

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

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Figure S1. *H. pylori* antibody distribution in mothers and children. Distribution of levels (ODR) of mothers (A) and children (B). The numbers above the bars reflect the percent within the total population.

Figure S2. Continuing acquisition of *H. pylori* by age. *H. pylori* positivity rates (%) in 2,516 children of Dutch and 1,951 children non-Dutch ethnicity at ages 5, 6, and 7. The p-value for trend is <0.05 in both groups.

Figure S3. Comparison of *H. pylori* and CagA status in 3,185 mothers and their children. The 3,185 children are grouped by *H. pylori* and CagA status. Each figure shows the percent (%) of children with an *H. pylori*-negative (Hp^-), CagA⁻ (Hp^+CagA^-), or CagA⁺ (Hp^+CagA^+) mother. *p < 0.05 by Chi-square test. The p-values reflect the difference between the corresponding *H. pylori* status of mother and child, and the two other groups.

Table S1. Comparison of the maternal and child characteristics between those included and those not included in the study among the 8,305 eligible subjects.

	Population without <i>H. pylori</i> data N=3,338 (40.2)	Population with <i>H. pylori</i> data N=4,467 (59.8)	p-value
<i>Child characteristics</i>			
Age	6.1 (0.5)	6.2 (0.6)	<0.01
Female sex	1,948 (50.8)	2,164 (48.4)	0.04
Gestational age at birth	39.7 (1.9)	39.8 (1.8)	0.06
Birth weight	3,383 (586)	3,413 (569)	0.26
Ethnicity			
Dutch	1,999 (55.2)	2,505 (57.6)	0.02
Surinamese	287 (7.9)	317 (7.3)	
Turkish	309 (8.5)	311 (7.2)	
Moroccan	241 (6.7)	256 (5.9)	
Dutch Antilles	118 (3.3)	141 (3.2)	
Cape Verdean	99 (2.7)	129 (3.0)	
Other	630 (17.4)	690 (15.9)	
Caesarean section	441 (13.1)	496 (12.9)	0.74
Breastfeeding	2,448 (90.9)	3,248 (92.5)	0.03
Day-care attendance 1 st year	1,043 (57.2)	1,548 (60.3)	0.04
<i>Maternal characteristics</i>			
Parity			
Nulliparous	2,077 (55.9)	2,370 (55.0)	0.45
Multipara	1,640 (44.1)	1,936 (45.0)	
Maternal education			
Low/medium	1,989 (58.3)	2,101 (51.8)	<0.01
High	1,420 (41.7)	1,955 (48.2)	

Values shown are absolute numbers (and percentages) for categorical variables, except that age, gestational age at birth, and birth weight are reported as the mean (and standard deviation).

Table S2. Missing data analyses in 4,467 children.

	Population with incomplete data on covariates* N= 3,039 (68.0)	Population with complete data on covariates N= 1,428 (32.0)	P-value	Missing (%) ^a
<i>Child characteristics</i>				
Age (SD)	6.36 (0.54)	6.03 (0.35)	<0.001	0
Female sex (%)	1,469 (48.3)	695 (48.7)	0.84	0
Gestational age at birth (SD)	39.6 (1.9)	40.0 (1.7)	<0.001	0.7
Birth weight (SD)				0.2
Ethnicity (%)			<0.001	2.6
Dutch	1,509 (51.7)	996 (69.7)		
Surinamese	247 (8.5)	70 (4.9)		
Turkish	250 (8.6)	61 (4.3)		
Moroccan	222 (7.6)	34 (2.4)		
Dutch Antilles	118 (4.0)	23 (1.6)		
Cape Verdean	108 (3.7)	21 (1.5)		
Other	467 (16.0)	223 (15.6)		
Caesarean section (%)	299 (12.3)	197 (13.8)	0.19	13.7
Breastfeeding (%)	1,917 (92.0)	1,331 (93.2)	0.18	21.4
Day-care attendance 1 st year (%)	637 (55.9)	911 (63.8)	<0.001	42.5
<i>Maternal characteristics</i>				
Parity (%)			<0.001	3.6
Nulliparous	889 (51.2)	866 (60.6)		
Multiparous	846 (48.8)	562 (39.4)		
Maternal education (%)			<0.001	9.2
Low/medium	1,562 (59.4)	539 (37.7)		
High	1,066 (40.6)	889 (62.3)		

*Data on ≥ 1 covariate is missing. ^aPercentage of missings per category.

Values shown are absolute numbers (and percentages) for categorical variables. Gestational age at birth and birth weight are reported as the mean (and standard deviation).

Table S3. Multivariate analysis of determinants for CagA-positivity in 438 *H. pylori*-positive children^a.

Age^b	0.93 (0.51-1.71)
Gender	
Female	0.87 (0.56-1.35)
Male	<i>Reference</i>
Ethnicity	
Dutch	<i>Reference</i>
Non-Dutch	2.48 (1.27-4.85)*
Breastfeeding^c	
Never	<i>Reference</i>
Partial	0.84 (0.31-2.25)
Exclusive	0.60 (0.20-1.87)
Educational level	
Primary/secondary	2.65 (1.33-5.28)*
Higher	<i>Reference</i>
Number of older siblings	
0	<i>Reference</i>
≥1	1.02 (0.66-1.60)
Day-care attendance^d	
No	<i>Reference</i>
Yes	0.62 (0.35-1.12)
Caesarian section	
No	<i>Reference</i>
Yes	1.66 (0.72-3.81)
Cumulative antibiotics exposure^e	
0 courses	<i>Reference</i>
1-2 courses	0.76 (0.34-1.73)

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≥3 courses 0.76 (0.35-1.67)

^aMultivariate logistic regression analysis comparing 142 CagA⁺ with 296 CagA⁻-children. Numbers are displayed as odds ratio (95% confidence interval) and represent the association with CagA⁺-children. All listed variables were entered into the multivariate analysis.

^bfor each additional year

^cData pertain to the first 4 months of life

^dData pertain to the first year of life

^eCumulative antibiotic exposure data combined until the age of 72 months.

* p < 0.05

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Table S4. Multivariate analysis of determinants associated with female gender^a.

Age^b	0.94 (0.82-1.07)
Ethnicity	
Dutch	<i>Reference</i>
Non-Dutch	1.04 (0.91-1.18)
Breastfeeding^c	
Never	<i>Reference</i>
Partial	1.00 (0.75-1.34)
Exclusive	1.03 (0.78-1.35)
Educational level	
Primary/secondary	1.17 (1.01-1.35)*
Higher	<i>Reference</i>
Number of older siblings	
0	<i>Reference</i>
≥1	0.90 (0.79-1.01)
Day-care attendance^d	
No	<i>Reference</i>
Yes	1.09 (0.92-1.29)
Caesarian section	
No	<i>Reference</i>
Yes	0.89 (0.73-1.08)
Cumulative antibiotic exposure^e	
0	1.30 (1.07-1.60)*
1-2 courses	1.06 (0.89-1.25)
≥3 courses	<i>Reference</i>

^aMultivariate logistic regression analysis comparing 2,164 females with 2,303 males. Numbers are displayed as odds ratio (and 95% confidence interval) and represent the association with female gender. All listed variables were entered into the multivariate analysis.

^bfor each additional year

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^cData pertain to the first 4 months of life
^dData pertain to the first year of life
^eCumulative antibiotic exposure data combined until the age of 72 months.
* p < 0.05

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Table S5 Multivariate analysis of determinants associated with Ceasarian section^a.

Age^b	1.01 (0.81-1.27)
Ethnicity (%)	
Dutch	<i>Reference</i>
Non-Dutch	0.98 (0.79-1.21)
Breastfeeding^c	
Never	1.98 (1.31-2.98)*
Partial	1.30 (0.95-1.78)
Exclusive	<i>Reference</i>
Educational level	
Primary/secondary	0.98 (0.69-1.30)
Higher	<i>Reference</i>
Number of older siblings	
0	1.81 (1.48-2.20)*
≥1	<i>Reference</i>
Day-care attendance^d	
No	<i>Reference</i>
Yes	1.34 (1.01-1.77)*
Cumulative antibiotic exposure^e	
0	<i>Reference</i>
1-2 courses	0.95 (0.69-1.30)
≥3 courses	1.00 (0.76-1.31)

^aMultivariate logistic regression analysis comparing Ceasarian section (n=589) with vaginal births (n=3,878). Numbers are displayed as odds ratio (95% confidence interval) and represent association with Ceasarian section. All listed variables were entered into the multivariate analysis.

^bfor each additional year

^cData pertain to the first 4 months of life

^dData pertain to the first year of life

^eCumulative antibiotic exposure data combined until the age of 72 months.

* p < 0.05

Table S6. Multivariate analysis of *H. pylori* status by ethnicity (European vs. non-European)^a.

	European	Non-European
Age^b	1.55 (1.07-2.24)*	1.09 (0.82-1.44)
Gender		
Female	1.50 (1.10-2.06)*	1.46 (1.12-1.92)*
Male	<i>Reference</i>	<i>Reference</i>
Breastfeeding^c		
Never	<i>Reference</i>	<i>Reference</i>
Partial	1.10 (0.58-2.12)	1.33 (0.57-3.11)
Exclusive	1.08 (0.51-2.27)	1.45 (0.64-3.29)
Educational level		
Primary/secondary	1.10 (0.76-1.60)	1.74 (1.13-2.69)*
Higher	<i>Reference</i>	<i>Reference</i>
Number of older siblings		
0	<i>Reference</i>	<i>Reference</i>
≥1	1.13 (0.81-1.56)	1.16 (0.87-1.54)
Day-care attendance^d		
No	<i>Reference</i>	<i>Reference</i>
Yes	1.22 (0.71-2.08)	0.77 (0.52-1.14)
Caesarean section		
No	<i>Reference</i>	<i>Reference</i>
Yes	0.90 (0.53-1.53)	0.69 (0.39-1.22)
Cumulative antibiotic use^e		
0	<i>Reference</i>	<i>Reference</i>
1-2 courses	1.24 (0.77-1.99)	0.92 (0.56-1.50)
≥3 courses	1.20 (0.74-1.93)	0.91 (0.56-1.48)

Maternal *H. pylori* status

<i>Hp</i> -	<i>Reference</i>	<i>Reference</i>
<i>Hp</i> +CagA-	2.16 (1.42-3.27)*	1.99 (1.31-3.04)*
<i>Hp</i> +CagA+	3.75 (2.14-6.59)*	1.95 (1.24-3.07)*

Multivariate logistic regression analysis comparing 2,848 subjects of European with 1,619 subjects of non-European ethnic background. Numbers are displayed as odds ratio (and 95% confidence interval) and represent association with *H. pylori* positivity. All listed variables were entered into the multivariate analysis.

^bfor each additional year

^cData pertain to the first 4 months of life

^dData pertain to the first year of life

^eCumulative antibiotic exposure data combined until the age of 72 months.

* $p < 0.05$

Table S7. Multivariate analysis of risk factors for *H. pylori* loss in 1,328 children with an *H. pylori*-positive mother^a.

Gender	
Female	<i>Reference</i>
Male	1.64 (1.21-2.23)*
Breastfeeding^b	
Never	1.38 (0.55-3.48)
Partial	1.10 (0.65-1.84)
Exclusive	<i>Reference</i>
Educational level	
Primary/secondary	<i>Reference</i>
Higher	1.78 (1.15-2.76)*
Number of older siblings	
0	1.37 (1.01-1.88)*
≥1	<i>Reference</i>
Day-care attendance^c	
No	<i>Reference</i>
Yes	1.30 (0.81-2.09)
Caesarian section	
No	<i>Reference</i>
Yes	1.58 (0.74-3.39)
Cumulative antibiotic exposures^d	
0	<i>Reference</i>
1-2 courses	1.08 (0.56-2.06)
≥3 courses	1.03 (0.58-1.83)

^aMultivariate logistic regression analysis comparing 1,117 *H. pylori*-negative children with 211 *H. pylori*-positive children. Numbers are displayed as odds ratio (and 95% confidence interval) and represent

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3 association with *H. pylori*-negative children (loss of *H. pylori*). All listed variables were entered into the
4 multivariate analysis.
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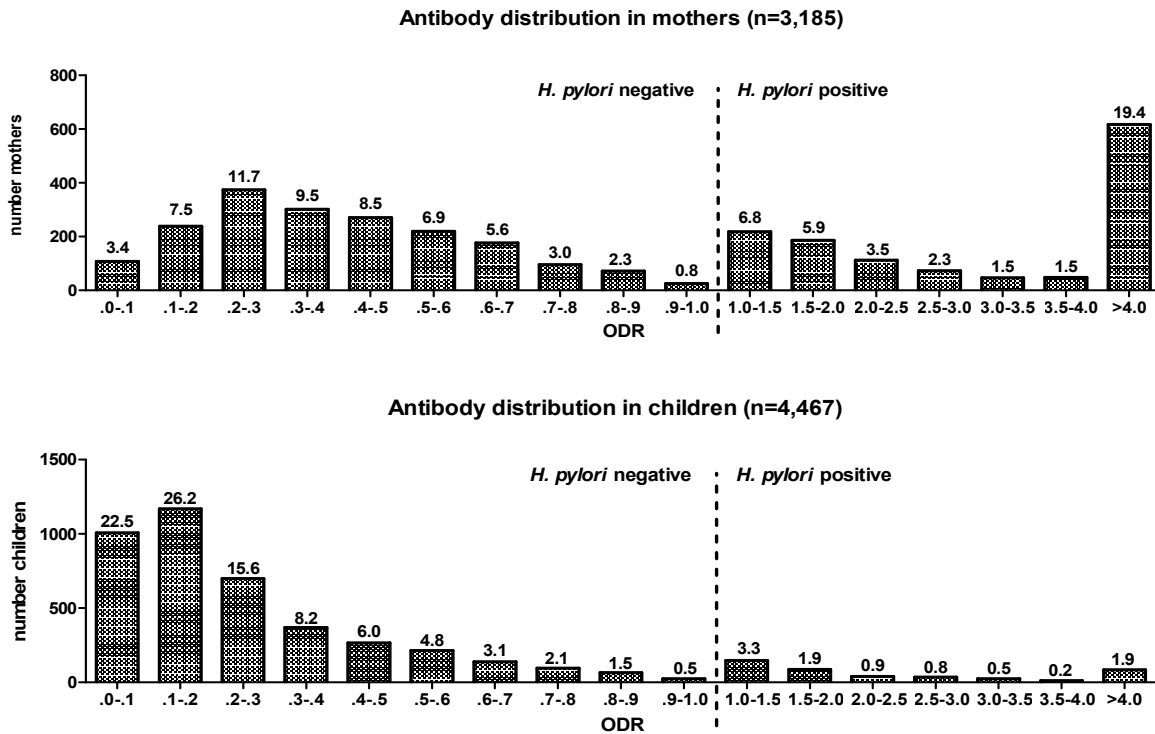
6 ^bData pertain to the first 4 months of life

7 ^cData pertain to the first year of life

8 ^dCumulative antibiotic exposure data combined until the age of 72 months.

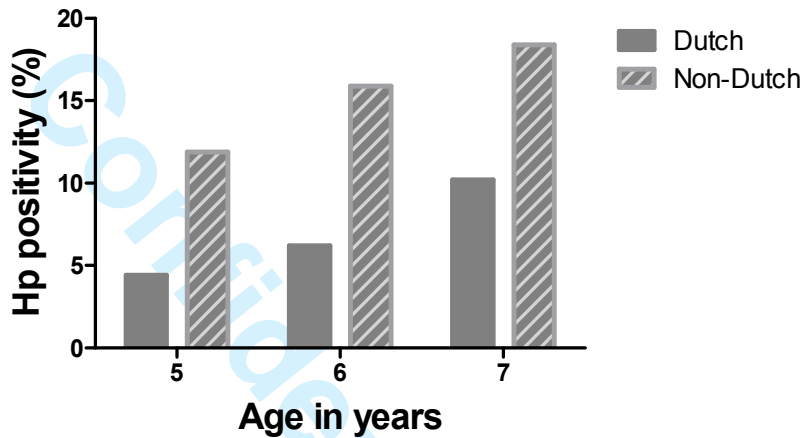
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Figure S1



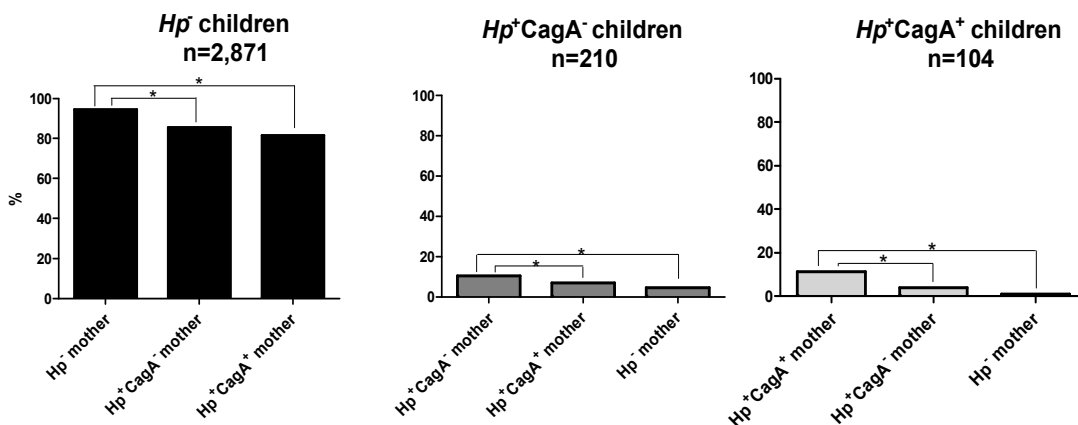
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Figure S2



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Figure S3



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