

# *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 [<sup>13</sup>C]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.

**Keywords**  
*Helicobacter pylori*  
Anaemia  
Latin America

Eradication of *Helicobacter pylori* infection has been recommended for patients with unexplained Fe-deficiency anaemia<sup>(1,2)</sup>. This recommendation is based on case reports, cross-sectional studies and limited data from intervention studies<sup>(3–37)</sup>. Fe deficiency is the most common nutritional deficiency in Latin American countries<sup>(38)</sup>. It is important to

establish whether *H. pylori*, which is common throughout the region<sup>(39–44)</sup>, 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

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6042-6054). During phase I of the project, the main outcomes were standardization of the [ $^{13}\text{C}$ ]urea breath test ( $^{13}\text{C}$ -UBT) for the diagnosis of *H. pylori* and assessment of the association between *H. pylori* and socio-economic, environmental and demographic factors. In phase II, the main outcome was assessment of possible associations between *H. pylori* and anaemia. The results obtained from six Latin American countries – Argentina, Bolivia, Brazil, Cuba, Mexico and Venezuela – are presented in the current article.

## 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,  $\alpha$  and  $\beta$  errors were set at 0.05 and 0.20, respectively, and relative risk at  $\geq 2.0$  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 'Superiora 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.

### Brazil

A sample size of 909 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 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.

### 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 35%. All urban and rural public schools in the community of Yaguajay were eligible for inclusion in the study and thirty-nine schools were randomly selected. A random sample of 996 children aged 6–14 years was then selected from these schools. Clinical records of school physicians were reviewed and only healthy children were eligible for inclusion. Children who had taken antibiotics, acid suppressants or Fe supplements during the previous month were excluded. Data collection was carried out in June 2006.

### 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  $^{13}\text{C}$ -UBT was used for detecting *H. pylori* as previously described<sup>(45)</sup>. 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  $^{13}\text{C}$ : $^{12}\text{C}$  ratio. Then, 50 mg of [ $^{13}\text{C}$ ]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, Zaandam, The Netherlands) on the day of collection.

A change of  $>3.5\%$  in the delta over baseline values was considered positive<sup>(45)</sup>.

### **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<sup>®</sup>; HemoCue Inc., Lake Forest, CA, USA). Blood samples were obtained by trained field workers. Anaemia was diagnosed according to WHO criteria<sup>(46)</sup> 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  $<2\ \mu\text{g/l}$  for children  $<5$  years of age and as  $<15\ \mu\text{g/l}$  for  $\geq 5$ -year-olds. For pregnant women ferritin cut-off was also  $<12\ \mu\text{g/l}$ . 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<sup>(47,48)</sup>.

### **Socio-economic characteristics**

Argentina, Brazil, Cuba, Mexico and Venezuela collected data on family income, level of education of the subjects (parents in the case of children), housing conditions (type of house, source of water, sanitation and crowding) and rural/urban residency.

### **Dietary Fe intake**

Argentina and Cuba conducted a 24 h dietary recall for the whole group or a sub-sample, respectively, from parents. Fe intakes were estimated for foods using a nutrient composition database (INFOODS) compiled by ARGENFOODS<sup>(49)</sup> from the FAO<sup>(50)</sup> and US Department of Agriculture food database<sup>(51)</sup>. For Brazil, weekly frequency of consumption of Fe-rich foods was assessed.

### **Anthropometric data**

Height and weight were recorded by trained field workers using anthropometers and calibrated scales. For adults, BMI  $\leq 18.5\ \text{kg/m}^2$  was classified as underweight; BMI =  $18.6$ – $24.9\ \text{kg/m}^2$  as normal weight; BMI =  $25.0$ – $29.9\ \text{kg/m}^2$  as overweight; and BMI  $\geq 30.0\ \text{kg/m}^2$  as obese. 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<sup>(52)</sup>. 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 *Trichiuris trichiuria*. 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  $^{13}\text{C}_2$  was based on having two identical IRMS units in each laboratory (Finnigan MAT GmbH and ThermoQuest Corp.) and identical protocols<sup>(45)</sup> 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  $\chi^2$  test. Crude and adjusted analyses were made through logistic regression (for anaemia) and linear regression (for Hb, ferritin and transferrin receptor levels) taking into account the natural clustering of the data as might occur according to PHU in Brazil and schools in Bolivia and Cuba. In all analyses, variables were considered to be possible confounders of the effect of *H. pylori* on the outcome if they were associated with both outcome and *H. pylori* with  $P < 0.20$ . Crude and adjusted odds ratios and  $\beta$  coefficients were obtained with 95% confidence intervals. 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  $^{13}\text{C}$ -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  $\beta$  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

**Table 1** *Helicobacter pylori* and anaemia prevalence and odds ratios for anaemia according to *H. pylori* in six countries in Latin America

| Country   | Target population                         | Sample size | H. pylori prevalence |            | Anaemia prevalence       |            |                          |            | OR crude | 95% CI | OR adjusted | 95% CI | P          |     |
|-----------|---|-------------|----------------------|------------|--------------------------|------------|--------------------------|------------|----------|--------|-------------|--------|------------|-----|
|           |   |             | %                    | 95% CI     | Among H. pylori-positive |            | Among H. pylori-negative |            |          |        |             |        |            |     |
|           |   |             |                      |            | %                        | 95% CI     | %                        | 95% CI     |          |        |             |        |            |     |
| Argentina | Gastroenterology patients aged 4–17 years | 307         | 25.1                 | 20.5, 30.2 | 14.5                     | 7.6, 24.3  | 10.2                     | 6.4, 15.1  | 0.3      | 1.50   | 0.66, 3.41  | 1.79†  | 0.75, 4.26 | 0.2 |
| Bolivia   | Schoolchildren aged 5–9 years             | 424         | 74.0                 | 69.3, 78.3 | 16.3                     | 12.3, 21.1 | 14.7                     | 8.6, 23.0  | 0.7      | 1.12   | 0.27, 4.68  | 1.25§  | 0.30, 5.16 | 0.6 |
| Brazil    | PHU users aged 18–45 years                | 1007        | 70.7                 | 68.0, 73.6 | 20.9                     | 18.0, 24.0 | 20.5                     | 16.2, 25.5 | 0.9      | 1.02   | 0.75, 1.39  | 0.94   | 0.70, 1.27 | 0.6 |
| Cuba      | Schoolchildren aged 7–12 years            | 996         | 47.7                 | 44.6, 50.8 | 10.9                     | 8.0, 13.7  | 10.3                     | 7.7, 12.9  | 0.73     | 1.06   | 0.74, 1.53  | 1.22¶  | 0.84, 1.77 | 0.3 |
| Mexico    | Pregnant women aged 14–32 years           | 71          | 59.2                 | 47.5, 70.1 | N/A†                     |            | N/A†                     |            | –        | N/A†   |             | N/A†   |            | –   |
| Venezuela | Schoolchildren aged 3–14 years            | 418         | 77.8                 | 73.7, 81.8 | 7.1                      | 4.3, 9.9   | 15.1                     | 7.7, 22.5  | 0.02     | 0.43   | 0.21, 0.87  | 0.81** | 0.37, 1.79 | 0.6 |

PHU, primary health-care unit; N/A, not applicable.  
 \*P value of the difference in Fe-deficiency anaemia prevalence between H. pylori-positive and H. pylori-negative individuals.  
 †Only two women had anaemia.  
 ‡Adjusted for number of children in household.  
 §Adjusted for age.  
 ||Adjusted for age, sex and skin colour.  
 ¶Adjusted for age, sanitation, helminths, father's education and hand-washing.  
 \*\*Adjusted for age.

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<sup>(4)</sup> and supported by a series of case reports<sup>(5–15)</sup>. Additional evidence was provided by five well-designed cross-sectional studies<sup>(16–20)</sup>, although two further studies failed to detect an association<sup>(53,54)</sup>. Four cross-sectional studies suggested that other indicators of low Fe status were associated with *H. pylori*<sup>(21–24)</sup>, and five publications involving selected groups of subjects also supported a link between *H. pylori* and Fe-deficiency anaemia<sup>(25–29)</sup>. 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 <sup>13</sup>C-UBT or faecal antigen test was not<sup>(55)</sup>. This contrasts with the findings of another large population study among Alaskan Natives<sup>(20)</sup>, 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<sup>(16)</sup>, a clear aetiology could be proposed. Others have suggested that a defect in Fe absorption may occur<sup>(27)</sup>. Plausible mechanisms could involve decrease of mucosal Fe absorption capacity due to reduced gastric acid output<sup>(56)</sup>, competition of the bacterium with the host for the dietary Fe supply<sup>(33)</sup>, reduction of the gastric juice vitamin C content in infected subjects<sup>(57)</sup>, increased hepcidin production from hepatocytes in response to IL-6 production associated with *H. pylori* gastritis<sup>(58)</sup> or sequestration of Fe in lactoferrin in the gastric mucosa<sup>(59)</sup>. However, a detailed study of Fe absorption using stable isotopes from Bangladesh did not show a difference in Fe absorption between *H. pylori*-colonized anaemic children and anaemic controls, and also failed to show any improvement in Fe absorption after *H. pylori* eradication<sup>(60)</sup>.

**Table 2** Crude and adjusted  $\beta$  coefficients for Hb level according to *Helicobacter pylori* in six countries in Latin America

| Country   | $\beta$ coefficient crude | 95% CI       | P     | $\beta$ coefficient adjusted | 95% CI      | P   |
|-----------|---------------------------|--------------|-------|------------------------------|-------------|-----|
| Argentina | 0.11                      | -0.42, 0.21  | 0.5   | 0.02*                        | -0.31, 0.35 | 0.9 |
| Bolivia   | 0.79                      | -5.24, 6.81  | 0.7   | 0.36†                        | -5.86, 6.58 | 0.9 |
| Brazil    | -0.17                     | -0.38, -0.05 | 0.1   | -0.07‡                       | -0.24, 0.11 | 0.4 |
| Cuba      | -0.62                     | -2.34, 1.10  | 0.5   | -1.17§                       | -3.09, 0.75 | 0.2 |
| Mexico    | -0.02                     | -0.54, 0.49  | 0.9   | -0.02                        | -0.54, 0.49 | 0.9 |
| Venezuela | 0.31                      | 0.10, 0.52   | 0.004 | -0.32¶                       | -0.24, 0.18 | 0.8 |

\*Adjusted for maternal education and crowding.

†Adjusted for age.

‡Adjusted for sex, skin colour, income, age and smoking.

§Adjusted for age, father's education and sanitation.

||There was no confounding by age.

¶Adjusted for age.

*H. pylori* is genetically diverse and type I strains, which are *cagA*<sup>+</sup> and secrete the vacuolating cytotoxin, are assumed to be the most virulent<sup>(61,62)</sup>, being associated with the presence of more severe disease<sup>(63-69)</sup>. Increased bacterial load could also promote gastric inflammation<sup>(70)</sup> 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<sup>(30-35)</sup>. A randomized controlled trial did suggest a role for *H. pylori* in causing anaemia among adolescents, but only included small numbers of subjects<sup>(36)</sup>. 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<sup>(37)</sup>, but an even larger open label study failed to demonstrate any improvement in Fe-deficiency anaemia attributable to *H. pylori* eradication<sup>(71)</sup>.

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<sup>(46)</sup>.

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.

**Table 3** Crude and adjusted  $\beta$  coefficients for iron status indicators (ferritin and transferrin receptors) according to *Helicobacter pylori* in four of the six countries studied in Latin America

| Country   | Ferritin                  |               |      |                              | Transferrin-receptors |     |                              |             |     |
|-----------|---------------------------|---------------|------|------------------------------|-----------------------|-----|------------------------------|-------------|-----|
|           | $\beta$ coefficient crude | 95% CI        | P    | $\beta$ coefficient adjusted | 95% CI                | P   | $\beta$ coefficient adjusted | 95% CI      | P   |
| Argentina | -5.76                     | -13.51, 2.00  | 0.2  | -6.07*                       | -13.74, 1.60          | 0.1 | -0.26                        | -0.94, 0.41 | 0.5 |
| Bolivia   | 10.10                     | 0.37, 19.82   | 0.05 | †                            | —                     | —   | -3.28                        | -8.28, 1.68 | 0.1 |
| Mexico    | -5.37                     | -21.65, 10.91 | 0.5  | †                            | —                     | —   | 0.12                         | -0.03, 0.27 | 0.1 |
| Venezuela | 2.93                      | -3.03, 8.88   | 0.3  | 2.93                         | -3.03, 8.88           | 0.3 | -1.53                        | -4.16, 1.09 | 0.3 |

\*Adjusted for age, Fe intake and BMI.

†There were no confounding variables.

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

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