Helicobacter pylori is not associated with anaemia in Latin America: results from Argentina, Brazil, Bolivia, Cuba, Mexico and Venezuela

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Abstract

Objective: To investigate the association between Helicobacter pylori infection and anaemia.

Design: Six cross-sectional studies. H. pylori infection was assessed by the [13C]urea breath test using MS or IR analysis. Hb was measured for all countries. Ferritin and transferrin receptors were measured for Argentina, Bolivia, Mexico, and Venezuela.

Setting: Health services in Argentina, Brazil and Mexico or public schools in Bolivia, Cuba and Venezuela.

Subjects: In Argentina, 307 children aged 4–17 years referred to a gastroenterology unit; in Bolivia, 424 randomly selected schoolchildren aged 5–8 years; in Brazil, 1007 adults (157 men, 850 women) aged 18–45 years attending thirty-one primary health-care units; in Cuba, 996 randomly selected schoolchildren aged 6–14 years; in Mexico, seventy-one pregnant women in their first trimester attending public health clinics; in Venezuela, 418 children aged 4–13 years attending public schools.

Results: The lowest prevalence of H. pylori found was among children in Argentina (25±1 %) and the highest in Bolivia (74±0 %). In Bolivia, Cuba and Venezuela children showed similar prevalence of H. pylori infection as in Brazilian and Mexican adults (range 47.5% to 81.8%). Overall anaemia prevalence was 11.3% in Argentina, 15.4% in Bolivia, 20.6% in Brazil, 10.5% in Cuba and 8.9% in Venezuela. Adjusted analyses allowing for confounding variables showed no association between H. pylori colonization and anaemia in any study. Hb, ferritin and transferrin receptor levels were also not associated with H. pylori infection in any country.

Conclusions: The present study showed no evidence to support the hypothesis that H. pylori contributes to anaemia in children, adolescents, adults or pregnant women in six Latin American countries.

Eradication of Helicobacter pylori infection has been recommended for patients with unexplained Fe-deficiency anaemia1,2). This recommendation is based on case reports, cross-sectional studies and limited data from intervention studies3–5). Fe deficiency is the most common nutritional deficiency in Latin American countries5,6). It is important to establish whether H. pylori, which is common throughout the region7–9), could account for the increased prevalence of anaemia, particularly among vulnerable groups including children and pregnant women.

In 2002 The International Atomic Energy Agency started an international multi-centre regional project (ARCAL-RLA...
Experimental methods

The present coordinated series of cross-sectional studies aimed to investigate the relationship between *H. pylori* and anaemia in Latin America. Coordinating meetings were held before defining protocols and primary outcome measures. Each centre produced specific protocols including secondary outcomes relevant to their own communities, and identified local populations at risk of Fe deficiency in whom *H. pylori* might be a contributing factor. Argentina, Bolivia, Cuba and Venezuela studied children, Brazil studied adults and Mexico included pregnant women. Full details of these study groups are given below. For sample size calculation, α and β errors were set at 0.05 and 0.20, respectively, and relative risk at 2 to 4 for all countries. A value of 15% was added to account for expected refusals and subject drop-out, and 30% to allow adequate controlling of confounding factors. The estimated local prevalences of *H. pylori* and anaemia were used to calculate the minimum sample size in each population.

Subjects

Argentina

A sample size of 150 was calculated estimating a ratio of *H. pylori*-positive to *H. pylori*-negative of 2:3 and prevalence of anaemia of 30% among unexposed (*H. pylori*-negative) children. A total of 307 children aged 4 to 17 years, referred to the Gastroenterology Unit of the ‘Superior Sor Maria Ludovica’ Children’s Hospital for evaluation of upper gastrointestinal symptoms (gastro-oesophageal reflux, symptoms of oesophagitis, dyspepsia and abdominal pain), were enrolled. The hospital is a tertiary-level health-care referral institution treating children with complex clinical problems from the Province of Buenos Aires. Forty per cent of the country’s population live in this province. The Gastroenterology Unit receives on average 600 patients per month. Subjects taking antibiotics or acid suppressants during the previous month or mineral supplements (including Fe) during the previous three months were excluded. Data collection was carried out from April 2006 to May 2007.

Bolivia

A sample size of 400 was calculated estimating a ratio of *H. pylori*-positive to *H. pylori*-negative of 1:1 and prevalence of anaemia of 15% among unexposed children. From a population of 24,121 children aged 5-8 years attending public schools in the city of Sucre, 424 children were randomly selected from four large schools (also selected at random), proportional to the number of students attending each school. Any children who were clinically unwell at recruitment were excluded. Field work took place from May 2006 to April 2007.

Cuba

A minimum sample size of 500 was calculated estimating a ratio of *H. pylori*-positive to *H. pylori*-negative of 2:3 and anaemia prevalence among *H. pylori*-negative subjects of 10%. The study was carried out in all thirty-one primary health-care units (PHU) within the National Health System, in the urban zone of Pelotas, between August 2006 and February 2007. Data collection in each PHU lasted approximately one week. The study included all adults aged 18 to 45 years who were users of the PHU and were in the waiting room for any reason on study days. The maximum age was defined as 45 years in order to avoid cases of anaemia due to malignancy. Only adults unable to complete the interview were excluded.

Mexico

A sample size of 182 was calculated estimating a ratio of *H. pylori*-positive to *H. pylori*-negative of 1:1 and frequency of anaemia of 20%. Women were recruited from public health sector clinics after their first contact visit for prenatal care and referred directly to the responsible researcher. All women enrolled in the study were in their first trimester of pregnancy. Volunteers who had taken vitamin or mineral supplements in the first trimester, antacids or antibiotics one month prior to the study were excluded. Data collection started in November 2006. To date, a total of seventy-one pregnant women aged 14–32 years have been enrolled in the study.

Venezuela

A sample size of 312 was calculated estimating a ratio of *H. pylori*-positive to *H. pylori*-negative of 3:1 and anaemia prevalence among *H. pylori*-negative subjects of 17%.
A total of 478 children aged 4–14 years from public schools in the community of Celio Celli were enrolled. The study zone is in the Municipality of Valencia and comprises eleven educational units with a total of 5691 students. The selection of the schools was made by intentional sampling allowing for schools with the highest number of students, acceptance of the study from parents and members of the educational community, and security conditions. Children taking antibiotics or acid suppressants during the two months before data collection were excluded. Data collection was carried out from May to September 2006.

**Determination of H. pylori infection**

The $^{13}$C-UBT was used for detecting *H. pylori* as previously described$^{45}$. Briefly, participants were instructed to fast for at least 6 h, after which two samples of exhaled air were collected by trained field workers to determine basal $^{13}$C:$^{12}$C ratio. Then, 50 mg of $[^{13}$C]urea (Cambridge Isotope Laboratories Inc., Andover, MA, USA) were ingested by each patient with an appropriate test meal. Bolivia and Cuba used IR spectroscopy to analyse breath samples, while all other countries used isotope ratio mass spectrometry (IRMS). For IRMS, samples were collected at 30 and 45 min after ingestion of the labelled solution, stored in hermetically sealed containers (Labco Limited, High Wycombe, UK) and isotope ratios measured using a mass spectrometer coupled to a gas chromatograph (Finnigan MAT GmbH and ThermoQuest Corp., Bremen, Germany). For IR spectroscopy, exhaled breath was collected 30 min after ingestion of labelled solution in gas-tight sample bags and analysed (FANci 2 or IRIS 2; Fischer Analysen Instrumente GmbH, Leipzig, Germany; Van Loenen Instruments, Zandam, The Netherlands) on the day of collection.

A change of $>3.5\%$ in the delta over baseline values was considered positive$^{45}$.

**Anaemia definition**

Argentina, Bolivia, Mexico and Venezuela measured Hb from venepuncture samples; in Brazil and Cuba, fingerpick samples were analysed using a portable haemoglobin meter (HemoCue Inc., Lake Forest, CA, USA). Blood samples were obtained by trained field workers. Anaemia was diagnosed according to WHO criteria$^{46}$ at the following Hb levels: children below 12 years, Hb < 11.5 g/dl or <12.7 g/dl for Bolivia in order to account for altitude (2750 m); pregnant women, Hb < 11 g/dl; non-pregnant women, Hb < 12 g/dl; and men, Hb < 13 g/dl.

**Fe status measure**

Serum ferritin levels were measured in Argentina and Venezuela by RIA (Diagnostic Systems Laboratories, Webster, TX, USA) and transferrin receptors by enzyme immunoassay (TF-94; Ramco Laboratories, Stafford, TX, USA). In Bolivia and Mexico, ferritin was measured by the Coat-A-Count Ferritin IRMA (Diagnostics Products Corporation, Los Angeles, CA, USA) and serum transferrin receptors by ELISA (R & D Systems Inc., Minneapolis, MN, USA). Samples were analysed in duplicate. Quality control was performed running three controls with known concentration of transferrin receptors in the assay (supplied in the kit). The values obtained were within the established ranges supplied with the kit. C-reactive protein (CRP) level was measured with the Protex-CR kit (Lafon Labs, Mexico).

Cut-off points for ferritin level were defined as $<2\mu$g/l for children $<5$ years of age and as $<15\mu$g/l for $\geq5$-year-olds. For pregnant women ferritin cut-off was also $<12\mu$g/l. The cut-off point for transferrin receptor level was defined as $>28\text{nmol/l}$ for all groups.

**Confounding variables**

A structured questionnaire was used to collect information on potential confounding factors. Pilot studies with these questionnaires were undertaken in each country before data collection began, to train field workers and develop study logistics.

**Demographic variables**

Age and sex of subjects were collected for all countries. In Brazil, skin colour as observed by the interviewer was recorded as this has previously been shown to be a marker of socio-economic status in this population$^{47,48}$. In Argentina and Cuba, children’s height and weight were recorded for all ages. In Bolivia, children’s height and weight were expressed as $Z$-scores relative to the 2000 Centers for Disease Control and Prevention age- and sex-appropriate standards$^{52}$. In Argentina and Cuba, children’s height and weight were expressed as $Z$-scores relative to the 2000 Centers for Disease Control and Prevention age- and sex-appropriate standards$^{52}$. Underweight and stunted were classified as $>2\text{sd}$ below median values for weight-for-age and height-for-age, respectively.
General health
In Brazil adults were asked about smoking and consumption of alcoholic beverages, previously diagnosed anaemia, and the presence of haemorrhoids or blood in faeces. For women, additional questions addressed reproductive and menstrual history.

Parasites
Cuba used microscopy of fresh faeces by the Kato–Katz method to identify intestinal parasites, specifically _Necator americanus_, _Ascaris lumbricoides_ and _Trichiurus trichuria_. All analyses were carried out by two microbiologists under the supervision of the principal investigator.

Quality control
Quality control of data collection was achieved through close supervision of the interviewers, review of the questionnaires and weekly meetings of the field workers with the research assistants at each study site. Quality control for 15CO2 was based on having two identical IRMS units in each laboratory (Finnigan MAT GmbH and ThermoQuest Corp.) and identical protocols in Argentina and Mexico. Analysis of the same samples on various occasions, with different combinations of subjects with values above or below 3-5% in the delta over baseline values, always classified positivity or negativity equally.

Statistical analyses
Prevalence of anaemia was compared between _H. pylori_-positive and -negative subjects using the χ2 test. Crude and adjusted analyses were made by logistic regression (for anaemia) and linear regression (for Hb) on individuals with transferrin receptor levels measured. Data from Mexico were used uniquely in linear regression analyses because only two of the seventy-one enrolled women presented anaemia. Analyses were performed using the STATA statistical software package version 9.0 (StataCorp., College Station, TX, USA).

All studies were approved by the relevant institutions’ Research Ethics Committee and Institutional Research Boards. All subjects or parents gave written informed consent for participation. All results on _H. pylori_ and blood parameters were sent to the responsible health facilities in Argentina, Brazil and Mexico so that the appropriate treatment could be given. In Bolivia and Venezuela results were given directly to families along with a referral letter to a paediatrician in the case of abnormal results. In Cuba children with anaemia were treated with Fe salts after the diagnosis.

Results
The overall non-response rate for the study was 10-4%, 0%, 8-1%, 1-5%, 0% and 11-6%, respectively, in Argentina, Bolivia, Brazil, Cuba, Mexico and Venezuela.

Prevalence of _H. pylori_ infection
Incomplete data rates for 13C-UBT were 0%, 13-0%, 2-9%, 0-5% and 12-1%, respectively, in Argentina, Bolivia, Brazil, Cuba and Venezuela. Table 1 compares the prevalence of _H. pylori_ with anaemia in each country. The lowest prevalence of _H. pylori_ found was among children in Argentina (25-1%), followed by Cuba (47-7%). Schoolchildren in Bolivia and Venezuela showed similar prevalence of _H. pylori_ as Brazilian and Mexican adults, ranging from 47-5% to 81-8%.

Prevalence of anaemia
Incomplete data rates for Hb measures were 16-3%, 0%, 2-3%, 0-5%, 0% and 2-0%, respectively, in Argentina, Bolivia, Brazil, Cuba, Mexico and Venezuela. Overall anaemia prevalence (95% CI) was 11-3% (7-4, 15-2%) in Argentina, 15-4% (11-9, 18-8%) in Bolivia, 20-6% (18-2, 23-2%) in Brazil, 10-5% (8-6, 12-5%) in Cuba and 8-9% (6-1, 11-6%) in Venezuela. Except for Mexico, the prevalence of anaemia in _H. pylori_-positive or -negative subjects was compared. There were no differences between colonized and non-colonized subjects in any country except for Venezuela, where a significant difference was observed with anaemia more prevalent in _H. pylori_-negative (15-1%) than in _H. pylori_-positive (7-1%) subjects.

Estimated risk of anaemia
For all countries except Venezuela, 95% confidence intervals of crude odds ratios of anaemia associated with _H. pylori_ included unity. In Venezuela, the crude odds ratio suggested _H. pylori_-colonized children had a decreased risk of anaemia. Adjusted analyses allowing for confounding variables identified as described above showed no change in risk of anaemia associated with _H. pylori_ in any study (Table 1).

Hb was also analysed as a continuous variable with respect to _H. pylori_ (Table 2). The crude β coefficients suggested that _H. pylori_-colonized children from Venezuela had elevated Hb levels, but no significant effect was observed in other countries. After adjusting for confounding variables, no significant effects were detected in any population.

Fe status
Low ferritin concentration was observed in 8-8%, 2-8% and 4-6%, respectively, of the children in Argentina, Bolivia and Venezuela; and in 12-7% of the pregnant women in Mexico. The prevalence of high transferrin receptor level was 31-5% and 57-2%, respectively, in
Helicobacter pylori and anaemia prevalence and odds ratios for anaemia according to H. pylori-positive and H. pylori-negative individuals.

Table 1

<table>
<thead>
<tr>
<th>Country</th>
<th>H. pylori prevalence</th>
<th>Anaemia prevalence</th>
<th>P</th>
<th>OR crude 95% CI</th>
<th>OR adjusted 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina Gastroenterology patients aged 40–65 years</td>
<td>25.9%</td>
<td>10.2%</td>
<td>6.4</td>
<td>1.50 (0.75–2.96)</td>
<td>2.06 (0.75–5.64)</td>
</tr>
<tr>
<td>Bolivia Schoolchildren aged 5–9 years</td>
<td>30.7%</td>
<td>12.0%</td>
<td>8.7</td>
<td>1.22 (0.68–2.16)</td>
<td>2.19 (0.68–8.07)</td>
</tr>
<tr>
<td>Brazil PHU users aged 18–45 years</td>
<td>70.8%</td>
<td>76.8%</td>
<td>4.9</td>
<td>1.90 (1.06–3.42)</td>
<td>2.25 (1.00–5.00)</td>
</tr>
<tr>
<td>Cuba Schoolchildren aged 9–12 years</td>
<td>29.6%</td>
<td>7.4%</td>
<td>3.9</td>
<td>1.33 (0.75–2.38)</td>
<td>1.79 (0.92–3.50)</td>
</tr>
<tr>
<td>Mexico Pregnant women aged 20–34 years</td>
<td>47.5%</td>
<td>24.7%</td>
<td>6.0</td>
<td>1.30 (0.70–2.43)</td>
<td>2.25 (1.00–5.00)</td>
</tr>
</tbody>
</table>

PHU, primary health-care unit; N/A, not applicable.

- *P* adjusted for number of children in household.
- **P** adjusted for number of children in household and skin colour.
- + Adjusted for age, sex, and skin colour.
- z Adjusted for age, ethnicity, geographical area, and hand-washing.

Bolivia and in Venezuela. In Argentina and Mexico, none of the studied subjects presented high transferrin receptor levels.

Table 3 summarizes the effect of H. pylori on serum ferritin and transferrin receptors. There was a negative trend of association in ferritin levels that did not achieve significance in Argentina and Mexico, and a positive crude association between H. pylori and transferrin receptor levels in Mexican pregnant women.

**Discussion**

Anaemia and H. pylori are both common throughout Latin America. The present coordinated series of studies, involving different geographical regions and including subjects from childhood to adulthood, detected no association between these two conditions.

A potential link between H. pylori and Fe-deficiency anaemia was first proposed in 1990(43) and supported by a series of case reports(5–15). Additional evidence was provided by five well-designed cross-sectional studies(16–20), although two further studies failed to detect an association(53,54). Four cross-sectional studies suggested that other indicators of low Fe status were associated with H. pylori(21–24), and five publications involving selected groups of subjects also supported a link between H. pylori and Fe-deficiency anaemia(25–29). Conflicting results have been obtained from some communities. A recent description of work undertaken several years ago in Alaska found that H. pylori IgG seropositivity was associated with Fe-deficiency anaemia, whereas in the same subjects, H. pylori detected by 13C-UBT or faecal antigen test was not(55). This contrasts with the findings of another large population study among Alaskan Natives(20) and encourages a cautious approach to interpreting the results of these data.

Several authors have suggested that H. pylori may cause Fe deficiency. In situations such as that described in Alaskan Natives, where a haemorrhagic gastritis was described in H. pylori-infected adults(16), a clear aetiology could be proposed. Others have suggested that a defect in Fe absorption may occur(27). Plausible mechanisms could involve decrease of mucosal Fe absorption capacity due to reduced gastric acid output(56), competition of the bacterium with the host for the dietary Fe supply(57), reduction of the gastric juice vitamin C content in infected subjects(58), increased hepcidin production from hepatocytes in response to IL-6 production associated with H. pylori gastritis(59), or sequestration of Fe in lactoferrin in the gastric mucosa(60). However, a detailed study of Fe absorption using stable isotopes from Bangladesh did not show a difference in Fe absorption after H. pylori eradication(61).
H. pylori is genetically diverse and type I strains, which are cagA⁺ and secrete the vacuolating cytotoxin, are assumed to be the most virulent[61,62], being associated with the presence of more severe disease[63-69]. Increased bacterial load could also promote gastric inflammation[70] which therefore could stimulate epithelial damage and increase disease severity. Most studies that have reported an association of H. pylori with Fe status have not been able to investigate the potential role of bacterial load or genotype.

Despite the supportive evidence summarized above, relatively few intervention studies have sought to establish whether H. pylori contributes directly to the development of Fe deficiency and anaemia. Six open studies have observed improvements in anaemia and Fe status associated with H. pylori eradication[30-35]. A randomized controlled trial did suggest a role for H. pylori in causing anaemia among adolescents, but only included small numbers of subjects[360]. A larger randomized study evaluating response of anaemia to different forms of Fe supplementation found that the presence of H. pylori had a negative effect on treatment success[357], but an even larger open label study failed to demonstrate any improvement in Fe-deficiency anaemia attributable to H. pylori eradication[71].

The available data thus suggest that H. pylori plays a role in the development of Fe-deficiency anaemia in certain individuals and selected populations, but falls short of providing firm evidence to support the conclusion drawn by several authors of the studies described above, that H. pylori causes Fe deficiency and is thus a significant public health concern.

Our studies demonstrate that H. pylori is common from early childhood in Latin America, particularly in communities such as that studied in Bolivia, where 74% of children were colonized. No significant associations between H. pylori and anaemia were detected in any study after allowing for confounding variables. In children from Venezuela, those colonized by H. pylori tended to have higher levels of Hb than controls. Analyses of ferritin and transferrin receptor levels also failed to produce conclusive or consistent results between different populations.

A source of criticism to our results is that not all countries have measured Fe status and that concomitant infectious diseases may have falsely increased the ferritin levels. This was only accounted for in Mexico and Venezuela. All subjects, however, were CRP-negative in Mexico and only a small number were positive in Venezuela, reinforcing serum ferritin as a valid indicator of Fe status in community studies of healthy individuals[460].

A potential source of bias in cross-sectional studies is inverse causality in which the exposure of interest is in fact the consequence not the cause of the studied outcome. This is not the case in the current studies, in which no association was found. The main feature to emerge from this combined data set is the lack of any single clear message concerning the relationship between H. pylori and anaemia. Sporadic associations (both positive and negative) were detected in individual populations, making it likely that if we extended our work, we would inevitably encounter a population in which H. pylori appeared to be associated with anaemia. This raises the possibility that the preponderance of cross-sectional studies describing an association between Fe deficiency and H. pylori over those failing to detect such an association may have been influenced by ‘positive finding’ publication bias.

Our coordinated approach, which is the principal strength of the current report, has produced no evidence to support the hypothesis that H. pylori contributes to anaemia in children, adolescents or adults throughout our region. We highlight the importance of presenting potentially exciting results, such as the trends of association between H. pylori and ferritin levels in Argentina and Mexico, in the context of less exciting (and by extension potentially less publishable) results from similar studies. In order to infer causality from associations uncovered in epidemiological reports, a key requirement is for consistency of results between different studies. The lack of association between H. pylori and anaemia in the present series of cross-sectional studies argues against this common gastric pathogen being a significant cause of anaemia in Latin America.
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### References


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**Table 3** Coefficients for iron status indicators (ferritin and transferrin receptor) in four of the six countries studied in Latin America

<table>
<thead>
<tr>
<th>Country</th>
<th>Ferritin</th>
<th>Transferrin-receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β coefficient adjusted</td>
<td>95% CI</td>
</tr>
<tr>
<td>Argentina</td>
<td>-0.04</td>
<td>-0.63, 0.57</td>
</tr>
<tr>
<td>Bolivia</td>
<td>0.26</td>
<td>0.02, 0.52</td>
</tr>
<tr>
<td>Mexico</td>
<td>0.28</td>
<td>0.24, 0.31</td>
</tr>
<tr>
<td>Venezuela</td>
<td>2.93</td>
<td>2.93, 2.94</td>
</tr>
</tbody>
</table>

*Adjusted for age, Fe intake and BMI.

**There were no confounding variables.

**There were no confounding variables.

*Adjusted for sex, number of other children in household, Fe intake and weight-for-age Z-scores.
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