Published in final edited form as:
Helicobacter. 2012 April ; 17(2): 96–106. doi:10.1111/j.1523-5378.2011.00919.x.

Non-invasive Genotyping of *Helicobacter pylori cagA, vacA*, and *hopQ* from Asymptomatic Children

Liviu A. Sicinschi[1,5], **Pelayo Correa**[1], **Luis E. Bravo**[6], **Richard M. Peek, Jr.**[1], **Keith T. Wilson**[1,4], **John T. Loh**[2], **Maria C. Yepez**[7], **Benjamin D. Gold**[9], **Dexter T. Thompson**[8], **Timothy L. Cover**[2,3,4], and **Barbara G. Schneider**[1]

[1]Division of Gastroenterology, Dept. of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232

[2]Division of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

[3]Department of Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

[4]Veterans Affairs Tennessee Valley Health Care System, Nashville, TN 37212, USA

[5]Department of Microbiology and Immunology, Holmes Regional Medical Center, Melbourne, FL 32901, USA

[6]Department of Pathology, School of Medicine, Universidad del Valle, Pasto, Colombia

[7]Centro de Estudios de Salud, Universidad de Nariño, Pasto, Colombia

[8]Division of Gastroenterology, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322, USA

[9]Children's Center for Digestive Healthcare, Pediatric Gastroenterology, Hepatology and Nutrition, Atlanta, GA 30342

Abstract

Background—*H. pylori* infection is usually acquired in childhood, but little is known about its natural history in asymptomatic children, primarily due to the paucity of non-invasive diagnostic methods. *H. pylori* strains harboring *cagA* and specific alleles of *hopQ* and *vacA* are associated with increased risk for gastric cancer. Many studies of *H. pylori* virulence markers in children have the bias that symptomatic subjects are selected for endoscopy, and these children may harbor the most virulent strains. Our aim: to genotype *cagA, hopQ* and *vacA* alleles in stool DNA samples of healthy Colombian children residing in an area with high incidence of gastric cancer, in order to avoid selection bias resulting from endoscopy.

Methods—*H. pylori* status of 86 asymptomatic children was assessed by ^{13}C-Urea Breath Test (UBT) and PCR. *H. pylori 16S rRNA, cagA, hopQ* and *vacA* genes were amplified from stool DNA samples and sequenced.

Results—UBT was positive in 69 (80.2%) of 86 children; in stool DNA analysis, 78.3% were positive by *16S rRNA* PCR. *cagA, vacA* and *hopQ* were detected in 66.1%, 84.6%, and 72.3% of stool DNA samples from *16S rRNA* positive children. Of the children's DNA samples which

Corresponding Author: Barbara G. Schneider, Ph.D. barbara.schneider@vanderbilt.edu phone: 615-343-3959 fax: 615-343-6229.

DISCLOSURE No author has conflicts of interest or financial arrangements that could potentially influence the described research.

revealed *vacA* and *hopQ* alleles, 91.7% showed *vacA* s1 and 73.7% showed type I *hopQ*. Type I *hopQ* alleles were associated with *cagA*-positivity and *vacA s1* genotypes (P<0.0001).

Conclusions—Using stool DNA samples, virulence markers of *H. pylori* were successfully genotyped in a high percentage of the asymptomatic infected children, revealing a high prevalence of genotypes associated with virulence. Type I *hopQ* alleles were associated with the presence of *cagA* and the *vacA* s1 genotype.

INTRODUCTION

Helicobacter pylori infection is primarily acquired in childhood and persists as an asymptomatic infection for decades in the majority of colonized individuals. Only a small fraction of infected persons develop clinically significant outcomes, such as peptic ulcer or gastric adenocarcinoma [1]. However, infection early in life has been linked to a greater risk for gastric cancer [2–4]. The epidemiology of infection with *H. pylori* is characterized by a linear increase in prevalence with age in Western industrial countries and by a steep rise in childhood, followed by a stable high rate in adults in developing countries. This pattern results in a large number of asymptomatic children being infected [5]. Colonization with *H. pylori* at an early age may be transient, and spontaneous elimination or loss of infection due to use of antibiotics may occur [6–8]. Studies in Peru and Nicaragua suggest that infection status in children within the first five years of life may change frequently with subjects losing and regaining the *H. pylori* bacterium [9, 10]. Other investigators have questioned the transient nature of infection in children, and raised the issue of reliability of diagnostic methods [11, 12].

The most widely used methods for diagnosis of *H. pylori* infection, such as culture and histology, are sensitive and highly specific tests, but they require invasive procedures, which are not indicated in asymptomatic children. Among non-invasive methods, serology, in spite of its high specificity, shows low sensitivity in children and may not always indicate current infection with *H. pylori*. The ^{13}C - Urea Breath Test (UBT), considered very sensitive and specific, requires expensive equipment and is not always routinely available in clinical settings. The Stool Antigen Test has been found to be useful in children; however, like the UBT, it indicates only the presence of the bacterium, but does not allow characterization of the *H. pylori* strains with which individuals are infected. Another option is the string test, which employs a minimally invasive non-endoscopic procedure for harvesting gastric juice samples [13–15]. This procedure requires the swallowing of a capsule attached to a string, which is left in the stomach for an hour; the capsule dissolves, and the string is drawn out and used for culture and/or PCR detection of *H. pylori* from the stomach. The diagnostic efficiency of the string test when used with PCR may be comparable with ^{13}UBT in detecting *H. pylori* infection, though results may vary depending on the performance and processing technique [16, 17]. The capsule is easily swallowed, but parents may hesitate to subject their children to this test, creating a selection bias. Molecular assays based on detection of *H. pylori* DNA and of *H. pylori* virulence markers in stool samples from children may be considered an alternative that avoids selection bias that may result when children must be subjected to endoscopy or other invasive techniques. While studies detecting the presence of *H. pylori* DNA in stool are common (reviewed in [18]; see also [19–29]), those characterizing virulence determinants, or markers associated with virulence, are fewer [30] and mostly concern *cagA* [31–34].

Several *H. pylori* genes, such as *cagA*, *vacA*, and *hopQ*, are associated with specific disease outcomes. The *cagA* gene and its encoded CagA protein are associated with peptic ulcer disease and with an increased risk of gastric adenocarcinoma [2, 35]. The *vacA* gene is present in all *H. pylori* strains, but only *vacA* s1 type strains secrete an active vacuolating

cytotoxin; the s1 allele is associated with a higher risk for peptic ulcer disease and gastric adenocarcinoma [35, 36]. The less well-studied *hopQ* gene encodes HopQ [37], an outer membrane protein (*omp-27)* that can modulate the adherence of some *H. pylori* strains to gastric epithelial cells [38] and thus may play an important role in the initial colonization and long-term persistence of the bacterium in the stomach. The *hopQ* gene is present in 2 forms: types I and II. *hopQ* type I alleles were found significantly more commonly in *cagA* positive *vacA* s1 strains from patients with peptic ulcer disease than in *cagA* negative *vacA* s2 strains from patients without ulcer disease [39–41]. *HopQ* allele prevalence and relationships with other disease-associated genes have not been studied in *H. pylori*-infected asymptomatic populations residing in areas of high gastric cancer incidence. The *16S rRNA* gene has been used previously for *H. pylori* identification in stool DNA samples from children and adults [8, 42].

Our previous studies of *H. pylori* infection in rural Colombian residents have relied on gastric biopsies from symptomatic adult volunteers [43, 44]. *H. pylori* strains from such biopsies have shown a high proportion of the disease-associated markers *cagA* and *vacA* s1 (from 87.0 to 90.4% and from 93.2 to 96.1% respectively). To reduce the possible selection bias in use of symptomatic subjects, in the present study, we aimed to investigate the prevalence of *H. pylori* infection and of the *cagA* gene, *vacA* genotypes, and *hopQ* genotypes using a non-invasive analysis of stool DNA samples from asymptomatic Colombian children residing in a high risk area for gastric cancer. In addition, we intended to characterize the diversity of *hopQ* alleles in healthy children and investigate a potential relationship of *hopQ* to *cagA* and *vacA* genotypes.

MATERIALS AND METHODS

Study population

The population consisted of 86 asymptomatic children (41 males; 45 females) ages 4.1–8.7 (mean 6.4±1.3) years, from 79 families. All children were from the rural villages of Nariño or Genoy, in the State (“departamento”) of Nariño, located at a high altitude in the Andes Mountains of southwestern Colombia. A high gastric cancer incidence has been documented in this area [45]. The children were predominantly mestizos of Spanish-Amerindian ancestry from rural agricultural families. Participants were recruited by door-to door outreach, by a social worker who obtained informed consent from the parents. The inclusion criterion for initial recruitment into the study was that the child be from 4 to 8 years old, and no children in this age range were excluded. None of the children had received antibiotics within the six weeks before stool collections. As part of an earlier study, all of the children had received a course of anti - *H. pylori* therapy either in 2001 or in 2004, at least six months before our first molecular investigation. All parents gave written consent for the study. The study was approved by the Ethics Boards of the Universities of Cali and Nariño, and Vanderbilt University Medical Center.

Materials

Stool samples were collected twice with an interval of six months: from 71 children at the first visit (August 2004) and from 83 children at the second visit (February 2005). Parents were asked to collect stool samples from children at home and bring samples to the hospital laboratory the same morning. Stool specimens were stored at −20°C until they were transported to Emory University in Atlanta, GA, USA, where DNA extraction was performed. Samples were stored on dry ice during transportation from Colombia to the USA.

H. pylori status

The *H. pylori* status of children was determined by the ^{13}C-UBT, which was administered at the same time points as stool samples were collected. All 86 children underwent UBT at the second visit, and 73 of 86 underwent UBT at the first visit. Urea-derived $^{13}CO_2$ was measured utilizing an automated infrared breath ^{13}C analyzer. The measured $^{13}CO_2/^{12}CO_2$ ratio was compared with a known ratio of a standard gas. The $^{13}CO_2$ values were corrected for the international Pee Dee Belemnite (PDB) standard, then averaged and expressed as relative delta per ml difference to the standard gas (δ(‰)). Δδ(‰) was used to express the difference between δ at time 30 min (t_{30}) and at baseline (t_0). Two nurses who were trained in this procedure collected all breath samples. The cut-off delta value of 5 per mille (δ≥5‰), as proposed by Logan and colleagues in their European standard protocol[46], was applied for all children in this study.

DNA extraction

DNA was extracted from stool specimens using the QIAamp® DNA Stool Mini Kit (Qiagen, Valencia, CA, USA), by the protocol for isolation of DNA from stool for pathogen detection, from 180–220 mg frozen stool. DNA from *H. pylori* reference strains J99 (ATCC 700824, *cagA* positive, *vacA* s1m1), Tx30a (ATCC 51932, *cagA* negative, *vacA* s2m2), J178 and J63 (isolated from patients from Nashville, TN [39] were used as controls for the presence of *cagA, vacA* s1 and s2, *hopQ* types I and II, respectively. The extraction method was validated previously [47].

PCR amplifications

Separate PCRs were performed for *16S rRNA*, type I *hopQ*, type II *hopQ, cagA* and *vacA* s region. Five sets of primers were used in the study (Table 1), all generating amplicons not exceeding 200 bp. For the amplification of the types I and II *hopQ* alleles, two sets of primers were designed to amplify segments of 187 and 160 bp, respectively, from the relatively conserved 3' end of the *hopQ* gene. Validation of our *hopQ* primers was performed by amplification of 13 *H. pylori* DNA samples from Colombian clinical strains using earlier published *hopQ* type I, region B (OP4070F, BA8705R) and *hopQ* type II (BA8363F, BA8364R) primers and by amplification of reference strains J178 (for type I *hopQ*) and J63 (for type II *hopQ*) [48]. Sequences obtained from amplified clinical and reference genotypes were determined (NCBI, BLAST) as belonging to type I *hopQ* or type II *hopQ* alleles. *cagA* fragments were amplified from the conserved region at the 5' end of the *cagA* gene using published primers [49]. To amplify *vacA* s regions, primers VA1F and V136R (the latter designed in this study) were selected, resulting in generation of fragments of 136 bp for the type s1 variants or fragments of 163 bp for type s2 variants [50]. Validation of V136R was performed similarly to that for *hopQ* primers. Fragments of 109 bp were amplified from the *16S rRNA* gene, using primers designed by Ho et al. [51].

PCR mixtures for *16S rRNA*, *cagA*, *hopQ I* and *hopQ II* consisted of 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mol/L $MgCl_2$, 400 μmol/L of each dNTP, 0.4 μmol of each forward and reverse primer, 0.12 μg/μl of bovine serum albumin, and 0.5 to 2.5 U of HotStar Taq DNA Polymerase, or ABI Hot Start DNA polymerase for the *vacA* assays, in a final volume of 25 μL. Higher concentrations of dNTP and primers were dictated by use of higher numbers of cycles for stool DNA amplifications. PCR mixtures and handling of samples was performed using protocols designed to reduce the risk of cross-contamination, including use of dedicated areas for pre- and post-PCR, use of plugged pipette tips, and UV irradiation of pipettors and other materials.

The PCR program for *16S rRNA*, *hopQ*, *cagA* and *vacA* amplifications was as follows: 15 min at 95°C, and 60 cycles consisting of 1 min at 94°C, 1 min at 55°C (or 52°C for *vacA* s),

and 1 min at 72°C, plus a final 3 min extension step at 72°C. Preliminary experiments using lower cycle numbers produced frequent failures of amplification, presumably due to the small amount of *H. pylori* DNA present. PCR products (10 μl of each sample) were electrophoresed in 2% agarose gels for 2 hours at 60 volts to confirm the presence of a single band of the expected size. Only samples producing such a band were submitted for sequencing. For quality control of PCR, positive and negative reagent control reactions, including a no-template control, were included in each experiment.

DNA Sequence analysis

Prior to sequencing, PCR products were purified using ExoSAP-it enzyme (Affymetrix, Cleveland, OH, USA). All sequencing for *cagA*, *vacA* and *hopQ* was performed on both strands. The *16S rRNA* PCR products were not sequenced. Sequencing was performed with an ABI 3130xl Genetic Analyzer, using BigDye 3.1 terminator chemistry. Sequencing primers were the same as those used for PCR amplification. Sequence construction and alignment were performed using Geneious Pro software, version 4.6.4. (Biomatters, Auckland, New Zealand).

All sequences were compared with reference sequences of *H. pylori* NCTC 11637 (GenBank AF202973), *H. pylori* 26695 (GenBank AE000511), *H. pylori* J99 (GenBank AE001439), *H. pylori* J178 (prototype of type I *hopQ* alleles [39], *H. pylori* J63 (prototype of type II *hopQ* alleles [39]. Each sequence was also compared with sequences from the same child recovered at a different time point (where DNA was available) and with sequences from other children. Discrimination of s1a, s1b, and s1c *vacA* genotypes was performed by comparing their sequences with previously published peptide sequences of the *vacA* s region in reference strains [49]. For purposes of sequence comparison, primer binding sites were omitted. For quality control, 18 randomly selected DNA samples were re-amplified and re-sequenced.

Statistical analysis

The two-sided P value for specificity and sensitivity of *16S rRNA* assays versus UBT, and of PCR assay for *vacA* s or *hopQ* results versus *16S rRNA* PCR results, as well as a P value for the relationship of types I and II *hopQ* alleles with *cagA* and *vacA* genotypes were calculated using Fisher's exact test. $P<0.05$ was accepted as statistically significant. Calculations were carried out using Interactive InStat software (JavaStat and GraphPad Software Inc, San Diego, CA).

RESULTS

H. pylori status

Fifty-three of 73 children were UBT positive at the first visit (72.6%) and 66 of 86 were UBT positive at the second visit (76.7%; Table 2). Five of 20 children who were UBT negative at the first visit converted to UBT positivity at the second visit. Additionally, of 13 children for whom UBT was unavailable at the first visit, 11 were UBT positive at the second visit. At either visit, sixty-nine (80.2%) of 86 children were classified as *H. pylori* positive by ^{13}C-UBT (Table 2).

PCR results

Our PCR assays with primers for *16S rRNA*, *cagA*, *vacA* and *hopQ* genes amplified the expected fragments of 109 bp for *16S rRNA*, 183 bp for *cagA*, 136 bp for *vacA* s1/163 bp for *vacA* s2, and 187 bp for type I *hopQ* /160 bp for type II *hopQ* from DNA preparations from the *H. pylori* J99, Tx30a, J178, and J63 reference strains. No amplification was observed for the no-template controls. Of 53 children who were UBT positive at the first visit, stool DNA

samples from 45 (84.9%) were positive for *16S rRNA*, and of 66 children who were UBT positive at the second visit, samples from 55 (83.3%) were positive for *16S rRNA* (Table 2). At either visit, 65 of 83 UBT positive (78.3%) children were classified as *H. pylori* positive by the *16S rRNA* PCR.

We compared the performance of PCR assays for *cagA*, *vacA* and *hopQ* versus *16S rRNA* PCR (Table 2). The *cagA* assay was positive in samples from 23 of 46 children with positive signals for *16S rRNA* at the first visit (50.0%), and in samples from 33 of 58 children with positive signals for *16S rRNA* at the second visit (56.8%, Table 2). At either visit, 43 of 65 (66.1%) children had *cagA* positive stool DNA samples. *vacA* s1 or s2 was found in 55 of 65 (84.6%); types I or II *hopQ* alleles were detected in samples from 47 of 65 (72.3%) children with positive signals for the *16S rRNA* assay.

Comparing overall the performance of *16S rRNA* assays to UBT (See Table 3), the sensitivity and specificity of the *16S rRNA* assay varied from 87.3% to 88.2% and from 85.0% to 95.0%, respectively. In samples from four children (one at the first visit and three at the second visit), the *16S rRNA* assay was positive, although the UBT was negative.

The sensitivity and specificity of *vacA* and *hopQ* marker gene assays compared to *16S rRNA* are shown in Table 4. The sensitivity and specificity of the *cagA* assay could not be assessed due to the fact that the *cagA* gene is not present in all *H. pylori* strains. In samples from 7 children, the *vacA* assay was positive at either time point in the presence of negative *16S rRNA* assays. Of those, 5 samples produced both gel signals and sequences for *vacA* s1, and 2 produced signals and sequences for *vacA* s2. For samples from 36 children with results for both *cagA* and *vacA* at either time point, all *cagA* positive signals were accompanied by *vacA* s1 signals. No sample with a *vacA* s2 signal was accompanied by a *cagA* positive signal. In samples from 9 children, the *hopQ* assay was positive at either time point in the presence of negative *16S rRNA* assays. Of those 9, 7 samples produced both gel signals and sequences for type I *hopQ*, and 2 produced signals and sequences for type II *hopQ*.

We examined the effect of age on associations between UBT and *vacA* or *hopQ* PCR assays by dividing the children into 2 groups: those younger than 6.0 years, and those 6.1 years or older. We found no significant difference between the two age groups.

We evaluated the prevalence of *H. pylori* infection and of the *cagA* gene and *vacA* s1 and type I *hopQ* alleles in children of three different age groups: 4.0 to 4.9 years, 5.0 to 6.9 years and 7.0 to 9.0 years. The *16S rRNA* gene was found 1.77 and 1.87 times more frequently in the second and third group, respectively, compared to the first group.

Diversity of *hopQ* and *vacA s* alleles in Colombian children

Type I *hopQ* alleles were found in DNA samples from 38 of 57 (66.6%) children with positive signals for *hopQ* PCR, and type II *hopQ* alleles were detected in samples from 15 (26.3%) children (Table 5). For the remaining 4 children (7.1%), signals for both types I and II alleles of *hopQ* were found. *vacA s* genotypes were detected from the stool DNA in 60 of the 83 tested children; the predominant *vacA* genotype was s1b, which was found in samples from 49 children (81.7%). The other genotypes detected were as follows: s1a for 3 children (5.0%), s2 for 5 children (8.3%) and s1 and s2 at different time points in samples from 3 children (5.0%).

Relationship between *hopQ* genotypes and *cagA* or *vacA* s alleles

The type I *hopQ* genotype was significantly associated with *cagA* positivity and *vacA* s1 genotypes at both visits, whereas the type II *hopQ* genotype was significantly associated

with *cagA* negative and *vacA* s2 genotypes (P=0.005 and P<0.0001 for *cagA*, and P<0.0001 for *vacA*, Table 6).

Sequencing results for infecting *H. pylori* strains

To learn to what extent sequencing revealed diversity in infecting strains, we examined all *cagA*, *vacA*, and *hopQ* sequences (from 42, 44, 36 and 16 children, respectively). From common polymorphisms in the portions of the genes sequenced, groups of sequences from the children's strains could be discriminated. Sequence identity for each gene in different children was consistent with harboring a common strain. The detection of sequence polymorphisms in each gene allowed us to discriminate different strains. The highest sequence diversity was observed for the *vacA* s2 fragments showing 3 different patterns in 7 children (43%), followed by type II *hopQ* fragments with 6 different patterns in 16 sequences (37%). No *H. pylori* sequences obtained from the children in the study matched those of the *H. pylori* strains 26695, 60190 or reference strain J99, Tx30A, J178, J63 sequences, used as PCR controls in this study. The 18 randomly selected DNA samples that were re-amplified and re-sequenced for the purpose of quality control of our assays produced identical sequences in all cases.

H. pylori infection at two time points

The presence of *H. pylori* infection was determined by detection of a positive result in at least one genotyping assay confirmed by sequencing. Analysis of results obtained from samples collected at two consecutive visits (August, 2004 and February, 2005) allowed us to note differences consistent with status changes between the two time points. Overall in samples from 29 children, sequences for one, two or three genes could be obtained at both time points. In samples from nine of those children (31%), sequences were different at the two time points; in samples from the remaining children (69%), the sequences of one, two or three genes at the two time points were identical. These findings are consistent with the idea that the majority of children (69%) maintained the same strain over a six month period. Considering the presence at least of one positive genotyping result for any of the four genes, eight children of 83 (9.6%) had results consistent with loss of strains, and 9 children (10.8%) showed results consistent with gain of *H. pylori* infection during the 6 month period. Mixed infection was found in 12 of 70 children (17.1%) as evidenced by PCR and sequencing.

DISCUSSION

Our study is the most extensive evaluation of markers of virulence in *H. pylori* infections in asymptomatic children from a high-risk population, which we have seen to date. First, the study revealed the high prevalence of *H. pylori* infection (80.2% by UBT and 78.3% by *16S rRNA* PCR) in this population. The high prevalence detected by UBT agrees with our results obtained previously by UBT in non-overlapping study populations of children from this same area of Colombia, where gastric cancer is common [52, 53]. Although published studies on *H. pylori* infection in children may differ in methodology, it is interesting to note that the prevalence we measured by both PCR and UBT exceeded those reported even for symptomatic children in some studies. Prevalence of *H. pylori* infection in Spanish children was only 53% as found by Real-Time PCR using gastric biopsies and PCR for the *23S RNA* gene [54].

Secondly, our study shows that even when the accrual of participants was not subjected to the potential bias of selection for clinically indicated endoscopy, the prevalence of markers of virulence in this population was high. The prevalence of the *cagA* gene and *vacA* s1 alleles associated with increased risk for gastric cancer were found in stool DNA samples from 66.1% and 91.7%, respectively, of *H. pylori* positive children investigated in our study,

percentages surprisingly close to those found in infections in symptomatic Colombian adult volunteers (for *cagA*, 87%, and for *vacA* s1 alleles, 93.2%), who may have volunteered due to concern about gastric complaints [43, 44]. These percentages are higher than those reported from children in other countries, even from studies of symptomatic children. In gastric biopsies from symptomatic Portuguese children from a population at high risk for gastric cancer, *cagA* was detected in 36.1%, and *vacA* s1 was detected in 32.7% of children [55]. Lower frequencies of both c*agA* (47%) and *vacA* s1 (58%) have been reported in gastric biopsy isolates from Mexican children with recurrent abdominal pain, but no peptic ulceration [56]. The high prevalence of virulent *H. pylori* in young children in the Colombian population we studied is likely to contribute to the high incidence of gastric cancer in adults in the area. The determination that infection by *H. pylori* is a risk factor for gastric cancer [57] highlights the importance of early detection of *H. pylori* virulence factors in children, especially in those residing in areas with a high risk for gastric cancer development.

We also examined the presence of *hopQ* gene alleles in infections in our population. Our findings revealed that the predominant allele of the *hopQ* gene was type I, found in samples from the majority (72.4%) of children compared to only 42.4% found earlier in symptomatic adults from another area of Colombia [58]. Our results also showed that type I *hopQ* genotypes were found in all cases with the *vacA* s1 genotype. Type I *hopQ* alleles were also significantly associated with *cagA*-positive status: 81% and 75% of children with type I *hopQ* positive strains also had *cagA* positive strains, at the two time points. These associations are in agreement with those found in studies by Cao et al. and Ohno et al. for *H. pylori* strains isolated from adult patients from Western countries [39, 58]. Predominance of type I *hopQ* alleles in children from the high risk area for gastric cancer also may be a factor contributing to geographic variation in gastric cancer in Colombia.

Our study demonstrates the feasibility of stool DNA amplification and genotyping of *16S rRNA* and *hopQ, cagA* and *vacA* markers in a single step PCR assay using a high number of amplification cycles. High cycle numbers in the PCR are generally avoided due to concern about artifacts; however, we have no indication that these occurred with the PCR conditions that we used. Complete reproducibility of results in independent PCRs and a very low percentage of *16S rRNA* PCR signals in the presence of negative UBTs (less than 3.6% “false positives”, if UBT is taken to be the standard for the *16S rRNA* assay) support the idea that we detected signals accurately. The low percentage of *cagA*, *vacA*, and *hopQ* PCR signals in the presence of negative *16S rRNA* PCR (from 2.4% in the *cagA* assay to 8.4% “false positives” in the *hopQ* assay, if *16S rRNA* PCR is taken to be the standard) also support the accuracy of our assays. Additionally, sequences of multiple positive *cagA*, *vacA* and *hopQ* assays in the absence of *16S rRNA* signals suggest that in some instances, it was the *16S rRNA* assay that was the false negative. In a previous study examining the presence of *cagA* and *vacA* in the stool DNA of other Colombian children, using PCR with the same primers, but without sequencing, we detected no false positive results when we tested DNA samples from *Escherichia coli, Enterobacter cloacae, Citrobacter koserii, Enterococcus fecalis, Klebsiella pneumoniae* and *Campylobacter jejuni subsp. jejuni* cultures added to the stool DNA [47]. The present study, in which the identity of the amplified fragments was confirmed by sequencing, also shows that only specific *H. pylori* DNA was amplified. Additionally, the inclusion of sequencing into the analysis brings the potential of tracking of strains, and confirms exclusion of contamination by reference strains.

We found considerable diversity of sequence in the genes we analyzed, but because other unexamined portions of the genes may contain additional polymorphisms, the variants we detected represent a minimum amount of diversity, and not a complete measure. Identical short sequences between strains are consistent with, but do not establish identity of strains.

Despite this limitation, the analyses of sequence diversity of sequences obtained from stool DNA samples allowed us to compare them at different time points, to identify children with identical gene fragments and possibly carrying the same strains, and to exclude laboratory DNA contamination with DNA from reference *H. pylori* strains. Addition of more and possibly longer amplicons to the analysis may increase the confidence with which particular *H. pylori* strains may be followed in asymptomatic children.

Because the sequences obtained allowed discrimination of *H. pylori* strains, our study provides some information that bears upon the question of dynamics of the infection in children. Sequence analysis of polymorphisms within the same segment length allowed us to determine that strains were different. Our findings are consistent with studies showing frequent gain and loss of *H. pylori* strains in children. Taneike et al. reported intrafamilial changes in infection in two 4 and 9 year old children with duodenal ulcer, as demonstrated by *rRNA* gene restriction pattern analysis and electron microscopy [59]. Other studies examining infection in Peruvian, Nicaraguan, German, Turkish and Japanese children [6, 9, 10, 60, 61] also found variation, using UBT, antigen-based stool assays or by the presence of IgG antibodies to *H. pylori*. Higher rates of acquisition and loss of infection reported by these authors (14% acquisition in Turkish children; 7% of acquisition and 35% loss of infection in German children) may be due to longer follow-up periods (6 years and 2 years respectively). The transient infection phenomenon may be an artifact of false positive tests, especially in cases of low prevalence of infection [8, 11, 62], or when using the ELISA test with stool DNA samples without molecular confirmation of positivity. In our study, UBT and our PCR assays for multiple genes all support the high prevalence of infection in the population of children we examined. Our control experiments, including comparison of sequences with our control strains, and replication, as discussed above, support that idea that the risk for false positive results in our study was low.

Our study has some limitations. Although it would have been interesting to compare the sensitivity of our analysis of stool DNA with results from analyses of *H. pylori* obtained from cultures of gastric biopsies from the same individuals, we were not able to obtain such materials from our set of children, for ethical reasons. Gastric juices from string tests from the same children were not available for comparison of sensitivity of the two methods. Our *vacA* s region assay was competitive, which means that in the presence of mixed s1/s2 infections, the larger s2 amplicon may be underrepresented. Due to the possibility of mixed infections, we cannot conclude that amplicons corresponding, for example, to *vacA* s1 and type I *hopQ* alleles in the same patient both originated from the same strain. For all amplifications, false negatives may occur, which is not surprising, considering the very small proportion of *H. pylori* DNA mixed with total DNA derived from normal enteric flora and epithelial cells of the gut. Because of the fragmentation inherent to stool DNA, we designed our primers to amplify short sequences. Both *hopQ* and *vacA* s1 primers were designed from relatively conserved regions of the respective *H. pylori* genes. However, in clinical *H. pylori* strains, variations in those regions are possible, and some negative results may occur due to mismatch of primer binding sites.

In conclusion, the present study based on stool DNA amplification and genotyping of *16S rRNA* and of *cagA, vacA* and *hopQ* genes reveals the high prevalence of disease-associated *H. pylori* infection in a group of healthy Colombian children living in a high risk area for gastric cancer. We found type I *hopQ* alleles in samples from the majority of children; this allelic type was associated with the presence of *cagA* and *vacA* s1. High percentages of disease-associated genes in *H. pylori* infected children may contribute further to the high incidence of gastric cancer in adults in the area. These noninvasive assays may prove to be useful for screening of asymptomatic and symptomatic individuals for early detection of *H. pylori* disease-associated genotypes in stool samples.

Acknowledgments

This work was supported by the National Institute of Health grants PO1CA028842, CA077955, CA116087, DK058587, DK053620, AI039657, AI068009 and the Office of Medical Research, and Department of Veterans Affairs. The authors acknowledge the devoted efforts of the Nariño's cohort staff Cristina Campo and Nancy Guerrero. We are thankful to Dr. Sean Moore, Division of Pediatric Gastroenterology of Vanderbilt University Medical Center for consultations on statistical questions. We thank Drs. Karen Goodman and Monica Sierra for helpful discussions. We greatly appreciate the participation of parents and children from the village of Nariño, Colombia.

References

1. NIH Consensus Conference. *Helicobacter pylori* in peptic ulcer disease. NIH Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Disease. JAMA. 1994; 272:65–9. [PubMed: 8007082]
2. Blaser MJ, Perez-Perez GI, Kleanthou TL, Cover RM, Peek RM Jr, Chyou PH, Stemmermann GN, Nomura A. Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res. 1995; 55:2111–5. [PubMed: 7743510]
3. Peek RM Jr. Blaser MJ. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. Nat Rev Cancer. 2002; 2:28–37. [PubMed: 11902583]
4. Blaser MJ, Kirschner D. The equilibria that allow bacterial persistence in human hosts. Nature. 2007; 449:843–9. [PubMed: 17943121]
5. Kikuchi S, Dore MP. Epidemiology of *Helicobacter pylori* Infection. Helicobacter. 2005; 10(Suppl 1):1–4. [PubMed: 16178964]
6. Rothenbacher D, Bode G, Brenner H. Dynamics of *Helicobacter pylori* infection in early childhood in a high-risk group living in Germany: loss of infection higher than acquisition. Aliment Pharmacol Ther. 2002; 16:1663–8. [PubMed: 12197846]
7. Goodman KJ, O'Rourke K, Day RS, Wang C, Nurgalieva Z, Phillips CV, Aragaki C, Campos A, de la Rosa JM. Dynamics of *Helicobacter pylori* infection in a US-Mexico cohort during the first two years of life. Int J Epidemiol. 2005; 34:1348–55. [PubMed: 16076858]
8. Haggerty TD, Perry S, Sanchez L, Perez-Perez G, Parsonnet J. Significance of transiently positive enzyme-linked immunosorbent assay results in detection of *Helicobacter pylori* in stool samples from children. J Clin Microbiol. 2005; 43:2220–3. [PubMed: 15872245]
9. Klein PD, Gilman RH, Leon-Barua R, Diaz F, Smith EO, Graham DY. The epidemiology of *Helicobacter pylori* in Peruvian children between 6 and 30 months of age. Am J Gastroenterol. 1994; 89:2196–200. [PubMed: 7977241]
10. Kehrt R, Becker M, Brosicke H, Kruger N, Helge H. Prevalence of *Helicobacter pylori* infection in Nicaraguan children with persistent diarrhea, diagnosed by the ^{13}C-urea breath test. J Pediatr Gastroenterol Nutr. 1997; 25:84–8. [PubMed: 9226533]
11. Nurgalieva ZZ, Opekun AR, Graham DY. Problem of distinguishing false-positive tests from acute or transient *Helicobacter pylori* infections. Helicobacter. 2006; 11:69–74. [PubMed: 16579835]
12. Perry S, Parsonnet J. Commentary: H. pylori infection in early life and the problem of imperfect tests. Int J Epidemiol. 2005; 34:1356–8. [PubMed: 16303814]
13. Kopanski Z, Schlegel-Zawadzka M, Witkowska B, Cienciala A, Szczerba J. Role of the enterotest in the diagnosis of the *Helicobacter pylori* infections. Eur J Med Res. 1996; 1:520–2. [PubMed: 9438153]
14. Perez-Trallero E, Montes M, Alcorta M, Zubillaga P, Telleria E. Non-endoscopic method to obtain *Helicobacter pylori* for culture. Lancet. 1995; 345:622–3. [PubMed: 7898181]
15. Velapatino B, Balqui J, Gilman RH, Bussalleu A, Quino W, Finger SA, Santivanez L, Herrera P, Piscoya A, Valdivia J, Cok J, Berg DE. Validation of string test for diagnosis of *Helicobacter pylori* infections. J Clin Microbiol. 2006; 44:976–80. [PubMed: 16517886]
16. Wang SW, Yu FJ, Lo YC, Yang YC, Wu MT, Wu IC, Lee YC, Jan CM, Wang WM, Wu DC. The clinical utility of string-PCR test in diagnosing *Helicobacter pylori* infection. HepatoGastroenterol. 2003; 50:1208–13.

17. Atherton JC. Non-endoscopic tests in the diagnosis of *Helicobacter pylori* infection. Aliment Pharmacol Ther. 1997; 11(Suppl 1):11–20. [PubMed: 9146786]
18. Kabir S. Detection of *Helicobacter pylori* DNA in feces and saliva by polymerase chain reaction: a review. Helicobacter. 2004; 9:115–23. [PubMed: 15068412]
19. Booka M, Okuda M, Shin K, Miyashiro E, Hayashi H, Yamauchi K, Tamura Y, Yoshikawa N. Polymerase chain reaction--restriction fragment length polymorphism analysis of clarithromycin-resistant *Helicobacter pylori* infection in children using stool sample. Helicobacter. 2005; 10:205–13. [PubMed: 15904478]
20. Lottspeich C, Schwarzer A, Panthel K, Koletzko S, Russmann H. Evaluation of the novel *Helicobacter pylori* ClariRes real-time PCR assay for detection and clarithromycin susceptibility testing of *H. pylori* in stool specimens from symptomatic children. J Clin Microbiol. 2007; 45:1718–22. [PubMed: 17392440]
21. Man SM, Zhang L, Day AS, Leach S, Mitchell H. Detection of enterohepatic and gastric helicobacter species in fecal specimens of children with Crohn's disease. Helicobacter. 2008; 13:234–8. [PubMed: 18665930]
22. Noguchi N, Rimbara E, Kato A, Tanaka A, Tokunaga K, Kawai T, Takahashi S, Sasatsu M. Detection of mixed clarithromycin-resistant and -susceptible *Helicobacter pylori* using nested PCR and direct sequencing of DNA extracted from faeces. J Med Microbiol. 2007; 56:1174–80. [PubMed: 17761479]
23. Queralt N, Bartolome R, Araujo R. Detection of *Helicobacter pylori* DNA in human faeces and water with different levels of faecal pollution in the north-east of Spain. J Appl Microbiol. 2005; 98:889–95. [PubMed: 15752335]
24. Rimbara E, Noguchi N, Yamaguchi T, Narui K, Kawai T, Sasatsu M. Development of a highly sensitive method for detection of clarithromycin-resistant *Helicobacter pylori* from human feces. Curr Microbiol. 2005; 51:1–5. [PubMed: 15971095]
25. Samie A, Obi CL, Barrett LJ, Powell SM, Guerrant RL. Prevalence of *Campylobacter* species, *Helicobacter pylori* and *Arcobacter* species in stool samples from the Venda region, Limpopo, South Africa: studies using molecular diagnostic methods. J Infect. 2007; 54:558–66. [PubMed: 17145081]
26. Sen N, Yilmaz O, Simsek I, Kupelioglu AA, Ellidokuz H. Detection of *Helicobacter pylori* DNA by a simple stool PCR method in adult dyspeptic patients. Helicobacter. 2005; 10:353–9. [PubMed: 16104952]
27. Stray-Pedersen A, Gaustad P, Stray-Pedersen B, Rognum TO. Detection rate of *Helicobacter pylori* stool antigen in newborn infants and small children. J Perinat Med. 2007; 35:155–8. [PubMed: 17343545]
28. Vecsei A, Innerhofer A, Binder C, Gizci H, Hammer K, Bruckdorfer A, Riedl S, Gadner H, Hirschl AM, Makristathis A. Stool polymerase chain reaction for *Helicobacter pylori* detection and clarithromycin susceptibility testing in children. Clin Gastroenterol Hepatol. 2010; 8:309–12. [PubMed: 20005978]
29. MacKay WG, Williams CL, McMillan M, Ndip RN, Shepherd AJ, Weaver LT. Evaluation of protocol using gene capture and PCR for detection of *Helicobacter pylori* DNA in feces. J Clin Microbiol. 2003; 41:4589–93. [PubMed: 14532187]
30. Falsafi T, Favaedi R, Mahjoub F, Najafi M. Application of stool-PCR test for diagnosis of *Helicobacter pylori* infection in children. World J Gastroenterol. 2009; 15:484–8. [PubMed: 19152455]
31. Bindayna KM, Al Baker WA, Botta GA. Detection of Helicobacter pylori *cagA* gene in gastric biopsies, clinical isolates and faeces. Indian J Med Microbiol. 2006; 24:195–200. [PubMed: 16912439]
32. Hirai I, Sasaki T, Kimoto A, Fujimoto S, Moriyama T, Yamamoto Y. Assessment of East Asian-type *cagA*-positive *Helicobacter pylori* using stool specimens from asymptomatic healthy Japanese individuals. J Med Microbiol. 2009; 58:1149–53. [PubMed: 19528144]
33. Russo F, Notarnicola M, Di Matteo G, Leoci C, Caruso ML, Pirrelli M, Caradonna M, Morandi L, Di Leo A. Detection of *Helicobacter pylori cagA* gene by polymerase chain reaction in faecal samples. Eur J Gastroenterol Hepatol. 1999; 11:251–6. [PubMed: 10333197]

34. Tanuma M, Rimbara E, Noguchi N, Boonyaritichaikij S, Kuwabara K, Fukunaga Y, Sasatsu M. Analysis of clarithromycin resistance and CagA status in Helicobacter pylori by use of feces from children in Thailand. J Clin Microbiol. 2009; 47:4144–5. [PubMed: 19794037]
35. van Doorn LJ, Figueiredo C, Sanna R, Plaisier A, Schneeberger P, De Boer W, Quint W. Clinical relevance of the *cagA*, *vacA*, and *iceA* status of *Helicobacter pylori*. Gastroenterology. 1998; 115:58–66. [PubMed: 9649459]
36. Atherton JC, Peek RM Jr. Tham KT, Cover TL, Blaser MJ. Clinical and pathological importance of heterogeneity in *vacA*, the vacuolating cytotoxin gene of *Helicobacter pylori*. Gastroenterology. 1997; 112:92–9. [PubMed: 8978347]
37. Sabarth N, Hurvitz R, Schmidt M, Zimny-Arndt U, Jungblut PR, Meyer TF, Bumann D. Identification of *Helicobacter pylori* surface proteins by selective proteinase K digestion and antibody phage display. J Microbiol Methods. 2005; 62:345–9. [PubMed: 15939494]
38. Loh JT, Torres VJ, Algood HM, McClain MS, Cover TL. *Helicobacter pylori* HopQ outer membrane protein attenuates bacterial adherence to gastric epithelial cells. FEMS Microbiol Lett. 2008; 289:53–8. [PubMed: 19065710]
39. Cao P, Cover TL. Two different families of *hopQ* alleles in *Helicobacter pylori*. J Clin Microbiol. 2002; 40:4504–11. [PubMed: 12454143]
40. Lehours P, Menard A, Dupouy S, Bergey B, Richy F, Zerbib F, Ruskone-Fourmestraux A, Delchier JC, Megraud F. Evaluation of the association of nine *Helicobacter pylori* virulence factors with strains involved in low-grade gastric mucosa-associated lymphoid tissue lymphoma. Infect Immun. 2004; 72:880–8. [PubMed: 14742532]
41. Oleastro M, Santos A, Cordeiro R, Nunes B, Megraud F, Menard A. Clinical relevance and diversity of two homologous genes encoding glycosyltransferases in *Helicobacter pylori*. J Clin Microbiol. 2010; 48:2885–91. [PubMed: 20554820]
42. Hirai I, Sasaki T, Fujimoto S, Moriyama T, Azuma T, Yamamoto Y. A method for assessment of *Helicobacter pylori* genotype using stool specimens. FEMS Immunol Med Microbiol. 2009; 56:63–6. [PubMed: 19484810]
43. Bravo LE, van Doorn LJ, Realpe JL, Correa P. Virulence-Associated Genotypes of *Helicobacter pylori*: Do they Explain the African Enigma? Am J Gastroenterol. 2002; 97:2839–42. [PubMed: 12425557]
44. Sicinschi LA, Correa P, Peek RMJ, Camargo MC, Delgado A, Piazuelo MB, Romero-Gallo J, Bravo LE, Schneider BG. *Helicobacter pylori* genotyping and sequencing using paraffin-embedded biopsies from residents of Colombian areas with contrasting gastric cancer risks. Helicobacter. 2008; 13:135–45. [PubMed: 18321303]
45. Correa P, Cuello C, Duque E. Carcinoma and intestinal metaplasia of the stomach in Colombian migrants. Journal of the National Cancer Institute. 1970; 44:297–306. [PubMed: 11515071]
46. Logan RP, S D, Bauer FE. The European ^{13}C-urea breath test for the detection of *Helicobacter pylori*. Eur J Gastroenterol Hepatol. 1990; 2:437–44.
47. Sicinschi L, Correa P, Bravo LE, Schneider BG. Detection and typing of H*elicobacter pylori cagA/vacA* genes by radioactive, one-step polymerase chain reaction in stool samples from children. J Microbiol Methods. 2003; 52:197–207. [PubMed: 12459240]
48. Cao P, Lee KJ, Blaser MJ, Cover TL. Analysis of *hopQ* alleles in East Asian and Western strains of *Helicobacter pylori*. FEMS Microbiol Lett. 2005; 251:37–43. [PubMed: 16102915]
49. van Doorn L-J, Figueiredo C, Sanna R, Pena S, Midolo P, Ng EWK, Atherton JC, Blaser MJ, Quint WGV. Expanding allelic diversity of *Helicobacter pylori vacA*. J Clin Microbiol. 1998; 36:2597–603. [PubMed: 9705399]
50. Atherton JC, Cao P, Peek RM. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific *vacA* types with cytotoxin production and peptic ulceration. J Biol Chem. 1995; 270:17771–7. [PubMed: 7629077]
51. Ho SA, Hoyle JA, Lewis FA, Secker AD, Cross D, Mapstone NP, Dixon MF, Wyatt JI, Tompkins DS, Taylor GR, et al. Direct polymerase chain reaction test for detection of *Helicobacter pylori* in humans and animals. J Clin Microbiol. 1991; 29:2543–9. [PubMed: 1723072]
52. Correa P. *Helicobacter pylori* infection and gastric cancer. Cancer Epidemiol Biomarkers Prevention. 2003; 12:238s–41s.

53. Camargo MC, Yepez MC, Ceron C, Guerrero N, Bravo LE, Correa P, Fontham ETH. Age at acquisition of *Helicobacter pylori* infection: Comparison of two areas with contrasting risk of gastric cancer. Helicobacter. 2004; 9:262–70. [PubMed: 15165263]
54. Agudo S, Alarcon T, Urruzuno P, Martinez MJ, Lopez-Brea M. Detection of *Helicobacter pylori* and clarithromycin resistance in gastric biopsies of pediatric patients by using a commercially available real-time polymerase chain reaction after NucliSens semiautomated DNA extraction. Diagn Microbiol Infect Dis. 2010; 67:213–9. [PubMed: 20542201]
55. Lopes AI, Palha A, Monteiro L, Olcastro M, Pelerito A, Fernandes A. *Helicobacter pylori* genotypes in children from a population at high gastric cancer risk: no association with gastroduodenal histopathology. Am J Gastroenterol. 2006; 101:2113–22. [PubMed: 16848806]
56. Gonzalez-Valencia G, Atherton JC, Munoz O, Dehesa M, la Garza AM, Torres J. *Helicobacter pylori vacA* and *cagA* genotypes in Mexican adults and children. J Infect Dis. 2000; 182:1450–4. [PubMed: 11023467]
57. IARC; Working Group on the Evaluation of Carcinogenic Risks to Humans. Schistosomes, liver flukes and Helicobacter pylori. IARC Monographs on the evaluation of carcinogenic risks to humans. International Agency for Research in Cancer, Lyon, France. 1994; 1994; 61:177–240.
58. Ohno T, Sugimoto M, Nagashima A, Ogiwara H, Vilaichone RK, Mahachai V, Graham DY, Yamaoka Y. Relationship between *Helicobacter pylori hopQ* genotype and clinical outcome in Asian and Western populations. J Gastroenterol Hepatol. 2009; 24:462–8. [PubMed: 19226380]
59. Taneike I, Tamura Y, Shimizu T, Yamashiro Y, Yamamoto T. *Helicobacter pylori* intrafamilial infections: Change in source of infection of a child from father to mother after eradication therapy. Clin Diagn Lab Immunol. 2001; 8:731–9. [PubMed: 11427419]
60. Kumagai T, Malaty HM, Graham DY, Hosogaya S, Misawa K, Furihata K, Ota H, Sei C, Tanaka E, Akamatsu T, Shimizu T, Kiyosawa K, Katsuyama T. Acquisition versus loss of *Helicobacter pylori* infection in Japan: results from an 8-year birth cohort study. J Infect Dis. 1998; 178:717–21. [PubMed: 9728540]
61. Ozen A, Ertem D, Pehlivanoglu E. Natural history and symptomatology of *Helicobacter pylori* in childhood and factors determining the epidemiology of infection. J Pediatr Gastroenterol Nutr. 2006; 42:398–404. [PubMed: 16641578]
62. Konno M, Fujii N, Yokota S, Sato K, Takahashi M, Mino E, Sugiyama T. Five-year follow-up study of mother-to-child transmission of *Helicobacter pylori* infection detected by a random amplified polymorphic DNA fingerprinting method. J Clin Microbiol. 2005; 43:2246–50. [PubMed: 15872250]

Table 1

PCR primers Used in This Study

Gene or Locus	Primer designation	Primers 5'→ 3'	Amplimer size (bp)	Reference
16S rRNA	Hpl F Hp2 R	CTGGAGAGACTAAGCCCTCC ATTACTGACGCTGATTGTGC	109	Ho et al., 1991
HP1177	hopQ I F R	ACGAACGCGCAAAAACTTTA TTGCCATTCTCATCGGTGTA	187	This study
HP1177	hopQII F R	ACAGCCACTCCAATCCAGAA AACCCCACCGTGGATTTTAG	160	This study
cagA	cagA F R	TTGACCAACAACCACAAACCGAAG CTTCCCTTAATTGCGAGATTCC	183	van Doorn et al 1998
vacA	VA1 F V136 R	ATGGAAATACAACAAACACAC CAACAATGGCTGGAATGAT	136/163	Atherton et al 1995 This study

Table 2

Detection of *H. pylori* Infection by UBT and by PCR for *16S* rRNA and for Markers of Virulence

Test	Positive results by									
	UBT[a]		PCR							
			16SrRNA		cagA		vacA		hopQ	
Visit	1	2	1	2	1	2	1	2	1	2
Positives/Tested (%)	53/73[b] (72.6)	66/86 (76.7)	45/53[c] (84.9)	55/66[c] (83.3)	23/46[d] (50.0)	33/58[d] (56.8)	30/46[d] (65.2)	36/58[d] (62.1)	27/46[d] (58.7)	41/58[d] (70.7)
Positives at either time point (%)	69/86 (80.2)		65/83 (78.3)		43/65 (66.1)		55/65 (84.6)		47/65 (72.3)	

[a] UBT considered positive when δ≥5.0%

[b] 13 children were not available for UBT at the 1st visit

[c] *16S rRNA* positive results of UBT positives

[d] marker gene assay positive results of *16S rRNA* positives.

Table 3

Comparative Performance of UBT and *16S rRNA* PCR

n(%)		
Total tested by UBT and 16S rRNA: →	**Visit 1**	**Visit 2**
	71[a]	**83**[b]
UBT+, 16S rRNA+	45 (63)	55 (66)
UBT−, *16S rRNA−*	19 (27)	17 (21)
UBT+, *16S rRNA−*	6 (8.5)	8 (9.5)
UBT−, *16S rRNA+*	1 (1.5)	3 (3.5)
p** value	0.000	0.000
PCR Sensitivity (%)	88.2	87.3
PCR Specificity (%)	95.0	85.0

[a] children from whom both stool DNA samples and UBT were available, or stool DNA samples for which both *16S rRNA* and virulence marker assay results were available

[b] from three children, stool DNA samples were unavailable.

Table 4

Relationship of *16S rRNA* PCR and Virulence Marker Gene PCR Results

n (%)						
Samples tested by *16S* and Virulence Marker Gene PCR[a]→	**cagA**		**vacA s**		**hopQ**	
	Visit 1	**Visit 2**	**Visit 1**	**Visit 2**	**Visit 1**	**Visit 2**
	71[b]	**83[c]**	**71[b]**	**82[d]**	**71[b]**	**83[b]**
16S rRNA+, Marker^{+}	26 (36)	33 (40)	36 (51)	36 (44)	32 (45)	41 (49)
16S rRNA−, Marker −	24 (34)	23 (28)	16 (23)	22 (27)	23 (33)	18 (22)
16S rRNA+, Marker −	19 (27)	25 (30)	15 (21)	21 (25)	13 (18)	17 (20.5)
16S rRNA−, Marker +	2 (3)	2 (2)	4 (5)	3 (4)	3 (4)	7 (8.5)
p value[e]	0.000	0.000	0.000	0.000	0.000	0.001
Marker PCR Sensitivity (%)	-[f]		70.6	63.2	71.1	82.0
Marker PCR Specificity (%)	-[f]		80.0	88.0	88.5	85.7

[a] Virulence Marker gene PCR assay=PCR for *cagA*, or *vacA* or *hopQ*.

[b] children from whom both stool DNA samples and UBT were available, or stool DNA samples for both *16S rRNA* and virulence marker assays were available;

[c] from three children, stool DNA samples were unavailable;

[d] stool DNA sample from four *16S rRNA* positive children were unavailable;

[e] p value from Fisher exact test;

[f] sensitivity and specificity of the *cagA* PCR assay cannot be determined, because not all *H. pylori* strains carry the *cagA* gene.

Table 5

Diversity of *hopQ* and *vacA* Genotypes at Two Time Points.

	Genotypes						
	***hopQ* (in 57 of 83 tested children)**			***vacA s* (in 60[a] of 83 tested children)**			
Allele	**type I**	**type II**	**type I+II**	**s1a**	**s1b**	**s2**	**s1+s2**
Visit 1	27	6	2	1	32	3	1
Visit 2	32	12	4	2	34	2	2
Children with same genotype at 2 time points	21	3	2	0	17	0	0
Total children with genotype:	38	15	4	3	49	5	3[b]
%→	66.6	26.3	7.1	5.0	81.7	8.3	5.0

[a] including cases negative for 16S rRNA gene;

[b] in three children, the *s1* and *s2* genotypes were found at different time points.

Table 6

Relationship between *hopQ* Alleles and *cagA* and *vacA*.

Gene or allele		hopQ type I	hopQ type II	Two-sided p value *
cagA	+	46 (60%)	2 (2.5%)	<0.0001
	−	13 (17%)	16 (20.5%)	
vacA[b]	s1	44 (86%)	2 (4%)	<0.0001
	s2	0 (0%)	5 (10%)	

* p value for difference using Fisher's exact test.