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Intergenerational change in *Helicobacter pylori* colonization in children living in a multi-ethnic Western population

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Abstract

Objective—*Helicobacter pylori (H. pylori)* colonization rates in childhood have declined in Western populations, but it is unknown whether this trend is similar in children of non-Western ethnic backgrounds, born in a Western country. We aimed to identify *H. pylori* status in children, and determine both mother-to-child transmission and risk factors for colonization.

Design—Antibodies against *H. pylori* and cytotoxin-associated gene A (CagA) were measured in children participating in a population-based prospective cohort study in Rotterdam, the Netherlands. Information on demographics and characteristics was collected using questionnaires.

Results—We analysed the serum of 4,467 children (mean age 6.2 years ±0.5 SD) and compared the results with the *H. pylori* status of their mothers (available for 3,185 children). Overall, 438 (10%) children were *H. pylori*-positive, of whom 142 (32%) were CagA-positive. Independent risk factors for colonization were: maternal *H. pylori* positivity (OR 2.12; 95%CI 1.62–2.77), non-Dutch ethnicity (OR 2.05; 95%CI 1.54–2.73), female gender (OR 1.47; 95%CI 1.20–1.80), and lower maternal education level (OR 1.38; 95%CI 1.06–1.79). Comparing mothers and children, we found an intergenerational decrease of 76% and 77% for $Hp^{+}CagA^{-}$ and $Hp^{+}CagA^{+}$-strains, respectively, consistent across all nine ethnic groups studied. Male gender, higher maternal educational level, and no older siblings, were independently associated with loss of *H. pylori*.

Conclusions—Although the highest *H. pylori* and CagA prevalence was found in children of non-Dutch ethnicities, the decreased colonization rates were uniform across all ethnic groups,

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COMPETING INTERESTS

None of the authors has any conflict of interest.

implying the importance of environmental factors in *H. pylori* transmission in modern cities, independent of ethnicity.

Keywords

children; epidemiology; *H. pylori*; transmission

INTRODUCTION

The gastric bacterium *Helicobacter pylori (H. pylori)* colonizes more than half of the human population. It usually induces the influx of inflammatory cells in the stomach wall, which is a major risk factor for peptic ulcer disease and gastric cancer [1, 2], and also is associated with diminished risk of oesophageal reflux and childhood-onset asthma [3, 4, 5], and possibly more resistance to infectious diseases [6, 7]. *H. pylori* colonization is usually acquired during early childhood, and in most cases persists unless eliminated by antibiotic treatment [8]. A recent study reported that the risk of *H. pylori* colonization was influenced by host genetics [9].

The prevalence of colonization differs between children and adults [10]. Several cross-sectional surveys in Western countries have shown that *H. pylori* prevalence increases with age [11, 12]. Since acquisition during adulthood is rare [13, 14], the higher prevalence in the elderly rather reflects a birth cohort effect with higher rates of childhood exposure to the organism in the past [1]. The current lower levels of exposure to *H. pylori* and consequent lower prevalence in children are believed to be due to improved hygiene, and active elimination by antibiotics, together contributing to declining transmission risk [1, 15].

However, a recent study in Dutch children reported similarity in the *H. pylori* prevalence in two subsequent birth cohorts [16], possibly indicating that determinants previously responsible for declining colonization in the past now have stabilized. One factor contributing to this trend is the altered composition of western populations; during recent decades, the populations of western cities have become multi-ethnic as a result of immigration, often from countries where *H. pylori* remains endemic. Recently, we reported large differences in colonization rates among pregnant women of different ethnic origins living in Rotterdam, the Netherlands [17], but whether these differences are reflected in their offspring was not determined. Analysis of *H. pylori* transmission and risk factors would allow better prediction of the future incidence of *H. pylori*-associated illnesses.

In this population-based prospective cohort study, we aimed to measure *H. pylori* status, as well as risk factors for colonization and transmission, in children living in a multi-ethnic Western urban population, and in relation to colonization of their mothers. Unexpectedly, we found a relatively uniform intergenerational decrease in *H. pylori* prevalence in all nine ethnic groups studies. We explore the factors associated with this broad change.

METHODS

Design and setting

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards. All participants live in the multi-ethnic Rotterdam, the second largest city in the Netherlands. The children were born between April 2002 and January 2006. The background, design, and aims of the Generation R study have been reported in detail [18]. In total, 8,305 children and their parents participated in the postnatal phase of the study (from birth onwards) (Figure 1). From this initial population, 6,690 children visited the research centre at the age of 6 years. During these visits, blood samples were collected from 4,593 (69%) children (see Table S1 comparing the children, with and without *H. pylori* data). Data on age, ethnicity, breastfeeding, day-care attendance, antibiotic use, and socioeconomic status of the mother were collected using questionnaires. The Generation R Study was approved by the Medical Ethical Committee of the Erasmus University Medical Centre, and parents of the children gave written informed consent.

Covariates

The Generation R cohort comprises a wide range of ethnic groups, reflecting Rotterdam's urban population as a typical Western city; the largest ethnic groups are of Dutch, Surinamese, Turkish, Moroccan, Dutch-Antilles, and Cape Verdean descent. Ethnicity is determined by country of birth of the child and its parents. According to the definition of Statistics Netherlands, a child was considered of non-Dutch ethnic origin if one of its parents was born abroad [19]. If both parents were born in two different countries outside the Netherlands, the country of the mother prevailed. Children of Dutch origin are considered as the native population. Participants with an ethnic background other than mentioned above are grouped together as European, Asian, African, or 'rest of the world', which included Central and South America (n = 95), Indonesia (n = 32), North America (n = 21), Asia, western (i.e. western ethnicity but parents had lived in Asia, n = 21), and Oceania (n = 9). Data on type of delivery was obtained by review of midwife and hospital registries. Exclusiveness of breastfeeding was categorized into three breastfeeding groups: never, non-exclusive breastfeeding until 4 months, or exclusive breastfeeding until 4 months. Data on day-care attendance was based on each child's first year of life. Maternal parity served as a proxy for the presence of older siblings in the family, which was categorized as either none or at least one older sibling. Use of antibiotics was assessed by questionnaire at the ages of 12, 24, 36, 48, and 72 months. Parents were asked whether their child had received antibiotics (for example, penicillin) during the past year. Based on these questions, we calculated the cumulative number of courses in the first 6 years of life and computed a categorical variable with 3 groups: never any course of antibiotics, 1–2 courses, and 3 or more courses. Data on antibiotic use were not validated by physician prescriptions or pharmacy records. The socioeconomic status of the children was defined according to the educational level of their mother on the basis of her highest level of completed education. The highest educational level was defined as completion of university or higher vocational training. Mothers were categorized as having a middle-low level of education if they had completed intermediate vocational training, or had completed education below that level.

Serological determinants

Serum samples from 4,467 children, obtained at the mean age of 6.2 (±0.4) years (range 4–8 years), were available for analysis (Figure 1). The main reasons for missing blood samples were non-consent of the parents and technical or logistic failure. Procedures for the collection and storage of serum samples have been described [20]. Samples were examined for *H. pylori* IgG antibody levels by enzyme-linked immunosorbent assay (ELISA), using whole cell antigens [21]. A separate ELISA was performed to determine serum IgG antibodies against a specific recombinant truncated cytotoxin-associated gene A (CagA) protein, as described [22]. Both ELISAs have been validated in children [23], and have been previously used in Dutch children [16]. All samples were examined in duplicate; for each, the optical density ratio (ODR) was calculated by dividing the optical density (OD) by the mean OD of the positive controls. *H. pylori*-positive samples were those having either an ODR ≥ 1 or a CagA-positive test result. The cut-off value for CagA positivity was ODR value ≥ 0.35 [22]. Only 13 children were found to be CagA-positive but *H.* pylori-negative (0.3% of *H. pylori*-negative children). Based on prior studies, these subjects were considered as truly *H. pylori* positive [24]. Data on the maternal *H. pylori* status was available for 3,185 children (71%). *H. pylori* antibody distributions for both children and their mothers are shown in Figure S1. Details regarding *H. pylori* colonization in the total cohort of mothers have been described [17].

Statistical analysis

Chi-square tests (categorical variables) and t-tests (continuous variables) were used to compare different variables with *H. pylori* status. Univariate analyses were performed to assess determinants associated with *H. pylori* presence. To study the individual effect of each potential determinant, each was tested separately, followed by a multivariate analysis corrected for all others. For all covariates, the percent of missing values within the population for analysis was lower than 10%, except for caesarean section, breastfeeding, day-care attendance, and antibiotic use. Missing data in the covariates (except maternal *H. pylori* status) were imputed with multiple imputations using chained equations, by which the most likely value for a missing response is selected [25]. Ten new datasets were created by imputation based on all covariates and outcomes in the model. Data from each separate imputation was analysed, after which results were combined. Except for breastfeeding and antibiotic exposure, no major differences in the direction or magnitude of the effect estimates were observed between analyses with imputed missing data and complete cases only. Only the results based on the pooled imputed datasets are presented in this manuscript. To identify the potential modifying effect of determinants included in the multivariate analyses, we evaluated statistical interaction by adding to the multivariate model the product term of an independent variable and subgroup (independent variable ^{x} subgroup) as covariate. The interaction was tested between ethnicity and day-care, ethnicity and gender, and ethnicity and maternal education level. We calculated the population attributable fraction (PAF) for *H. pylori* disappearance in children with an *H. pylori*-positive mother, using adjusted odds ratios estimated from logistic regression models [26]. All measures of associations are presented as Odds Ratios (OR) with their 95% Confidence Intervals (CI).

Statistical analyses were performed using IBM SPSS Statistics 21.0 for Windows (SPSS IBM, Armonk, New York, USA).

RESULT

H. pylori prevalence

The serum of 4,467 children was analysed (Figure 1). Table 1 summarizes both the observed population characteristics as well as the imputed data, stratified by *H. pylori* status. Overall, 438 (10%; 95% CI 8.9–10.7%) children were *H. pylori*-positive, of whom 142 (32%; 95% CI 28.0–36.8%) also were CagA-positive. In all children of non-Dutch ethnicity, the colonization rate was significantly higher than that in children of Dutch ethnicity (Figure 2A). Higher *H. pylori* colonization rates were observed in children of either Dutch or non-Dutch ethnicity of older age (Figure S2). The proportion of CagA-positivity amongst *H. pylori*-positive children varied widely between ethnic groups (Figure 2B). The lowest proportions were found in children with Dutch or other European ethnicities (16% and 14%, respectively).

Risk factors for *H. pylori* colonization

In univariate analyses, a child's *H. pylori* positivity was associated with an *H. pylori-positive* mother (OR 3.22; 95% CI 2.52–4.12), and non-Dutch ethnicity (OR 2.99; 95% CI 2.32–3.86). Female gender, age, breastfeeding, presence of older siblings, antibiotic exposure, and lower maternal educational level also were positively associated with *H. pylori* status, whereas day-care attendance was negatively associated (Table 1).

Using multivariate analysis (Figure 3), we identified the following independent risk factors for *H. pylori* positivity in a child: maternal *H. pylori* positivity [(CagA-positive mother OR 2.25; 95% CI 1.61–3.16) (CagA-negative mother OR 2.05; 95% CI 1.53–2.74)], non-Dutch ethnicity (OR 2.04; 95% CI 1.53–2.72), female gender (OR 1.47; 95% CI 1.20–1.81), and lower maternal education level (OR 1.37; 95% CI 1.06–1.78). A separate multivariate analysis to examine risk for CagA-positivity amongst all *H. pylori-positive* children revealed independent associations with lower educational level of mother (OR 2.65; 95% CI 1.33–5.28), and non-Dutch ethnicity (OR 2.48; 95% CI 1.27–4.85) (Table S3). Compared with males, we found female gender independently associated with never having had exposure to antibiotics (OR 1.30; 95% CI 1.07–1.60) and lower educational level of the mother (OR 1.17; 95% CI 1.01–1.35) (Table S4). Caesarian section was not significantly associated with *H. pylori* colonization. Comparison of C–section with vaginal birth revealed independent associations with no breastfeeding (OR 1.98; 95% CI 1.31–2.98), nulliparity (OR 1.81; 95% CI 1.48–2.20), and day-care attendance (OR 1.34; 95% CI 1.01–1.77) (Table S5).

A stratified analysis by ethnicity was performed (Dutch *vs.* non-Dutch), based on the significantly lower *H. pylori* colonization rate in children of Dutch ethnicity compared to all other subjects (Figure 3, and Table S6 for comparison of European *vs.* non-European). Differences in the odds ratios for *H. pylori* colonization were observed for gender, educational level of mother, and day-care attendance. There was no evidence for effect modification by ethnicity for the associations of gender, educational level of mother, and day-care attendance with *H. pylori* colonization (p-value for interactions >0.05).

Comparison of *H. pylori* colonization in mothers and their children

Data on the *H. pylori* status of mother was available for 3,185 (71%) children (Table 2). The *H. pylori* positivity rate in mothers (mean age of 30.5 ±5.0 years) was 42%. An *H. pylori*-positive mother was associated with an *H. pylori*-positive child (OR 3.22; 95% CI 2.52–4.12). Of the 1,328 children with an *H. pylori-positive* mother, 211 (15.9%) were *H. pylori-positive*, compared to 103 (5.5%) of the 1,857 children with an H. pylori-negative mother. As a result, 33% (n=103) of all *H. pylori-positive* children had a mother who tested *H. pylori-negative.* The median antibody titer in these children was significantly lower (1.43; 2.5–97.5th percentile 0.74–6.68) than in children with an *H. pylori*-positive mother (2.11; 2.5–97.5th percentile 0.56–15.68). In children of non-Dutch ethnicity, the proportion *H. pylori-positive* children with an *H. pylori-negative* mother was 22% compared to 55% of children with Dutch ethnicity ($p<0.001$). Table 2 shows the associations between mother and child's *H. pylori* colonization rates by strain type. Children born from *H. pylori-negative* mothers were significantly less likely to be colonized at age 6 with either a CagA-negative or CagA-positive *H. pylori* strain than children born from *H. pylori-positive* mothers. Conversely, children born from *H. pylori*$^{+}$CagA^{-} mothers were more likely to be colonized with the same strain, and for children with *H. pylori*$^{+}$CagA^{+} mothers, the OR for carrying the same strain was 6.74 (95% CI 4.52–10.05).

Overall, the *H. pylori* prevalence decreased 76% comparing mothers and their children. A significant decline in *H. pylori* prevalence was observed across all nine ethnic groups studied (Figure 4). This decline was consistent for both *H. pylori*$^{+}$CagA^{-} and *H. pylori*$^{+}$CagA^{+}-strains (Figure 4A and 4B). The overall decline rate for males (−80%) was slightly higher than for females (−72%), which was consistent across all ethnic groups. Multivariate analysis of the loss of *H. pylori* in children with an *H. pylori-positive* mother (n = 1,328) revealed male gender (OR 1.64; 95% CI 1.21–2.23), higher maternal education level (OR 1.78; 95% CI 1.15–2.76), and no older siblings (OR 1.37; 95% CI 1.01–1.88) independently associated with an *H. pylori-negative* child (Table S7). The proportion of the *H. pylori* decline attributable to male gender (21%), having no older siblings (14%), and higher maternal education level (14%), were all significant (Figure 5).

DISCUSSION

In this multi-ethnic population-based cohort, we found highly variable *H. pylori* colonization rates in six-year old children, with both the prevalence of *H. pylori* and the proportion of CagA-positive strains higher in children of non-Dutch ethnicity. Independent of ethnic background, maternal *H. pylori* colonization was the strongest risk factor for *H. pylori-positivity* in their offspring. Our study design made it possible to compare *H. pylori* colonization in children directly with that of their mothers, showing essentially identical intergenerational reductions for both *H. pylori*$^{+}$CagA^{-} and *H. pylori*$^{+}$CagA^{+}-strains.

The overall colonization rate of 10% differs from that found in a previous study performed in the Netherlands [16]. This recent study of 545 Dutch children between 7 and 9 years old, which used the same ELISA as did we, found an *H. pylori* positivity rate of 9% (95% CI 6.6–11.4%) [16], a prevalence slightly higher than that measured in children of Dutch ethnicity in our study (6%). This difference may reflect a continuing decline in colonization,

or may be due to the different study designs, or the younger age of children in our study. The latter may contribute, as our data suggest continuing acquisition of *H. pylori* at least until the age of 7, consistent in children of Dutch and non-Dutch ethnicities. The higher *H. pylori* prevalence in children of non-Dutch ethnicity confirms findings of other studies [27, 28]. When comparing previous studies, the exact colonization rate of particular ethnic groups may differ due to differences in age or selected populations, but nevertheless it is clear that subjects of non-Western ethnicity comprise risk groups for *H. pylori* colonization within multi-ethnic populations of Western cities.

The intergenerational decrease was of the same magnitude among all different ethnic groups, resulting in the same birth cohort effect in all groups. The decline in children with an *H. pylori*-positive mother can be partially attributed to male gender, lack of older siblings, and higher educational level of mother. The lack of older siblings may reduce horizontal transmission of *H. pylori* within a family. Others found the number of siblings within a family rather than birth order independently associated with *H. pylori* colonization [29]. Nevertheless, our findings imply that environmental factors and living conditions of the country in which a child is raised have a major impact on transmission, irrespective of ethnicity. The consistent decline across all ethnic groups support the hypothesis that in contemporary Dutch society, and probably elsewhere as well, there are highly prevalent factors that interfere with the early life acquisition and-or maintenance of *H. pylori*. Besides the involvement of socio-economic status, family size, and other living conditions, possible candidates include the widespread use of antibiotics, particularly in young children. The effect of antibiotic monotherapy on *H. pylori* status is limited [30], but repeated antibiotic exposure may eventually result in eradication. Another possibility could be the run-off of antibiotics from farms where antibiotic-intensive husbandry is being practiced, which may contaminate surface and drinking water [31]. In contrast to the use of antibiotics in humans, the Dutch consumption of antibiotics per animal exceeds the consumption of all European countries, and despite the prohibition of antibiotics as growth promoters, the use remained stable [32]. There is no strong evidence of contaminated drinking water; in a recent screening of superficial groundwater used for the production of drinking water, none of the veterinary pharmaceuticals have been observed, and no concentrations of related compounds were observed above the threshold of toxicological concern [32]. In a separate survey of human pharmaceuticals, clindamycin (5 ng/L) and erythromycin (10 ng/L) residuals were found in surface water, but not in produced drinking water [32]. Regardless of its cause, the clinical consequences of this rapid disappearance may have opposite effects: a fall in prevalence of the later life expression of gastric and duodenal ulcer disease, and gastric carcinoma [33, 34], but a rise in earlier life-expressed atopy, asthma, and reflux-related disorders [11], since epidemiological studies have shown inverse associations of these disorders with *H. pylori* colonization [3, 5].

The association of specific *H. pylori* types in mother and child provides further evidence supporting a role for maternal inheritance in early life transmission, shown in molecular typing studies [35]. A recent German study that included the *H. pylori* status of parents and siblings in a multivariate model, showed that only maternal infection was associated with *H. pylori* positivity in the children [28]. Despite this important maternal role, we found that one

third of all positive children had an apparently *H. pylori*-negative mother. This proportion was even higher (>50%) in children of Dutch ethnicity, implying the involvement of other transmission sources, such as fathers and siblings [35]. An alternative hypothesis is that some maternal *H. pylori* colonisations were missed, due to lack of complete sensitivity of the assay, or post-natal acquisition of the organism.

Especially in a multi-ethnic population, children may become colonized with *H. pylori* by acquiring the bacterium from persons of ethnicities with higher *H. pylori* prevalence, e.g., in day-care facilities. However, a recently published meta-analysis found no significant effect of day-care attendance on *H. pylori* colonization (summary OR 1.12; 95% CI 0.82–1.52) [36]. Nevertheless, a Portuguese study of 1,047 children reported increasing *H. pylori* prevalence with cumulative attendance in day-care centres [37]. Our stratified analysis of ethnicity revealed opposite trends for the relation between *H. pylori* colonization and day-care attendance. Such observations suggest that child-to-child transmission in a day-care setting may be more likely for children of Dutch ethnicity where children of non-Dutch ethnicity could serve as transmission sources.

Remarkably, female gender was found to be associated with *H. pylori* colonization; a possible explanation may be the higher antibiotic exposures we observed in males. This may also explain the higher rate of decline we found in boys compared to girls. The positive associations of Caesarian section with nulliparity, and no breastfeeding, confirm prior observations [38], but that mothers who underwent Caesarian section were more likely to use day-care suggests that mode of delivery correlates with other lifestyle aspects.

An important strength of this study is that we had a large multi-ethnic study population drawn from the general population of Rotterdam; since immigration is common in many western countries, our findings may be more broadly applicable. An additional strength is the use of maternal data on *H. pylori* colonization, which provides insight into mother-to-child transmission.

This study has some limitations, including missing data for several characteristics and potential risk factors for colonization, which may have biased the outcome. However, we performed the final analyses after a multiple imputation procedure, considered useful to deal with missing data, as it requires the fewest assumptions and reduces potential bias when missing data are not random [25]. A second limitation was lack of data on *H. pylori* colonization in fathers and siblings, precluding examination of their potential roles in transmission. A third limitation is that data on antibiotic exposures were not validated by pharmacy records, nor was information available on specific types. Finally, although both ELISAs have been validated in adults and children, including Dutch adults, and have been used in previous studies in Dutch children [16], they have not been separately validated in Dutch children.

In conclusion, we found relatively high *H. pylori* colonization rates in children of non-Dutch ethnicity who were born and raised in a western city. Regardless of ethnicity, maternal *H. pylori* type was an important predictor for a child's *H. pylori* type. The high and consistent

intergenerational decline in *H. pylori* prevalence irrespective of ethnicity and sex points toward very common exposures fuelling this phenomenon.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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SUMMARY BOX

1. What is already known about this subject?
   - *H. pylori* prevalence of children living in Western countries is low.
   - Maternal *H. pylori* status is an important transmission source for *H. pylori* colonization in their children.
   - Migrant communities in Western populations constitute risk groups for *H. pylori* colonization.
2. What are the new findings?
   - A high intergenerational decline in *H. pylori* prevalence was found, comparing mothers with their children, with nearly identical rates (76% and 77%) for $Hp^{+}CagA^{-}$ and $Hp^{+}CagA^{+}$/strains, respectively.
   - The intergenerational drop in *H. pylori* prevalence was uniform in nine separate ethnicities.
   - Risk factors for *H. pylori* positivity are mostly the same among diverse ethnic groups.
   - Our data suggest a continuing acquisition of *H. pylori* at least to age 7.
3. How might it impact on clinical practice in the foreseeable future?
   - The maternal-child linkage is to some degree predictive of *H. pylori* positivity in a child, which affects risk of subsequent diseases.

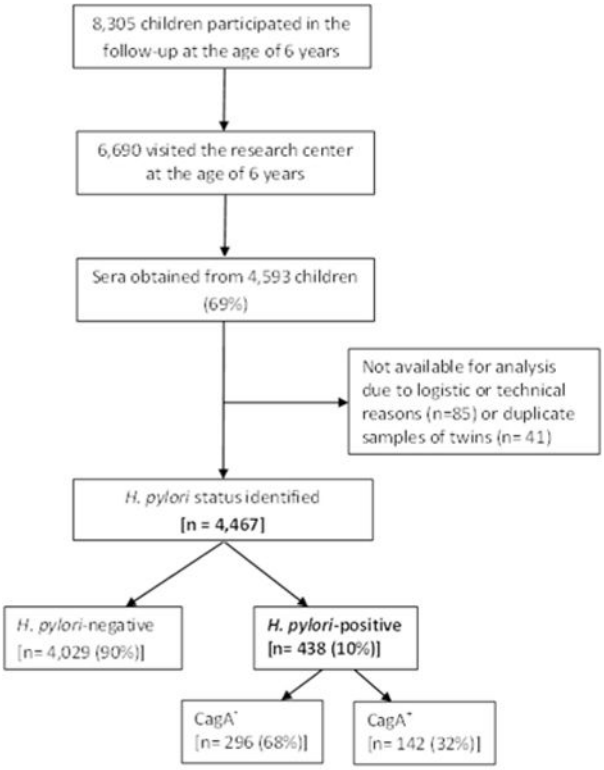


Figure 1. Definition of the study population

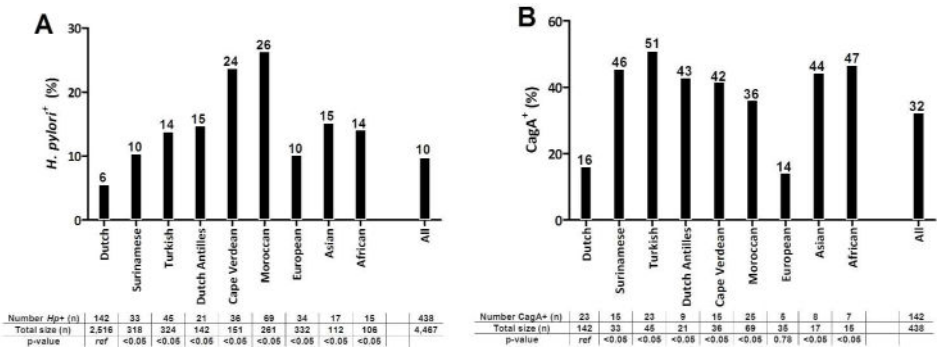


Figure 2. Prevalence of *H. pylori* and CagA-positivity in 4,467 children according to ethnic group

Panel A: *H. pylori* prevalence, by ethnic group. Panel B: Proportion of CagA-positive strains amongst the *H. pylori-positive* children, by ethnic group.

Numbers are either percent (above bars) or absolute numbers (Tables).

P-values reflect differences between children of Dutch ethnicity (reference group) and any other group, using Chi-square test. Children classified as 'rest of the world' are not shown in this figure. Their *H. pylori*-positivity rate is 12.6% (26 of 206), and proportion of CagA-positive strains is 42% (11 of 26).

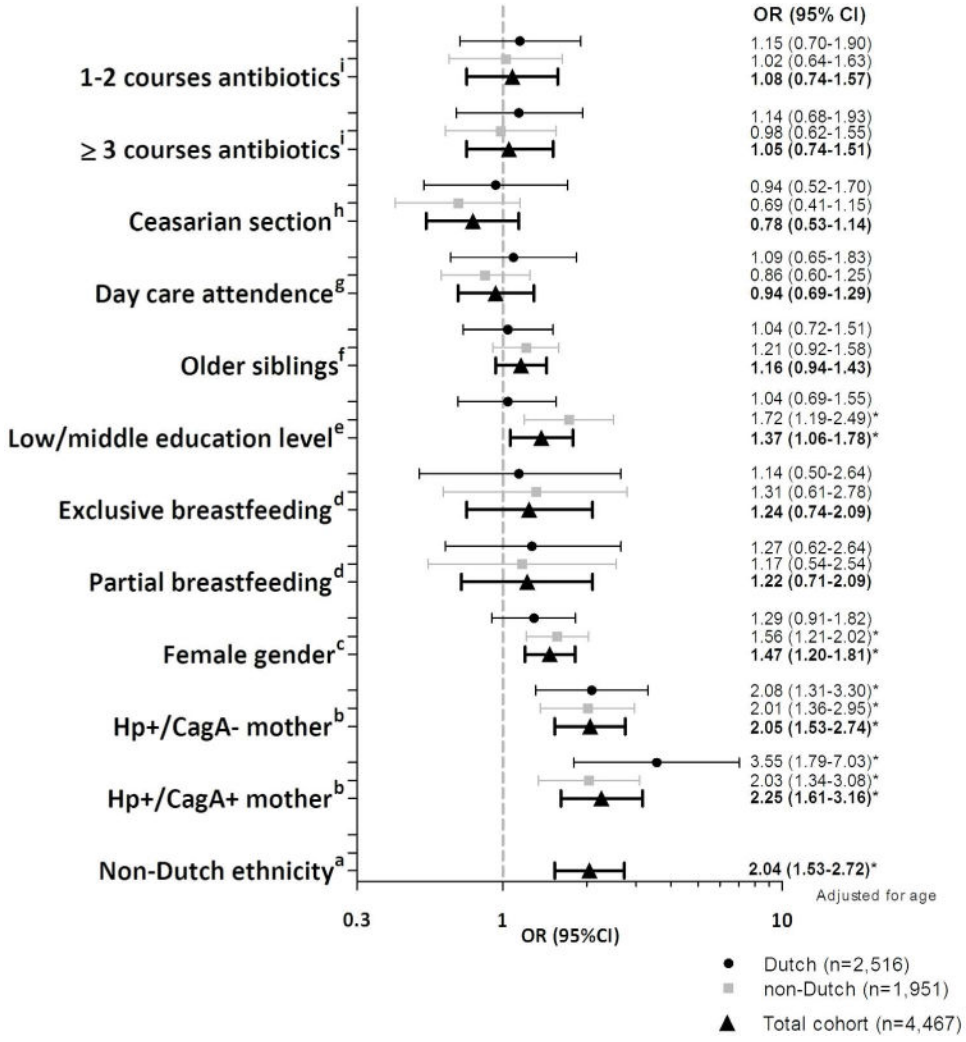
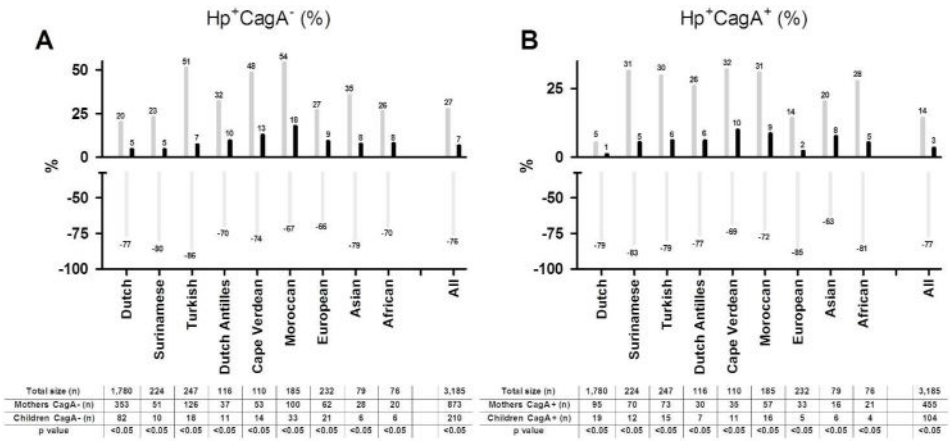


Figure 3. Multivariate analyses of determinants associated with *H. pylori* colonization in the total population, and comparing Dutch and non-Dutch origins

All listed variables are entered into the logistic regression model. Odds ratios (ORs) and 95% confidence intervals (CIs) are adjusted for age and express the association with *H. pylori* positivity. The ORs for the total population are in bold. Reference groups: [a]*H. pylori*-negative mother, [b]Dutch ethnicity, [c]male gender, [d]no breastfeeding, [e]higher educational level, [f]no older siblings, [g]no day-care attendance, [h]vaginal birth, [i]never exposed to antibiotics.

* $p < 0.05$.



	Dutch	Surinamese	Turkish	Dutch Antilles	Cape Verdean	Moroccan	European	Asian	African	All
Total size (n)	1,780	224	247	116	110	185	232	79	76	3,185
Mothers CagA- (n)	353	51	126	37	53	100	62	28	20	873
Children CagA- (n)	82	10	18	11	14	33	21	6	6	210
p value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

	Dutch	Surinamese	Turkish	Dutch Antilles	Cape Verdean	Moroccan	European	Asian	African	All
Total size (n)	1,780	224	247	116	110	185	232	79	76	3,185
Mothers CagA+ (n)	95	70	73	30	35	57	33	16	21	455
Children CagA+ (n)	19	12	15	7	11	16	5	6	4	104
p value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Figure 4. Prevalence and differential rates of *H. pylori*-strains in 3,185 mothers and their children by ethnic group

A: $CagA^{-}$ strains B: $CagA^{+}$ strains. Numbers are either percentages (above or below bars) or absolute numbers (Tables). Light grey bars reflect the percent differences between mothers and their children. The p-values reflect differences between the positivity rates in mothers (dark grey bars) compared to their children (black bars). Subjects categorized in 'rest of the world'-group are not shown as a separate group, but included in 'All' category.

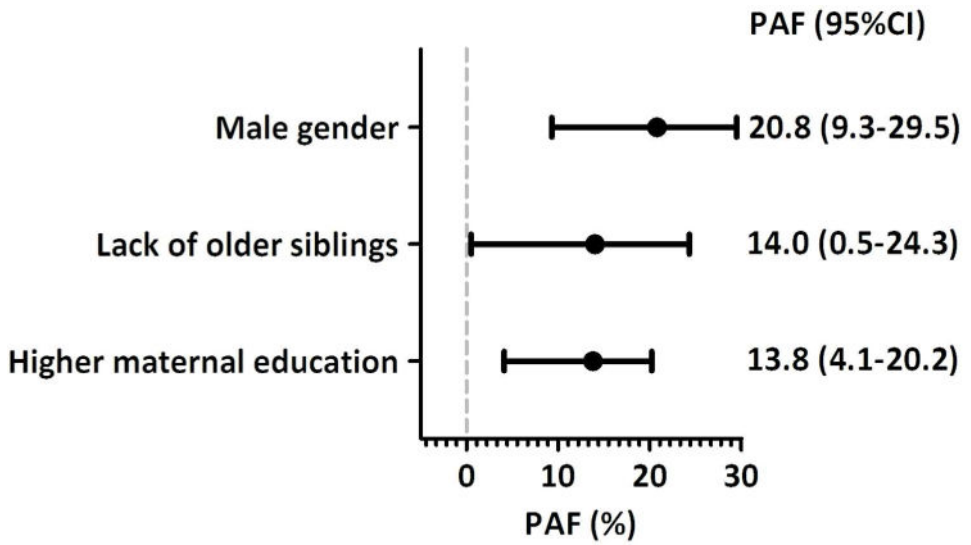


Figure 5. Population-attributable fraction (PAF) for decline in *H. pylori* colonization in 1,328 children with an *H. pylori*-positive mother

PAF was calculated from adjusted ORs (aOR) derived from multivariable logistic regression model comparing *H. pylori-negative* children (n = 1,117) with *H. pylori*-positive children (n = 211), additionally adjusted for caesarean section, breastfeeding, day-care attendance and cumulative antibiotic exposures. PAF, population-attributable fraction; CI, confidence interval

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Table 1

Characteristics of the 4,467 children and their mothers, categorized according to *H. pylori* status.

Child characteristics	Observed	Imputed	*H. pylori*$^{-}$ n = 4,029	*H. pylori*$^{+}$ n = 438	Univariate OR (95% CI)
Female sex (%)	2,164 (48.4)	2,164 (48.4)	1,916 (47.6)	248 (56.6)	1.44 (1.18–1.76)*
Mean age sera taken, years (SD)	6.2 (0.4)	6.2 (0.4)	6.2 (0.01)	6.4 (0.03)	1.66 (1.39–1.98)*
Ethnicity (%)					
Dutch	2,505 (56.1)	2,516 (56.3)	2 ,374 (58.9)	142 (32.4)	1.0
Surinamese	317 (7.1)	318 (7.1)	285 (7.1)	33 (7.5)	1.95 (1.29–2.94)*
Turkish	311 (7.0)	324 (7.3)	279 (6.9)	45 (10.3)	2.64 (1.71–4.07)*
Moroccan	256 (5.7)	261 (5.8)	192 (4.8)	69 (15.8)	6.01 (4.27–8.44)*
Dutch Antilles	141 (3.2)	142 (3.2)	120 (3.0)	21 (4.8)	2.96 (1.78–4.92)*
Cape Verdean	129 (2.9)	151 (3.4)	115 (2.9)	36 (8.2)	5.20 (3.31–8.16)*
Other:					
European	331 (7.4)	332 (7.4)	298 (7.4)	34 (7.8)	1.92 (1.28–2.89)*
Asian	111 (2.5)	112 (2.5)	95 (2.4)	17 (3.9)	3.01 (1.73–5.23)*
African	100 (2.2)	106 (2.4)	91 (2.3)	15 (3.4)	2.63 (1.31–5.30)*
Rest of the world[a]	148 (3.3)	206 (4.6)	180 (4.5)	26 (5.9)	2.11 (0.74–6.00)
Data missing	*118 (2.6)*				
Caesarean Section (%)					
No	3,358 (75.2)	3,878 (86.8)	3,483 (86.4)	395 (90.2)	1.0
Yes	496 (11.1)	589 (13.2)	546 (13.6)	43 (9.8)	0.70 (0.47–1.02)
Data missing	*613 (13.7)*				
Breastfeeding[b] (%)					
Never	264 (5.9)	388 (8.7)	356 (8.8)	32 (7.3)	1.0
Partial	1,857 (41.6)	2,925 (65.5)	2,623 (65.1)	302 (68.9)	1.34 (0.73–2.43)
Exclusive	723 (16.2)	1,154 (25.8)	1,050 (26.1)	104 (23.7)	1.14 (0.64–2.03)
Data missing	*1,623 (36.3)*				
Day-care attendance[c] (%)					
No	1,019 (22.8)	2,110 (47.2)	1,849 (45.9)	261 (59.6)	1.0
Yes	1,548 (34.7)	2,357 (52.8)	2,180 (54.1)	177 (40.4)	0.57 (0.44–0.75)*

Child characteristics	Observed	Imputed	*H. pylori*$^{-}$ n = 4,029	*H. pylori*$^{+}$ n = 438	Univariate OR (95% CI)
Data missing	*1,900 (42.5)*				
Number of older siblings (%)					
0	2,370 (53.1)	2,425 (54.3)	2,219 (55.1)	206 (47.0)	1.0
≥1	1,936 (43.3)	2,042 (45.7)	1,810 (44.9)	232 (53.0)	1.38 (1.13–1.69)*
Data missing	*161 (3.6)*				
Antibiotic exposure (%)					
6–11 months					
No	1,800 (40.3)	2,728 (61.1)	2,467 (61.2)	261 (59.6)	1.0
1–2 courses	879 (19.7)	1,390 (31.1)	1,263 (31.3)	127 (29.0)	0.95 (0.68–1.32)
≥3 courses	105 (2.4)	350 (7.8)	300 (7.4)	50 (11.4)	1.51 (0.82–2.77)
Data missing	*1,683 (37.7)*				
12–23 months					
No	1,638 (36.7)	2,337 (52.3)	2,114 (52.5)	223 (50.9)	1.0
1–2 courses	1,111 (24.9)	1,702 (38.1)	1,541 (38.2)	161 (36.8)	0.99 (0.74–1.32)
≥3 courses	195 (4.4)	428 (9.6)	374 (9.3)	54 (12.3)	1.35 (0.86–2.11)
Data missing	*1,523 (34.1)*				
24–35 months					
No	1,817 (40.7)	2,642 (59.1)	2,404 (59.7)	238 (54.3)	1.0
1–2 courses	886 (19.8)	1,521 (34.0)	1,364 (33.9)	156 (35.6)	1.16 (0.87–1.56)
≥3 courses	105 (2.4)	305 (6.8)	260 (6.5)	44 (10.0)	1.71 (0.99–2.95)
Data missing	*1,659 (37.1)*				
36–47 months					
No	1,977 (44.3)	2,820 (63.1)	2,566 (63.7)	254 (58.0)	1.0
1–2 courses	763 (17.1)	1,253 (28.1)	1,131 (28.1)	122 (27.9)	1.09 (0.79–1.50)
≥3 courses	75 (1.7)	395 (8.8)	333 (8.3)	62 (14.2)	1.80 (0.91–3.57)
Data missing	*1,652 (37.0)*				
60–71 months					
No	2,956 (66.2)	3,303 (73.9)	2,999 (74.4)	304 (69.4)	1.0
1–2 courses	760 (17.0)	921 (20.6)	829 (20.6)	93 (21.2)	1.10 (0.83–1.46)
≥3 courses	73 (1.6)	243 (5.4)	202 (5.0)	41 (9.4)	1.95 (1.03–3.67)*
Data missing	*678 (15.2)*				

Child characteristics	Observed	Imputed	*H. pylori*$^{-}$ n = 4,029	*H. pylori*$^{+}$ n = 438	Univariate OR (95% CI)
Cumulative antibiotic use					
Never	529 (11.8)	940 (21.0)	861 (21.4)	78 (17.8)	1.0
1–2 courses	1,162 (26.0)	1,715 (38.4)	1,564 (38.8)	151 (34.5)	1.07 (0.73–1.56)
≥3 courses	828 (18.5)	1,813 (40.6)	1,604 (39.8)	209 (47.7)	1.44 (1.01–2.06)*
Data missing	*1,948 (43.6)*				
Maternal characteristics					
Maternal education level (%)					
Primary, or secondary	2101 (47.0)	2,438 (54.6)	2,127 (52.8)	311 (71.0)	2.20 (1.76–2.75)*
Higher	1955 (43.8)	2,029 (45.4)	1,902 (47.2)	127 (29.0)	1.0
Data missing	*411 (9.2)*				
Mean age sera taken, years (SD)	30.5 (5.0)	30.5 (5.0)	31.3 (4.6)	29.4 (5.4)	0.92 (0.91–0.94)*

Values are means (and standard deviation), absolute numbers (and percentages) or odds ratio (and 95% confidence interval). Missing data on maternal *H. pylori* and CagA status were not imputed.

[a] Includes subjects from Central and South America (n = 95), Indonesia (n = 32), North America (n = 21), Asia, western (n = 21), and Oceania (n = 9).

[b] Data until 4 months of life

[c] Data completed from the first year of life. * $p < 0.05$

Table 2

H. pylori colonization by strain type in 3,185 mother-child pairs[a].

		Child's *H. pylori* status		
		Hp− (n= 2,871)	**Hp+CagA− (n=210)**	**Hp+CagA+ (n=104)**
Mother's H. pylori status	Hp− [n= 1,857 (%)] OR (95% CI)	1,754 (94.5) *Reference*	86 (4.6) **0.44 (0.33–0.59)***	17 (0.9) **0.12 (0.07–0.21)***
	Hp+CagA− [n= 873 (%)] OR (95% CI)	746 (85.5) *Reference*	92 (10.5) **2.22 (1.67–2.95)***	35 (4.0) 1.45 (0.95–2.19)
	Hp+CagA+ [n= 455 (%)] OR (95% CI)	371 (81.5) *Reference*	32 (7.0) 1.21 (0.82–1.79)	52 (11.5) **6.74 (4.52–10.05)***

[a]Values shown are absolute numbers and their percentages in relation to maternal *H. pylori* status. The odds ratios and 95% confidence intervals represent the association between the reference group (children without *H. pylori*) and the other groups.

*$p < 0.05$