.DR BINEYAM TAYE (Orcid ID : 0000-0002-5583-9941)

Article type : Original Article-Epidemiology of Allergic Disease

Association between infection with *H. pylori* and atopy in young Ethiopian Children: a longitudinal study

Bineyam Taye[1∞], Fikre Enquselassie[2], Aster Tsegaye[3], Alemayehu Amberbir[4], Girmay Medhin[5], Andrew Fogarty[6], Karen Robinson[7] & Gail Davey[8]

1. Colgate University, Department of Biology, Hamilton, NY, USA
2. School of Public Health, College of Health Sciences, Addis Ababa University, Ethiopia.
3. School of Allied Health Sciences, College of Health Sciences, Addis Ababa University, Ethiopia.
4. Dignitas International, Malawi.
5. Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia.
6. Division of Epidemiology and Public Health, University of Nottingham, UK.
7. Nottingham Digestive Diseases Biomedical Research Centre, School of Medicine, University of Nottingham, UK.
8. Wellcome Trust Centre for Global Health Research, Brighton & Sussex Medical School, UK.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cea.12995

This article is protected by copyright. All rights reserved.

Accepted Article

Email address:

∞Bineyam Taye: btaye@colgate.edu

Fikre Enquselassie: fikreens@yahoo.com

Aster Tsegaye: tsegayeaster@yahoo.com

Alemayehu Amberbir**:** alamwo1@yahoo.com

Girmay Medhin: gtmedhine@yahoo.com

Andrew Fogarty: Andrew.Fogarty@nottingham.ac.uk

Karen Robinson: karen.robinson@nottingham.ac.uk

Gail Davey: g.davey@bsms.ac.uk

∞Corresponding author, Colgate University, Department of Biology, 214 Olin Hall, 13 Oak Dr. Hamilton, NY, 13346, USA, Phone: 315-228-7398, e-mail: btaye@colgate.edu

Abstract

Background: Epidemiological evidence from developed countries indicates that *Helicobacter pylori* infection correlates with a reduced risk of atopy and allergic disorders, however limited data are available from low-income countries.

Objective: We examined associations between *H. pylori* infection in early childhood and atopy and reported allergic disorders at the age of 6.5 years in an Ethiopian birth cohort.

This article is protected by copyright. All rights reserved.

Accepted Article

Methods: A total of 856 children (85.1% of the 1006 original singletons in a population-based birth cohort) were followed up at age six and half years. An interviewer-led questionnaire administered to mothers provided information on demographic and lifestyle variables. Questions on allergic disease symptoms were based on the International Study of Asthma and Allergies in Children (ISAAC) core allergy and environmental questionnaire. Serum samples were analysed for total IgE levels and anti-*H. pylori* cytotoxin associated gene A (CagA) IgG antibody using commercially available ELISA kits. Stool samples were analysed for *H. pylori* antigen using a rapid immunochromatographic test. The independent effects of *H. pylori* infection (measured at age 3, 5 and 6.5 years) on prevalence and incidence of atopy and reported allergic disorders (measured at age 6.5 years) were determined using multiple logistic regression.

Results: In cross-sectional analysis, current *H. pylori* infection at age 6.5 years was inversely, though not significantly, related to prevalence of atopy and 'any allergic condition' at age 6.5 years. However detection of *H. pylori* infection at any point up to age 6.5 years was associated with a significantly reduced odds of both atopy and 'any allergic condition' (adjusted OR AOR, 95% CI, 0.54; 0.32 to 0.92, p=0.02, and 0.31; 0.10 to 0.94, p=0.04, respectively). In longitudinal analyses, *H. pylori* infection at age 3 was inversely associated with incidence of atopy (AOR, 95% CI, 0.49; 0.27 to 0.89, p=0.02). Furthermore, among *H. pylori* infected children, those with a CagA+ strain had a more pronounced reduction in odds of atopy (AOR=0.35 vs. 0.63 for CagA+ vs. CagA-) and this reduction reached borderline significance.

Conclusion: These data are consistent with the hypothesis that early exposure to *H. pylori* is inversely associated with atopy and allergic conditions. A possible modest protective association against atopy was observed in those infected with a more virulent CagA+ strain of *H. pylori*.

This article is protected by copyright. All rights reserved.

Accepted Article

Key words: *Helicobacter pylori*, atopy, allergic disorders, birth cohort, Ethiopia

Accepted Article

Introduction

The prevalence of IgE-mediated atopy and allergic diseases has been increasing considerably over the past 30 years, and this is particularly apparent in developed countries(1, 2). In conjunction with evidence from other developing and transitional societies (3, 4), this indicates that factors associated with urbanization and/or relative affluence are likely to be responsible for these emerging allergic disorders. A potential explanation for this was given by Strachan, who first proposed the hygiene hypothesis (5). The 'hygiene hypothesis' (now revised as the 'old friends hypothesis') (6) postulates that the rise of allergic disease in recent decades is attributable to a decrease in exposure to certain microbes and infections during childhood, and has gained wide acceptance (7). Microbial infections, which are less prevalent in populations with improved lifestyles and better hygiene, may play a protective role in the aetiology of asthma and allergy (8, 9). Several viral and parasitic pathogens, including influenza viruses and helminths have been reported to play a role in protection against asthma and allergy (10, 11). *Helicobacter pylori* is a common bacterium, which is estimated to be present in the stomach of around half the world's population. It usually colonises the gastric mucosa from early childhood, where it persists lifelong unless treated with antibiotics. It is the major cause of peptic ulceration and gastric cancer, however disease occurs in only 10-15% of cases and the infection usually remains asymptomatic. *H. pylori* has co-evolved with humans over thousands of years, however the prevalence of the infection (particularly amongst children in developed countries) has been declining rapidly over the last two decades (12-14). Important epidemiologic evidence for the role of *H. pylori* in the hygiene hypothesis has recently emerged, and the infection has been associated with a reduced risk of allergic disorders including asthma (15), wheeze (16), eczema (17-19) , and atopy (20-22). This association has also been reported to be stronger with more pathogenic strains of *H. pylori* which express the virulence factor cytotoxin

This article is protected by copyright. All rights reserved.

associated gene A (CagA) (23, 24). Infection with these strains reportedly induces a higher-level T-helper 1 (Th1) cellular immune response (24, 25), which may counterbalance the pro-allergic Th2 response. CagA+ strains usually also co-express the most active form of the vacuolating cytotoxin A (VacA), and this is important in the induction of immunosuppressive regulatory T cell (Treg) responses (26). Higher frequencies of IL-10-secreting Tregs were shown to be present in the peripheral blood of *H. pylori*-infected patients in the UK, and serum IgE concentrations were lowest when there was a strong Treg response. Mechanistic *in vitro* experiments showed that the systemic IL-10+ Treg response is likely to play an important role in *H. pylori*-mediated protection against allergy in humans (26). A number of studies with mouse models of allergy and asthma have very convincingly demonstrated that *H. pylori* infection is protective and that VacA-induced Tregs play a major role (27-29). Despite this convincing evidence, however, others have reported no epidemiological association between the infection and asthma (30, 31) or atopy (31, 32). This inconsistency may be attributed to the way in which allergic disease is defined or the heterogeneity of the study designs and study populations.

Our recent meta-analysis, using 16 observational studies showed a significant protective effect of *H. pylori* infection against objectively measured atopy (33). However, most studies included in this analysis were cross-sectional, with only two prospective studies (34, 35), based in adult high-income country populations, and lacked data on children from low-income countries. This is important as infection with *H. pylori* is usually acquired very early in childhood (36), when the immune system is developing. It is possible that timing of exposure to *H. pylori* infection may be important in modulating its effects, and any long-term protection that arises from effects occurs during the crucial early period of immune development.

Accepted Article

This article is protected by copyright. All rights reserved.

Our previous work on the Butajira Birth Cohort in rural Ethiopia demonstrated an inverse association of *H. pylori* infection with skin sensitization to either *D. pteronyssinus* or cockroach allergen (37) measured by skin prick test at age 5. Although this method provides a convenient test for atopy in epidemiological studies (38), and the allergens used are believed to be predominant in Ethiopian populations(8), this approach will not necessarily identify all cases of atopy. It is therefore important to consider atopy as defined in terms of total serum IgE, since it has been suggested that this provides an overall estimate of allergic disorders (39, 40). We therefore expanded our previous observations by collecting additional data on total serum IgE levels when the child reached 6.5 years, in order to investigate a possible association between atopy (defined by a raised total serum IgE level) and *H. pylori* infection. Additionally, and for the first time in this cohort, we also tested whether the presence of a CagA+ *H. pylori* strain was associated with atopy and allergic conditions.

METHODS

Study Setting and Design

A detailed description of the original Butajira birth cohort study has been published previously (17, 41). Briefly, the birth cohort is nested in the Butajira Demographic Surveillance Site (42) which covers a sample of nine rural and one urban administrative units in and around the town of Butajira in Southern Ethiopia (43). Between July 2005 and February 2006, all women in the DSS aged 15–49 and in their third trimester of pregnancy were identified by the DSS fieldworkers and invited to participate in the study. Of the 1,234 eligible women, 1,065 were recruited (86% of those eligible) and all live singleton babies born to these women (n = 1006) were followed-up as a birth cohort (figure 1).

Accepted Article

This article is protected by copyright. All rights reserved.

Measurement and Data collection

At age 6.5 follow-ups visits took place, where the same project data collectors visited the child at home and collected information related to allergic outcomes. Questions on allergic disease symptoms were based on the International Study of Asthma and Allergies in Children (ISAAC) core allergy and environmental questionnaire (44), and included wheeze (*'In the last 12 months has your child had wheezing or whistling in their chest?*'), asthma (*'In the last 12 months has your child had asthma*?'), hay fever *('In the last 12 months has your child had problems with sneezing or running nose (when not affected by cold or flu), or problems with itchy watery eyes?')* and eczema (*'In the last 12 months has your child ever had an itchy skin rash which has affected the skin creases, eg, front of the elbow, behind the knees, the front of the ankles, around the neck, or around the eyes?')*. The questionnaire also asked about various potential confounders including environmental factors (sanitation and water supply, household roof/wall/floor type, indoor smoking, presence of animals, insecticide use, cooking facilities), asthma and allergy in the family, household size, child's sleeping place, siblings, birth order, and child's use of medication (use of paracetamol and antibiotics).

In addition to the questionnaire data, at the age 3, 5 and 6.5 year visits, mothers were also asked to collect a faecal sample from their child using a leak-proof plastic container. The samples were then transported for analysis in the Butajira Health Center laboratory to ascertain the child's *H. pylori* and intestinal parasite infection status. Furthermore, at the 6.5 year visit, a 5 ml blood sample was collected from each child using a vacutainer tube. Serum samples were separated within 2 hrs. of collection at Butajira hospital and then transported to the Ethiopian Public Health Institute (EPHI) Research Laboratory, Addis Ababa for serum CagA and IgE ELISA analyses.

This article is protected by copyright. All rights reserved.

Accepted Article

Laboratory analyses

H. pylori status

H. pylori status was evaluated using the commercially available SD Bioline *H. pylori* stool antigen test (Standard Diagnostics, Inc) according to the manufacturer's instructions. A portion of faeces (approximately 50mg) from each stool sample was mixed with assay diluent solution, until the sample had been dissolved, before allowing the suspension to settle for 5 minutes at room temperature. 100 µL of the liquid phase of the sample was placed on the *H. pylori Ag* test strip. The test results were checked after 15 minutes, where one red line indicated a negative result and a double red line indicated a *H. pylori* positive result.

Additionally, all faecal samples were examined qualitatively using the modified formol-ether concentration method to ascertain the child's intestinal parasites infection status.

H. pylori CagA status

Serum anti-CagA IgG antibody status was assessed using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Genesis Diagnostics Ltd, UK), as per the manufacturer's instructions. The optical densities of the wells were measured using a microplate reader at 450nm. Values above the 6.25 U/ml standard were defined as positive. According to the manufacturer, the sensitivity and specificity of this kit are 96.3% and 96.7%, respectively.

Accepted Article

This article is protected by copyright. All rights reserved.

Accepted Article

Total IgE analysis

Total IgE levels were measured using a commercially available ELISA kit (Omega diagnostic, UK), according to the manufacturer's instructions. The optical densities (ODs) of the standards, positive control and samples were measured using a microplate reader at 450nm. Elevated IgE levels were determined based on the manufacturer's recommended threshold by age range. For this study total IgE levels greater than 144 IU/ml for age 5-10 were considered as the cut-off value to define atopy.

Outcome definition

Atopy was defined in this study as an elevated total serum IgE. A total serum IgE concentration of ≤ 144 IU/ml for children aged 5-10 years was considered to be normal based on reference ranges provided by the manufacturer's recommended threshold by age range.

'Any allergic condition' was defined as a positive response to one or more of the questions on asthma, hay fever and eczema in the past 12 months, at years 6.5 follow-up.

Statistical analysis

Data were double entered into EpiData 3.1 (EpiData, Denmark). The datasets were cleaned, coded and merged ready for analysis using Stata 12 (Statacorp, College Station, Texas, USA).

Prior to investigating the association between *H. pylori* infection and atopy, univariate analyses were used to identify the possible confounders. Variables that were associated with both exposure and outcome variables in the crude analysis using statistical significance at p value <0.2 were considered to be possible confounders. Additionally, we included variables previously shown to be associated with atopy and /or elevated total IgE in the literature; child's sex (45), smoking (46), antibiotic use (47) and intestinal parasite status (48)

This article is protected by copyright. All rights reserved.

We estimated odds ratios (ORs) associated with *H. pylori* positivity for atopy and 'any allergic condition' using multivariate logistic regression. The ORs were adjusted for *a priori* and potential confounders listed in Table S1. The same approach was used in a separate set of analyses to assess the effect of CagA *H. pylori* strain infection on atopy and self-reported allergic symptoms for all available children at year 6.5, by creating a new exposure variable with categories representing different combinations of *H. pylori* and Cag A exposure status: 'Negative' (negative for both *H. pylori* and CagA strain), '*H. pylori positive and CagA Negative* ' (positive for *H. pylori* but negative for Cag A strain) and '*H. pylori positive and CagA positive'* ***(***positive for both *H. pylori* and CagA strain). At age 6.5 year follow up visit, the prevalence of self-reported allergy symptoms was low, ranging from 0.5% for eczema and hay fever to 2.2% for wheeze, we therefore create a new outcome variable 'Any allergic condition at age 6.5' (i.e. positive response either to wheeze, or eczema, or hay fever in the past 12 months, at years 6.5 follow-up).

For longitudinal analysis of incident atopy between ages 3 and 6.5, those children without atopy (defined as 'negative to any skin sensitization test (SPT), i.e. skin prick test to either *Dermatophagoides pteronyssinus* or cockroach allergen <3mm diameter) at age 3 were selected for analysis and incident atopy defined as a raised total serum IgE level at the year 6.5 follow up. Furthermore, children with self-reported allergic symptoms such as wheeze; eczema and hay fever at ages 3 (positive response to wheeze, eczema, and hay fever in the past 12 months, at years 3, and ever at year 3) were excluded for analysis of incident atopy at the year 6.5 follow up.

Covariates were kept in the model if they changed the coefficient of exposure (*H. pylori* infection) by > 10% or if they were independently associated with the outcome at $p < 0.10$. Probability values < 0.05 were considered statistically significant for main effects

This article is protected by copyright. All rights reserved.

Accepted Article

Sensitivity analysis was done to compare the distribution of demographic and life style variables between study subject who have complete outcome data (i.e. “complete-case”) and the "all respondents" populations.

Ethical Approval

The study was approved by the Institutional Review Board (IRB) of Addis Ababa University, College of Health Sciences, Ethiopia, and the University Of Nottingham Medical School Ethics Committee. Written, informed consent was obtained from the mothers after they have been clearly informed about the study, and in keeping with the requirements of the College of Health Sciences IRB all women and their children were reimbursed for health care costs. Children were also requested to give assent and were informed of their right to refuse to participate in the study and to withdraw at any time during the study without jeopardizing their right of access to other health services. Invasive procedures such as collection of blood samples were fully explained to parents and children, and were carried out using sterile disposable materials

Results

Description of cohort participants followed-up at age 6.5 years

A detailed description of the cohort at years 1, 3, and 5 is reported elsewhere (17, 37, 43). At 6.5 years, a total of 856 singleton children were successfully followed-up (85.1% of the original cohort at birth, and 99.3% of those available at year 5 follow-up), of whom 713 had total serum IgE measurement and 848 had *H. pylori* data at the 6.5 year follow up visit (Fig 1).

Accepted Article

This article is protected by copyright. All rights reserved.

Demographic and lifestyle characteristics of study participants at 6.5-year follow-up visit

Of children enrolled at the 6.5 year follow up visit, 51.2% (434/848) were male and the majority, 88.2% (748/848) were from a rural area. Maternal demographic characteristics showed that 47.4% (402/848) of the mothers belonged to the Meskan ethnic group, 78.4% (665 /848) were Muslim, 71.6% (607/848) were illiterate and 83.8% were housewives (709/848). Most mothers (61.3%, 520/848) reported using piped water as their primary drinking source. Selected early life characteristics at 2 months and 1 year of age showed that 57.5% had been vaccinated at 2 months and only 11.7 (99/848) had received vitamin A supplementation at 1 year (online supplementary: Table S1).

Comparing the distribution of demographic and life style variables between all study subjects and those who have complete outcome data (i.e. "all respondents" and “complete-case”), found similar distribution patterns in relation to demographic and life style characteristics (online supplementary: Table S2).

Prevalence of atopy and allergic conditions among children followed up at age 6.5

The prevalence of atopy among children enrolled at the 6.5-year follow up visit was 18.1% (129/713), with a higher proportion among rural (20.0%) than urban (6.1%) residents, $p<0.05$ (Table 2). Using an ISAAC core allergy and environmental questionnaire, wheezing was reported in 2.2% (18/806) of children whilst eczema and hay fever was reported in less than 1% of the children (Table 1). When any combination allergic condition considered, the prevalence reached 3.0% (24/806), with a similar proportion among urban and rural children, (3.1% vs. 3.0% in urban and rural, respectively, $p= 0.93$, Table 1).

Accepted Article

This article is protected by copyright. All rights reserved.

Accepted Article

Potential confounders of atopy

The main outcome variable showed no statistically significant association with most demographic and lifestyle variables, including child's sex, history of vaccination, use of antibiotics, and intestinal parasite infection at age 6.5. Neither were family history of allergic conditions (maternal and paternal), common environmental factors such as household roof type, indoor smoking, insecticide use, and child's sleeping place significantly associated with atopy. However, atopy was significantly associated with place of residence, indoor cooking, presence of large animal(s) inside the house, and higher numbers of older siblings (Table S3).

Association between *H. pylori* infection and atopy or allergic conditions at age 6.5

The prevalence of atopy was lower in children currently infected with *H. pylori* (15.2%) than in those not infected (18.6%), but this did not achieve statistical significance (p = 0.46). In multivariate analysis adjusted for *a priori* confounders, *H. pylori* infection at age 6.5 was inversely, though not significantly, related to prevalence of atopy and 'any allergic condition' at age 6.5 years (adjusted OR, 95% CI, 0.87; 0.45 to 1.70, p=0.69). When the analysis was restricted to those children who had *H. pylori* infection at age 3, 5 and 6.5 years, or those who were ever infected up to age 6.5, a more pronounced reduction in odds of atopy and 'any allergic condition' was demonstrated. This reduction reached statistical significance (adjusted OR, 95% CI, 0.54; 0.32 to 0.92, p=0.02, and 0.31; 0.10 to 0.94, p=0.04, respectively Tables 2&3).

This article is protected by copyright. All rights reserved.

Association between CagA+ *H. pylori* infection with atopy and allergic condition at age 6.5

Separate logistic regression models related atopy (outcome) to the individual estimates of CagA *H. pylori* infection status at age 6.5 years (exposure). These showed a non-significant reduction in the odds of atopy among *H. pylori*+ CagA- children (Adjusted OR=0.63, 95%CI, 0.21 to 1.87, p=0.63) compared to non-infected children. Children infected with CagA+ strains of *H. pylori* showed a more pronounced reduction in odds of atopy and reached a borderline significant association (Adjusted OR=0.35, 95%CI, 0.12 to 1.01, p=0.051) (Table 4).

A broadly similar pattern, but non-significant inverse association was also observed between exposure to CagA+ *H. pylori* and any combination of self-reported allergic symptoms ('any allergic condition') at age 6.5 (Adjusted OR=0.73, 95%CI, 0.10 to 5.56, p=0.76) (Table 5).

Effects of *H. pylori* infection on incidence of atopy between ages 3 and 6.5: Longitudinal Analysis

Table 6 presents the models for the longitudinal analysis between *H. pylori* infection and atopy Data are presented for the population without atopy (defined as 'negative to any skin sensitisation test (SPT)) at age 3 to examine the effect of *H. pylori* infection at age 3 on the new onset of atopy (defined as raised total serum IgE level) at age 6.5. *H. pylori* infection at age 3 was inversely associated with incident atopy, after control for *a priori* and identified confounders (adjusted OR, 95% CI, 0.49; 0.27 to 0.89, p=0.02) (Table 6). Adjustment for other potential confounders did not materially change the odds ratio.

Accepted Article

This article is protected by copyright. All rights reserved.

Discussion

This study provides further evidence from a low-income, population-based birth cohort that current *H. pylori* infection at age 6.5 is inversely, though not significantly, related to prevalence of atopy (defined as a raised total IgE level). Any previous exposure to *H. pylori* infection up to age 6.5 was significantly associated with a 46% decreased odds of atopy. We also explored the effects of CagA+ strains of *H. pylori* infection for the first time in this cohort at the age 6.5-year follow-up visit. Infection with a CagA+ *H. pylori* strain was associated with a 65% decreased risk of atopy, and reached borderline statistical significance. In longitudinal analyses, infection with *H. pylori* at age 3 was associated with a 51% decreased risk of incident atopy at age 6.5 years.

We do not think that our findings result from selection bias for several reasons. First, the study cohort maintained high follow-up proportions throughout the entire study period: 85.1% of the original cohort at birth, and 99.3% of those available at year 5 provided data at subsequent follow-up. Secondly, children who had IgE measurement results did not differ with regard to environmental, demographic and life style variables from those who had no IgE measurements. We also used a highly sensitive and specific *H. pylori* stool antigen test (49). In addition, the key outcome (atopy) was defined using age-specific total IgE cutoffs, though the sensitivity and specificity of serum total IgE determination still remains controversial (50, 51). With normal values differing in their range, an upper limit of 144 IU/ml for total IgE levels in children is generally accepted to distinguish atopics from non-atopics (51).

The role of total IgE as a biomarker for atopy and allergic disease has been suggested in several studies (39, 51-53). Two cross-sectional studies by Burrows *et al* (39) and Sunyer *et al* (52), reported an independent effect of total IgE on asthma using the data from Tucson and the European Community Respiratory Health Survey (ECRHS) study, respectively. Others demonstrated a strong positive relationship between markers of the atopic syndrome and serum total IgE levels in children (53), and suggested total IgE as a tool to discriminate those with and without atopy (51).

This article is protected by copyright. All rights reserved.

Accepted Article

Despite these observations, however, elevated IgE levels are occasionally observed in disorders like parasitic infection (48), myeloma (54), and chronic inflammatory bowel diseases (55). Even though these conditions remain a possibility, in this cohort all study participants were apparently healthy children, and the magnitude of intestinal parasitosis was reported very low at age 3 (17). This suggests the role of intestinal parasites in modulating the immune system and increasing serum IgE levels is likely to be low in this study cohort.

In this study, the exposure variable representing current *H. pylori* infection (being positive for stool antigen test) at age 6.5 was inversely, though not significantly, related to prevalence of atopy (defined as raised total IgE level). Any previous exposure up to age 6.5 (suggestive of early age exposure), was significantly associated with a 46% decreased risk of atopy (defined as raised total IgE level). Other studies have also shown no cross-sectional associations between *H. pylori* infection and raised total IgE among older children (age 7.1-15.0 years) in Finland (56), and in young adults in Germany and Spain (57); the latter study reported a similar magnitude of effect estimate as our study (OR =0.88, vs. 0.87 in the current study) (57). Failure to detect a significant protective effect of infections on the risk of atopy and allergic disease at age 6.5 might be related to the age of the population being studied and/or the time of infection. Early life infection is considered to have a stronger protective effect due to the fact that programming of Th-cell memory against allergens commonly occurs during early childhood (58); the critical time period during which immunomodulation with long-lasting effects is considered most successful, probably even within the first 2 years of life (59). Thus, infections occurring later in childhood, may have shifted risk estimates towards no effect among the children studied, i.e., could have masked potentially protective effects. A study in Japan reported strong inverse associations between *H. pylori* infection and serum IgE level in younger than older adults (60).

Accepted Article

This article is protected by copyright. All rights reserved.

Similar patterns of significant inverse associations between any previous *H. pylori* infection up to age 6.5, but not at age 6.5, with an outcome variable 'any allergic condition' (defined as a positive response to one or more of asthma, hay fever and eczema) also fit with previous observations from a large population based study in the United States, by Chen and Blaser (15), who used data from the 1999-2000 *National Health and Nutrition Examination Survey* (NHANES), and stratified their analysis according to participants age (3–19 years). This revealed a strong inverse association between *H. pylori* infection and onset of asthma among children age 2-4 years old (OR: 0.32), but this inverse association became weaker and non-significant when the analysis was done among children age greater than 5 years (OR:0. 74).

The findings of this study must, however, be interpreted with caution. Although the atopy outcome was measured objectively using serum IgE, measures of wheeze, eczema, hayfever and asthma were based on maternal questionnaire reporting, and hence are susceptible to misclassification and recall bias. The questionnaire was based on the widely used and validated ISAAC symptoms questionnaire (44) that has been successfully used in previous studies in adults in Butajira (61), in under-five children (62, 63) and in older age groups in Jimma, Ethiopia (8, 64), increasing the validity of our findings.

This study also provides a borderline significant inverse association between colonization with *H. pylori* CagA+ strains and atopy (defined as raised total IgE) at age 6.5 (Adjusted OR=0.35, 95%CI, 0.12 to 1.01, p=0.051). Most of the available observational studies did not investigate the role of CagA strains on allergic disorders in general, and on atopy (defined as raised total IgE) in particular. Only one study from the US National Health and Nutrition Examination Survey (23) reported an inverse association between colonization with *H. pylori* CagA+ strains and skin sensitization to pollen and moulds (as measured by SPT).

This article is protected by copyright. All rights reserved.

Accepted Article

Although it may be difficult to compare the current finding due to the difference in outcome measurements (Total IgE vs. SPT to specific allergens), previous studies indicated a good correlation between an elevated total IgE and SPT in predicting atopy in children (65, 66). Comparing SPT to total serum IgE levels, Fajraoui *et al.* found that the two tests agree in 80% of cases (67). Matricardi *et al.* followed total and allergen- specific IgE levels in children from birth to 13 years of age (68). This study revealed that the evolution of total IgE was extremely heterogeneous but was parallel with that of allergen-specific IgE from the age of 5 onwards.

The hypothesis that more virulent CagA+ strains of *H. pylori*, may have a stronger protective effects against asthma and allergic diseases has been reported in a few epidemiological and animal studies (23, 24, 26). Although we found a borderline significant association in our study, the magnitude and direction of observed effect estimate was in the expected pattern, and our analyses were limited by insufficient statistical power as a result of the low prevalence of *H. pylori* infection at age 6.5 years. The low prevalence of *H. pylori* infection at the age 6.5 follow-up visit in this cohort may be linked to spontaneous elimination of the bacterium (69), or better attention to health issues in older children, or use of antibiotics for other common diseases (70). Further study is warranted to understand the exact reason of declining *H. pylori* prevalence in older children in Ethiopia.

In conclusion, our study among young children from a low-income birth cohort provides further evidence that any exposure of *H. pylori* up to age 6.5, suggestive of early colonization, is inversely associated with atopy and any allergic condition. A modest inverse association with atopy was observed among children infected with the more virulent CagA+ strains of *H. pylori*.

This article is protected by copyright. All rights reserved.

Accepted Article

Acknowledgments

We gratefully thank the mothers and children in the birth cohort who generously provided information, and the project data collectors and the laboratory technicians for their commitment during the fieldwork. The study was supported by the National Institute for Health Research (NIHR), through the NIHR Biomedical Research Centre at Nottingham University Hospitals NHS Trust and the University of Nottingham. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Competing interests

We declare that we do not have any conflicts of interest.

Authors' contributions

BT conceived and designed the study and collected data in the field and performed data analysis, and drafted the manuscript. AA and GM participated in data collection, assisted with the design, performed analysis, interpretation of data and the critical review of the manuscript. GD, FE, AT, KR and AF participated in study design and interpretation of data, helped in drafting the manuscript and critically reviewed the manuscript. All authors read and approved the final manuscript.

Accepted Article

This article is protected by copyright. All rights reserved.

Accepted Article

References

1. Kosunen TU, Hook-Nikanne J, Salomaa A, Sarna S, Aromaa A, Haahtela T. Increase of allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish population and a possible relationship to Helicobacter pylori infections. Clin Exp Allergy. 2002;32(3):373-8.

2. Asher MI, Montefort S, Bjorksten B, Lai CK, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet (London, England). 2006;368(9537):733-43.

3. Woolcock AJ, Green W, Alpers MP. Asthma in a rural highland area of Papua New Guinea. Am Rev Respir Dis. 1981;123(5):565-7.

4. Chan-Yeung M, Zhan LX, Tu DH, Li B, He GX, Kauppinen R, et al. The prevalence of asthma and asthma-like symptoms among adults in rural Beijing, China. Eur Respir J. 2002;19(5):853-8.

5. Strachan DP. Hay fever, hygiene, and household size. Bmj. 1989;299(6710):1259-60.

6. Rook GA. Regulation of the immune system by biodiversity from the natural environment: an ecosystem service essential to health. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(46):18360-7.

7. Strachan DP. Family size, infection and atopy: the first decade of the "hygiene hypothesis". Thorax. 2000;55(1):S2-10.

8. Scrivener S, Yemaneberhan H, Zebenigus M, Tilahun D, Girma S, Ali S, et al. Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study. Lancet (London, England). 2001;358(9292):1493-9.

9. Matricardi PM, Rosmini F, Riondino S, Fortini M, Ferrigno L, Rapicetta M, et al. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. Bmj. 2000;320(7232):412-7.

10. Wilson MS, Taylor MD, Balic A, Finney CA, Lamb JR, Maizels RM. Suppression of allergic airway inflammation by helminth-induced regulatory T cells. J Exp Med. 2005;202(9):1199-212.

11. Kitagaki K, Businga TR, Racila D, Elliott DE, Weinstock JV, Kline JN. Intestinal helminths protect in a murine model of asthma. J Immunol. 2006;177(3):1628-35.

12. Atherton JC, Blaser MJ. Coadaptation of Helicobacter pylori and humans: ancient history, modern implications. J Clin Invest. 2009;119(9):2475-87.

13. Banatvala N, Mayo K, Megraud F, Jennings R, Deeks JJ, Feldman RA. The cohort effect and Helicobacter pylori. J Infect Dis. 1993;168(1):219-21.

14. White JR, Winter JA, Robinson K. Differential inflammatory response to Helicobacter pylori infection: etiology and clinical outcomes. Journal of inflammation research. 2015;8:137-47.

This article is protected by copyright. All rights reserved.

15. Chen Y, Blaser MJ. Helicobacter pylori colonization is inversely associated with childhood asthma. J Infect Dis. 2008;198(4):553-60.

16. Holster IL, Vila AM, Caudri D, den Hoed CM, Perez-Perez GI, Blaser MJ, et al. The impact of Helicobacter pylori on atopic disorders in childhood. Helicobacter. 2012;17(3):232-7.

17. Amberbir A, Medhin G, Erku W, Alem A, Simms R, Robinson K, et al. Effects of Helicobacter pylori, geohelminth infection and selected commensal bacteria on the risk of allergic disease and sensitization in 3-year-old Ethiopian children. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2011;41(10):1422-30.

18. Herbarth O, Bauer M, Fritz GJ, Herbarth P, Rolle-Kampczyk U, Krumbiegel P, et al. Helicobacter pylori colonisation and eczema. J Epidemiol Community Health. 2007;61(7):638-40.

19. McCune A, Lane A, Murray L, Harvey I, Nair P, Donovan J, et al. Reduced risk of atopic disorders in adults with Helicobacter pylori infection. Eur J Gastroenterol Hepatol. 2003;15(6):637-40.

20. Pessi T, Virta M, Adjers K, Karjalainen J, Rautelin H, Kosunen TU, et al. Genetic and environmental factors in the immunopathogenesis of atopy: interaction of Helicobacter pylori infection and IL4 genetics. Int Arch Allergy Immunol. 2005;137(4):282-8.

21. von Hertzen LC, Laatikainen T, Makela MJ, Jousilahti P, Kosunen TU, Petays T, et al. Infectious burden as a determinant of atopy-- a comparison between adults in Finnish and Russian Karelia. Int Arch Allergy Immunol. 2006;140(2):89-95.

22. Seiskari T, Kondrashova A, Viskari H, Kaila M, Haapala AM, Aittoniemi J, et al. Allergic sensitization and microbial load--a comparison between Finland and Russian Karelia. Clin Exp Immunol. 2007;148(1):47-52.

23. Chen Y, Blaser MJ. Inverse associations of Helicobacter pylori with asthma and allergy. Arch Intern Med. 2007;167(8):821-7.

24. Reibman J, Marmor M, Filner J, Fernandez-Beros ME, Rogers L, Perez-Perez GI, et al. Asthma is inversely associated with Helicobacter pylori status in an urban population. PloS one. 2008;3(12):29.

25. Wang SK, Zhu HF, He BS, Zhang ZY, Chen ZT, Wang ZZ, et al. CagA+ H pylori infection is associated with polarization of T helper cell immune responses in gastric carcinogenesis. World journal of gastroenterology. 2007;13(21):2923-31.

26. Hussain K, Letley DP, Greenaway AB, Kenefeck R, Winter JA, Tomlinson W, et al. Helicobacter pylori-Mediated Protection from Allergy Is Associated with IL-10-Secreting Peripheral Blood Regulatory T Cells. Frontiers in immunology. 2016;7:71.

27. Arnold IC, Dehzad N, Reuter S, Martin H, Becher B, Taube C, et al. Helicobacter pylori infection prevents allergic asthma in mouse models through the induction of regulatory T cells. J Clin Invest. 2011;121(8):3088-93.

This article is protected by copyright. All rights reserved.

Accepted Article

28. Oertli M, Sundquist M, Hitzler I, Engler DB, Arnold IC, Reuter S, et al. DC-derived IL-18 drives Treg differentiation, murine Helicobacter pylori-specific immune tolerance, and asthma protection. The Journal of clinical investigation. 2012;122(3):1082-96.

29. Engler DB, Reuter S, van Wijck Y, Urban S, Kyburz A, Maxeiner J, et al. Effective treatment of allergic airway inflammation with Helicobacter pylori immunomodulators requires BATF3-dependent dendritic cells and IL-10. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(32):11810-5.

30. Jun ZJ, Lei Y, Shimizu Y, Dobashi K, Mori M. Helicobacter pylori seroprevalence in patients with mild asthma. Tohoku J Exp Med. 2005;207(4):287-91.

31. Fullerton D, Britton JR, Lewis SA, Pavord ID, McKeever TM, Fogarty AW. Helicobacter pylori and lung function, asthma, atopy and allergic disease--a population-based cross-sectional study in adults. Int J Epidemiol. 2009;38(2):419-26.

32. Baccioglu A, Kalpaklioglu F, Guliter S, Yakaryilmaz F. Helicobacter pylori in allergic inflammation--fact or fiction? Allergol Immunopathol. 2008;36(2):85-9.

33. Taye B, Enquselassie F, Tsegaye A, Medhin G, Davey G, Venn A. Is Helicobacter Pylori infection inversely associated with atopy? A systematic review and meta-analysis. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2015;45(5):882-90.

34. Seiskari T, Viskari H, Kaila M, Haapala AM, Koskela P, Hyoty H. Time trends in allergic sensitisation and Helicobacter pylori prevalence in Finnish pregnant women. Int Arch Allergy Immunol. 2009;150(1):83-8.

35. Cullinan P, Harris JM, Newman Taylor AJ, Jones M, Taylor P, Dave JR, et al. Can early infection explain the sibling effect in adult atopy? Eur Respir J. 2003;22(6):956-61.

36. Malaty HM, El-Kasabany A, Graham DY, Miller CC, Reddy SG, Srinivasan SR, et al. Age at acquisition of Helicobacter pylori infection: a follow-up study from infancy to adulthood. Lancet. 2002;359(9310):931-5.

37. Amberbir A, Medhin G, Abegaz WE, Hanlon C, Robinson K, Fogarty A, et al. Exposure to Helicobacter pylori infection in early childhood and the risk of allergic disease and atopic sensitization: a longitudinal birth cohort study. Clin Exp Allergy. 2014;44(4):563-71.

38. Oryszczyn MP, Annesi I, Neukirch F, Dore MF, Kauffmann F. Relationships of total IgE level, skin prick test response, and smoking habits. Ann Allergy. 1991;67(3):355-8.

39. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. The New England journal of medicine. 1989;320(5):271-7.

40. Pearce N, Pekkanen J, Beasley R. How much asthma is really attributable to atopy? Thorax. 1999;54(3):268-72.

Accepted Article

This article is protected by copyright. All rights reserved.

41. Hanlon C, Medhin G, Alem A, Tesfaye F, Lakew Z, Worku B, et al. Impact of antenatal common mental disorders upon perinatal outcomes in Ethiopia: the P-MaMiE population-based cohort study. Trop Med Int Health. 2009;14(2):156-66.

42. Santos IS, Boccio J, Davidsson L, Hernandez-Triana M, Huanca-Sardinas E, Janjetic M, et al. Helicobacter pylori is not associated with anaemia in Latin America: results from Argentina, Brazil, Bolivia, Cuba, Mexico and Venezuela. Public Health Nutr. 2009;12(10):1862-70.

43. Medhin G, Hanlon C, Dewey M, Alem A, Tesfaye F, Worku B, et al. Prevalence and predictors of undernutrition among infants aged six and twelve months in Butajira, Ethiopia: the P-MaMiE Birth Cohort. BMC Public Health. 2010;10(27):1471-2458.

44. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. The European respiratory journal. 1995;8(3):483-91.

45. Omenaas E, Bakke P, Elsayed S, Hanoa R, Gulsvik A. Total and specific serum IgE levels in adults: relationship to sex, age and environmental factors. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 1994;24(6):530-9.

46. Carsin AE, Zock JP, Jarvis D, Basagana X, Heinrich J, Toren K, et al. Serum total immunoglobulin E is a surrogate of atopy in adult-onset asthma: a longitudinal study. International archives of allergy and immunology. 2013;160(4):387-92.

47. von Mutius E, Illi S, Hirsch T, Leupold W, Keil U, Weiland SK. Frequency of infections and risk of asthma, atopy and airway hyperresponsiveness in children. The European respiratory journal. 1999;14(1):4-11.

48. Kojima S, Yokogawa M, Tada T. Raised levels of serum IgE in human helminthiases. The American journal of tropical medicine and hygiene. 1972;21(6):913-8.

49. Vaira D, Malfertheiner P, Megraud F, Axon AT, Deltenre M, Hirschl AM, et al. Diagnosis of Helicobacter pylori infection with a new non-invasive antigen-based assay. HpSA European study group. Lancet. 1999;354(9172):30-3.

50. Ricci G, Capelli M, Miniero R, Menna G, Zannarini L, Dillon P, et al. A comparison of different allergometric tests, skin prick test, Pharmacia UniCAP and ADVIA Centaur, for diagnosis of allergic diseases in children. Allergy. 2003;58(1):38-45.

51. Tu YL, Chang SW, Tsai HJ, Chen LC, Lee WI, Hua MC, et al. Total serum IgE in a population-based study of Asian children in Taiwan: reference value and significance in the diagnosis of allergy. PloS one. 2013;8(11):e80996.

52. Sunyer J, Anto JM, Castellsague J, Soriano JB, Roca J. Total serum IgE is associated with asthma independently of specific IgE levels. The Spanish Group of the European Study of Asthma. The European respiratory journal. 1996;9(9):1880-4.

53. Stromgaard S, Thomsen SF, Fenger M, Backer V. Predictors of Serum Total IgE in a Random Sample of 7-17 Year Old Children. ISRN allergy. 2011;2011:169859.

Accepted Article

This article is protected by copyright. All rights reserved.

54. Helby J, Bojesen SE, Nielsen SF, Nordestgaard BG. IgE and risk of cancer in 37 747 individuals from the general population. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2015;26(8):1784-90.

55. Levo Y, Shalit M, Wollner S, Fich A. Serum IgE levels in patients with inflammatory bowel disease. Annals of allergy. 1986;56(1):85-7.

56. Virta M, Pessi T, Helminen M, Seiskari T, Kondrashova A, Knip M, et al. Interaction between CD14-159C>T polymorphism and Helicobacter pylori is associated with serum total immunoglobulin E. Clin Exp Allergy. 2008;38(12):1929-34.

57. Uter W, Stock C, Pfahlberg A, Guillen-Grima F, Aguinaga-Ontoso I, Brun-Sandiumenge C, et al. Association between infections and signs and symptoms of 'atopic' hypersensitivity--results of a cross-sectional survey among first-year university students in Germany and Spain. Allergy. 2003;58(7):580-4.

58. Holt PG. Parasites, atopy, and the hygiene hypothesis: resolution of a paradox? Lancet. 2000;356(9243):1699-701.

59. von Hertzen LC. Puzzling associations between childhood infections and the later occurrence of asthma and atopy. Ann Med. 2000;32(6):397-400.

60. Imamura S, Sugimoto M, Kanemasa K, Sumida Y, Okanoue T, Yoshikawa T, et al. Inverse association between Helicobacter pylori infection and allergic rhinitis in young Japanese. J Gastroenterol Hepatol. 2010;25(7):1244-9.

61. Davey G, Venn A, Belete H, Berhane Y, Britton J. Wheeze, allergic sensitization and geohelminth infection in Butajira, Ethiopia. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2005;35(3):301-7.

62. Dagoye D, Bekele Z, Woldemichael K, Nida H, Yimam M, Hall A, et al. Wheezing, allergy, and parasite infection in children in urban and rural Ethiopia. American journal of respiratory and critical care medicine. 2003;167(10):1369-73.

63. Haileamlak A, Dagoye D, Williams H, Venn AJ, Hubbard R, Britton J, et al. Early life risk factors for atopic dermatitis in Ethiopian children. The Journal of allergy and clinical immunology. 2005;115(2):370-6.

64. Yemaneberhan H, Bekele Z, Venn A, Lewis S, Parry E, Britton J. Prevalence of wheeze and asthma and relation to atopy in urban and rural Ethiopia. Lancet (London, England). 1997;350(9071):85-90.

65. Milavec-Puretic V, Lipozencic J, Zizic V, Milavec D. Correlation among skin prick test, total and specific IgE UniCAP tests in atopic patients from Zagreb, Croatia. Acta dermatovenerologica Croatica : ADC. 2004;12(4):257-60.

66. Lee MH, Cheong JH, Koh YY, Noh CI, Choi JY, Yun YS. The Relationship between Total Serum IgE, Allergen-Specific IgE, and Skin Prick Test in Children with Atopic Asthma. Korean J Pediatr. 1999;42(3):403-11.

Accepted Article

This article is protected by copyright. All rights reserved.

67. Fajraoui N, Charfi MR, Khouani H, Abouda M, Kerkenil Y, Zouari B. [Contribution of serum total immunoglobulin E measurement in the diagnosis of respiratory allergic diseases]. La Tunisie medicale. 2008;86(1):32-7.

68. Matricardi PM, Bockelbrink A, Gruber C, Keil T, Hamelmann E, Wahn U, et al. Longitudinal trends of total and allergen-specific IgE throughout childhood. Allergy. 2009;64(7):1093-8.

69. Granstrom M, Tindberg Y, Blennow M. Seroepidemiology of Helicobacter pylori infection in a cohort of children monitored from 6 months to 11 years of age. Journal of clinical microbiology. 1997;35(2):468-70.

70. Broussard CS, Goodman KJ, Phillips CV, Smith MA, Fischbach LA, Day RS, et al. Antibiotics taken for other illnesses and spontaneous clearance of Helicobacter pylori infection in children. Pharmacoepidemiology and drug safety. 2009;18(8):722-9.

Fig. 1 The study cohort

Supporting Information

Accepted Article

This article is protected by copyright. All rights reserved.

Accepted Article

Table 1. Prevalence of self-reported allergic symptoms and atopy at age 6.5 years according to place of residence, Butajira Birth Cohort Study, Ethiopia.

	Place of residence				
Variables	**Overall N(%)**	**Urban n(%)**	**Rural n(%)**	**Crude OR¥¥¥ (95 % CI)**	**P-value**
Wheeze (N= 806)	18(2.2)	2(2.1)	16(2.3)	0.92(0.21 to 4.08)	0.92
Eczema (N= 806)	4(0.5)	1(1.0)	3(0.4)	2.45(0.26 to 24.1)	0.43
Hay-fever (N= 806)	4(0.5)	0(0.0)	4(0.6)	NA	NA
Any allergic condition¥ (N=806)	24(3.0)	3(3.1)	21(3.0)	1.06(0.31 to 3.62)	0.93
Atopy ¥¥ (N=713)	129(18.1)	6(6.1)	123(20.0)	0.26(0.11 to 0.60)	<0.01

¥Any allergic condition' was defined as a positive response either to wheeze, or eczema, or hay fever in the past 12 months, at years 6.5 follow-up

¥¥ Atopy was defined as total serum IgE level > 144IU/ml (according to the manufacturer's recommended threshold by age)

¥¥¥ Crude odds ratio (OR) was calculated using binary logistic regression

This article is protected by copyright. All rights reserved.

Table 2. Associations between Atopy at age 6.5 years and *H. pylori* exposures from age 3-6.5 years, Butajira birth cohort study, Ethiopia. Cross sectional analysis (N=713).

	Atopy at age 6.5 years¥						
Variables	**Overall N(%)**	**Yes n(%)**	**No n(%)**	**Crude OR (95 % CI)**	**P-value**	**Adjusted OR¥¥¥¥ (95 % CI)**	**P-value**
Exposure to *H. pylori* at age 6.5							
No	634(88.9)	118(18.6)	516(81.4)	1		1	
Yes	79 (11.1)	12(15.2)	67(84.8)	0.78(0.41-1.49)	0.46	0.87(0.45 to 1.70)	0.69
Exposure to *H. pylori* up to age 6.5¥¥ (N=560)							
Never infected	165(29.5)	32(19.4)	133(80.6)	1		1	
Infected at any age up to age 6.5	393(70.5)	43(10.9)	352(89.1)	0.51(0.31-0.84)	<0.01	0.54(0.32 to 0.92)	0.02
Persistent exposure of *H. pylori*¥¥¥							
No	467(95.3)	72(15.4)	395(84.6)	1		1	
Yes	23(4.7)	3(13.0)	20(87.0)	0.82(0.24-2.84)	0.76	0.93(0.26 to 3.39)	0.92

¥ Atopy was defined as total serum IgE level > 144 IU/ml (according to the manufacturer's recommended threshold by age)

¥¥ *Exposure of H pylori infection at any age from 3-6.5 years*

¥¥¥ *H. pylori positive* at both time points (3.5 & 6.5 years of age)

¥¥¥¥ *Adjusted for area of residence, history of maternal allergy, number of siblings and maternal education using multiple logistic regression analysis.*

This article is protected by copyright. All rights reserved.

Table 3. Associations between allergic conditions at age 6.5 years and *H. pylori* exposure from age 3-6 years, Butajira birth cohort study, Ethiopia. Cross sectional analysis

	Any allergic conditions at age 6.5 years¥						
Variables	**Overall N (%)**	**Yes N (%)**	**No N (%)**	**Crude OR (95 % CI)**	**P-value**	**Adjusted OR¥¥¥¥ (95 % CI)**	**P-value**
Exposure to *H. pylori* at age 6.5 (N=797)							
No	716(89.8)	21(2.9)	695(97.1)	1		1	
Yes	81(10.2)	1 (1.2)	80(98.8)	0.41(0.06 to 3.12)	0.39	NA	NA
Exposure to *H. pylori* up to age 6.5¥¥ (N=559)*							
Never infected	194(34.7)	9(4.6)	185(95.4)	1		1	
Infected at any age up to age 6.5	365(65.3)	5(1.4)	360(98.6)	0.29(0.09 to 0.86)	0.03	0.31 (0.10 to 0.94)	0.04
Persistent exposure to *H. pylori*¥¥¥ (N=559)							
No	537(96.1)	12(2.2)	525(97.8)	1		NA	
Yes	22(3.9)	1(4.5)	21(95.5)	1.98(0.25 to 15.98)			

¥*'Any allergic condition' was defined as a positive response to one or more of asthma, hay fever and eczema in the past 12 months, at years 6.5 follow-up*

¥¥ Exposure *to H. pylori infection at any age from 3-6.5 years*

¥¥¥ *H. pylori positive* at both time points (3.5 and 6.5 years of age)

¥¥¥¥ *Adjusted for area of residence, maternal education and household size using multiple logistic regression analysis.*

This article is protected by copyright. All rights reserved.

Accepted Article

Table 4. Associations between atopy at age 6.5 years and CagA *H. pylori* exposure at 6.5 years, Butajira birth cohort study, Ethiopia. Cross sectional analysis

	Atopy at age 6.5 years [a]						
Variables	**Overall N (%)**	**Yes N (%)**	**No N (%)**	**Crude OR (95 % CI)**	**P-value**	**Adjusted OR[e] (95 % CI)**	**P-value**
***H. pylori* CagA status [b]**							
Negative	629(88.2)	121(19.2)	508(80.8)	1	-	1	-
[c]*H.**pylori +/ CagA***	31(4.3)	4 (12.9)	27(87.1)	0.62(0.21 to 1.81)	0.38	0.63(0.21 to 1.87)	0.63
[d] CagA +	53(7.4)	4(7.5)	49(92.5)	0.34 (0.12 to 0.96)	0.04	0.35(0.12 to 1.01)	0.051

[a] Atopy was defined as total serum IgE level : > 144 IU/ml (according to the manufacturer's recommended threshold by age)

[b] Exposure *of CagA strain H. pylori infection at 6.5 years,* [c] *H. pylori* positive but CagA strain negative, [d] *Positive for both H. pylori and* CagA *strain*

[e] *Adjusted for place of residence, history of maternal allergy, and number of older sibling, , using multivariate logistic regression analysis.*

This article is protected by copyright. All rights reserved.

Table 5. Associations between **allergic conditions** at age 6.5 years and CagA *H. pylori* exposure at 6.5 years, Butajira birth cohort study, Ethiopia. Cross sectional analysis (N=799)

	Allergic conditions at age 6.5 years¥						
Variables	**Overall N (%)**	**Yes N (%)**	**No N (%)**	**Crude OR (95 % CI)**	**P-value**	**Adjusted OR¥¥¥ (95 % CI)**	**P-value**
***H. pylori* CagA status ¥¥**							
Negative	716(88)	21(2.9)	695(97.1)	1	-	1	-
H. ***pylori + /CagA -***	34(4.1)	2 (5.9)	32(94.1)	2.07(0.46 to 9.21)	0.34	2.01 (0.45 to 9.07)	0.36
CagA +	49(6.1)	1 (2.0)	48(98.0)	0.69 (0.09 to 5.23)	0.72	0.73(0.10 to 5.56)	0.76

¥*'Any allergic condition' was defined as a positive response to one or more of asthma, hay fever and eczema in the past 12 months, at yeas 6.5 follow-up*

¥¥ Exposure to *CagA strain H. pylori infection at 6.5 years*

¥¥¥ *Adjusted for sex, place of residence, number of older siblings and, history of maternal and paternal allergy, using multivariate logistic regression analysis.*

This article is protected by copyright. All rights reserved.

Accepted Article

Table 6. Models for longitudinal association between atopy at age 6.5 and exposure to *H. pylori* infection at age 3 years, Butajira birth cohort study, Ethiopia (N=444)

	Atopy incident at age 6.5 years¥						
Variables	**Overall N (%)**	**Yes N (%)**	**No N (%)**	**Crude OR (95 % CI)**	**P-value**	**Adjusted OR¥¥¥ (95 % CI)**	**P-value**
Exposure to *H. pylori* at age 3 (N=444) ¥¥							
No	256(57.7)	48(18.8)	208(81.2)	1		1	
Yes	188(42.3)	18(9.6)	170(90.4)	0.46(0.26 to 0.82)	<0.01	0.49(0.27 to 0.89)	0.02

¥ *Atopy was defined as total serum IgE level > 144 IU/ml (according to the manufacturer's recommended threshold by age)*

¥¥ Exposure to *H. pylori infection at age 3 years*

¥¥¥ Adjusted for gender, area of residence, and number of siblings using multiple logistic regression analysis.

This article is protected by copyright. All rights reserved.

Accepted Article

Accepted Article

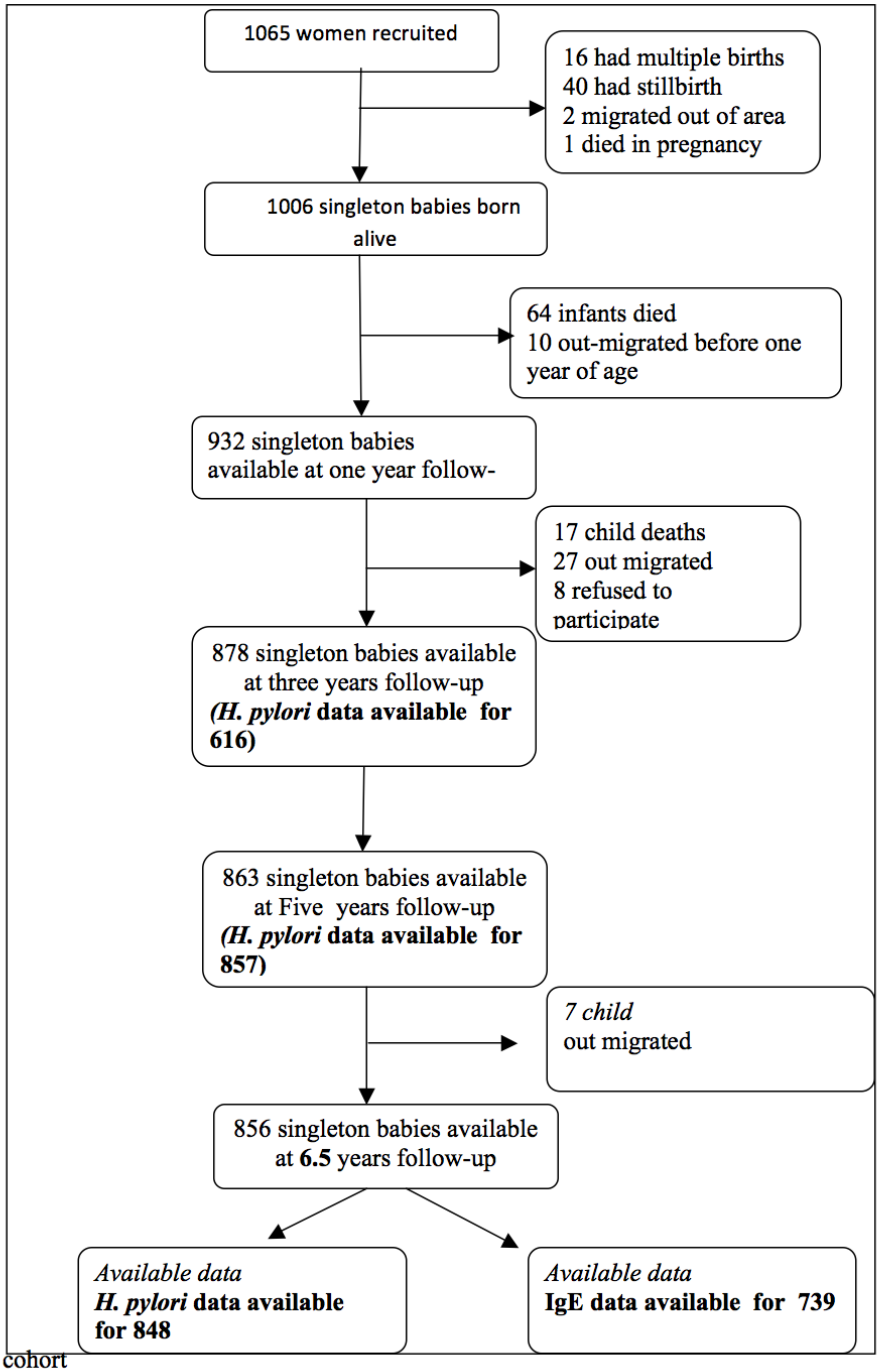

Fig. 1 The study cohort

This article is protected by copyright. All rights reserved.

Table S1. Selected demographic, life style and early childhood characteristics of children followed up at 6.5 years, Butajira birth Cohort, Ethiopia (N=848).

Variables	Number	Percent
Sex		
Male	434	51.2
Female	414	48.8
Place of residences		
Urban	100	11.8
Rural	748	88.2
Ethnicity		
Meskan	402	47.4
Mareko	111	13.1
Silti	200	23.6
Others	135	15.9
Religion		
Orthodox Christian	121	14.3
Muslim	665	78.4
Catholic	10	1.2
Protestant	52	6.1
House hold size		
1-3	20	2.8
4-6	378	53.7
7+	306	43.5
Maternal age in years Mean (SD)	27.1 (6.27)	
Maternal age group in years		
15-24	316	37.3
25-34	134	41.7
35-44	133	15.7
Maternal education		
None	607	71.6
Informal only	83	9.8
Formal	158	18.6
Maternal occupation		
Housewife	709	83.8

Farming and related	27	3.2
Trading and related	96	11.3
Other	14	1.7
History of vaccination at 2 months		
Vaccinated	485	57.5
Not vaccinated	359	42.5
Birth weight		
Normal	483	91.3
Low (<2.5kg)	46	8.7
History of any vaccination at 1 year		
Had some vaccination	823	97.6
Not vaccinated at all	20	2.4
Sanitary conditions*		
Poor	156	18.4
Good	692	81.6
Water source		
River	179	21.2
Well	134	15.8
Pipe	520	61.5
Spring	13	1.5
Vitamin A supplementation at 12 months		
Yes	99	11.7
No	747	88.3

***Poor sanitary condition was defined as not having a toilet facility and not having safe water**

Table S2. Comparison of the distribution of demographic and lifestyle characteristics those seen at 6.5 years and all respondent, Butajira Birth Cohort study, Ethiopia

Variables	All respondent (N=848) N (%)	Case complete (N=739) N (%)
Sex		
Female	414 (48.8)	361 (48.8)
Male	434 (51.2)	378 (51.2)
Place of residence		
Rural	748 (88.2)	653 (88.4)
Urban	100 (11.8)	86 (11.6)
Ethnicity		
Meskan	402 (47.4)	339 (45.9)
Mareko	111 (13.1)	103 (13.9)
Silti	200 (23.6)	173 (23.4)
Others	135 (15.9)	124 (16.8)
Religion		
Muslim	665 (78.4)	571 (77.3)
Christian	183 (21.6)	168 (22.7)
Maternal education		
None	607 (71.6)	535 (72.4)
Informal only	83 (9.8)	70 (9.5)
Formal	158 (18.6)	134 (18.1)
Maternal occupation		
Housewife	709 (83.6)	615 (83.2)
Farming and related	28 (3.3)	26 (3.5)
Trading and related	96 (11.3)	84 (11.4)
Other	15 (1.8)	14 (1.9)
Maternal age		
15-24	316 (37.3)	275 (37.2)
25-34	399 (47.1)	350 (47.4)
35-44	133 (15.7)	114 (15.4)
Water source		
River or Spring	192 (22.7)	166 (22.5)
Well	134 (15.8)	118 (16.0)
Pipe	520 (61.3)	453 (61.5)
History of vaccination[∞]		
Vaccinated	359 (42.5)	307 (41.7)
Not vaccinated	485 (57.5)	429 (58.3)
Household crowdedness[∞∞]		
1-2	469 (55.3)	399 (54.0)
3-4	298 (35.1)	266 (36.0)
5-12	81 (9.6)	74 (10.0)
Intestinal parasitosis		

Yes	218 (26.5)	200 (27.1)
No	604 (73.5)	539 (72.9)
Use of Antibiotics		
Yes	115(14.2)	105 (14.7)
No	703(85.8)	610 (85.3)

, ∞ measured at 2month, ∞∞ measured in person/household

Table S3. Distribution of potential confounders and associations with atopy among children followed at age 6.5 years, Butajira Birth Cohort, Ethiopia (N=713)

Variables	**Overall N (%)**	**Atopy**[¥]	**Atopy Crude OR (95 % CI)** [¥¥]
		Yes (%)	
Sex			
Male	369(51.8)	74 (20.1)	1.32(0.89 to 1.94)
Female	344(48.2)	55(16.0)	1
Place of residence			
Urban	614(86.1)	123(20.0)	0.26(0.11 to 0.60)*
Rural	99(13.9)	6 (6.1)	1
Maternal education			
No formal	580(81.3)	112(19.3)	1.63(0.94 to 2.83)
Formal	133(18.7)	17 (12.8)	1
Maternal occupation			
Housewife	585(80.9)	110(18.8)	0.64(0.28 to 1.45)
Farming and related	19(2.6)	1 (5.3)	0.15(0.02 to 1.33)
Trading and related	89 (12.3)	14 (15.7)	0.51(0.19 to 1.38)
Other	30(4.1)	8 (26.7)	1
History of vaccination[∞]			
Vaccinated	418(59.0)	84(20.1)	1.41(0.95 to 2.10)
Not vaccinated	291(41.0)	44(15.1)	1
Breastfeeding at yr 3			
Yes	77(10.8)	14(18.2)	1.01(0.54 to 1.86)
No	520(89.2)	115(18.1)	1
Household Size			
1-3	20(2.8)	2 (1.6)	1
4-6	378(53.7)	65(17.2)	1.87(0.42 to 8.25)
7+	306(43.5)	60(19.6)	2.19(0.49 to 9.72)
Any STH [∞∞]			
Yes	193(27.3)	26(13.5)	0.63 (0.39 to 1.02)
No	514(72.7)	101(19.6)	1
Maternal history of allergy			
Yes	43(6.1)	11(25.6)	1.62 (0.79 to 3.29)
No	661(93.9)	116(17.5)	1
Paternal history of allergy			
Yes	32(38.5)	5 (15.6)	0.84 (0.32 to 2.12)
No	672(61.5)	122(18.2)	1
Child received antibiotic			
Yes	104(14.8)	21 (20.2)	1.18 (0.69 to 1.98)
No	600(85.2)	106(17.7)	1
Household roof types			

Thatched	491(69.7)	96(19.6)	1.43(0.92 to 2.22)
Corrugated iron	213(30.3)	31(14.6)	1
Smoking in the house hold			
Yes	77(10.9)	17(22.1)	1.33(0.75 to 2.37)
No	627(89.1)	110(17.5)	1
Insecticide use in house			
Yes	476(67.6)	83(19.3)	0.88(0.59 to 1.33)
No	228(32.4)	44(17.4)	1
Place of cooking			
Inside the house	513(72.9)	103(20.1)	1.75(1.10 to 2.78)*
Outside the house	191(29.1)	24(12.6)	1
Use of charcoal/kerosene in the house			
Yes	458(65.1)	80(17.5)	0.89(0.60 to1.34)
No	246(34.9)	47(19.1)	1
Large animals inside the house			
Yes	574(81.5)	115(20.0)	2.46(1.32 to 4.61)*
No	130(18.5)	12(9.2)	1
Child's sleeping place			
Bed	40(5.7)	5 (12.5)	1
Mattress	177(25.3)	19(10.7)	0.84(0.29 to 2.41)
Grass matting	483(69.0)	103(21.3)	1.89(0.73 to 4.97)
No of older siblings			
0	99 (14.1)	8(6.3)	1
1-3	381(54.1)	71(18.6)	2.61(1.21 to 5.61)*
≥4	224(31.8)	48(21.4)	3.10(1.41 to 6.84)*

∞ *measured at 2month*, ∞∞ *Any Soil-Transmitted Helminths (STHs) parasites*

¥ *Atopy was defined as total serum IgE level> 144 IU/ml (according to the manufacturer's recommended thresholds*

¥¥ OR(95% CI) calculated for *atopy* outcome at age 6.5 and covariates

*P value <0.2