III). In paper II a crude association was noted between infection at any time and living with a single parent at 11 years of age. No association was seen between living with a single parent and concurrent infection status at 2 and 4 years of age, respectively (Table 12), or among the 10-12-year-olds in paper III.

Day-care centres and schools (paper II and III)

No association was seen between day-care centre attendance and *H. pylori* seropositivity among children in Sweden. In paper II, attendance of day-care centres was reported for 59% and 67%, respectively, for the participating 2- and 4-year-olds with no association between day-care centre attendance and *H. pylori* seropositivity at either age (Table 12).

In paper III, day-care in some form had been attended by 605/678 (89%) children. In total, 533 (77%) children had attended a day-care centre. An analysis of children born in Scandinavia, irrespective of parental origin, resulted in an OR of 1.1 (95% CI 0.4-3.1) for children attending day-care centres as compared to those who had been exclusively looked after at home, when adjusted for gender, household SES, family size and family origin. The corresponding OR for children born in Scandinavia also having parents born in a low prevalence area was 1.8 (0.2-15.6).

Exposure opportunity at school (paper III) had no apparent importance for the likelihood of being *H. pylori* infected, over and above the likelihood determined by exposure opportunity within the family. The seroprevalence among children born in Scandinavia with parents also born in low preva-

lence areas was 1.6%, 1.6% and 4.5% when the seroprevalence among the classmates was <10%, 10-29%, ≥30%, respectively. However, the corresponding ORs, adjusted for gender, household SES and family size, were 1.0 (reference), 0.9 (95% CI 0.1-5.5) and 1.1 (95% CI 0.1-10.8), respectively.

Table 12 *(paper II).*
Potential risk indicators for *H. pylori* infection in Swedish children with a majority of the parents also born in Sweden

Factor	*H. pylori* infected* n/total	%	Crude OR# (95% CI)
Gender			
Boys	15/150	10	1.0
Girls	25/144	17	1.9 (1.0-3.8)
History of ulcer in the mother			
No	36/232	16	1.0
Yes	2/5	40	3.6 (0.4-25)
Living with a single parent at 11 years			
No	26/208	13	1.0
Yes	10/38	28	2.5 (1.0-5.7)
Day-care centre at 2 years of age			
No	6/56♣	11	1.0
Yes	15/133♣	11	1.1 (0.4-2.9)
Day-care centre at 4 years of age			
No	2/36♣♣	6	1.0
Yes	11/134♣♣	8	1.5 (0.3-7.2)
Use of antibiotics during childhood			
<5 courses	26/173	15	1.0
5-9 courses	8/69	12	0.7 (0.3-1.7)
≥10 courses	5/21	24	1.8 (0.6-5.2)
Use of antibiotics during childhood	Seroreverters/ total Hp+		
<5 courses	22/26	85	1.0
5-9 courses	7/8	88	1.3 (0.1-13)
≥10 courses	3/5	60	0.3 (0.03-2.2)

*Seropositive children at one or several samplings during follow-up
♣ Seropositive children at 2 years of age
♣♣ Seropositive children at 4 years of age

Antibiotic treatments during childhood (paper II and III)

In paper III, children born to parents with a presumed low prevalence of *H. pylori* were reported to have a considerably higher antibiotic consumption than children to parents who were likely to have a medium or high prevalence of *H. pylori* (Table 13). However, in analyses stratified by parental origin, there was virtually no association between reported use of antibiotics and *H. pylori* infection, indicating that it was a result of confounding.

The lack of an association between antibiotics and infection status was also seen in an adjusted analysis restricted to 501 children born in Scandinavia. With no reported use of antibiotics as reference, the OR for infection was 1.0 (95% CI 0.3-3.6), 0.7 (95% CI 0.2-3.0) and 0.8 (95% CI 0.2-3.3) for 1-5, 6-10 and ≥11 courses, respectively.

A question on use of antibiotics during the 6 months preceding blood sampling was answered by 638 (82%) of the children. Only 15% had taken antibiotics during the period, reflecting the lower consumption in 9-13-year-olds as compared to infants and toddlers. The same pattern, i.e. absence of an association between use of antibiotics and *H. pylori* infection, was seen also in this analysis.

Also in paper II, crude analyses of estimated use of antibiotics during childhood and *H. pylori* seroprevalence showed no pattern of association (Table 12). Clearance of infections was not found to be associated with use of antibiotics in childhood (Table 12).

Table 13 *(paper III).*
History of use of antibiotics and *H. pylori* risk in children by family origin[∂]

	All children		Medium prevalence area [1]		High prevalence area [2]	
Factor	Hp+ %	OR* (95% CI)	Hp+ %	OR* (95% CI)	Hp+ %	OR* (95% CI)
Use of antibiotics[◗]						
None	30	1.0	35	1.0	50	1.0
1-5	13	0.4 (0.2-0.8)	19	0.3 (0.1-1.8)	50	0.9 (0.3-2.5)
6-10	11	0.3 (0.1-0.7)	19	0.6 (0.1-4.8)	59	2.0 (0.3-12.7)
≥11	9	0.3 (0.1-0.6)	22	0.9 (0.1-9.3)	47	0.9 (0.2-3.4)

[◗] Reported use of antibiotics in childhood based on answers from 586 children
[1] Eastern Europe, Asia or South America
[2] Africa or Middle East
* Mantel-Haenszel χ^2-test, odds ratios adj. for gender, household SES and family size
[∂] No estimate could be calculated for the strata of children originating from the low prevalence areas since there were no seropositive subjects in the reference category

Multivariate modelling (paper III)

With the purpose to disentangle independent effects of studied determinants, a multivariate analysis of risk factors for *H. pylori* infection at age 10-12 years was restricted to children originating from medium and high prevalence areas. This was made since the low seroprevalence among children with parents born in low prevalence areas prevented from meaningful estimations of risk factors. Socio-economic status of the household, family size, country of birth of parents and age at arrival in Sweden were independently associated with *H. pylori* status (Table 14). The risk estimates were not affected when gender, age, use of antibiotics and *H. pylori* prevalence in the school class were included in the model.

Table 14 *(paper III)*
Multivariate modelling of risk factors for *H. pylori* infection among 247 school children with parents born in medium and high prevalence areas

	H. pylori infected		
Factor	n/total	%	Adjusted OR* (95% CI)
Origin of parents			
Medium high prevalence area [1]	24/103	23	1.0
High prevalence area [2]	79/144	55	3.8 (1.9-7.5)
Age at arrival in Sweden			
<4 years (or born in Sweden)	57/174	33	1.0
≥4 years of age	41/67	61	4.2 (2.1-8.5)
Family size			
2-3 persons	15/62	24	1.0
4-5 persons	52/127	41	1.6 (0.7-3.4)
≥6 persons	33/47	70	3.6 (1.4-9.5)
Household SES			
White-collar worker	16/72	22	1.0
Blue-collar worker, self-employed, unemployed	84/173	49	4.1 (1.9-8.7)

[1] At least one of the parents born in Eastern Europe, South America or Asia
[2] At least one of the parents born in Africa or the Middle East
* GENMOD procedure, adjusted for all other variables in the table

Abdominal symptoms in children

Abdominal pain (paper II and IV)

In paper II, unspecified abdominal pain during childhood was reported for 71/256 (28%) children. Unspecified abdominal pain during the preceding 6 months was reported for 116/256 (45%) and recurrent abdominal pain (RAP) was reported for 32/208 (15%) children.

Girls were significantly more likely to have reported any type of abdominal pain than the boys. No association was seen between family size, living with a single parent or use of antibiotics and abdominal symptoms.

For children infected, at any time during follow-up, unspecified abdominal pain during childhood was twice as often reported as for the uninfected children when gender was taken into account, OR 2.2 (95% CI 1.0-4.4). Correspondingly, children infected at any time also tended to have reported RAP more often at 11 years of age, OR 2.0 (0.8-4.6). The infection prevalence at 11 years (3%) was too low to allow for statistical calculations.

In paper IV, any abdominal pain during the six months prior to the study was reported for 440/695 (63%) children. RAP according to Apley was reported for 88 (13%) children and weekly abdominal pain was reported for 76 (11%), with an overlap in 46 children.

Any kind of abdominal pain was significantly more common in girls as compared to boys and inversely related to SES of the household. Age, within the narrow range in this study, was found to be an important factor for the likelihood of reporting weekly abdominal pain, with an adjusted 50% lower risk among children aged 12-13 than among 9-11-year-olds. Immigrant background, family size and use of antibiotics were not associated with occurrence of abdominal symptoms in this population.

In paper IV, where a number of possible confounders were controlled for, *H. pylori* infection was not accompanied by an increased risk for abdominal pain, neither overall, nor for RAP (Table 15). On the contrary, the adjusted OR indicated a 50% lower risk of any abdominal pain among *H. pylori* infected children, compared to the uninfected. The prevalence of *H. pylori* was 19% (17/88) and 16% (95/607) in children with and without RAP, respectively, giving an adjusted OR of 1.0 for RAP among infected children.

RAP was less frequently reported among children infected with Type 1 strains than among uninfected children, while RAP was nearly twice as common in children presenting with a Type 2 strain (Table 15). The risk deficit among carriers of Type 1 strains was driven mainly by children with VacA-positive strains (adjusted OR for RAP=0.3; 95% CI 0.1-0.8), while those harbouring CagA-positive strains (VacA-positive or negative) had an

adjusted OR of 0.7 (95% CI 0.3-1.6). For no other symptom did CagA- and VacA-specific associations differ importantly (data not shown).

Weekly abdominal pain was reported in 16% of infected and 10% of uninfected children, with an adjusted OR of 1.5 (0.7-3.3) (Table 15). In our adjusted model, the risk for weekly abdominal pain was three times as high among children infected with a Type 2 strain than among uninfected children. No increased risk was seen among children with a Type 1 strain (Table 15).

Table 15. Associations between *H. pylori* infection, by strain type, and recent abdominal pain and reflux symptoms in school children

	Type of strain		N	Symptom (%)	OR* (95% CI)
Any abdominal pain	Hp-		583	65.9	1.0
	Hp+	Any type	112	50.0	0.5 (0.3-0.8)
		Type 1[a]	64	50.0	0.4 (0.2-0.8)
		Type 2[b]	28	57.1	1.0 (0.4-2.6)
RAP	Hp-		583	12.2	1.0
	Hp+	Any type	112	15.2	1.0 (0.5-2.1)
		Type 1[a]	64	7.8	0.3 (0.1-1.1)
		Type 2[b]	28	21.4	1.9 (0.7-5.5)
Weekly abdominal pain	Hp-		583	10.0	1.0
	Hp+	Any type	112	16.1	1.5 (0.7-3.3)
		Type 1[a]	64	10.9	0.8 (0.3-2.3)
		Type 2[b]	28	25.0	3.0 (1.1-8.3)
Any acid regurgitation [#]	Hp-		555	29.9	1.0
	Hp+	Any type	107	25.2	0.5 (0.3-0.9)
		Type 1[a]	59	17.0	0.2 (0.1-0.5)
		Type 2[b]	28	46.4	1.6 (0.7-3.8)

*GENMOD procedure, adjusted for gender, age, SES, family size and immigrant background

[#] Missing data n=33 (4.7%)

[a] CagA- and VacA-positive strain

[b] CagA- and VacA-negative strain

Gastrointestinal symptoms

When compared to the uninfected, children infected with *H. pylori* reported significantly less acid regurgitation, especially those colonised by Type 1 positive strains (Table 15). When only frequent reflux symptoms (at least once a week) were considered, we instead observed a slightly increased prevalence, though statistically non-significant, among infected children.

The prevalence of disturbed bowel habits was not related to presence of *H. pylori* infection or specific strains (data not shown).

Accuracy of non-invasive diagnostic methods (paper V)

Serology

When using a ^{13}C-UBT, previously validated among children, as the reference, the adjusted upper limit of normal values for our in-house ELISA yielded a sensitivity of 97.8%, a specificity of 95.8%, a PPV of 97.8%, a NPV of 95.8% and a performance index (PI) of 97.2% in the present population of school children with mixed ethnic backgrounds.

Immunoblot

When evaluated according to the manufacturer's criteria, the commercially available Helico Blot 2.0 had a sensitivity of 97.8%, a specificity of 93.8% and a PI of 96.5% with ^{13}C-UBT as reference. The performance of the individual immunoreactive bands is shown in Table 16. The best PI for the individual immunoreactive bands was obtained with the 26.5 kDa (98.6%), 30 kDa (95.7%) and 19.5 kDa (91.5%) antigens, while the CagA, VacA and 35 kDa antigens had a corresponding PI of only 68-78%. None of the combinations of bands had a higher PI than the evaluation criteria suggested by the manufacturer.

Table 16. Performance of each immunoreactive band and immunoblot reaction relative to ^{13}C-UBT in school children

Immunoreactive Bands (kDa)	Sens. %	Spec. %	PPV %	NPV %	PI %*
116	69.9	93.8	95.6	61.6	78.0
89	60.2	100	100	56.5	73.8
35	54.8	95.8	96.2	52.3	68.8
30	96.8	93.8	96.8	93.8	95.7
26.5	98.9	97.9	98.9	97.9	98.6
19.5	88.2	97.9	98.8	81.0	91.5
Immunoblot reaction♣	97.8	93.8	96.8	95.7	96.5

* Performance Index (PI): percentage of children correctly classified [(number of true positive + number of true negative)/total number of children tested]

♣ Immunoblot reaction according to the manufacturers' instruction.

^{13}C-UBT

Considering that the cut-off limits for the UBT had been established in a limited series of paediatric patients, it seemed reasonable to investigate the performance of the breath test against the immunoblot. With Helico Blot 2.0 as reference, the ^{13}C-UBT yielded a sensitivity of 96.8% (91/94), a specificity of 95.7% (45/47), a PPV of 97.8% (91/93) and a NPV of 93.8% (45/48). The PI between tests was 96.5%.

Distribution of *H. pylori* strains

Since the mother seems to be of major importance for the transmission of *H. pylori* infection we investigated the prevalence of specific immunoreactive bands in children by maternal geographic origin (Table 17).

The overall prevalence was 73% for CagA, 59% for VacA and 57% for the presence of CagA and VacA antibodies (Type 1 strains). Antibodies to the 35 kDa band were noted in 57% of the children. The highest antibody prevalence was seen for the 26.5 and 30 kDa antigens in all groups.

A significantly lower ($p<0.002$) prevalence of antibodies to the 19.5 and the 26.5 kDa was noted among children with Scandinavian and Western European mothers. For the heavier molecular weight antigens, no significant difference in prevalence was found by maternal origin.

Table 17. The percentage prevalence of *H. pylori* infection and specific immunoreactive bands in school children according to maternal origin

Place of birth of mother	Infected children/ total	19.5 kDa %	26.5 kDa %	30 kDa %	35 kDa %	89 kDa %	116 kDa %
Scandinavia, W Europe	11/481	55	82	91	45	45	55
East Europe	12/41	92	100	100	67	58	92
S America	8/22	62	100	100	50	62	62
Asia	5/20	100	100	100	60	100	100
Mid East	42/67	95	100	100	45	55	67
Africa	34/52	91	100	97	74	62	79
*p**	0.001	0.002	0.002	0.4	0.2	0.4	0.2

* Mantel-Haenszel χ^2 test for general associations

GENERAL DISCUSSION - Methodological considerations

Study design

Case-control and cohort studies

Studies I and II were designed as a retrospective cohort study, originally being a vaccination trial, with outcome data collected, supplemented by additional exposure information, during the follow-up period (paper II).

For paper III and IV, a cross-sectional case-control study design was chosen. This study design can be conceptualised as the case-control analogue of the general population follow-up study (Rothman et al. 1998b) and is suitable for exposures that represent stable personal characteristics, such as SES, *H. pylori* and family background. The study design was considered to be acceptable when the outcome, here being *H. pylori* infection and abdominal pain, respectively, was expected to be relatively common in the study base (dos Santos Silva 1999).

Community-based studies

All studies in this thesis are community-based and thus reflecting the situation in the respective source populations. For paper I-II the source population was a child population, with parents fluent in Swedish, residing in the catchment areas of 93 child health care centres. For paper III-IV it was all 10-12-year old school children in the catchmnet areas of 11 schools, irrespective of ethnic and social background. One advantage with the community-based study design is that it is not affected by the potential selection bias arising from hospital-based case-control studies, where the controls may be selected due to factors, known or unknown, in children treated at that hospital.

Participation

Statistical analyses for paper I and II were based on serial serological data from 294/305 (96%) identifiable children. At 11-12 years of age 263 (86%) children and their parents answered our questionnaire and 208 (68%) children accepted a further blood sample. The 86% response rate for the questionnaire must be considered a high since the questionnaire was offered 7 years after the last contact with these families. However, the >30% drop-out rate for the 11-year blood sample could represent a problem if it was related to the outcome, i.e. *H. pylori* status.

For paper III and IV, we were aware that several children might deny blood sampling at this particular age. The present outcome, being returned questionnaires from 756/858 (88%) children and a blood sample donated

from 695 (81%), we thus regard as a high participation rate. This high rate was obtained by extensive efforts to motivate the invited pupils for participation (see Subjects and Methods). In paper V, 107/133 (80%) of seropositive and borderline cases and 34/37 (92%) of seronegative controls volunteered for the follow-up.

Precision

Precision (or reliabililty) of measures of associations in epidemiological studies corresponds to the reduction of random error, mainly depending on the sample size. To provide information about the precision of our relative risk estimates (paper II-IV) we used 95% confidence intervals. A statistically significant result, however, does not rule out that chance has accounted for the finding, but tells us that such a chance variation is unlikely.

For paper II, no formal sample size calculations preceded the study. The lack of an association between explanatory variables and *H. pylori* infection is likely to be a result of the low prevalence of infection and relatively homogeneous living conditions (i.e. family size and density of living) for Swedish-born young adults and their children. It could also have been a result of confounding by unmeasured SES. The small difference in *H. pylori* prevalence noted for exposed and unexposed groups in combination with the sample size resulted in low power of the study, thus making Type II errors likely.

The sample size of the cross-sectional study (paper III) was based on a power calculation indicating that we would be able to detect a difference in *H. pylori* prevalence of at least 10% with 90% power if the lower prevalence was 5% and the α-level was 0.05. To compensate for the possibility of a 30% non-participation rate 858 children were invited. In order to improve efficiency, study schools were also selected to give a wide range of exposure, i.e. *H. pylori* infection, among the children.

Internal validity

Internal validity in epidemiological studies corresponds to the reduction of systematic errors. Three general types of systematic errors, i.e. biases, can be identified: selection bias, confounding and information bias.

Selection bias

Selection bias is introduced in a study as a result from procedures that are used to select subjects and from factors that influence study participation.

In paper I-II, the possibility that loss to follow-up at 11 years of age might have been related to either presence/absence of abdominal pain in the child or socio-economic conditions of the family and thus possibly also to *H. pylori* status in the child cannot be entirely excluded.

In paper III-IV, the concern about selection bias was to some extent allayed since 61 children answering the questionnaire but refusing a blood sample were practically identical to their participating peers with regard to age, gender, background variables and reported symptoms. Also, the high participation rate (81%), regardless of background, allay concerns about important selection bias in these studies.

Confounding

In any study, the exposed and unexposed may differ with regard to other factors that affect the risk of developing the disease (in our case contracting the infection or developing GI symptoms). Such a factor mixes with the effect of the studied factor and may introduce an error (i.e. an over- or underestimation), when measuring the effect of the exposure by comparing the disease occurrence in the exposed and unexposed. If so the factor is called a confounder (Table 18). If confounding is not controlled for, distortion of the results will be introduced.

Table 18. Criteria for a confounding factor (Rothman et al. 1998c)

To introduce confounding, a factor must:
a) be a risk factor for the disease
b) be associated with the exposure under study in the source population
c) not be an intermediate step in the casual path between the exposure and the disease

Even though the exact route of transmission of *H. pylori* infection is not known, a number of risk indicators and thus possible confounders have been identified. Likewise, abdominal pain in children may be associated with a number of sociodemographic characteristics, even though it is uncertain if socio-economic factors are themselves causal in the aetiology of RAP and other abdominal pain.

For paper III-IV information on known and potential confounders, such as age, gender, SES, family size, ethnic background and age at arrival in Sweden, were collected and adjusted for in the statistical analyses. The lack of information about SES prohibited proper adjustments and might have left some confounding untreated in paper II.

Different approaches used to control for confounding were: 1) multivariate modelling (paper III-IV) and 2) stratified statistical analyses (paper III). The

latter was used to control for confounding by geographic origin when estimating the effects of SES, family size, cumulated use of antibiotics and day-care attendance on *H. pylori* infection.

Information bias

Information bias in a case-control study is introduced when the information on exposure is affected by being a case or a control. This awareness may result in differential over- or underestimation of exposures in the past (recall bias) leading to an over- or underestimation of the estimated association. Nondifferential misclassification, on the other hand, introduces bias that may lead to an underestimation of the measured association.

Possible misclassification of exposures

In our case-control study (paper III), any misclassification in the questionnaires must have been nondifferential vis-à-vis *H. pylori* status since all questionnaires were turned in before blood samples were collected. Such misclassification would lead only to conservative estimates of any association (Rothman et al. 1998c).

A general problem with cross-sectional and case-control studies is the assessment of exposure in the past. Most of our main variables were, however, invariable personal characteristics, e.g. ethnicity and age at arrival in Sweden.

Age at immigration to Sweden and family size, might have been affected by some misclassification. This could have been due to difficulties in understanding the questions, but this problem was minimised by offering the questionnaire in the eight most commonly understood languages. Any minor misclassifications would have been nondifferential and thus leading to an underestimation of the measured association.

The classification of socio-economic status has been validated for Swedish citizens (Official Statistics of Sweden, 1999), and it is frequently used in Swedish epidemiological research. For more recently arriving immigrants, whose children were particularly likely to be infected with *H. pylori*, this validation has never been made.

The information on antibiotic usage may represent a problem. There is a possibility that misclassification of antibiotic use varies with the socio-economic level of the parents, and thus indirectly with the outcome (*H. pylori* status). The direction of any possible bias due to this partly differential bias is difficult to predict and may have resulted in an under- or overestimation of the true association.

An attempt to collect or validate the information on use of antibiotics from medical records was unfeasible. Many of the children had

medical records at different general practitioners and hospitals, for immigrating children possibly also in a number of towns before settling down in the study area. Moreover, such an endeavour could only have shed light on the false positive rate.

Possible misclassification of the outcome/disease

In case-control studies, the effect of nondifferential misclassification of the outcome resembles that of the nondifferential misclassification of the exposure, producing a reduction of the relative risk estimate.

All serological analyses performed to detect *H. pylori* infections in the present studies (paper II and III) were made blind to any exposure. Furthermore, a validation study was performed (paper V) to assure the accuracy of our tests in the present child population (paper III). The validation resulted in an adjusted cut-off at an OD value of 0.22 with a sensitivity of 97.8% and a specificity of 95.8%.

However, one needs to remember that for *H. pylori* infection there is no real gold standard for both sensitivity and specificity. This was illustrated by our finding of variable breath test results as well as two negative UBT results in a seropositive girl who happened to undergo gastroscopy (due to her celiac disease) and who had a sparse *H. pylori* growth from biopsies. False positive UBT cannot be entirely excluded either, due to the presence of urease producing bacteria in the oral cavity (Hamlet et al. 1995), possibly demonstrated by a UBT positive but serology negative girl in our control group.

Truly false positive reactions by serology could be due to cleared *Helicobacter*, either as a result of eradication or spontaneous clearance, previously noted in paediatric populations (Kumagai et al. 1998, Malaty et al. 1999). Although none of the children had received *H. pylori* eradication treatment, antibiotic therapy for other causes could theoretically have resulted in eradication. Positive serology after successful eradication has been noted to be more long-standing by immunoblot than by ELISA (Sörberg et al. 1997), even though the opposite finding has been reported in a Japanese study of children (Kato et al. 2000).

To further improve the detection of cases and to reduce the number of false positive subjects (Feldman et al. 1995), the three noninvasive tests (paper V) were combined to identify the cases for paper III-IV.

In paper I-II the same ELISA was used as in paper III-V, but a cut-off level had been established in adults, likely to lead to a lower sensitivity in young children. The use of the higher cut-off level was chosen since we preferred to safeguard a high specificity rather than a higher sensitivity.

For the data on abdominal symptoms (paper II and IV), including recurrent abdominal pain (RAP), we used different methods to obtain the information on RAP in the two studies.

For paper II, all parents of participating children were interviewed about any occurrence of RAP in the child. One nurse, who had been trained to identify RAP, made all 208 interviews. For paper IV, all information on frequency and intensity of abdominal pain was collected in a questionnaire. Transformations of these data were made to identify children fulfilling the criteria for RAP.

The two methods resulted in similar prevalences of RAP that are consistent with the literature. This would indicate that our data on recurrent abdominal pain reported for school children are reliable.

In both studies, any misclassification of abdominal symptoms must be nondif-ferential vis-à-vis *H. pylori* status since all answers were collected before blood samples were drawn. Again, such misclassification would dilute any associations between the exposure and the symptom (Rothman et al. 1998c) and could conceivably have contributed to some false negative results for weak associations.

Causality or reversed causality

A cause of a disease may be defined as an event or condition that preceded the disease and without which the disease would not have occurred at all or would not have occurred until later (Rothman et al. 1998a). A possible disadvantage with the cross-sectional case-control study design may be the concern about the opposite situation, i.e. reversed causation (des Santos Silva, 1999).

However, we did not have to worry about this in paper III, since none of our main variables (age, gender, country of origin, age at arrival in Sweden, family size and SES) were likely to be a result of the infection.

The lack of knowledge on a temporal association between the exposure to antibiotics and *H. pylori* infection could have been a problem (paper III). There may be reversed causation; i.e. increased use of antibiotics in *H. pylori* infected. If so we would have underestimated the possible protective role of antibiotics on *H. pylori* infection.

In the study of the association between *H. pylori* infection and abdominal symptoms (paper IV) the cross-sectional design limits the interpretation of causality since the time sequence of events cannot be established. It is, for instance, conceivable that frequent symptoms, perhaps with associated vomiting and health care contacts, may have facilitated subsequent transmission of *H. pylori.*

GENERAL DISCUSSION - Interpretation and implications of the observations

Incidence and age at acquisition

Our serological follow-up from 6 months to 11 years of age showed that acquisition of *H. pylori* infection occurs early in life also in a developed country, i.e before 4 years of age. We could also demonstrate that transient infections are common in early life, thus leaving only 3% of the 11-year-olds infected (paper I). The generally low seroprevalence among these children is likely to reflect that these children came from families with a low exposure opportunity at home.

The findings of early acquisition and higher infection rates at younger ages are consistent with the findings in the literature. Incidence rates as high as the one noted by us, i.e. 13 per 100 person-years between 18 and 24 months, has been reported from less developed parts of the world (Klein et al. 1994, Lindkvist et al. 1999a and 1999b) and among Turkish immigrants in Germany (Rothenbacher et al. 2000). Seroepidemiological reports from developed parts of the world have shown more moderate incidence rates among pre-school and school children (Ashorn et al. 1995, Rehnberg-Laiho et al. 1998, Kumagai et al. 1998, Fawcett et al. 1998, Malaty et al. 1999) and also among infants (Ashorn et al. 1996).

The differences in results between studies from developed parts of the world could be due to differences in the diagnostic methods used. Since serological methods may result in a lower sensitivity in younger children, it is plausible that we, on basis of the current sensitivity, could have missed one or two cases of infection in our study (paper I and II). A lower specificity of a test, on the other hand, might have result in an overestimation of prevalence especially in populations where the true prevalence is low (Cockburn et al. 1997). Given the high reliability of our serology, as confirmed by concordant test results in the two sera obtained with a two-week-interval at 24 months of age, the high incidence noted between 18 and 24 months of age is not likely to be due to chance.

The infection prevalence of 2-3% among 10-12-year-old children with both parents from a low prevalence area (paper I and III) is likely to reflect a birth cohort effect (Banatvala et al. 1993, Cullen et al. 1993). If so the noted low prevalence among the children would be consistent with the relatively low prevalence of 20-30% reported for Swedish adults of similar age as the parents in the present studies (Agréus et al. 1995, Bergenzaun et al. 1996).

Risk factors for infection

The result from our school-based cross-sectional study (paper III) provide support for the hypothesis that exposure opportunity within the family is a strong determinant for the probability of being *H. pylori* infected in Western societies. Among children born in Scandinavia *H. pylori* seroprevalence seemed to be approximately proportional to the prevalence in the populations from which their parents originated. For children born into families with low exposure opportunity the low seroprevalence was unaffected by high exposure opportunity at day-care centres and at school.

Our study (paper III) confirmed previously established risk factors for *H. pylori* infection, such as low SES and large family size (Mendall et al. 1992, Staat et al. 1996, Malaty et al. 1996, Rothenbacher et al. 1998, Torres et al. 1998, Dominici et al. 1999), but only among children with appreciable exposure opportunity within the family. It also confirmed the observations that the *H. pylori* prevalence in children originating from less developed parts of the world is high even though they are born in countries with a low infection prevalence (Banatvala et al. 1995, Lanciers et al. 1996, Rothenbacher et al. 1998a). It is conceivable that the socio-economic factors in our study were markers of parental *H. pylori* prevalence rather than having a direct effect on the probability of transmission at any given exposure level, even though the mode of transmission was not studied in detail.

Our findings, which are likely to be representative for children of the same age in Sweden and highly developed Western societies, may however not be directly generalizable to developing or developed countries with a high prevalence of *H. pylori*. If the exposure intensity is large enough, it is conceivable that others than family members may infect the children. This is illustrated by our observation that the time spent by the child in a medium or high *H. pylori* prevalence area was independently linked to the probability of being seropositive (paper III). The latter observation may, however, also be explained by the fact that worse living conditions and poorer sanitation in the country of origin, by means of shared exposure to environmental reservoirs, may have further enhanced the transmission of the organism within the family.

Transmission

Our finding that *H. pylori* prevalence is unaffected by high exposure opportunity at day-care centres and schools for children born into families with low exposure opportunity is indicating indirectly that the family is the milieu where most of the transmission of *H. pylori* takes place (paper II-III). Thus,

little or no transmission seems to occur outside the family in the Swedish setting even if the child is exposed at ages when he/she is susceptible.

The effect of both parental origin and socio-economic status on a child's probability of being seropositive was considerably stronger when the mother's status was considered than when the father was taken into consideration (paper III). This observation is consistent with a predominant mother-to-child transmission pattern. Also, our observation of a higher seroprevalence at any time during childhood in children of mothers who had reported a history of a peptic ulcer (paper II), which has been convincingly linked to *H. pylori* infection, would indirectly support mother-to-child transmission.

Our findings are in line with previously reported epidemiological and molecular evidence of intrafamilial transmission (Drumm et al. 1990, Rothenbacher et al. 1999, Raymond et al. 1998, Elitsur et al. 1999a). Since extrafamilial sources of the infection are contradicted in our study (paper III), it is unlikely that the microorganism is introduced into the family by any of the children, at least in low prevalence areas. This does not, however, exclude that transmission between siblings within the family might occur (Goodman et al. 2000).

Close child-to-child contacts outside the family, typically occurring at day-care centres or at school, that has previously been proposed to be an important mechanism for transmission (Webb et al. 1994, Mendall et al. 1995, Parsonnet 1995), did not seem to result in high rates of seroconversion in the present setting. Studies to confirm or refute our observation are so far lacking.

The respective role of the faecal-oral, oral-oral and gastro-oral routes of transmission has been discussed by a number of authors as indicated in the introductory remarks. With the information available from our studies we are not able to separate the effects of these mechanisms.

The high level of sanitation in Sweden, with controlled treatment of sewage water, indoor water closets as a rule etc., would imply that the faecal-oral route is less likely to play a substantial role in the present setting. Overt domestic crowding is generally not seen in the Swedish setting, although overcrowding cannot be ruled out for the time spent by some of the families in a refugee camp when entering Sweden nor for families with 6-10 members living in one flat even with high Swedish standards.

Bedsharing, which does not seem to be associated with socio-economic status in Western societies (Whitaker et al. 1993), has been suggested to play a role in close person-to-person contact transmission of

the infection (Whitaker et al. 1993, Webb et al. 1994). However, a prerequisite for transmission of the organism as a result of bedsharing is the presence of the bacteria in the family member with whom the bed is shared. The theory with bedsharing however, fits with our data indicating that exposure opportunity within the family is a strong determinant for the probability of contracting *H. pylori* infection in childhood in Western societies.

The gastro-oral route, which has been suggested to play a role in child-to-child transmission and especially at times of acute gastrointestinal tract infections with vomiting (Leung et al. 1999, Parsonnet et al. 1999), could not be evaluated in the present studies. However, any mechanism of transmission of the organism, gastro-oral or faecal-oral, at times of gastroenteritis in the family would fit with our noted pattern *of H. pylori* infections also in the Swedish society.

A common source of infection that is shared by the family has been suggested as an alternative mode to intrafamilial transmission (Malaty et al. 1991, Nwokolo et al. 1992, Bamford et al. 1993, van der Ende et al. 1996). An environmental source of the infection cannot be unequivocally refuted based on our findings, but seems less likely. Close person-to-person contacts, especially between mother and child, appear to play the major role in *H. pylori* transmission among children in Sweden.

Use of antibiotics during childhood

We found that antibiotic use for minor childhood morbidity does not importantly affect *H. pylori* colonisation. This practically also rules out confounding by antibiotics. The latter was suggested in another study among children in a Western setting (Rothenbacher et al. 1998b), that however did not adjust for parental origin.

One reason for an apparent absence of effect by casual antibiotics could be self-treatment with antibiotics sold over the counter, as seen in some parts of the world. Such drugs are not available in Sweden, where all antibiotics have to be prescribed by doctors. Our analysis restricted to children born in Sweden confirmed the general finding of no association between antibiotic consumption and *H. pylori* seroprevalence (paper III).

Recurrent abdominal pain (RAP)

Overall, *H. pylori* infection was not accompanied by an increased occurrence of abdominal pain in our cross-sectional school-based study among 10-12-year-old children (paper IV). Instead, abdominal pain was significantly less common among infected children. This deficit appeared to be driven by a strong inverse association between presence of Type 1 strains and abdominal pain. Conversely, and unexpectedly, children infected with Type 2 strains were at increased risk for having both RAP and abdominal pain at least once a week. For the latter symptom the risk showed 3-fold and statistically significant increase.

The overall absence of association between *H. pylori* infection and RAP in school children is consistent with the only community-based study of school children performed previously (O´Donohoe et al. 1996). A number of clinical studies have explored the possible relationship between *H. pylori* infection and RAP. Macarthur et al. (1999) reported a low prevalence of *H. pylori* (<5%) both in cases with RAP and controls seen by Canadian primary care paediatricians, and concluded that *H. pylori* infection could not be a major cause of RAP. Studies of RAP patients undergoing upper endoscopies have yielded divergent results (Chong et al. 1995, Blecker et al. 1996, Hardikar et al. 1996, Wewer et al. 1998). Conflicting results could, however, reside in selection bias and confounding by background factors.

Abdominal pain during the most recent six months was reported for 63% of our school children, which is comparable with a reported 70% prevalence of gastrointestinal (GI) symptoms among German pre-school children (Bode et al. 1998b). Despite the differences between that study and ours, both found an inverse association between *H. pylori* infection and GI symptoms. The explanation for these findings is unclear since GI symptom rates of 60-70% are unlikely to represent organic disorders in a community-based sample of children of pre-school or school age.

Our study, which used both ELISA and immunoblot for identifying *H. pylori* infection, enabled us to identify specific *H. pylori* strains (paper IV). To our knowledge, this is the first study of *H. pylori* strains and their relationships to symptoms in a community-based sample of school children. Abdominal pain during the preceding 6 months, no matter which criteria was used to define the effect, were less often reported for children infected with Type 1 strains as compared to uninfected children. Our finding is consistent with previous observations of a high prevalence of CagA-positive and VacA-positive strains in asymptomatic children (Elitsur et al. 1999b, Mitchell et al. 1999). A 2-year follow-up study with upper endoscopies in

French asymptomatic children, infected with *H. pylori,* found that children with CagA-positive strains did not become symptomatic more often than did the children with CagA-negative strains. They noted, however, that microscopic changes progressed more rapidly in CagA-negative than in CagA-positive children (Ganga-Zandzou et al. 1999).

Other studies, based on children referred for upper endoscopies due to GI symptoms, reported worse symptoms and more severe gastritis among children with CagA-positive strains (Husson et al. 1995, Elitsur et al. 1999b, Lerro et al. 2000). It is unclear to what extent selection to gastroscopy may have affected the findings since only a small proportion of all children with abdominal symptoms undergoes endoscopy. It is conceivable that this group of children with the most severe symptoms have an aetiology that might be *H. pylori* related and also different from that of the more trivial abdominal complaints captured in our study.

In conclusion, our community-based study supports previous observations indicating no positive association overall between *H. pylori* infection and common, trivial abdominal symptoms or RAP in non-consulting school children. The inverse association between Type 1 strain infections and risk for abdominal pain and the novel positive association between presence of Type 2 infections and frequent abdominal pain, indicate that a role of *H. pylori* in the aetiology of paediatric gastrointestinal symptoms cannot be unequivocally refuted.

Gastroesophageal reflux symptoms

Somewhat unexpectedly, we observed that reflux symptoms were significantly less common among *H. pylori* infected than uninfected children (paper IV). Also this deficit appeared to be driven by an inverse association between presence of Type 1 strains and reflux symptoms. On the contrary, children infected with Type 2 strains were at increased risk for having reflux symptoms.

Similar findings of a negative association between *H. pylori* infection and esophagitis have been reported from two previous studies based on upper endoscopies in children with RAP (Ashorn et al. 1993, Roma et al. 1999). The findings might also support previous claims of an inverse relationship between *H. pylori* infection and risk of GERD in adults (Labenz et al. 1997, Vicari et al. 1998).

Accuracy of serology in epidemiological H. pylori research

Our study evaluating non-invasive methods to screen for *H. pylori* infection in school children has shown that serology by ELISA is an useful diagnostic method to identify *H. pylori* infection, provided that an adjusted cut-off value is used. When adjusted, the present in-house ELISA with sonicated *C. jejuni* in the serum dilution buffer to remove cross reacting antibodies, as well as the commercial immunoblot kit (Helico Blot 2.0), had a high diagnostic validity in a paediatric population.

However, all evaluation of diagnostic tests will depend on the performance of the chosen reference method. For the present evaluation we had chosen a validated ^{13}C-UBT as reference, since UBT has been recommended a reference method for epidemiological purposes (Hawtin 1999) where endoscopies would not be feasible. One needs to remember that there is no real gold standard for both sensitivity and specificity in *H. pylori* infection as discussed earlier, however.

Our result of the highest accuracy of 98.6% for the low- molecular weigh band (26.5 kDa), rather than any of the high-molecular weigh bands, when using the immunoblot is consistent with findings in the literature. The 26 kDa band has been described to be the first to appear after a primary infection with *H. pylori* in childhood (Mitchell et al. 1996b) soon followed by other low-molecular weight bands. Two early Danish studies suggested a combination of the 19- to 36-kDa band to identify *H. pylori* positive patients (Andersen et al. 1992 and 1995). This was supported by the high accuracy for the low-molecular weight bands as seen in our study as well as in a French study of paediatric patients (Raymond et al. 2000).

Our results indicating validity also for serological *H. pylori* diagnostic methods, after adequate adjustments for age and geographic origin, is of importance for forthcoming epidemiological studies as well as clinical purposes, particularly for follow-up after treatment. Like in adults, the decision to use one test rather than another depends on the clinical circumstances, the reported parameters reflecting the performance of the test in a given population, cost and convenience. A prerequisite, however, is a validation in the given population that may differ both in age and geographic origin.

Distribution of immunoreactive bands according to ethnic origin

Our use of immunoblot in all identified ELISA seropositive children (paper V) allowed for an analysis of specific immunoreactive bands according to geographic origin. A marked heterogeneity in distribution of all bands, statistically significant for the low-molecular weight bands, was an interesting finding among children with mixed geographic background but living in the same country.

Our finding of a variable prevalence of CagA antibodies in children with an origin in different parts of the world seemed to reflect published prevalences for children living in corresponding areas (Mitchell et al. 1997, Kato et al. 2000, Karaczewska et al. abstract 1997). The 55% CagA positivity prevalence noted by us in children with mothers born in Scandinavia is similar to the rate of 54% in asymptomatic *H. pylori* infected children in North America (Elitsur et al. 1999b), and to the 43% in French children undergoing upper endoscopy due to abdominal disorders (Raymond et al. 2000). The finding is also consistent with recent reports comparing CagA and VacA expression in adults of different ethnic belonging in different parts of the world (Campbell et al. 1997, Perez-Perez et al. 1997, Webb et al. 1999).

The cause of the noted differences in distribution of immunoreactive bands remains unclear. It might, however, reflect differences in infecting strains, in antibody response patterns or in age at infection. Furthermore, the finding might be of importance for the choice of antigens in serological assays and needs further studies. If antigen differences are of importance in serological and seroepidemiological investigations, then they could result in variable performance of assays and divergent results of studies performed in different ethnic populations.

Prevention and interventions in childhood

A test and treat approach for limiting the burden of *H. pylori* infection in the adult population has been suggested. The approach has, however, not been approved by European and Canadian paediatricians (Sherman et al. 1999, Drumm et al. 2000). The decision was mainly based on the lack of a specific clinical picture indicating a need for *H. pylori* screening and no evidence to suggest a link between *H. pylori* gastritis and abdominal pain in children in the absence of ulcer disease.

Thus, the only indication for active intervention in the paediatric patient so far agreed upon is an identified peptic ulcer. A need of a consensus on the importance of intervention in children in relation to the risk of developing cancer has, however, been proposed (Drumm et al. 2000). It is noteworthy that for adults a consensus has been achieved recommending *H. pylori* eradication in first degree relatives of gastric cancer patients (Maastricht II Consensus, 2000). This group could include also children.

The results of our large community-based study indicating an overall negative association between *H. pylori* infection and abdominal pain in otherwise healthy school children support the above mentioned consensus statements on the management of *H. pylori* infection in children.

The inverse association between Type 1 strain infections and risk for abdominal pain and/or acid regurgitation, noted by us and others, and the novel positive association between presence of Type 2 infections and frequent abdominal pain, however, indicate that a role of *H. pylori* in the aetiology of paediatric gastrointestinal symptoms cannot be unequivocally refuted. A possible association between infection and abdominal pain for a subset of children with severe symptoms could not be excluded and this warrants further investigations.

Before any active interventions can be recommended, further knowledge is needed on the role of specific infecting strain as well as host related factors that are currently less well understood.

For some time it has been believed that after the establishment of *H. pylori* infection the organism was to persist in the stomach for life. Based on current knowledge about transient *H. pylori* infections in childhood, the assumption has been revised. The present awareness about frequent transient infections as well as the possibility of frequent reinfections early in life, which both may vary between populations, has implications for the research and management of childhood *H. pylori* infections.

From an epidemiological research perspective, the age at acquisition will be a key when unveiling the dominating mode or modes of

transmission. As indicated above, these modes may vary between populations with different exposure opportunity in their environment. Also, the evidence of a self-resolving form of *H. pylori* infection makes it necessary to distinguish between factors associated with the chronic infection and those associated with an acquisition at younger ages.

If further studies would indicate that populations would benefit from test and treat approaches already in childhood, then school children might be a good target group for active interventions against *H. pylori* infection, at least in Western societies. At this age and in this setting, the acquisition rate of *H. pylori* infection has been shown to be low, given that the child has a low exposure opportunity at home. Also, antibiotic eradication treatments would not be unnecessarily used, as might be the case if treating younger children in whom transient infections are common.

The results presented in this thesis also showed that accurate non-invasive diagnostic methods for *H. pylori* detection could be developed for paediatric populations. Adjustments for age and a validation within the specific population should be made.

However, *H. pylori* infection is highly endemic predominantly in areas were active treatment of the infection could not be afforded or even feasible due to high reinfection rates. This may also be the case for minorities residing in parts of the world were the *H. pylori* prevalence has started to decline already before the introduction of antibiotic treatments. A potential vaccine, for treatment or prevention, may not be developed in a long time. Also, a vaccine might be too expensive for the populations with the most pronounced need. Thus, it seems, as efforts should be aimed at primary prevention.

To design preventive measures with the goal of limiting the transmission of the infection, it is important to understand when and where the infection is acquired. After narrowing in on time and place, it may be possible to identify circumstances that favour the transmission and to unveil the mechanism. Evidences suggest that early childhood is the critical time for acquisition of the infection. The findings indicating that mother-to-child transmission is an important mechanism for the spread of *H. pylori* may be valuable when devising future strategies aimed at accelerating the disappearance of the infection in high prevalence populations.

CONCLUSIONS

Helicobacter pylori infection is acquired early in life, i.e. before 4 years of age, also in a developed country. The acquisition of infection in childhood, however, does not always result in a persistent infection.

Major differences in *H. pylori* prevalence are seen among Swedish children of different ethnic origin and indicate that exposure opportunity within the family is a strong determinant for childhood *H. pylori* infection.

Low socio-economic status and large family size are risk factors for infection among Swedish children having appreciable exposure opportunity at home. Also the time spent by the child in the country of origin seem to matter for the risk of contracting *H. pylori* infection early in life.

Close contacts between children outside the family, such as those at day-care centres and in school classes with a high *H. pylori* prevalence, does not increase the risk of *H. pylori* infection in children in Sweden, pointing at intrafamilial transmission as the major route of transmission.

Intrafamilial transmission of *H. pylori* infection is likely to be dominated by mother-to-child transmission.

Use of antibiotics during childhood does not seem to be related to the prevalence of *H. pylori* infection when family origin is taken into account.

H. pylori infection is not associated with an increased risk of recurrent abdominal pain (RAP) or gastrointestinal symptoms among 10-12-year-olds.

Type 1 strain infections seem to be inversely associated with abdominal pain and reflux symptoms among school children, while Type 2 infections might increase the risk of reflux symptoms, RAP and weekly abdominal pain.

Serology by ELISA and Immunoblot, as well as ^{13}C-UBT, were demonstrated to be useful diagnostic methods for *H. pylori* infection in children provided that adjusted cut-offs are used.

The presence of specific *H. pylori* immunoreactive bands might vary according to geographic origin also for children living in the same country.

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