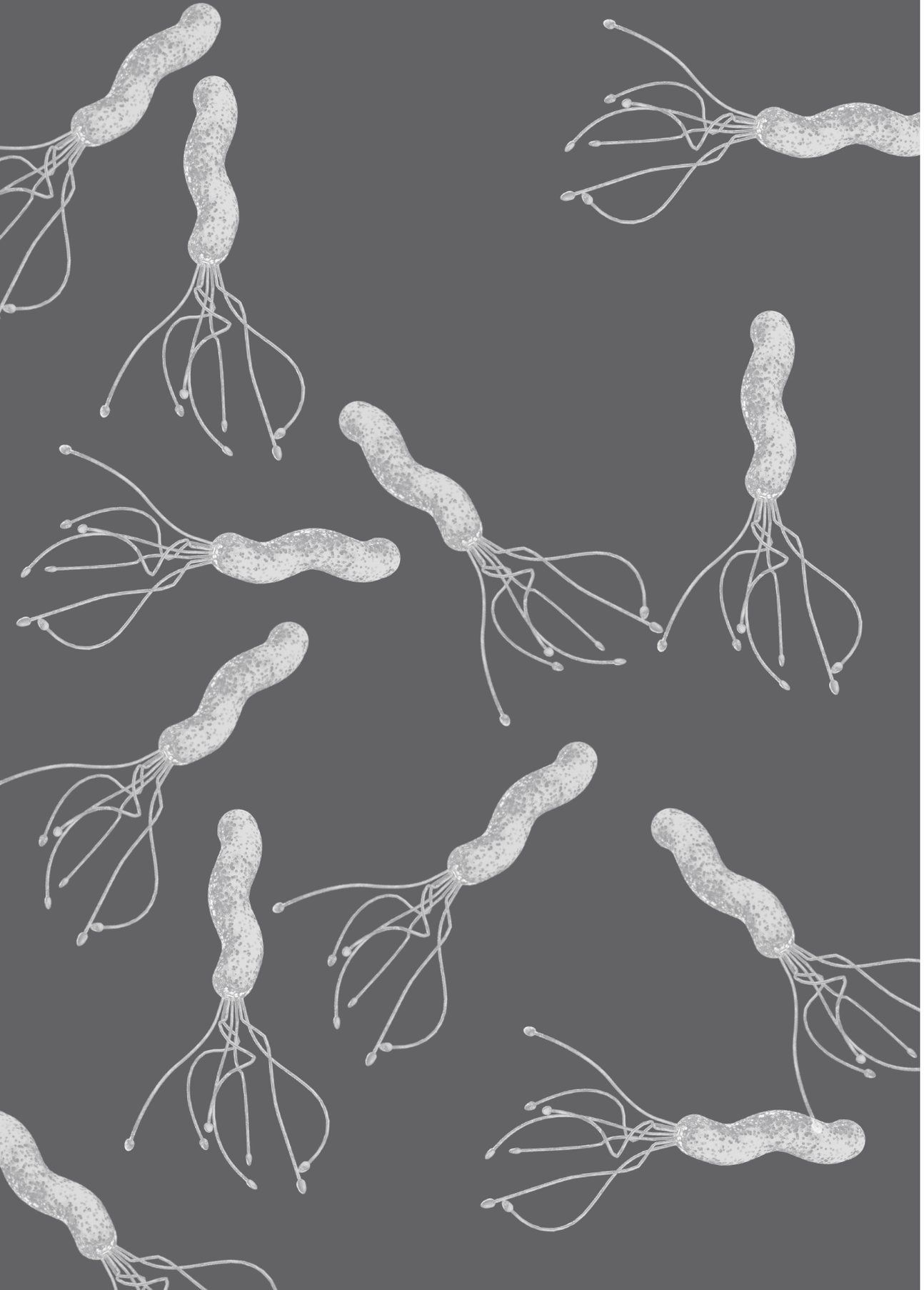
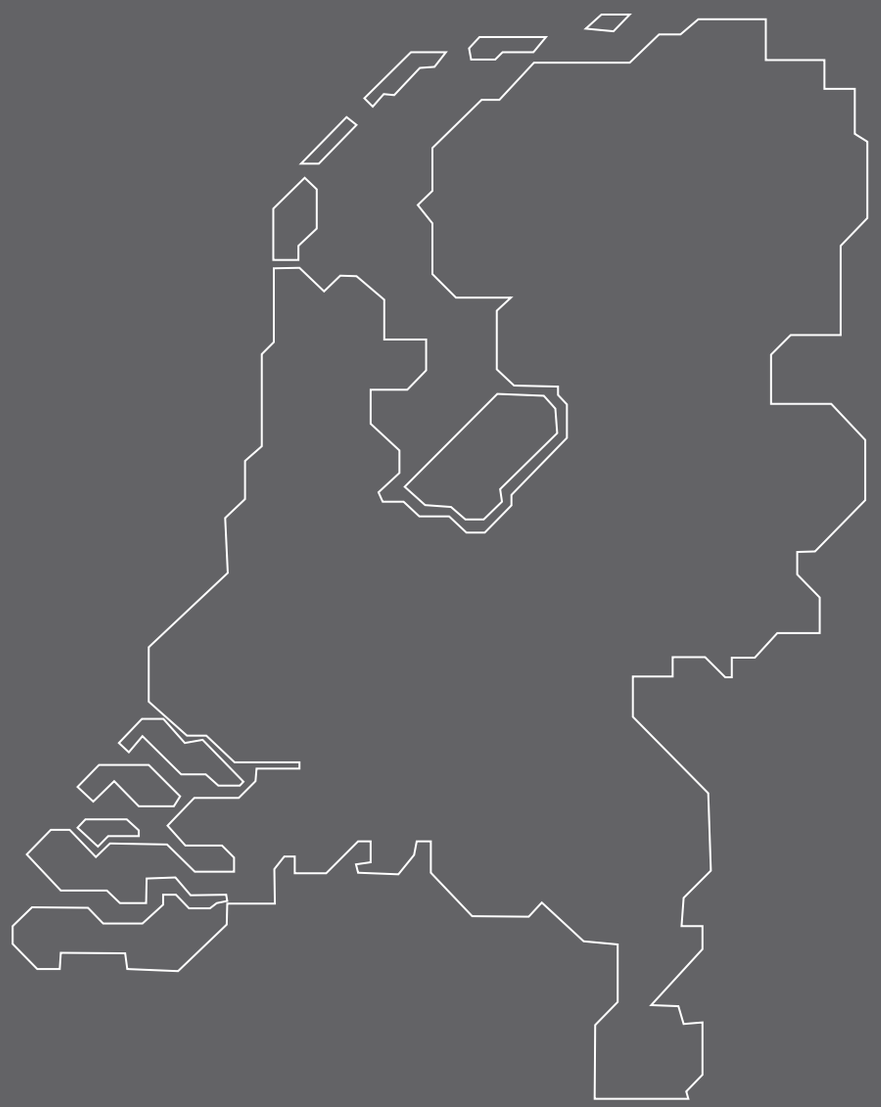


SECTION B

PREVALENCE



CHAPTER 4

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

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European Journal of Gastroenterology and Hepatology 2007; 19:213-216

ABSTRACT

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

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

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

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

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

INTRODUCTION

Helicobacter pylori (*Hp*) infection is acquired early in life within families [1, 3] and is recognized as a causative agent of gastritis, ulcer disease, gastric carcinoma and mucosa-associated lymphoid tissue-lymphoma [2]. In industrialized countries the prevalence of the infection is much lower than in developing countries and the rate of infection is related to factors such as ethnic origin. The Netherlands form a multicultural community: in 1997 1,063,987 inhabitants belonged to ethnical minorities; 279,958 of which originated from Turkey, 232,991 from Morocco and 287,219 from Suriname [4]. Data from Dutch adults show a prevalence of *H. pylori* infection of 33% to 50% [5, 6]. In Turkish immigrants with reflux-esophagitis the prevalence of *Helicobacter pylori* infection is as high as 61% (vs. 33% in Dutch adults with reflux-esophagitis) [7].
As there are no data on the frequency of *H. pylori* infection in young infants in the Netherlands, the objective of this study was to investigate its prevalence in this group of children.

METHODS

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

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

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

(* = p<0,001)

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

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

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

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

RESULTS

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

DISCUSSION

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

surgery[12] or suspected viral infections[14]. In addition, as shown by Roosendaal *et al* [14], *H. pylori* infection rates in Dutch children have continuously declined over the last decades, demonstrating a persistent birth cohort effect. This decline will result in a very low prevalence of *H. pylori* infection in the Dutch population during the next few years. One question is whether our population is representative for the general Dutch population of 2-4 years of age. Possibly, this is not the case, since we selected the sera from all the children with non-Dutch parents and from all the children with celiac disease. On the other hand, the total study group is big enough to be considered representative for our region. Moreover, the results of our study (1.2% *H. pylori* infection) may represent an underestimation of the true prevalence of *H. pylori* infection, because we used the determination of serum antibodies against *H. pylori* to identify the infection. Although our technique was a home made ELISA with a well-known high sensitivity and specificity for *H. pylori* infection in adults, the sensitivity of serological tests may be lower in children under the age of six [11]. On the other hand, our home made Elisa contains the extract of 6 *H. pylori* strains [10] , which improves the sensitivity and specificity and to cover infrequent strains of *H. pylori*. In addition, our home made ELISA has already been used in two other studies in children [15, 16], showing that the increase in sensitivity with age is not significant.

A recent collaborative European study using the kit Pyloritest EIA-G III (Orion Diagnostics, Espoo, Finland), also with a cut-off of 0.32 AI, has shown that serology can have excellent performance among children[17], but the authors observed a trend in better performance with increasing age or with lowering the cut-off. If we assume a lower cut-off of positivity for our young children (usually 30% lower than the cut-off for adults for other ELISA tests), in our group there were only 39 children with a cut-off higher than 0.20 AI, giving a frequency of positivity of 3%, which still is very low.

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

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

the results of an Italian study of 81 children with celiac disease [21].

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

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

Acknowledgments

We thank JDBM Verhoeff, medical student, who assisted us in selecting the serum samples.

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