Early Helicobacter pylori colonisation: the association with growth faltering in The Gambia

J E Thomas, A Dale, J E G Bunn, M Harding, W A Coward, T J Cole, L T Weaver

Arch Dis Child 2004;89:1149–1154. doi: 10.1136/adc.2002.015313

Background: Helicobacter pylori is one of the commonest causes of chronic infection of mankind, yet the natural history of acute infection is poorly understood. Some studies suggest that gastric colonisation with Helicobacter pylori is associated with suboptimal nutrition and growth in childhood.

Aims: To describe the clinical features of early H pylori colonisation and assess its role in the development of infant malnutrition and growth faltering.

Methods: Two consecutive prospective longitudinal cohort studies were conducted at the Medical Research Council Laboratories in a rural community in The Gambia, West Africa. The first birth cohort of 125 infants was followed by a second of 65 children from the same community. H pylori colonisation was detected by sequential 13C urea breath tests, and infant growth was monitored by serial measurements.

Results: Children with early H pylori colonisation became significantly lighter, shorter, and thinner than their peers in late infancy. The association was found in both cohorts. No socioeconomic or demographic confounding variables were identified to explain this, and the weight deficit was no longer detectable when the children were aged 5–8 years.

Conclusions: Results suggest that H pylori colonisation in early infancy predisposes to the development of malnutrition and growth faltering, although the effect did not persist into later childhood.

SUBJECTS AND METHODS

Two prospective longitudinal cohort studies were undertaken at the Medical Research Council (MRC) Overseas Research Station in Keneba, The Gambia. Details may be found in our earlier publications.5 10–14 The community are rural subsistence farmers living in extended families in three villages (total population approximately 3000), drawing water either from shallow surface wells (two villages) or from communal taps served by a deep borehole (one village). Infants were all breast fed throughout their first year, and weaning foods were introduced at around 3 months of age. Throughout the studies, primary health care for mothers and children was provided, including a full infant immunisation schedule, infant surveillance clinics, primary care clinics, and emergency medical cover. In addition to treatment of infectious diseases and oral rehydration therapy for acute diarrhoea, supplemental food was provided under supervision to children who developed severe growth faltering.

With parental consent, resident children were enrolled in one or other of two cohort studies. The first ran from 1991 to 1993, and included 125 children from all three villages, who were either aged <3 months at study outset, or were born during the first year. This included 115 children who also had gastric acid output measured, and who formed the subject of an earlier report.5 Subjects were studied from age 3 months, at intervals of three months thereafter. Children in the second cohort were recruited at birth from one village, and the sampling interval was modified after preliminary analysis of data from cohort 1. Recruitment began after completion of the first study, and between 1993 and 1994, 65/81 eligible infants were enrolled. These children were studied from age 4 weeks for one year, at approximately four weekly intervals. Detailed socioeconomic and demographic information was collected for all participating families, by field workers fluent in the local languages. In both cohorts, the principal reasons for children not joining the study were the withholding of...
parental consent for involvement, or mother and infant moving away from the village. All clinic attendances and antibiotic prescriptions for study children were recorded, and weekly retrospective records of diarrhoeal morbidity and vomiting illness were completed by trained field workers who interviewed mothers.

**13C-urea breath tests**

For both cohorts, a 13C-urea breath test was performed on study days as previously described, using a protocol that has been validated in young children in this population. Briefly, a baseline breath sample was obtained from children shortly after awakening, using a face mask and expired breath reservoir. Immediately thereafter, a drink containing 50 mg 13C-urea (99 atom % excess, Cambridge Isotopes, MA, USA), 50 mg naturally abundant urea, 0.5 mg/kg lactulose elixir (BP), and a test meal of 5 g glucose polymer (Polycose, Abbott Laboratories, UK) dissolved in an appropriate amount of water was given. Further breath samples were collected 30 minutes later.

Breath samples were stored in 20 ml vacutainers (Becton Dickinson, UK) and transported to Cambridge, UK. Carbon dioxide was cryogenically separated from the samples using an automated system, and analysed by gas isotope ratio mass spectrometry (SIRA 10, VG Isotech, UK). Isotopic enrichment was expressed as Craig corrected δ (%) relative to the international standard Pee Dee Belemnite limestone (PDB), using the formula:

$$\delta = \left( \frac{R_{\text{sample}}}{R_{\text{PDB}}} - 1 \right) \times 10^3,$$

where $R$ is the isotopic ratio (13C:12C) of the sample or standard.

The baseline corrected 13C enrichment was defined as the difference in isotopic enrichment between the baseline breath sample and at 30 minutes thereafter. An appropriate cut-off baseline enrichment to distinguish between positive and negative results of 5.47 δ‰ rel PDB was calculated as previously described and validated. Measurements of weight (Seca electronic scales), and length or height (Harpenden stadiometer, Hollain Ltd, UK), mid upper arm and head circumference, and sub-scapular skin fold were made at the same clinic visit.

**Statistical analysis of growth**

Longitudinal height and weight records were used to measure growth by reference to two sets of standard measurements. Standard deviation (SD) scores for weight and length were calculated for each child by reference to the National Center for Health Statistics (NCHS) standard values. These values were initially used to compare weights and height at appropriate ages between subjects with different urea breath test results by ANOVA, in order to gain an impression of any association between *H pylori* and nutritional state. The data for each cohort were further analysed by repeated measures analysis of variance assuming quadratic and cubic age trends in cohorts 1 and 2 respectively. A difference in growth between subjects with early consistent positive or sequential negative breath tests was tested for within the group by age interaction.

In order to account for any confounding age associated deterioration in growth performance when compared to NCHS standards, data were further analysed using an approach that corrected for local growth performance and assessed potential independent confounding factors. Height and weight data collected at child welfare clinics in this community over four years (including the recruitment period for the first cohort), were used to produce local growth standards by the LMS method. These local standards were used to calculate height and weight-for-age SD scores for children relevant to locally expected growth performance.

season of birth has a significant effect on growth in this community. The relation between birth weight, infant growth, and season of birth is essentially sinusoidal, so that we were able to allow for the effect of season of birth on growth by deriving two correction factors from fitting a sine wave to a large data set of anthropometric measurements (including data collected in this study):

$$x_1 = \sin \left( \frac{(m - 0.5) \times \pi}{6} \right)$$

and

$$x_2 = \cos \left( \frac{(m - 0.5) \times \pi}{6} \right),$$

where $m = \text{month of birth} (1 \ldots 12)$.

These two terms were included in the regression analysis which was used to assess the possible role of *H pylori* colonisation in infant growth, accounting for expected local growth performance, season of birth, and occurrence of diarrhoeal disease by multiple linear regression analysis using weight or height Z score for age as the outcome measures.

In 1999 all available children from both cohorts were weighed and had their height measured. Subjects were thus between 5 and 8 years old at follow up, and 122/190 (64%) attended for follow up weight and height measurement. Five children had died between 1995 and 1999, and the remaining children had left the villages.

**RESULTS**

Morbidity from infectious diseases and antibiotic prescription were high. A clinical diagnosis of respiratory infection was the commonest indication for prescribing antibiotics. Lower respiratory tract infections were diagnosed among 59% of cohort 1 and 74% of cohort 2, while upper respiratory infections were diagnosed among 48% of cohort 1 and 49% of cohort 2. Antibiotics were prescribed for skin infections for 9% of cohort 1 and 29% of cohort 2. Chloramphenicol was given to only five children, and metronidazole was prescribed to 14 children. One child died, suddenly from an unexplained cause. Acute diarrhoea was common, with an incidence of 6.7 episodes per child year. Persistent diarrhoea, of more than one week’s duration, occurred on 13 occasions. Bloodstained diarrhoea was recorded on at least one occasion among 13% of cohort 1 and 11% of cohort 2. Supplemental food was administered under clinic supervision to 14 children for a mean of five weeks, for poor weight gain complicating recovery from diarrhoeal disease.

In cohort 1, 48/125 children (38%) had sequential positive urea breath tests at three month intervals throughout the first year of life. Thirty one children (25%) had sequential negative urea breath tests until age 1, and the remaining 46 children (37%) had both positive and negative results. In the second cohort, 28/65 (43%) had sequential positive urea breath tests from age 3 months, 11/65 infants (17%) had consecutive negative urea breath tests throughout their first year, and the remaining 26/65 (40%) had both positive and negative results. This classification of children into three
groups, those with sequential positive tests, those with sequential negative tests, and those with mixed results, was used for further analyses described below.

Birth during the dry season (that is, not the “hungry” season) was associated with an increased likelihood of developing early positive breath tests. For most socioeconomic and demographic variables tested (table 1), the 95% confidence interval of the OR for a positive urea breath test included unity. The only exception was a marginally increased OR among children of a wage earning mother (OR 1.18, 95% CI 1.03 to 1.35). Neither diarrhoeal morbidity nor episodes of vomiting were associated with positive urea breath tests, and there were no significant associations between antibiotic usage and breath test result.

Birth weight was not significantly associated with subsequent breath test results in either cohort. In both cohorts, children with sequential positive urea breath tests from age 3 months became lighter than children with negative breath tests (figs 1 and 2). This weight deficit in children with consecutive positive breath tests, compared to those with repeated negative breath tests, first became significant at age 6 months (weight-for-age Z scores at 6 months: means cohort 1: −0.869 and −0.392 respectively, 95% CI for differences between means −0.019 to −0.935; and means cohort 2: −1.155 and −0.658 respectively, 95% CI for differences between means −0.179 to −1.073).

Repeated measures analysis of variance showed marginally significant size differences in cohort 1; children with positive breath tests from age 3 months had lower values for both length and weight Z score (difference 0.35 for length, p = 0.04; and 0.30 for weight, p = 0.05). In cohort 2, but not cohort 1, both length and weight decelerated dramatically faster in infants with positive breath test from 3 months age (difference in rate of fall for length Z score 0.063 per month, and for weight Z score 0.078 per month, both p < 0.0001).

Rates of weight gain were compared using local growth standards. Multiple regression analyses were performed to assess the independent effects of season of birth, H pylori status, and influence of diarrhoeal disease on gain in weight and height over three month intervals (tables 2 and 3). The association between positive urea breath tests from 3 months of age, and weight and height faltering between 3 and 6 months of age, remained significant after accounting for local growth patterns, season of birth, and level of diarrhoeal disease in the analysis. Between 9 and 12 months of age the situation changed, and developing H pylori colonisation at this age was associated with an increased weight velocity compared to other children from the same community, while diarrhoeal disease had an independent negative effect on weight gain (table 2).

Other anthropometric measurements exhibited similar trends. Significant differences later in the first year of life were detected in both cohorts between subjects with positive breath tests from age 3 months and controls for measures of mid upper-arm circumference (cohort 1 at 9 months, ANOVA groups comprising all positive breath tests (mean 128.7 mm), all negative breath tests (mean 135.8 mm), or mixed results (mean 131.8 mm); total DF = 104, F = 3.829, p = 0.025; cohort 2 at 6 months ANOVA groups comprising all positive breath tests from age 3 months; those with consecutive negative breath tests throughout infancy; and the remaining children with mixed results. Children in the first cohort were studied at 12 week intervals.

### Table 1: Odds ratios for selected variables for the development of H pylori colonisation by age 6 months

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Both cohorts of children</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotics prescribed by age 6 months</td>
<td>175</td>
<td>1.23 (0.91 to 1.67)</td>
</tr>
<tr>
<td>Born in dry season</td>
<td>175</td>
<td>2.59 (1.67 to 4.02)</td>
</tr>
<tr>
<td><strong>Cohort 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resident village 1</td>
<td>110</td>
<td>0.95 (0.57 to 1.58)</td>
</tr>
<tr>
<td>Resident village 2</td>
<td>110</td>
<td>1.03 (0.88 to 1.2)</td>
</tr>
<tr>
<td>Resident village 3</td>
<td>110</td>
<td>1.0 (0.81 to 1.23)</td>
</tr>
</tbody>
</table>

Other variables examined but not tabulated (95% CI odds ratios include unity) included: adult and child occupational and sleeping density; number of beds; type of bed; house construction; sources of water; variety and number of domestic animals; material possessions; consumption of purchased consumables; original tribe and education of either parent; cultivation of specific crops; sale of domestic animals.
DISCUSSION

This study suggests an association between early \textit{H pylori} colonisation and subsequent development of infant growth faltering. This association was found in two successive population cohorts.

Direct validation of the urea breath test in children aged less than 6 months has proved difficult. In three previous endoscopic reports we have described the common occurrence of \textit{H pylori} colonisation in Gambian infants,\textsuperscript{7-10} Gambian isolates of \textit{H pylori} appear typical of strains associated with disease throughout the world.\textsuperscript{11} Our previous endoscopic studies have shown that Gambian infants with positive urea breath tests have \textit{H pylori} colonisation,\textsuperscript{12} provided that an age appropriate cut-off is used for the breath test. We have derived a statistical approach for determining this,\textsuperscript{13} whereas other authorities have accounted for differences in CO\textsubscript{2} production in young children to produce age appropriate cut-off values.\textsuperscript{14} Additional evidence of IgG seroconversion from our previous studies\textsuperscript{15} suggests that children with consecutive positive breath tests throughout the first year are those with early \textit{H pylori} colonisation. Conversely, those with consecutive negative breath tests have remained colonisation-free. The children with a mixture of positive and negative results, including several with negative following positive breath tests, are impossible to classify with respect to \textit{H pylori} at present.

In The Gambia, \textit{H pylori} colonisation in infancy is common, as in many other developing countries.\textsuperscript{16-18} This contrasts with the situation in industrialised countries, where colonisation appears to occur later in childhood. It is well established that the risks of both early \textit{H pylori} colonisation and infant malnutrition are inversely related to socioeconomic status.\textsuperscript{19} Within our study community we were unable to identify socioeconomic or demographic variables associated with the risk of early \textit{H pylori} colonisation. The only variable tested to achieve marginal significance was “mother earning a wage”, which due to the large number of comparisons made could be a false positive. The lack of effect exerted by socioeconomic factors could be due to the small variability in these variables, but shows that the association in early infancy between \textit{H pylori} and growth faltering is not attributable to confounding.

Infant growth was poor when compared to NCHS standards, despite the provision of good primary health care, effective medical treatment of acute childhood illness, and the provision of nutritional supplements to the children with the poorest growth performance. This in itself would be expected to dilute the impact of any individual factor adversely affecting nutrition. The provision of medical support resulted in a low infant mortality (1/190 children died during these studies, although a further five died subsequently) and a lower level of persistent diarrhoea compared to that previously reported from this community.\textsuperscript{20} Acute diarrhoeal disease and poor infant growth remained common problems. A decrease in weight velocity occurred over the same time interval as \textit{H pylori} colonisation in both cohorts, and a decrease in height velocity occurred shortly thereafter. Significant deficits in other anthropometric measures in both cohorts indicate that this decreased growth was secondary to undernutrition. In the second cohort, in which we observed the highest early incidence of \textit{H pylori} and subsequently the worst infant growth, head circumference at age 1 year was a mean of 8 mm less among children who had become colonised with \textit{H pylori} by age 6 months, suggesting significant malnutrition.

Accounting for season of birth, and using local growth standards to remove any bias that might arise from the age associated worsening of growth performance of Gambian children when compared to NCHS standards, did not remove

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Mean weight-for-age and height-for-age Z scores (relative to NCHS values) for children up to age 1 year from cohort 2. Children are divided into three groups: those with consecutive positive urea breath tests from age 3 months; those with consecutive negative breath tests throughout infancy; and the remaining children with mixed results. Children in the second cohort were studied at four week intervals.}
\end{figure}
the association between early *H pylori* colonisation and growth faltering. We uncovered no evidence that *H pylori* colonisation occurred in children who were already malnourished, but instead observed *H pylori* colonisation occurred in children who were already malnourished, and who subsequently exhibited a decline in growth performance. This could not be explained by any increase in diarrhoea or other infectious disease morbidity.

Significant growth faltering relative to local standards was more common among children with early colonisation. Developing *H pylori* colonisation later in the first year of life was not associated with the development of significant growth faltering. Indeed, those children who did not develop *H pylori* colonisation until between 9 and 12 months showed an increased weight velocity compared to their peers (table 2), suggesting that a delay in the onset of *H pylori* colonisation is associated with improved growth performance in this community. The deterioration in growth performance was transient, and follow up measurements taken several years later failed to reveal any persistent growth effect subsequent to early *H pylori* colonisation (fig 1), suggesting that catch-up growth occurred naturally in this community. This implies that the association between *H pylori* and growth is age specific, and may explain why growth faltering accompanying *H pylori* colonisation in childhood is more commonly reported from longitudinal23 rather than cross sectional studies.34 Despite this, several studies from throughout the world have shown an association between *H pylori* and growth faltering in childhood.2 1 A recent nested case-control study from Peru, which relied on IgG seroconversion to estimate the age of onset of *H pylori* colonisation, also suggested that growth slowing following acute *H pylori* infection in early childhood was age dependent.24

The temporal association between *H pylori* colonisation and the onset of growth faltering in Gambian infants supports our hypothesis that *H pylori* predisposes infants to the development of malnutrition and growth faltering. The mechanism by which this may occur has not been elucidated, but may include transient loss of the gastric acid barrier during vulnerable periods, such as during the introduction of weaning foods.2 The differences in mean weight-for-age associated with *H pylori* colonisation in both cohorts, of more

### Table 2

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Variable</th>
<th>Regression coefficient</th>
<th>SE coefficient</th>
<th>t ratio</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight-age Z score at 3 months</td>
<td>Seasonal factor x1</td>
<td>0.058</td>
<td>0.012</td>
<td>4.71</td>
<td>0.001</td>
</tr>
<tr>
<td>Weight-age Z score at 6 months</td>
<td>Seasonal factor x2</td>
<td>0.054</td>
<td>0.017</td>
<td>3.12</td>
<td>0.007</td>
</tr>
<tr>
<td>Weight-age Z score at 9 months</td>
<td>Seasonal factor x3</td>
<td>0.052</td>
<td>0.015</td>
<td>3.38</td>
<td>0.001</td>
</tr>
<tr>
<td>Weight-age Z score at 12 months</td>
<td>H pylori at 6 months</td>
<td>0.251</td>
<td>0.016</td>
<td>15.39</td>
<td>0.001</td>
</tr>
<tr>
<td>Height-for-age Z score at 6 months</td>
<td>Seasonal factor x1</td>
<td>0.155</td>
<td>0.009</td>
<td>16.94</td>
<td>0.001</td>
</tr>
<tr>
<td>Height-for-age Z score at 9 months</td>
<td>Seasonal factor x2</td>
<td>0.162</td>
<td>0.008</td>
<td>19.96</td>
<td>0.001</td>
</tr>
<tr>
<td>Height-for-age Z score at 12 months</td>
<td>H pylori at 9 months</td>
<td>0.16</td>
<td>0.009</td>
<td>17.87</td>
<td>0.001</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Variable</th>
<th>Regression coefficient</th>
<th>SE coefficient</th>
<th>t ratio</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height-for-age Z score at 6 months</td>
<td>Seasonal factor x1</td>
<td>0.078</td>
<td>0.008</td>
<td>10.05</td>
<td>0.001</td>
</tr>
<tr>
<td>Height-for-age Z score at 9 months</td>
<td>Seasonal factor x2</td>
<td>0.072</td>
<td>0.007</td>
<td>9.99</td>
<td>0.001</td>
</tr>
<tr>
<td>Height-for-age Z score at 12 months</td>
<td>H pylori at 12 months</td>
<td>0.054</td>
<td>0.009</td>
<td>5.74</td>
<td>0.001</td>
</tr>
</tbody>
</table>
than 0.5 SD at 9 months, was sufficient to adversely affect expected morbidity and mortality from other illnesses in this community at this age.

It is becoming increasingly clear that \textit{H. pylori} colonisation in infancy is common throughout the developing world. We observed significant infant growth faltering, and although a growth deficit per se did not persist into middle childhood, poor nutrition in infancy may lead to long term adverse health consequences. There is therefore a need for intervention studies designed to test the hypothesis that \textit{H. pylori} colonisation at critical vulnerable ages may lead to malnutrition and growth faltering among infants in countries such as The Gambia.

ACKNOWLEDGEMENTS
We would like to thank the residents of Keneba, Manduara, and Kanton Kunda, and the field workers, particularly Mustapha Sanyang and Baba Jabateh for their help.

\textbf{Authors’ affiliations}
M Harding, W A Coward, T J Cole, MRC Human Nutrition Research, Cambridge, UK
J E Thomas, Department of Child Health, University of Newcastle upon Tyne, UK
A Dale, J E Bunn, MRC Keneba, The Gambia, West Africa
L T Weaver, Department of Child Health, University of Glasgow, U.K.
Funding: This work was supported by grants from the Medical Research Council of Great Britain and the Thrasher Research Fund
Study protocols were approved by the Joint MRC and Gambian Government Ethical Committee, and by a meeting of village elders.

\section*{REFERENCES}
Early *Helicobacter pylori* colonisation: the association with growth faltering in The Gambia

J E Thomas, A Dale, J E G Bunn, M Harding, W A Coward, T J Cole and L T Weaver

*Arch Dis Child* 2004 89: 1149-1154
doi: 10.1136/adc.2002.015313

Updated information and services can be found at:
http://adc.bmj.com/content/89/12/1149

**These include:**

**References**
This article cites 23 articles, 9 of which you can access for free at:
http://adc.bmj.com/content/89/12/1149#BIBL

**Email alerting service**
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Topic Collections**
Articles on similar topics can be found in the following collections
- Childhood nutrition (712)
- Childhood nutrition (paediatrics) (396)
- Child health (3922)
- Infant health (811)
- Infant nutrition (including breastfeeding) (405)
- Epidemiologic studies (1818)
- Malnutrition (176)

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/