Article:
Jaganath, D, Saito, M, Gilman, RH et al. (8 more authors) (2014) First detected Helicobacter pylori infection in infancy modifies the association between diarrheal disease and childhood growth in Peru. Helicobacter, 19 (4). 272 - 279. ISSN 1083-4389

https://doi.org/10.1111/hel.12130

First detected *Helicobacter pylori* infection in infancy modifies the association between diarrheal disease and childhood growth in Peru

Devan Jaganath MD MPH[*] (1), Mayuko Saito MD PhD[*] (2), Robert H Gilman MD DTMH (1, 2, 3), Dulciene MM Queiroz MD DPhil (4), Gifone A Rocha MD (4) Vitaliano Cama PhD (5), Lilia Cabrera (2), Dermot Kelleher MD FRCP (6), Henry J Windle PhD (6), Jean E Crabtree DPhil FRCPath (7), William Checkley MD PhD (1, 2).

1. Program in Global Disease Epidemiology and Control, Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, USA.
2. Biomedical Research Unit, A.B. PRISMA, Lima, Peru.
3. Department of Microbiology, Universidad Peruana Cayetano Heredia, Lima, Peru
4. Laboratory of Research in Bacteriology, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.
5. Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.
6. Department of Clinical Medicine, Trinity College Dublin, Ireland.
7. Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK.

* Co-first authors

Running title: Infant *H. pylori* infection and growth outcomes

Corresponding Author:

William Checkley, MD, PhD

Division of Pulmonary and Critical Care

School of Medicine

Johns Hopkins University

1800 Orleans Ave Suite 9121

Baltimore, MD 21205

E-mail: wcheckl1@jhmi.edu

Word Count

Abstract: 227

Text: 3,081

Keywords: child; diarrhea; growth; *Helicobacter pylori*

ABSTRACT

Background: In endemic settings, *Helicobacter pylori* infection can occur shortly after birth, and may be associated with a reduction in childhood growth.

Materials and Methods: This study investigated what factors promote earlier age of first *H. pylori* infection and evaluated the role of *H. pylori* infection in infancy (6-11 months) vs. early childhood (12-23 months) on height. We included 183 children near birth from a peri-urban shanty town outside of Lima, Peru. Fieldworkers collected data on socioeconomic status (SES), daily diarrheal and breastfeeding history, antibiotic use, anthropometrics and *H. pylori* status via carbon 13-labeled urea breath test up to 24 months after birth. We used a proportional hazards model to assess risk factors for earlier age at first detected infection, and linear mixed-effects models to evaluate the association of first detected *H. pylori* infection during infancy on attained height.

Results: 140 (77%) were infected before 12 months of age. Lower SES was associated with earlier age at first detected *H. pylori* infection (low vs. middle-to-high SES HR 1.59, 95% CI 1.16, 2.19; *P*=0.004), and greater exclusive breastfeeding was associated with reduced likelihood (HR 0.63, 95% CI 0.40-0.98, p = 0.04). *H. pylori* infection in infancy was not independently associated with growth deficits (*P* =0.58). However, children who had their first detected *H. pylori* infection in infancy (6-11 months) vs. early childhood (12-23 months) and who had an average number of diarrhea episodes per year (3.4) were significantly shorter at 24 months (-0.37 cm, 95% CI, -0.60, -0.15 cm; *P* =0.001).

Discussion: Lower SES was associated with a higher risk of first detected *H. pylori* infection during infancy, which in turn augmented the adverse association of diarrheal disease on linear growth.

INTRODUCTION

In resource-limited settings, the prevalence of *Helicobacter pylori* infection can be >80% (1). First *H. pylori* infection and colonization has been shown to occur in the beginning months of life (2-4). A longitudinal study in Bangladesh found that 49% of children were infected by 2 years of age based on stool antigen (5). Infection may occur several times as children spontaneously clear the infection and become re-infected (1). For example, a longitudinal study on the United States-Mexico border found that 77% had subsequently negative tests and 19% were re-infected (6). Similarly, a small study in the southwest United States among Native Americans found that 15.9% of children experienced a transient infection. Risk factors associated with *H. pylori* infection in childhood relate to living conditions that may increase the risk of waterborne or feco-oral transmission, including low socioeconomic status (SES) and not boiling feeding bottles (7).

The effect of recurrent infection on child health is poorly understood. Transient reduction in acid secretion may negatively affect growth through promotion of diarrheal disease (8). Cross-sectional and longitudinal studies have shown that acute *H. pylori* infection is associated with growth deficits in children that can persist for months after infection (9-12). In Bangladesh, *H. pylori* positive children were more likely to be infected with multiple enteric pathogens (5). However, the mechanistic relationship between diarrhea and *H. pylori* infection and their combined association on childhood growth remains to be elucidated.

To examine further the role of infant first detected *H. pylori* infection on growth, we evaluated what factors may influence earlier age of first infection among a cohort of

children in a shanty town outside Lima, Peru. We then assessed if acute first detected infection before the age of 12 months (i.e., infancy) compared to 12-23 months (i.e., early childhood) was 1) directly associated with growth deficits, or 2) modified the association of diarrheal disease on growth.

MATERIALS AND METHODS

Study setting

We conducted the study in Pampas de San Juan Miraflores and Nuevo Paraíso, two peri-urban shanty towns with high population density, 25 km south of central Lima (13, 14). These peri-urban communities are comprised of 50,000 residents, the majority of whom are immigrants from rural areas of the Peruvian Andes who settled nearly 35 years ago and later claimed unused land on the outskirts of Lima. In the last two decades Pampas has undergone many economic and social developments. In 1989, most homes were temporary structures constructed of wooden poles and woven thatch, without water or sewage lines. Currently, >85% of homes were constructed from brick or cement with in-home water and sewage lines (15). The study was approved by the European Union Ethics Committee, A.B. PRISMA and Universidad Peruana Cayetano Heredia, in Lima, Peru, and the Bloomberg School of Public Health, Johns Hopkins University, in Baltimore, USA. Mothers or caregivers provided written informed consent.

Study design

The study team keeps an ongoing vital status and birth census database in the study areas. Based on a sampling frame of all pregnant women from May 2007 to February 2011, we randomly selected pregnant women in Peru to participate in the study. Exclusion criteria included: children with severe disease that required hospitalization, severe chronic illness, child of a multiple pregnancy, birth weight less than 1500 grams and intentions to move during the period of the study. Of 304 eligible children, 11 did not have available anthropometric data, 95 did not complete 500 days of surveillance and

11 did not have a baseline socioeconomic status (SES) data or carbon 13-labeled (C-13) urea breath test results (UBT) (Figure 1). Our analysis included a total of 187 children with complete data. Because almost all (>97%) children became infected with *H. pylori* in the first two years of life, we sought to compare first detected infection in the first 12 months (6-11 months) vs. the second 12 months of life (12-23 months). Consequently, four children who were never infected during this period were excluded, for a final sample of 183 children.

We followed children from birth until 24 months. At enrollment, field workers asked caretakers to complete a questionnaire on socioeconomic and living conditions, including floor material, number of inhabitants, number of rooms, water source and storage, and durable assets. Field workers visited the household daily and obtained history on breast feeding and diarrheal symptoms, including consistency of feces, loss of appetite and intake of any antibiotics. Height (recumbent length) was measured weekly for the first three months, then twice per month between 3 months and one year of age, and then monthly thereafter. Height was measured with a locally made wooden platform with sliding footboard.

Starting at 6 months of age, C-13 UBT was repeated on average of every three months as previously described (16). C-13 UBT was performed after at least four hours of fasting. The children drank 100 mL of water containing 50 mg of C-13 urea (Eurisotop ≥ 99.00% chemical purity, Paris, France) through a straw. Trained staff collected breath samples using a face mask with unidirectional valve into a breath bag, and sent them to the Laboratory of Research in Bacteriology (LPB), Belo Horizonte, Brazil, where they

were analyzed within one month after sample collection with a non-dispersive infrared spectrometer (Wagner Analysen Tecnik, Bremen, Germany).

An increase in the ratio of C-13 to C-12 between the baseline sample and 30 minute sample delta over baseline values ≥4% were considered indicative of active infection, while <4% were considered negative (16). The cutoff value adopted was based on the results of our previous study evaluating 908 randomly selected samples from the same population (16). In that study, we showed an excellent agreement between C-13 UBT and an ELISA using a pool of monoclonal antibodies to detect *H. pylori* antigens in stool (kappa coefficient of 0.93; 95% CI 0.91-0.96), which points to a good accuracy of each test for the diagnosis of *H. pylori* infection in young children.

Definitions

In children aged ≥3 months, a diarrheal episode began if the child had ≥3 liquid or semi-liquid (loose) stools in 24 hours and if the mother reported diarrheal symptoms on that day. Because exclusive breastfeeding is associated with a greater frequency of loose stools, we used a more conservative definition of diarrheal episodes for young infants. Specifically, we defined a diarrheal episode in children aged <3 months as ≥6 loose stools in 24 hours with maternal report of symptoms on that day. We defined resolution of a diarrheal episode if there were <3 loose stools for children aged ≥3 months or <6 loose stools for children aged <3 months for two consecutive days. The age of first *H. pylori* infection was the age in months of the first positive result by UBT: *H. pylori* infection in infancy was defined as the first positive result before 12 months of age. Height was reported in centimeters to the nearest millimeter. We calculated Z-scores

according to the WHO MGRS standard (17). We calculated a SES score using principal component analysis (PCA) per the DHS wealth index (18, 19) using the following variables: number of rooms in the home, number of rooms used as bedrooms, number of persons that sleep in the same bedroom, primary source of water, location of feces elimination, and ownership of a refrigerator, radio, black/white TV, color TV, DVD/VHS player, stereo, telephone and animals for breeding or pets. The first principle component was stratified into tertiles low, middle and high.

Biostatistical methods

We performed proportional hazards regression to evaluate factors associated with earlier age of first detected *H. pylori* infection. Namely, we evaluated the role of SES and exclusive breastfeeding in the first six months, adjusting for differences in sex, height, diarrheal episodes, and antibiotic use. We utilized the Andersen-Gill model to account for time-varying covariates (20). Events were time to first *H. pylori* infection, and the time metric was age in months. The four children who did not have *H. pylori* infection were included in this analysis, as administratively censored. Variables included sex, SES level, and percent of visits with reported exclusive breastfeeding in the first 6 months (as a measure of early weaning) as fixed covariates, and attained HAZ, attained days of diarrhea, and attained days of antibiotics as time varying covariates during periods between anthropometric visits.

We used a linear mixed effects model to determine the effect of first instance of *H. pylori* infection on growth in height. To evaluate height across age, we used truncated polynomial splines at 3, 6, 12, and 18 months, with a random intercept and

slope to model heterogeneity across children, and a first order continuous autoregressive error to model serial correlation (See Online Supplement). We assessed goodness-of-fit by examining standardized residuals, a variogram of residuals and pairwise scatterplots of the residuals at multiple time points (21). Potential confounders included in the model were those that may have an impact on longitudinal growth, namely sex, percent of exclusive breastfeeding in the first 6 months, cumulative number of diarrheal episodes between birth and 24 months, antibiotic use in the two weeks prior to first detected infection, and SES tertile. In the final model, interaction terms were included between SES and age, and between infant *H. pylori* infection and diarrhea. Analyses were conducted on STATA 12 (StataCorp, College Station, USA) and R (www.r-project.org).

RESULTS

Participant Characteristics

Of 183 children with *H. pylori* infection before 24 months of age, 140 (77%) had their first detected infection in infancy. We summarize baseline characteristics in Table 1. At enrollment, 17 (9%) children were stunted, but none were wasted. Thirty-seven percent were in the lowest SES tertile and 86% had 24-hour access to water in the home. Household characteristics were similar between children infected by *H. pylori* before or after 12 months of age (Table 1). On bivariate analysis, children who had their first detected *H. pylori* infection after 12 months were on average 1.5 cm taller than children whose first infection was in infancy (median 53.2 cm vs. 51.7 cm, $P = 0.04$).

Risk factors for early *H. pylori* acquisition

187 children (183 positive for *H. pylori* and 4 negative for *H. pylori* by UBT in the first 2 years of life) contributed 1,752 child-months. We found that the earlier age to *H. pylori* infection was greater in children in the lowest SES tertile (Figure 2, log-rank test $P = 0.03$). Adjusting for sex, breastfeeding, attained HAZ, attained days of diarrhea and attained days on antibiotic, the Andersen-Gill model suggested that low SES tertile was associated with a significantly earlier time to first detected *H. pylori* infection compared to those in the middle or highest SES tertiles in the first 24 months after birth (Table 2; Hazard Ratio (HR)=1.59, 95% Confidence Interval (CI) 1.15, 2.19; $P = 0.004$). Greater percent of reported exclusive breastfeeding in the first 6 months was associated with reduced likelihood of earlier infection (HR=0.63, 95% CI 0.40, 0.98; $P = 0.04$).

Association of *H. pylori* infection in infancy on linear growth

Among the 183 children who were infected before 24 months, 7,251 measures of height were taken (range 22-44 per child). Lower SES significantly reduced growth velocity (P =0.01), so we included an interaction term between SES and age in our final model (See Online Supplement). In addition, children who had the mean number of diarrheal episodes (mean=6.8, SD=4.4) in the first 24 months of life had a significant reduction in height of 0.27 cm (95% CI -0.47, -0.06, P =0.01) compared to those without diarrheal disease.

First detected *H. pylori* infection in infancy (6-11 months) vs. early childhood (12-23 months) did not have an independent association on height compared to infection in early childhood, after adjusting for SES, breastfeeding, antibiotic use prior to infection, and cumulative diarrheal episodes (P =0.58). Instead, we found a significant, adverse interaction between *H. pylori* infection in infancy and diarrheal on height (P =0.03). Specifically, children with *H. pylori* infection in infancy vs. early childhood and who had the mean number of diarrheal episodes had a 0.37 cm reduction in height over 24 months compared to children who had their first infection in early childhood (Table 3; 95% CI -0.60, -0.15 cm, P =0.001). This deficit corresponded to a 0.11 reduction in standard deviations in both boys and girls at 24 months.

DISCUSSION

In this prospective cohort of children 0 to 24 months in Peru, we found that factors including low socioeconomic status and reduced exclusive breast feeding in the first six months were associated with earlier age of first detected *H. pylori* infection. While *H. pylori* infection during infancy vs. early childhood was not directly related to growth deficits, it may modify the effects of diarrheal disease on height.

The relationship between childhood *H. pylori* infection and socioeconomic status has been well established, with lower levels predicting higher prevalence of infection (22-24). At the same time, improvements in living conditions have been associated with reduced prevalence (23). However, the majority of these studies are retrospective, investigating how early life influenced adult chronic infection (24, 25). A study in Bangladesh found a relationship between parental illiteracy and infection in the first two years of life (5). A longitudinal study in the Gambia did not find an association between socioeconomic status and early colonization, but suggested that their population was too homogenous to see an effect (2). We found that lower socioeconomic status was associated with earlier age of first infection, independent of sex, breastfeeding, days of diarrheal disease and antibiotic use, and height-for-age. This is consistent with past studies in Peru that demonstrated that risk of infection occurs early in children, and that socioeconomic factors such as water source have a strong association with the probability of infection (26, 27). Because of the limited sample size, there was insufficient power to assess more specific proximal factors related to low SES associated with earlier age of *H. pylori*. Further research is required to elucidate particular determinants of early infection and possible interventions for prevention.

We also found that greater exclusive breast feeding in the first six months was associated with a reduced likelihood of earlier age at first infection. A meta-analysis found that breast-feeding led to an overall 32% reduction in odds of *H. pylori* infection (28). However, they were unable to assess a dose-response based on the studies evaluated. A study in Bangladesh was unable to find an association between exclusive breast feeding and mixed-feeding and *H. pylori* infection in the first two years of life (5). In our analysis, we found that greater percent of exclusive breast feeding in the first 6 months was associated with 37% reduction in likelihood of earlier age of first infection. It has been hypothesized that breast milk provides IgA antibodies specific to *H. pylori*, and may prevent gut adherence (29-31). For example, in the Gambia, early colonization was prevented in infants who had urease-specific IgA antibodies (29). In the context of our findings, further research that identifies how to delay *H. pylori* infection may also reduce deficits in childhood growth.

Other longitudinal studies in resource-poor settings have suggested that *H. pylori* infections in childhood can affect growth. A study in the Gambia found that early colonization in the first few months of birth by *H. pylori* was associated with height and weight deficits (2). In Ecuador, new *H. pylori* infections were associated with a 9.7 mm/year reduction in growth velocity among children with an average age of 19 months (12). In Colombia, a longitudinal study found that new infection in older children (12 to 60 months) was associated with a 0.24 cm cumulative reduction in height (10). In our study, while bivariate analysis suggested a height deficit in children infected in infancy, we did not find an independent relationship between age at first detected *H. pylori* infection and height. However, it is important to note that our exposure comparison was

first detected infection in 6-11 months vs. 12-23 months. These past studies show that *H. pylori* infection even after 12 months can impact growth, and so we may not expect to see direct differences between these groups.

We found that having the average number of diarrheal episodes was associated with a significant reduction in height at 24 months. The relationship between childhood diarrheal disease and growth faltering is well characterized (32, 33). Peruvian children who had diarrheal disease during 10% of their first 24 months were associated with 1.5 cm growth deficit compared to children without diarrheal episodes (34). Moreover, the odds of stunting at 24 months was found to be proportional to additional diarrheal episodes in a multi-country analysis (35). In our study, we found that the average number of diarrheal disease episodes over 24 months was associated with a 0.27 cm reduction in height at 24 months (i.e., a reduction of approximately 0.08 standard deviations). This association is smaller than past studies, and reflects how development in this community, such as improved access to clean water and sanitation, can reduce the comorbidities of disease in similar settings.

The mechanistic relationship between *H. pylori* and diarrheal disease has yet to be established. Several studies suggest that diarrheal disease is a mediator of the association of acute *H. pylori* infection on growth, through a transient hypochlorhydria that may reduce innate barriers and promote diarrheal disease (36, 37). For example, a longitudinal cohort in Peru among children 6 months to 12 years found that new *H. pylori* infection was associated with greater frequency and length of diarrheal disease compared to those who were either uninfected or persistently infected (38). However, chronic infection may behave differently; a prospective study in Thailand did not find a

role of chronic *H. pylori* infection on incidence of childhood diarrhea (39). Other studies actually suggest that chronic *H. pylori* infection is protective against diarrheal disease through mechanisms such as a non-specific immune response to chronic inflammation, greater secretion of IgA, and *H. pylori*-produced antimicrobial peptides (40).

The findings of this study suggest that first detected *H. pylori* infection in infancy is associated with greater adverse effects of diarrheal disease on growth. We found that children who had the average number of diarrheal episodes and were first infected by *H. pylori* during infancy had a significant reduction in height of 0.37 cm at 24 months. The interaction between diarrheal disease and early *H. pylori* infection may occur because *H. pylori* promotes more severe forms of disease. A case-control study in Bangladesh found that while *H. pylori* was not associated with greater prevalence of cholera, it was related to life-threatening disease among those who had not been exposed to cholera before (41). *H. pylori* infection has also been associated with micronutrient deficiencies (8, 42, 43), which may affect the ability to defend against diarrheal disease. Finally, first infection with *H. pylori* may just be a marker of enteric enteropathy (44, 45). Specifically, children who acquire *H. pylori* at an earlier age are also those who may have an earlier burden of enteropathogenic infections, with or without diarrhea, which in turn affects growth adversely. Further research is required to better understand the mechanisms, but our findings suggest that not only mediation, but also interaction, could be considered in the relationship between diarrheal disease and early first *H. pylori* infection.

This study has the advantages of being a prospective cohort study that followed children for up to 24 months in a resource-limited setting with high prevalence of *H.*

pylori infection. Past studies in Latin America have focused primarily on children greater than one year of age, and thus were able to investigate the epidemiology of *H. pylori* in infancy. Moreover, repeated measurements in our study allowed for longitudinal data analysis to more accurately model growth in children, while also allowing time-varying covariates in the time to event analysis. Our study also has some potential shortcomings. First, the high prevalence of *H. pylori* infection in the 24 months of follow-up did not allow comparison with those who were not infected during this time. Also, food intake data was not collected to evaluate for differences in nutrition beyond anthropometry. We would have benefited from a larger sample size to evaluate proximal factors such as water source and fecal elimination on early age of infection. Testing for *H. pylori* began at 6 months of age, and so first infection may have occurred earlier. While those who had their first infection after 12 months may have had fewer tests and thus less likely to be found positive earlier, any potential misclassification would lead to a more conservative estimate. Moreover, our analysis of first detected *H. pylori* infection does not distinguish transient from persistent infection; however, past studies support that young children have a high rate of transient infection (6, 46), and so age of first infection may serve as a marker of when recurrent infections begin. Lastly, we limited our sample to the group of children with at least 500 days of surveillance. While it is possible that we may have introduced a bias by restricting our analysis to this group of children, we believe that the bias is likely small because our analyses are based on internal comparisons using longitudinal data with a reasonable length of follow-up.

In our setting where infection with *H. pylori* is endemic, we found that there was a high burden of infant *H. pylori* infection. Socioeconomic status was a strong predictor of

early first infection, suggesting that interventions that target improved living conditions and prevention of early infection may reduce growth faltering. At the same time, exclusive breastfeeding has the potential to delay early acquisition and promote growth. Compared to children who were first infected in the second 12 months of life, infant first infection may modify the association of diarrhea on growth. Further research is required to greater understand the potential mediation or interaction of *H. pylori* and diarrheal disease.

Disclosures: The authors have no conflicts of interest to disclose.

Funding: The project was funded under the Sixth Framework Program of the European Union, Project CONTENT (INCO-CT-2006-032136).

AUTHOR CONTRIBUTIONS

All authors were involved in the study design and writing of the manuscript. Devan Jaganath and William Checkley conducted statistical analysis and interpretation, and drafted the manuscript. Mayuko Saito, Robert Gilman, Dermot Kelleher, Henry Windle and Jean Crabtree share equal responsibilities in study design and conduct, in data management and in drafting of manuscript. Mayuko Saito, Lilia Cabrera, Vitaliano Cama, Robert Gilman and William Checkley were responsible for the study conduct and data management. Dulciene Quieroz and Gifone Rocha were responsible for urea breath test processing and interpretation of findings. William Checkley had ultimate oversight over study design and administration, analysis and writing of this manuscript.

ACKNOWLEDGEMENTS

We would like to thank the participants and staff of the CONTENT study in Peru. We are also grateful to Maria Luiza E. Scarabelli and Marina do Amaral from the Laboratory of Research in Bacteriology (LPB), Belo Horizonte, Brazil. We thank Dr. Robert Black for his thoughtful comments.

REFERENCES

1. Suerbaum S, Michetti P. *Helicobacter pylori* infection. *New Engl J Med*. 2002; 347(15): 1175-86.

2. Thomas JE, Dale A, Bunn JE, et al. Early *Helicobacter pylori* colonisation: the association with growth faltering in The Gambia. *Arch Dis Child*. 2004; 89(12): 1149-54.

3. Klein PD, Gilman RH, Leon-Barua R, et al. The epidemiology of *Helicobacter pylori* in Peruvian children between 6 and 30 months of age. *Am J Gastroenterol*. 1994; 89(12): 2196-200.

4. Bassily S, Frenck RW, Mohareb EW, et al. Seroprevalence of *Helicobacter pylori* among Egyptian newborns and their mothers: A preliminary report. *Am J Trop Med Hyg*. 1999; 61(1): 37-40.

5. Bhuiyan TR, Qadri F, Saha A, Svennerholm AM. Infection by *Helicobacter pylori* in Bangladeshi Children From Birth to Two Years Relation to Blood Group, Nutritional Status, and Seasonality. *Pediatr Infect Dis J*. 2009; 28(2): 79-85.

6. Goodman KJ, O'Rourke K, Day RS, et al. Dynamics of *Helicobacter pylori* infection in a US-Mexico cohort during the first two years of life. *Int J Epidemiol*. 2005; 34(6): 1348-1355.

7. Muhsen K, Jurban M, Goren S, Cohen D. Incidence, age of acquisition and risk factors of *Helicobacter pylori* infection among Israeli Arab infants. *J Trop Pediatr*. 2012; 58(3): 208-213.

8. Windle HJ, Kelleher D, Crabtree JE. Childhood *Helicobacter pylori* infection and growth impairment in developing countries: a vicious cycle? *Pediatrics*. 2007; 119(3): e754-9.

9. Patel P, Mendall M, Khulusi S, et al. *Helicobacter pylori* infection in childhood: risk factors and effect on growth. *BMJ*. 1994; 309(6962): 1119-23.

10. Mera RM, Correa P, Fontham EE, et al. Effects of a new *Helicobacter pylori* infection on height and weight in Colombian children. *Ann Epidemiol*. 2006; 16(5): 347-51.

11. Mera RM, Bravo LE, Goodman KJ, et al. Long-term effects of clearing *Helicobacter pylori* on growth in school-age children. *Pediatr Infect Dis J*. 2012; 31(3): 263-6. .

12. Egorov AI, Sempertegui F, Estrella B, et al. The effect of *Helicobacter pylori* infection on growth velocity in young children from poor urban communities in Ecuador. *Int J Infect Dis*. 2010; 14(9): e788-91.

13. Checkley W, Gilman RH, Black RE, et al. Effects of nutritional status on diarrhea in Peruvian children. *J Pediatr*. 2002; 140(2): 210-8.

14. Bennett A, Epstein LD, Gilman RH, et al. Effects of the 1997-1998 El Nino episode on community rates of diarrhea. *Am J Public Health*. 2012; 102(7): e63-9.

15. Sterling R, Miranda JJ, Gilman RH, et al. Early anthropometric indices predict short stature and overweight status in a cohort of Peruvians in early adolescence. *Am J Phys Anthropol.* 2012; 148(3):451-61.

16. Queiroz DM, Saito M, Rocha GA, et al. *Helicobacter pylori* infection in infants and toddlers in South America: concordance between [13C]urea breath test and monoclonal H. pylori stool antigen test. *J Clin Microbiol*. 2013; 51: 3735-40.

17. De Onis M. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. World Health Organization; 2006.

18. Rutstein SO, Johnson K, MEASURE ORCM. The DHS wealth index: ORC Macro, MEASURE DHS; 2004.

19. Vyas S, Kumaranayake L. Constructing socio-economic status indices: how to use principal components analysis. *Health Policy Plan*. 2006; 21(6): 459-68.

20. Andersen PK, Gill RD. Cox's regression model for counting processes: a large sample study. *The annals of statistics*. 1982; 10(4): 1100-20.

21. Diggle PJ. An approach to the analysis of repeated measurements. *Biometrics*. 1988: 959-71.

22. Malaty HM, Kim JG, Kim SD, et al. Prevalence of *Helicobacter pylori* infection in Korean children: inverse relation to socioeconomic status despite a uniformly high prevalence in adults. *Am J Epidemiol*. 1996; 143(3): 257-62.

23. Tkachenko MA, Zhannat NZ, Erman LV, et al. Dramatic changes in the prevalence of *Helicobacter pylori* infection during childhood: a 10-year follow-up study in Russia. *J Pediatr Gastroenterol Nutr*. 2007; 45(4): 428-32.

24. Webb PM, Knight T, Greaves S, et al. Relation between infection with Helicobacter pylori and living conditions in childhood: evidence for person to person transmission in early life. *BMJ*. 1994; 308(6931): 750-3.

25. Fall CH, Goggin PM, Hawtin P, et al. Growth in infancy, infant feeding, childhood living conditions, and Helicobacter pylori infection at age 70. *Arch Dis Child*. 1997; 77(4): 310-4.

26. Klein PD, Graham DY, Gaillour A, et al. Water source as risk factor for *Helicobacter pylori* infection in Peruvian children. Gastrointestinal Physiology Working Group. *Lancet*. 1991; 337(8756): 1503-6.

27. Hulten K, Han SW, Enroth H, et al. *Helicobacter pylori* in the drinking water in Peru. *Gastroenterology*. 1996; 110(4): 1031-5.

28. Chak E, Rutherford GW, Steinmaus C. The role of breast-feeding in the prevention of *Helicobacter pylori* infection: a systematic review. *Clin Infect Dis*. 2009; 48(4): 430-7.

29. Thomas JE, Bunn JE, Kleanthous H, et al. Specific immunoglobulin A antibodies in maternal milk and delayed *Helicobacter pylori* colonization in Gambian infants. *Clin Infect Dis*. 2004; 39(8): 1155-60.

30. Bhuiyan TR, Saha A, Lundgren A, Qadri F, Svennerholm AM. Immune responses to Helicobacter pylori infection in Bangladeshi children during their first two years of life and the association between maternal antibodies and onset of infection. *J Infect Dis*. 2010; 202(11): 1676-84.

31. Clyne M, Thomas J, Weaver L, Drumm B. In vitro evaluation of the role of antibodies against *Helicobacter pylori* in inhibiting adherence of the organism to gastric cells. *Gut*. 1997; 40(6): 731-8.

32. Mondal D, Minak J, Alam M, et al. Contribution of enteric infection, altered intestinal barrier function, and maternal malnutrition to infant malnutrition in Bangladesh. *Clin Infect Dis*. 2012; 54(2): 185-92.

33. Walker CL, Rudan I, Liu L, et al. Global burden of childhood pneumonia and diarrhea. *Lancet*. 2013; 12(13): 60222-6.

34. Checkley W, Epstein LD, Gilman RH, et al. Effects of acute diarrhea on linear growth in Peruvian children. *Am J Epidemiol*. 2003; 157(2): 166-75.

35. Checkley W, Buckley G, Gilman RH, et al. Multi-country analysis of the effects of diarrhea on childhood stunting. *Int J Epidemiol*. 2008; 37(4): 816-30.

36. Sullivan PB, Thomas JE, Wight DG, et al. *Helicobacter pylori* in Gambian children with chronic diarrhea and malnutrition. *Arch Dis Child*. 1990; 65(2): 189-91.

37. Dale A, Thomas JE, Darboe MK, et al. *Helicobacter pylori* infection, gastric acid secretion, and infant growth. *J Pediatr Gastroenterol Nutr*. 1998; 26(4): 393-7.

38. Passaro DJ, Taylor DN, Meza R, et al. J. Acute *Helicobacter pylori* infection is followed by an increase in diarrheal disease among Peruvian children. *Pediatrics*. 2001; 108(5): E87.

39. Isenbarger DW, Bodhidatta L, Hoge CW, et al. Prospective study of the incidence of diarrheal disease and *Helicobacter pylori* infection among children in an orphanage in Thailand. *Am J Trop Med Hyg*. 1998; 59(5): 796-800.

40. Cohen D, Shoham O, Orr N, et al. An inverse and independent association between *Helicobacter pylori* infection and the incidence of shigellosis and other diarrheal diseases. *Clin Infect Dis*. 2012; 54(4): e35-42.

41. Clemens J, Albert MJ, Rao M, et al. Impact of infection by *Helicobacter pylori* on the risk and severity of endemic cholera. *J Infect Dis*. 1995; 171(6): 1653-6.

42. Harris PR, Serrano CA, Villagran A, et al. *Helicobacter pylori*-associated hypochlorhydria in children, and development of iron deficiency. *J Clin Pathol*. 2012; 25: 25.

43. Lahner E, Persechino S, Annibale B. Micronutrients (Other than iron) and *Helicobacter pylori* Infection: A Systematic Review. *Helicobacter*. 2012; 17(1): 1-15.

44. Humphrey JH. Child undernutrition, tropical enteropathy, toilets, and handwashing. *Lancet*. 2009; 374(9694): 1032-5.

45. Kosek M, Haque R, Lima A, et al. Fecal markers of intestinal inflammation and permeability associated with the subsequent acquisition of linear growth deficits in infants. *Am J Trop Med Hyg*. 2013; 88(2): 390-6.

46. Xia HH, Talley NJ. Natural acquisition and spontaneous elimination of *Helicobacter pylori* infection: clinical implications. *Am J Gastroenterol*. 1997; 92(10): 1780-7.

Table 1. Participant Characteristics of Children at Enrollment

Variable	Total (N= 183)	*H. pylori* Infection 6-11 Months (n = 140)	*H. pylori* Infection 12-23 Months (n = 43)	*P*-value
Enrollment age, median months (IQR)	0.69 (0.46-1.02)	0.69 (0.46-0.92)	0.92 (0.49-1.35)	0.01
Male Sex, n (%)	92 (50)	70 (50)	22 (51)	0.89
Height, median cm (IQR)	52 (50.3-53.7)	51.7 (50.25-53.45)	53.2 (50.5-55.3)	0.04
Weight, median grams (IQR)	4020 (3502-4494)	3978 (3510.5-4405)	4330 (3494-4816)	0.07
Low Socioeconomic Status	68 (37)	57 (41)	11 (26)	0.07
Number stunted at enrollment, n %)	17 (9)	11 (8)	6 (14)	0.23
Number of Rooms in Home, median (IQR)	3 (2-4)	3 (2-4)	3 (2-4)	0.18
Percent of exclusive breastfeeding in first six months, median (IQR)	70.7 (33.8-85.4)	66.2 (34.8-85.2)	71.3 (23.6-86.5)	0.88
Water source in home for 24 hours, n (%)	158 (86)	120 (86)	38 (88)	0.77
Source of Water for Child Feeding, n (%)				
Pipe in House	43 (24)	36 (26)	7 (17)	0.26
Bottled Water	3 (2)	3 (2)	0	
Didn't use water for feeding child	134 (74)	99 (72)	35 (83)	

Table 2. Proportional Hazards Regression of Age at First *H. pylori* Infection

Characteristic (n = 187)	Hazard Ratio	95% Confidence Interval	*P*-value
Male Sex (female is reference)	0.88	0.61, 1.27	0.50
Low Socioeconomic Status (mid-to-high is reference)	1.59	1.16, 2.19	0.004
Height-for-Age Z Score	1.01	0.87, 1.16	0.91
Percent Exclusive Breast Feeding in first 6 months	0.63	0.40, 0.98	0.04
Days of Diarrhea	1.20	0.91, 1.57	0.18
Days of Antibiotic Use	0.64	0.36, 1.14	0.13

Table 3. Linear Mixed-Effects Model of Height (cm) by 24 months

Characteristic (n = 183)	Coefficient	95% Confidence Interval	*P*-Value
Male vs. Female	1.53	0.97, 2.09	<0.001
Percent exclusive breast feeding in first 6 months	0.04	-0.82, 0.90	0.92
Antibiotic use within two weeks of first infection	2.33	-0.40, 5.06	0.10
First detected *H. pylori* Infection at 6-11 months without diarrheal episodes	0.19	-0.48, 0.85	0.58
Average number of diarrheal episodes			
If first *H. pylori* infection at 6-11 months	-0.37	-0.60, -0.15	0.001
If first *H. pylori* infection at 12-23 months	0.08	-0.30, 0.46	0.68

Figure 1. Flowchart of Children Included in Analysis of the Association of *H. pylori* Infection on Childhood Growth.

Figure 2. Cumulative Incidence Function of First *H. pylori* Acquisition, by Socioeconomic Status (Low, Middle, High).

ONLINE SUPPLEMENT

To evaluate the association of *H. pylori* on longitudinal growth (height), we constructed linear mixed effects model as outlined below. The time metric, t, was age in months, and the dependent variable was height in centimeters. The fixed-effects component utilized truncated polynomial cubic splines, with cubic knots at 3, 6, 12, and 18 months. A random slope (b_0) and intercept (b_1) were added to account for heterogeneity in growth across children. In addition, we added a continuous autoregressive error of order 1 (i.e., $\rho^{(|t_ij - t_ik|)} \ for j \neq k$) to account for serial correlation as a consequence of repeated measurements of height within a child. The final model is shown below:

$$[height]_ij = \beta_0 + [b_0i + \beta]_1 t_ij + b_1i\, t_ij + \beta_2 [t_ij]^2 + \beta_3 [t_ij]^3 + [\beta_4 (t_ij - 3)]_+$$

$$\begin{pmatrix} b_0 \\ b_1 \end{pmatrix} \sim \text{MVN}\left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} g_{11} & g_{21} \\ g_{12} & g_{22} \end{bmatrix}\right)$$

$$\varepsilon_{ij} \sim N\left(\begin{bmatrix} 0 \\ \vdots \\ 0 \end{bmatrix}, \sigma^2 \begin{bmatrix} 1 & \dots & \rho^{|t_{i1} - t_{im_i}|} \\ \vdots & \ddots & \vdots \\ \rho^{|t_{im_i} - t_{i1}|} & \dots & 1 \end{bmatrix}\right)$$

Where:

t = age in months for child *i* and measurement *j*

Male = 1 if male, 0 if female

Breastfeeding = Percent of visits in the first 6 months where the caretaker reported exclusive breast feeding

SES Status = Socioeconomic status tertile by principal components analysis (PCA) score, 1= low SES, 2 = middle SES, 3 = high SES

Diarrheal Episodes = Cumulative number of diarrheal episodes

Early *H. pylori* infection = First infection by *H. pylori* between 0 and 11 months as 1, 0 if not

Antibiotic Use in Last Two Weeks = Any use of antibiotics in the two weeks prior to first detected *H. pylori* infection as 1, 0 if not

We added interaction terms for SES status and age, as well as Early *H. pylori* infection and Diarrheal Episodes. To evaluate the association of growth over the 24 months period, age was scaled to 24 months. In addition, we sought to evaluate the association of the mean number of diarrheal episodes on height, and so scaled the number of diarrheal episodes to the mean of 6.75. Below are the coefficients of the final model, without scaling:

Variable	**Coefficient**	**95% CI**	**P-Value**
Age	4.59	4.24, 4.95	<0.001
Age^2	-0.31	-0.47, -0.15	<0.001
Age^3	0.001	-0.02, 0.02	0.92
Knot (Age - 3)^3	0.02	-0.002, 0.05	0.07
Knot (Age - 6)^3	-0.02	-0.03, -0.02	<0.001
Knot (Age - 12)^3	-0.002	-0.004, 0.0003	0.09
Knot (Age - 18)^3	-0.004	-0.007, -0.0002	0.04
Male vs. Female	1.53	0.97, 2.09	<0.001
Breastfeeding	0.04	-0.82, 0.90	0.92
Socioeconomic Status			
Low	1.00	-	-
Middle	-0.47	-1.17, 0.22	0.18
High	0.02	-0.67, 0.72	0.95
Interaction of Socioeconomic Status and Age			
Low	1.00	-	-
Middle	0.05	0.008, 0.09	0.02
High	0.05	0.01, 0.10	0.01
Diarrheal Episodes	0.01	-0.05, 0.07	0.68
Early *H. pylori* Infection	0.19	-0.48, 0.85	0.58
Interaction of Early *H. pylori* Infection and	-0.07	-0.13, -0.005	0.03

Diarrheal Episode			
Antibiotic Use in Last 2 weeks	2.33	-0.40, 5.06	0.10