EPIDEMIOLOGY

Prevalence of *Helicobacter pylori* at oral and gastrointestinal sites in children: evidence for possible oral-to-oral transmission

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Acquisition of *Helicobacter pylori* occurs mainly in childhood. However, the mode of transmission remains unclear. To help elucidate this, 100 children attending for upper gastrointestinal endoscopy were investigated for the presence of *H. pylori* at various sites. *H. pylori* was detected in antral gastric biopsies by the rapid urease test (13 patients), culture (13 patients), histology (15 patients) and PCR (20 patients). Gastric juice was positive for *H. pylori* in 3 patients by culture and 11 patients by PCR. The dental plaque from 68% of gastric biopsy-positive patients (as determined by culture or PCR) and 24% of gastric biopsy-negative patients was positive for *H. pylori* by PCR. The presence of *H. pylori* in dental plaque was significantly associated with the presence of this organism in the stomach. *H. pylori* was detected by PCR in the faeces of 25% of gastric biopsy-positive children sampled. *H. pylori* was not cultured on any occasion from the oral cavity or faeces. The evidence from this study suggests that oral-to-oral transmission may be a possible mode of spread of *H. pylori* in children.

Introduction

Most *Helicobacter pylori* infections are acquired early in life, both in developed and developing countries. However, the natural history of *H. pylori* infection is poorly understood, with spontaneous eradication and subsequent re-infection probably occurring in childhood. The potential for spread of the organism *via* a person-to-person route may, therefore, be greater in children than in adults [1–3].

In children, *H. pylori* is the major cause of antral gastritis and duodenal ulcer disease [4]. Although chronic abdominal pain is the main presentation, there is no significant difference between infected and non-infected children investigated because of this or any other symptom [5]. Further studies of *H. pylori* infection in children may lead to a better understanding of the disease process. As regards prevalence of *H. pylori*, 10–20% of children may be infected in the UK, whereas in developing countries this may be up to 80% [6, 7]. Little information is available within specific

paediatric populations compared with adults, and the prevalence of *H. pylori* at both gastric and non-gastric sites within children does not appear to have been studied.

The infectious dose of *H. pylori* and reservoirs of the organism must be considered to determine the relative importance of the three proposed routes of transmission: oral–oral, gastro–oral and faecal–oral. It is possible that all three may play a part in some populations. The oral cavity has been proposed as a reservoir of *H. pylori* infection and a source of re-infection following successful eradication from the stomach. The organism can certainly be detected in the mouth, but there is conflicting evidence to support the theory that it can colonise a specific niche within the oral cavity [8]. The apparent low prevalence of *H. pylori* in the mouth may be due to both a transient presence and an uneven distribution of the organism.

It has been suggested that gastric juice may play an important role in the natural route of transmission [9] as gastritis, especially the acute stage, is often accompanied by increased episodes of intermittent reflux [10] which may facilitate the passage of viable organisms into the mouth, from where they may be transmitted to other individuals. *H. pylori* DNA can be

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detected in the gastric juice of gastric biopsy-positive individuals by PCR [11–13] but the reported detection of viable organisms in this fluid varies greatly [14–16]. As most *H. pylori* infection is acquired in childhood, gastro–oral spread may be more significant in children, in whom exposure to gastric contents through vomit is more common, but information on this age group is lacking. Limited findings from fingerprint analysis of *H. pylori* DNA from the mouth and stomach of individual patients have demonstrated that the same strain can occur at both sites [17–19].

The aim of the present study was to determine the prevalence of *H. pylori* in the stomach, gastric juice, oral cavity and faeces of paediatric patients attending the paediatric gastro-enterology clinic of a London children's hospital. Immunomagnetic separation was used to increase the sensitivity and specificity of culture and PCR.

Materials and methods

Study subjects and sample collection

A total of 100 patients (45 male, 55 female; age 5 months to 16 years; mean age 8 years; 47 Caucasian, 22 Bengali, 15 Afro-Caribbean, 7 Turkish, 9 mixed origin) attending Queen Elizabeth Hospital for Sick Children for investigation by upper gastrointestinal tract endoscopy for various gastric problems was studied. Patients who had previously received anti-*H. pylori* therapy or were taking acid-suppressing medication were excluded from the study. Before endoscopy, an oral swab, rotated around the inside of the oral cavity, and a rectal swab were collected. Pooled supragingival dental plaque was obtained with two sterile toothpicks passed around the tooth margins in two quadrants of the mouth. Each sample was placed in 1 ml of Brain Heart Infusion (BHI) Broth (Oxoid CM225). At the beginning of the endoscopy procedure, gastric juice (0.5–10 ml, median 1 ml, mean 1.83 ml) was aspirated from the fundic pool of each patient through the endoscope and collected *via* a sputum trap. The pH of the gastric juice was measured with pH test strips (Sigma) and immediately neutralised with 0.67 M Tris buffer (pH 7.4) if necessary. One antral gastric biopsy was taken for the rapid urease test (RUT; CLO/‘Campylobacter-like organism’ test, Delta West Ply, Bentley, Australia). In this test the biopsy is placed within the small disk of agar in the test strip to detect the presence of the *H. pylori* urease enzyme, indicated by a yellow to pink colour change. Histological examination of two formalin-fixed antral gastric biopsies stained with standard haematoxylin and eosin (H&E) was performed. Two additional antral gastric biopsies were taken and placed in 1 ml of glucose 20% w/v for culture and DNA extraction.

Sample processing

Gastric biopsies were homogenised in a sterile tissue grinder. Oral, rectal and dental plaque samples were vortex mixed for 30 s before processing. Gastric juice samples were centrifuged at 13 000 rpm in a micro-centrifuge for 5 min at room temperature and the deposit was concentrated to a volume of 1 ml. All samples were concentrated by immunomagnetic separation (IMS) with polyclonal anti-*H. pylori* IgG before culture and PCR [8].

Bacterial culture

A 100- μ l volume of each sample was cultured in duplicate on non-selective BHI agar (Oxoid CM375) containing defibrinated horse blood 5%, and BHI agar containing *H. pylori* selective supplement (Oxoid SR147). Plates were incubated micro-aerobically (Campylobacter gas generating kit, Oxoid BR60) at 37°C for 5 days. Suspected colonies of *H. pylori* were identified by Gram's stain and positive reactions for catalase, oxidase and urease [20].

PCR amplification

Chromosomal DNA was extracted from all samples by the cetyltrimethyl-ammonium bromide (CTAB) method according to the DNA miniprep protocol of Wilson [21]. The primers HPU1 and HPU2 [22] were then used to amplify a 411-bp internal fragment of the urease A gene of *H. pylori*. This assay has been assessed previously for its specificity for the urease A gene of *H. pylori* and found not to cross-react with other *Helicobacter* species or known oral urease-producing organisms [23]. The reaction mixture (100 μ l) contained 0.4 μ M of each primer, 0.05 mM of each deoxynucleotide (Pharmacia), 5 μ l of target DNA and 2 U of *Thermus aquaticus* (Taq) DNA polymerase (Pharmacia) in 10 $\times$ reaction buffer. The amplification consisted of an initial denaturation of target DNA at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, primer annealing at 45°C for 1 min and extension at 72°C for 2.5 min. The final cycle included a further 5 min at 72°C to ensure full extension of the product. PCR reaction mixtures were electrophoresed through agarose 1% gels. *H. pylori* NCTC 11637 which had been incubated in Tris buffer (0.67 M) at room temperature for 2 h was also subjected to DNA extraction followed by PCR to determine the minimum number of bacteria detectable by this method. *H. pylori* was detected by PCR at all dilutions tested to a level of 10 cfu/ml. The cytotoxin-associated gene, *cagA*, of *H. pylori* was also detected with primers specific for this gene. The *cagA* gene, carried by *c.* 60% of strains expressing the *cagA* protein, is considered to be a marker of virulence as it is correlated with the production of the vacuolating cytotoxin, VacA [24].

Results

Analysis of gastric biopsy samples

H. pylori was detected in antral gastric biopsies by CLO, culture, PCR and histology in 13%, 13%, 20% and 15% of patients, respectively (Table 1). Overall, *H. pylori* was detected in the stomachs of 22% of children. These were all positive by either PCR or culture, or both. The *cagA* gene was found in 7 (35%) of those 20 patients who were gastric biopsy PCR-positive by the urease A method.

Histological analysis of the biopsy material revealed that 42 patients had varying degrees of chronic inflammation, 17 patients had mild or moderate inflammation, 11 had mild atrophy and one patient with *H. pylori* showed mild intestinal metaplasia. Biopsies from the remaining 58 patients were histologically normal.

Symptoms

A total of 43 children presented with recurrent abdominal pain and 17 had pain accompanied by regular vomiting (Table 2). Reflux was the major symptom in 10 patients and 6 were being investigated for coeliac disease, 8 for food allergies and 5 for suspected eating disorders. A further 11 patients who had previously been treated for *H. pylori* infections had experienced a recurrence of their original symptoms, mainly recurrent abdominal pain. All but 2 of these 11 patients were originally diagnosed and treated elsewhere for their *H. pylori* infection. All 11 children were asymptomatic and *H. pylori*-negative when checked 6–8 weeks after the initial treatment. Half of the *H. pylori*-positive children presented with recurrent abdominal pain, and of those children presenting with this symptom, 11 (26%) of 43 were *H. pylori* positive. Presentation with recurrent abdominal pain as the main symptom was not significantly associated with the presence of *H. pylori* in the stomach (χ^2 analysis; $p < 0.2$).

Macroscopic findings

The findings of macroscopic examination of the oesophagus, stomach and duodenum during endoscopy

and their relationship with *H. pylori* infection are shown in Table 3. There were no abnormalities in any of the areas examined in 57 patients. In nine patients mild inflammation of the gastric antrum was seen. Ten patients showed nodularity in the antrum, but no visible inflammation. Another patient had erosions over the entire antrum. Of 13 patients with oesophagitis, two showed severe ulceration and a further two had severe reflux. One patient had an abnormally shaped stomach which prevented correct stomach emptying. Duodenal inflammation was seen in three patients, duodenal ulceration in another three patients and ulceration of the duodenal bulb in a further three patients. None of the patients had a gastric ulcer. Most of the 22 *H. pylori* gastric antrum-positive patients had a normal (6; 27%), slightly inflamed (5; 23%) or nodular (6; 27%) gastric antrum on endoscopic examination. There was a significant association between the presence of *H. pylori* in the stomach and abnormal endoscopy findings (χ^2 analysis; $p < 0.01$).

cagA gene status

The *cagA* gene was detected in 7 (35%) of 20 gastric biopsy PCR-positive patients. Table 2 shows that three of those patients who were previously *H. pylori*-positive were *cagA*-positive (their previous *cagA* status was not known). The symptoms of the other *cagA*-positive patients were recurrent abdominal pain (2), reflux (1) and suspected food allergy (1). Both duodenal ulcer *H. pylori*-positive patients were *cagA*-positive. Two patients with mild antral inflammation were *cagA*-positive, as were two with a very nodular antrum and one whose gastric antrum was macroscopically normal. The *cagA* gene was detected in the gastric biopsy of only one patient who was *cagA*-negative at other PCR-positive sites.

Oral samples

H. pylori was not cultured from the dental plaque of any child. Urease A PCR of the dental plaque demonstrated that 36 children were *H. pylori*-positive. *H. pylori* was detected by this method in the dental plaque of 15 (68%) of 22 gastric biopsy-positive patients and 21 (24%) of 88 gastric biopsy-negative patients. The presence of *H. pylori* in dental plaque

Table 1. Detection of *H. pylori* in samples obtained from 100 pre-treatment paediatric patients

Sample	n	Percentage of samples positive by				
		RUT	Histology	Culture	Urease A PCR	<i>cagA</i> *
Gastric antral biopsy	100	13	15	13	20	35
Gastric juice	100	NA	NA	3	11	36
Dental plaque	100	NA	NA	0	36	17
Oral swabs	100	NA	NA	0	23	17
Faeces	52	NA	NA	0	8	0

NA, not applicable.

**cagA* is expressed as percentage of urease A PCR positives.

Table 2. Presenting symptoms, *H. pylori* infection of the stomach and the presence of the *cagA* gene in 100 paediatric patients

Symptoms	n	<i>H. pylori</i> -positive	<i>cagA</i> -positive
RAP	43	11	2
RAP prev HP	11	3	3
RAP and vomiting	17	2	0
Reflux	10	4	1
Possible coeliac disease	6	1	0
Food allergy	8	1	1
Eating disorder	5	0	0
Total	100	22	7

RAP, recurrent abdominal pain; RAP prev HP, patients with recurrent abdominal pain who previously were *H. pylori*-positive.

was significantly associated with the presence of the organism in the stomach (χ^2 analysis; $p < 0.001$). The *cagA* gene was detected in 6 (17%) of 36 dental plaque PCR-positive samples, and in only one of these patients was the gastric biopsy *cagA*-negative.

As with the dental plaque samples, *H. pylori* was never cultured from oral swabs. Urease A PCR of these samples found that 23 patients were *H. pylori*-positive. The *cagA* gene was detected in four (17%) of these PCR-positive samples. Of the 23 patients who had a positive oral swab, 22 were also positive in dental plaque, and six had a positive gastric biopsy. There was no significant association between the presence of *H. pylori* in oral swabs and the stomach (χ^2 analysis; $p < 0.5$).

Faecal samples

Faecal samples were collected from only 52 children; none was culture-positive and 4 (8%) were urease A PCR-positive. The *cagA* gene was not detected in any of these four samples. Only 12 of these patients were gastric biopsy-positive, and one of the PCR-positive faecal samples was from a gastric biopsy-negative patient who had a positive dental plaque and oral swab. Therefore, three of the gastric biopsy-positive patients

from whom faecal samples were collected were *H. pylori*-positive at this site.

Gastric juice

Gastric juice samples were collected from all 100 children. These ranged in volume from 0.5 to 10 ml (median 1 ml). *H. pylori* was detected in the gastric juice of 3 patients by culture and 11 patients by PCR, all of whom were gastric biopsy *H. pylori*-positive. The *cagA* gene was detected in 4 (36%) of the 11 PCR-positive gastric juice samples, all four of which were from gastric biopsy *cagA*-positive patients.

The quantity of *H. pylori* cultured ranged from 4700 to 9200 cfu/ml gastric juice (mean 6533 cfu/ml). Analysis of the gastric juice samples collected from the 22 gastric biopsy-positive patients showed that 15 of these had a pH of 7, were clear in appearance and had a volume of 0.5–1 ml.

Overall analysis

Ten patients were *H. pylori*-positive at one site only (one gastric biopsy, eight dental plaque samples and one oral swab). Mixed infections were indicated in only two patients who carried both *cagA*-positive and *cagA*-negative strains at different sites. Of the gastric biopsy-positive children, 32% were Bengali who were found to be 3.92 times more likely to be positive at this site than Caucasians (logistic regression odds ratio). Afro-Caribbean and Turkish children were 4.2 (5 of 15 positive) and 11.2 (4 of 7 positive) times more likely to be gastric biopsy-positive than Caucasians (5 of 47 positive), respectively. Positive dental plaque samples were predominantly found with Caucasian and Bengali children (36% and 31%, respectively), whereas almost half (48%) of all positive oral swabs were from Caucasians. Bengalis were 5 and 2.6 times more likely to be gastric juice- and dental plaque-positive than Caucasians, respectively. Afro-Caribbeans were 5.6 and 3.9 times more likely to be gastric juice- and dental plaque-positive than Caucasians, respectively.

Twice as many male than female children were gastric biopsy-positive (68% compared with 34%). Positive gastric juice, dental plaque and oral swabs were almost evenly split between males and females. Males were 3.6 (gastric biopsy), 1.6 (gastric juice), 1.7 (dental plaque) and 1.5 (oral swab) times more likely to be *H. pylori*-positive than females. The most prevalent age group, 6–8 years, had the largest proportion of gastric biopsy-positive patients (36%). The highest proportion of dental plaque-positive children (28%) fell within the 9–11 years age group, who were twice as likely to be positive at this site than 0–2 year olds, and 1.4 times more likely to be oral swab positive than 0–2 year olds.

Table 3. Endoscopy findings, *H. pylori* infection of the stomach and the presence of the *cagA* gene in 100 paediatric patients

Endoscopy findings	n	<i>H. pylori</i> -positive	<i>cagA</i> -positive
Normal	57	6	1
Mild antral inflammation	9	5	2
Nodular antrum	10	6	2
Antral erosions	1	0	0
Oesophagitis	13	1	0
Duodenal ulcer	3	2	2
Duodenal bulb ulcer	3	1	0
Duodenal inflammation	3	1	0
Abnormal stomach shape	1	0	0
Total	100	22	7

Discussion

H. pylori was detected in the stomachs of 22% of children in this study group, of whom 35% were *cagA*-positive. No other published studies appear to have examined the prevalence of *H. pylori* at gastric and non-gastric sites in children by culture and PCR, as well as RUT and histology. Most paediatric studies have analysed *H. pylori* status by non-invasive techniques, and so the results of this study are not directly comparable to those of others. However, the results of the present study do show similar prevalence levels to other studies conducted in developed countries. Perri *et al.* [25] found that 28% of Belgian children (age range 4.5–18.5 years) were ¹³C-urea breath test-positive. In the USA, 25% of children from a range of ethnic backgrounds (age range 6–19 years) were *H. pylori* seropositive [26]. However, Elitsur *et al.* [27] found that 40% of rural and urban children (age range 1–20 years) from Western Virginia were *H. pylori* seropositive. In Japan, seropositivity was found in 41% of those under 1 year old, 9% of those aged 1–2 years and 35% of children aged 15–19 years [28]. A study of London schoolchildren, aged 4–13 years, found that 17% were *H. pylori* seropositive [7]. Few studies have evaluated the *cagA* status of *H. pylori*-infected children, but the results of this study compare well with the findings of Husson *et al.* [29], in whose study group 40% of children were *cagA*-positive.

Male children were 3.6 times more likely to be gastric biopsy-positive than females. The reason for this is not clear. Generally, others have found no gender difference in *H. pylori* seroprevalence, except in certain ethnic groups in which males showed a higher seroprevalence [30, 31]. Bengali, Afro-Caribbean and Turkish children were all more likely to be *H. pylori* gastric biopsy-positive than Caucasian children. The socio-economic status of these children may have been a significant factor, but was not assessed in this study.

The prevalence of *H. pylori*, as determined by PCR, at the other sites tested was 11%, 36%, 23% and 8% in gastric juice, dental plaque, oral swabs and rectal swabs, respectively. *H. pylori* was not cultured from any of the oral cavity or faecal samples examined here, despite the use of immunomagnetic separation, which had a minimum detection level of 10 organisms by both culture and PCR. In a study of 19 asymptomatic Bangladeshi schoolchildren, Banatvala *et al.* [23] found that the dental plaque of 58% of subjects was positive for *H. pylori* by PCR. This figure may be higher than found in the present study because of a small sample size and choice of only Bangladeshi subjects. Although a much larger number of dental plaque-positive children were gastric biopsy-negative compared with adults (58% *versus* 22%), there was a significant association between the presence of *H. pylori* in dental plaque and the stomach. The presence of *H. pylori* in oral swabs from the children was not significantly

associated with the presence of the organism in the stomach. This may be due to the sites and number of samples taken.

The prevalence of *H. pylori* found in the rectal swabs of the children (8%) is lower than that found by Thomas *et al.* [32] who cultured *H. pylori* from the faeces of 39% of children aged 3–27 months. Although a visible amount of faecal material was collected on every rectal swab, analysis of a specific larger stool sample may have resulted in a higher level of detection of *H. pylori*. However, of those *H. pylori* gastric biopsy-positive patients sampled, 25% were rectal swab-positive.

H. pylori was detected in the gastric juice of only 11 children by PCR and 3 children by culture. This low level of detection was largely due to the difficulty in collecting a gastric juice sample. Only 5 of the samples collected from the 22 gastric biopsy-positive children were true gastric juice samples and contained no bile. However, the quantity of *H. pylori* cultured from the gastric juice of children (mean 6533 cfu/ml) was almost 10³-fold higher than in adults (median 18 cfu/ml) [16]. This may indicate that when *H. pylori* is present, children have a greater load of viable organisms in their stomach available for transmission *via* the mouth than adults.

In the present study, most children presented with recurrent abdominal pain, with 50% of *H. pylori*-positive children complaining of this symptom. Many studies have analysed the link between abdominal symptoms and carriage of *H. pylori* and found, as this study did, no clear association. A study of 945 pre-school children in Germany found that the 13.4% of infected children had even fewer gastrointestinal symptoms compared with uninfected children [5]. Blecker *et al.* [33] concluded that no specific symptoms were associated with *H. pylori* infection in 36 children compared with 107 *H. pylori*-negative children. The majority of patients in the present study were histologically and endoscopically normal (58% and 57%, respectively). Severe gastritis and gastric ulcers were not found in any of the children, but three had duodenal ulcers and a further three had ulcers on the duodenal bulb. It is known that active gastritis and ulcers are rare in children [34]. Nodularity of the antrum was seen in 10% of children. This state, not seen in adults, is thought to represent a local immunological response in the gastric mucosa. Of those *H. pylori*-positive children, most were found to have a normal gastric mucosa, mild antral inflammation or a nodular antrum (27%, 23% and 27%, respectively). Gottrand *et al.* [35] also found that 27% of infected children had a normal gastric mucosa. However, in the present study abnormal endoscopy findings were significantly associated with the presence of *H. pylori* in the stomach. Gastrointestinal symptoms of the parents of the children in the present study were not

assessed. Brenner *et al.* [36] found a significant association between maternal history of ulceration and *H. pylori* infection in children.

In conclusion, the present study of 100 paediatric patients has shown that 22% of those examined were *H. pylori* gastric biopsy-positive, and that the organism was present in the mouths and faeces of both gastric biopsy-positive and gastric biopsy-negative patients. *H. pylori* was not cultured from the oral cavity or faecal samples, indicating that a viable reservoir of the organism outside the stomach is unlikely. However, the PCR results do show that the organism was present in the mouth and faeces of 68% and 25% of those gastric biopsy-positive children sampled, respectively. The evidence from this study indicates that oral–oral transmission may be the predominant mode of spread of *H. pylori* in children. Although only a relatively small number of faecal samples was analysed, the results suggest that in this population of children, faecal–oral transmission may play a lesser role in spread of the organism.

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