CHAPTER 6

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

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ABSTRACT

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

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

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

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

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

INTRODUCTION

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

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

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

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

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

METHODS

Study design and population

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

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

Sample collection and stool tests

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

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

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

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

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

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

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

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

RESULTS

In total 150 infants (82 male and 68 female) aged 3-24 months were enrolled (table 2). The SES was low for 32 (21.4%), medium for 113 (75.3%) and high for 5 (3.3%) of the children.

Four stool samples tested *Hp*-positive at the first sampling (2 females), resulting in an overall *Hp*-prevalence of 2.7%. All positive samples originated from the groups aged 3-6 and 6-9 months, leading to a prevalence of 8% for this subgroup. The youngest infected child was aged 3 months and 4 days. Three of the positive children belonged to the lowest SES and 1 to the medium SES.

Two years after the first sampling, the *Hp* status was followed-up. Unfortunately, due to a big fire in the subdistrict, 27 children originally tested negative had moved elsewhere and attempts to trace their actual addresses remained unsuccessfully. In addition, one *Hp* positive tested child died from pneumonia and 10 *Hp* negative tested children could not be traced, so in total 112 children were available for the follow-up study. All samples in the follow-up were *Hp*-negative, including 3 of the children who were previously tested positive. Those 3 children had never been treated with antibiotics. None of their parents reported abdominal pain, ulcer or gastric carcinoma, nor underwent abdominal surgery.

DISCUSSION

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

Serologic tests are unreliable in young children and have revealed disappointing results with respect to the diagnostics of acute *Hp*-infection, since the antibody

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

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

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

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

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

Strengths and limitations of our study

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

CONCLUSION

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

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