JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2001, p. 212–216 Vol. 39, No. 1
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.1.212–216.2001

Characteristics of *Helicobacter pylori* Infection in Jamaican Adults with Gastrointestinal Symptoms

MICHIE HISADA,[1]* MICHAEL G. LEE,[2] BARRIE HANCHARD,[2] MARILYN OWENS,[3] QUNSHENG SONG,[3] LEEN-JAN VAN DOORN,[4] ALAN F. CUTLER,[5] AND BENJAMIN D. GOLD[3]

Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland[1]; Departments of Medicine and Pathology, University of the West Indies, Kingston, Jamaica[2]; Division of Pediatric Gastroenterology and Nutrition, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia[3]; Delft Diagnostic Laboratory, Delft, The Netherlands[4]; and Mt. Sinai Hospital, Detroit, Michigan[5]

Received 8 August 2000/Returned for modification 16 September 2000/Accepted 27 October 2000

***Helicobacter pylori* infection is common in Jamaica. Describing its epidemiology in a population-based study depends largely on serology, but serologic assays have not been validated in this population. To address this issue, we examined the presence of *H. pylori* infection in 30 sequential adult patients with gastroduodenal symptoms by three biopsy-based methods (rapid urease test, histology, and culture) as well as by one research and two commercial enzyme-linked immunosorbent assays (ELISAs). A patient was considered *H. pylori* positive if the organism was detected by at least one biopsy-based method. Eighteen (60%) of the 30 patients were *H. pylori* positive by these criteria, whereas 21 (70%) were seropositive for *H. pylori* immunoglobulin G by our research ELISA. The presence of *H. pylori* infection in patients with gastric cancer and those with chronic gastritis was missed by biopsy-based methods but was detected by serologic assays. This observation indicates that serologic assays may be better suited for the detection of this infection in a population in which *H. pylori*-associated pathology is prevalent. The performance of our research ELISA in detecting biopsy-based *H. pylori*-positive cases was excellent, with a sensitivity and specificity of 100% and 75%, respectively. Molecular genotyping of the isolates revealed that the predominant *H. pylori* genotypes in this cohort of Jamaicans were *cagA*$^+$ *vacA* slb-m1, and *iceA2*. The validated serologic assay enables us to interpret epidemiologic data from population-based studies in Jamaica by comparison to those from other populations.**

Helicobacter pylori is a common human gastric pathogen causing chronic gastritis and duodenal ulcers (6, 12). There is strong evidence that *H. pylori* infection is also associated with gastric cancers and gastric lymphomas (6, 13). Both the prevalence of *H. pylori* infection and the incidence of gastric cancer are higher in Asia, South America, and the Caribbean than in Europe and the United States. The prevalence of *H. pylori* infection is also higher among blacks than among caucasians in the United States (10). Because *H. pylori* infection persists for life in the absence of treatment (6), its clinical sequelae continue to present a major public health burden in areas in which this bacterium is endemic.

One of the challenges in epidemiologic studies of *H. pylori* infection has been the population-specific performance of serologic assays, which has made it difficult to interpret existing data across populations. Variations in bacterial genotype, antigen selections for the immunoassays employed, and host immune responses may affect the performance of serologic assays and their suitability for particular populations.

In the present study of Jamaican patients, we evaluated the performance of two commercial enzyme-linked immunosorbent assays (ELISAs) for immunoglobulin G (IgG) antibody to *H. pylori* and a research ELISA which had been validated in epidemiologic investigations of populations from diverse geographic regions (5, 7, 26). We also describe here the relationship of *H. pylori* infection to clinical and pathological findings and the molecular genotypes of Jamaican *H. pylori* strains.

MATERIALS AND METHODS

Study subjects. We evaluated 30 sequential adult patients who underwent diagnostic gastroduodenoscopy for various upper gastrointestinal symptoms at the Gastroenterology Service of the University Hospital of the West Indies, Kingston, Jamaica. Patients with the following backgrounds were excluded from this study: history of cardiac, neurological, or pulmonary diseases precluding safe procedure; immunodeficiency; and/or antibiotic therapy during the month before the procedure. Antisecretory medications were withheld from these patients for at least 2 weeks before endoscopy. No patients had previously received *H. pylori* eradication therapy. The study protocol was approved by the institutional review boards of the National Cancer Institute and the University Hospital of the West Indies. Written informed consent was obtained from all patients. Experienced nurses collected demographic and clinical data at initial study enrollment, before *H. pylori* infection status was determined. From each subject, a total of seven biopsy specimens were obtained during endoscopy; two samples (fundus and antrum) of each were submitted for histopathological analysis, rapid urease test, and primary culture. One additional biopsy specimen was obtained from the duodenum. Biopsy specimens for histopathological analysis were fixed in 10% buffered formaldehyde and embedded in paraffin for sectioning. Other biopsy specimens were placed into a sterile cryovial (Nalgene 1 ml) with Trypticase soy medium and 20% glycerol and were frozen until used. In addition, 10 ml of blood was drawn from each subject. Serum samples were stored at −70°C until subsequently used.

Laboratory methods. (i) Rapid urease test and histological analyses. The rapid urease test (CLO-test; Trimed Laboratories, Draper, Utah) was performed on fresh biopsy specimens in the endoscopy suite, following the manufacturer's specifications. The results were read by an experienced nurse and confirmed by a physician (M.G.L.). Biopsy specimens were assessed for the presence of inflammation, as well as for *H. pylori* (via hematoxylin-eosin and Warthin-Starry stains), by an experienced pathologist (B.H.), who was blinded to the rapid

* Corresponding author. Mailing address: Viral Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd., EPS 8008, Rockville, MD 20852. Phone: (301) 435-4729. Fax: (301) 402-0817. E-mail: mh280i@nih.gov.

urease test, *H. pylori* culture, and serologic results. Six sections per biopsy sample were inspected using the high-power (40×) objective. When no organisms were identified at this magnification, the sections were further examined with an oil immersion objective (100×). Specimens were graded according to the updated Sydney classification system for gastritis (1). Scores of ≥1 in the "mononuclear cell component" indicate chronic gastritis, with the normal (score = 0) being the presence of two to five mononuclear cells per high-power field (40×) in the lamina propria.

(ii) Primary culture. After inoculation onto both selective (Skirrows; Sigma, St. Louis, Mo.) and nonselective (brain heart infusion agar with 5% sheep blood; Centers for Disease Control and Prevention, Atlanta, Ga.) solid-medium plates, incubations at 37°C for 5 to 10 days under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2) were performed until small, translucent colonies consistent with the morphology of *H. pylori* were obtained. Biochemical analyses for catalase, oxidase, and urease were performed. A dark-field examination with a flagellum stain was used to confirm the presence of *H. pylori* at each site before freezing and storage of specimens (20).

(iii) Serology. Over 70 *H. pylori* strains of various genotypes were utilized for the development of the research ELISA (5, 7). Outer membrane proteins and whole-cell antigens from a single strain and pooled strains were employed in the initial assay development process (5, 7). In addition, in order to attain improved assay performance, both outer membrane proteins and whole-cell protein antigens of *H. pylori* isolates obtained from the Jamaican patients in the present study were purified and employed in our research ELISA. Bacteria were grown overnight in *Brucella* broth (Life Technologies, Gaithersburg, Md.) with 10% fetal bovine serum (Sigma), 5 μg of trimethoprim/ml, and 10 μg of vancomycin (Sigma)/ml. Antigen extraction and protein isolation were done by gentle freeze-thaw sonication (Misonix Sonicator XL-2015; Heat Systems Inc., Farmingdale, N.Y.) (7, 15). A standard protein assay (Pierce, Rockford, Ill.) was used to determine the accurate and reproducible quantity of solid-phase antigen for our microtiter research ELISA (17).

Cross-reactivities and specificities of *H. pylori* whole-cell antigens have been described previously (7, 17). Optical density (OD) values at a wavelength of 492 nm were determined in triplicate for each biopsy-confirmed control patient serum, using a standard 96-well microtiter plate ELISA spectrophotometer (Fisher Scientific, Pittsburgh, Pa.). The mean OD values were then calculated. The ELISA cutoff values were derived using known *H. pylori*-positive and -negative control sera as previously described (5, 7).

IgG antibodies to *H. pylori* in the 30 Jamaican patients were tested by this research ELISA in two independent laboratories by investigators blinded to the patient's clinical data (B.D.G., M.O., Q.S., and A.F.C.). As mentioned above, the assays were repeated using microtiter plates coated with various quantities of whole-cell antigens obtained from the Jamaican *H. pylori* strain isolated from the culture-positive patients in the present series. The same patient samples were also tested, in a blinded fashion, by two commercial immunoassays, FlexSure (SmithKline Diagnostics Inc., San Jose, Calif.) and HM-CAP (Enteric Products Inc., Stony Brook, N.Y.), in two independent laboratories (Mt. Sinai Hospital, Detroit, Mich., and EPI, Inc., Stony Brook, N.Y.). The validity of these commercial assays for use in the clinical setting has been evaluated previously (16, 17).

(iv) Molecular genotyping. Genomic DNA from *H. pylori* was isolated under standard conditions (24). Analyses of vacuolating cytotoxin gene *vacA* (s and m regions) and of cytotoxin-associated gene *cagA* were performed by PCR techniques, using one microliter of DNA from an *H. pylori* culture lysate (21). PCR products from the *vacA* s and m regions as well as from *cagA* were analyzed simultaneously by reverse hybridization, using a line probe assay (19). Allele-specific PCR assays were used for analysis of *iceA1* and *iceA2*. Among *iceA2* strains, different subtypes were distinguished on the basis of the size of the *iceA2*-specific amplimer, as described in detail elsewhere (4). *IceA* amplimers were examined by electrophoresis on a 2% agarose gel according to standard procedures (18).

Statistical analysis. For the purpose of this analysis, *H. pylori* positivity was determined by the detection of the organism by one or more biopsy-based methods. Multiple biopsy sections were examined by histopathologic methods in order to minimize false-negative results due to sampling errors. Samples giving indeterminate OD values in ELISAs were considered *H. pylori* negative. Concordance of the results of the research ELISA and those of two commercial assays and intraassay variability (reproducibility) were assessed by using Spearman's correlation coefficient. Concordance of the biopsy-based *H. pylori* positivity and seropositivity determined by the research ELISA was examined by the χ^2 test or Fisher's exact test. Sensitivity of serologic assays was determined as the proportion of biopsy-positive patients who are also seropositive; specificity was measured as the percentage of biopsy-negative patients who were also serologically negative. *P* values were two sided, with statistical significance set at the 0.05 level.

RESULTS

Thirty symptomatic gastroendoscopic patients (17 males and 13 females) were enrolled in the study. The mean age of these patients was 53 (range, 23 to 84). Twenty-five (84%) of the 30 patients were black. Overall, *H. pylori* was detectable by one or more biopsy-based methods in 18 (60%) of the 30 patients. Among these 30 patients, the *H. pylori* positivity values determined by culture, rapid urease test, and histologic examination were 50%, 50%, and 40%, respectively. Overall, 21 (70%) of the 30 patients were positive for *H. pylori* IgG antibodies by our research ELISA.

Correlation among the triplicate OD values of the research ELISA was greater than 0.95 ($P = 0.0001$), suggesting excellent assay reproducibility. The results obtained by the three serologic assays were concordant for >70% of the samples. The sensitivity and specificity of the research ELISA were determined to be 100% (18 of 18) and 75% (9 of 12), respectively, in an analysis that used biopsy-based *H. pylori* positivity as the "gold standard." The sensitivities and specificities of two commercial assays, calculated in the same manner, were identical (100% and 50%, respectively). Additionally, we employed proteins from *H. pylori* isolates obtained from Jamaican patients in our research ELISA and compared its performance with that of the original research ELISA and two commercial assays. The research ELISA using Jamaican *H. pylori* antigens did not demonstrate any increased accuracy compared with the others.

Twenty-eight of the 30 patients had biopsy specimens evaluable by histopathologic methods (Table 1). Specimens from two patients with gastric cancer were not evaluable by the Sydney scoring method. Histopathology consistent with a diagnosis of chronic gastritis was present in 19 (68%) of the 28 evaluable patients, of whom 17 had gastric atrophy and 7 exhibited metaplasia. One patient with metaplasia also had a gastric cancer. Of 21 patients who were *H. pylori* antibody positive by our research ELISA, three were *H. pylori* negative by all biopsy-based methods. Two of these three patients had gastric adenocarcinomas; the other had normal histology (Sydney score = 0/9).

H. pylori was successfully isolated and cultured from 15 patients, including 2 patients who did not have detectable *H. pylori* by either the rapid urease test or histologic examination of the biopsy specimen (JHP022 and JHP028). As shown in Table 2, the *cagA*$^+$ *iceA2 vacA* slb-ml genotype was found in 7 of the 15 patients (JHP002, -008, -011, -013, -019, -025, and -028). Two subjects were concurrently infected with a *vacA* sla strain, a genotype common in North America, Europe, and Australia but rare in South America (23, 25), in addition to a predominant slb strain. The *vacA* s genotype appeared to be absent from three isolates. Twelve of 15 (80%) isolated strains were *cagA* positive. Only one strain exhibited an *iceA1* genotype.

DISCUSSION

The epidemiology of *H. pylori* infection in the Caribbean islands remains an important agenda for public health investigation because of the high prevalence of this infection and its

TABLE 1. Correlations among anti-*H. pylori* IgG ELISAs, rapid urease test, histology, primary culture, and corresponding Sydney score of 30 Jamaican patients who underwent gastroendoscopy at the University of the West Indies

Patient ID	Result with *H. pylori* detection method[a]:						Sydney score[b]			
	Research ELISA	HM-CAP	FlexSure	Rapid urease test	Histology	Primary culture[c]	Gastritis activity	Gastric inflammation	Atrophy	Metaplasia
JHP002	+	+	+	+	+	+	2/3	2/3	2/3	0/3
JHP005	+	+	+	+	+	−	2/3	2/3	2/3	1/3
JHP007	+	+	+	−	−	−	0/3	0/3	0/3	0/3
JHP008	+	+	+	+	+	+	2/3	2/3	2/3	0/3
JHP009	+	+	+	+	+	+	1/3	1/3	1/3	0/3
JHP011	+	+	+	+	−	+	1/3	1/3	1/3	0/3
JHP012	+	+	+	+	+	−	2/3	2/3	2/3	0/3
JHP013	+	+	+	+	−	+	1/3	2/3	1/3	1/3
JHP014	+	+	+	+	+	+	2/3	2/3	2/3	1/3
JHP015	+	+	+	+	−	+	0/3	1/3	1/3	1/3
JHP016	+	+	+	−	−	−	ND[e]	ND	ND	ND[d]
JHP017	+	+	+	+	+	+	1/3	2/3	1/3	0/3
JHP018	+	+	+	−	+	−	0/3	1/3	0/3	0/3
JHP019	+	+	+	+	+	+	1/3	1/3	1/3	0/3
JHP020	+	+	+	+	+	+	2/3	3/3	2/3	2/3
JHP021	+	+	+	+	+	+	2/3	2/3	2/3	0/3
JHP022	+	+	+	−	−	+	0/3	0/3	0/3	0/3
JHP025	+	+	+	+	−	+	1/3	2/3	2/3	1/3[d]
JHP027	+	+	+	−	−	−	ND	ND	ND	ND[d]
JHP028	+	+	+	−	−	+	1/3	2/3	2/3	0/3
JHP030	+	ND	ND	+	+	+	1/3	2/3	1/3	1/3
JHP001	±	+	+	−	−	−	0/3	0/3	0/3	0/3
JHP004	±	+	−	−	−	−	0/3	2/3	1/3	0/3
JHP006	±	−	−	−	−	−	0/3	0/3	0/3	0/3
JHP010	±	−	−	−	−	−	0/3	0/3	0/3	0/3
JHP024	±	+	+	−	−	−	0/3	0/3	0/3	0/3
JHP003	−	−	−	−	−	−	0/3	0/3	0/3	0/3
JHP023	−	−	−	−	−	−	0/3	0/3	0/3	0/3
JHP026	−	−	+	−	−	−	0/3	0/3	0/3	0/3
JHP029	−	−	−	−	−	−	0/3	1/3	0/3	0/3

[a] +, positive result; −, negative result; ±, indeterminate value (considered negative for *H. pylori*).
[b] The Sydney score was determined for samples taken from both the antrum and the body of the stomach. If the two scores differed, the worse of the two was recorded.
[c] We were unable to isolate *H. pylori* from some of the *H. pylori*-positive patients because of contamination by oral flora, gastric biopsy sampling errors, or other undetermined causes. Thus, failure to detect *H. pylori* by primary culture did not prove the absence of *H. pylori*.
[d] Subject had gastric adenocarcinoma. The Sydney score was not applicable for cancerous tissue from JHP016 and JHP027 submitted for evaluation.
[e] ND, not determined.

association with gastric cancer. Although a few studies have evaluated *H. pylori* infection in this population by utilizing commercially available serologic assays, the resultant data are difficult to interpret because of differences in the performance of serologic assays across different populations. To understand *H. pylori* transmission and disease pathogenesis in this part of the world, the development of a validated, sensitive and specific serologic assay is critical. A serologic assay has an important advantage over endoscopy-based methods for large population-based epidemiologic studies because it is noninvasive and easily employed.

In the present study, we evaluated the performance of our research ELISA in two independent laboratories, using data from patients with gastrointestinal symptoms, and found excellent reproducibility and minimal intralaboratory variation for our research ELISA results. While the sensitivities of all three serologic assays were perfect, the specificity of the research ELISA was higher than those of the two commercial assays, indicating that the use of our research ELISA would minimize false-negative results. The accuracy of our research ELISA in the biopsy-based detection of *H. pylori* has been previously validated in many asymptomatic and symptomatic populations around the world in the same manner, with sensitivities of 89 to 96% and specificities of 92 to 97% (5, 7, 26). The use of a Jamaican *H. pylori* antigen in our research ELISA in the present study did not increase the accuracy of the assay, further

TABLE 2. Molecular analysis of *H. pylori* isolates from 15 Jamaican patients

H. pylori genotype (subtype)[a]				No. of patients
vacA s	*cagA*	*iceA1*	*iceA2*	
slb	+	−	+ (229)	7
slb(a)[b]	+	−	+ (334)	2
−	+	−	−	2
slb	+	+	−	1
slb	−	−	+ (229)	1
slb	−	−	+ (334)	1
−	−	−	+ (334)	1

[a] All isolates were of genotype *vacA* m1. −, negative; +, positive.
[b] Infection with mixed strains, with predominantly slb.

confirming an excellent performance of our research ELISA for various *H. pylori* strains across different populations.

In our series, 18 (60%) of the 30 patients were positive for *H. pylori* by one or more biopsy-based methods, whereas 21 (70%) of our patients were positive by serologic assay. The seroprevalence of 70% is similar to previously reported values for *H. pylori* infection in Jamaica and Barbados (2, 8, 9). Two of the three discordant results of *H. pylori* infection status determined by serologic and biopsy-based methods were seen in gastric cancer patients with chronic atrophic changes. In both patients, all biopsy-based methods were negative for *H. pylori*, while serology was positive. These observations are consistent with the hypothesis that *H. pylori* may be no longer detectable in tissue in the presence of chronic gastric atrophy. In previous studies, the presence of gastric atrophy, which is thought to precede gastric carcinoma, has been observed to result in a decrease of the *H. pylori* load and a subsequent decline in levels of IgG antibodies to *H. pylori* (11, 14). Colonization of *H. pylori* is also at times less dense and has a different distribution (i.e., antrum versus body predominant) in achlorohydric patients, including those treated with acid inhibitors, resulting in false-negative results of either the rapid urease test, histological examination, or primary culture (3). In addition, sampling errors during biopsy may result in false negatives.

Thus, in clinical settings in which a high prevalence of *H. pylori*-related pathology is expected, any one biopsy-based method may not be sufficient to reliably detect infection. In such instances, the use of additional confirmatory methods, such as breath tests and stool antigen detection tests, in identifying truly *H. pylori*-infected cases may be recommended. The breath test exploits the urease enzyme produced by *H. pylori*, with *H. pylori* infection status being determined by detection of ^{13}C- or ^{14}C-labeled CO_2 in the expired air subsequent to ingestion of ^{13}C- or ^{14}C-labeled urea. The stool antigen test detects *H. pylori* DNA in the stool by using *H. pylori*-specific antibodies in an enzyme immunoassay. Although these noninvasive tests are highly sensitive and specific, they are logistically difficult to employ in large population-based studies. In contrast, our serologic test was found to be easy to employ and detected all biopsy-based *H. pylori*-positive cases as well as cases with *H. pylori*-associated pathology which could not have been detected by any biopsy-based method. Furthermore, only one patient with positive serologic results in our series had no apparent histopathological abnormalities, suggesting that the probability of having a false-positive serologic result is low. Taken together, serologic tests appear to be a better tool than biopsy-based methods for detecting *H. pylori* infection in epidemiologic studies of populations in which this bacterium is endemic.

The molecular characteristics of *H. pylori* infection in Jamaica had not been well described prior to this study. Our investigation indicated that the predominant *H. pylori* strain in Jamaica, including the one isolated from a patient with gastric adenocarcinoma (JHP025), has the genotype *cagA*$^+$ *iceA2 vacA* slb-ml. The predominance of the *cagA*$^+$ *vacA* slb-ml genotype is consistent with the findings for other populations from Central and South America. Two subjects were concurrently infected with a *vacA* sla strain, a genotype rare in South America but common elsewhere, including North America, Europe, and Australia (23, 25). The lack of a *vacA* s genotype in three isolates in the present study is currently unexplained but may have been caused by the existence of additional *vacA* s molecular variants, ones not readily detectable by the PCR-line probe assay. Recent studies suggest that the genotype of *H. pylori* potentially correlates with the severity of gastroduodenal disease associated with this infection and that the genotype distribution differs by geographic region (6, 22). The association of the observed genotypes and their impact on disease manifestation remains to be described further in a larger patient series.

In sum, the present study demonstrates that serum antibodies to *H. pylori* are a useful marker for epidemiologic studies of this infection in Jamaica and that the performance of our research ELISA for this purpose is excellent. Because levels of antibodies against *H. pylori* may wane after bacterial eradication or under conditions resulting in gastric atrophy and hypo- or achlorohydria, analysis of serum samples collected prior to diagnosis to establish causal associations is desirable. Thus, the pathogenesis of *H. pylori* and the risk of its transmission in Jamaica and elsewhere need to be evaluated in a prospective study.

ACKNOWLEDGMENTS

We are indebted to Andrea Reynolds, Dawn McNaughton, and Donna Simpson for technical assistance; to Beverley Cranston for administrative assistance; to Norma Kim for preparing data for analysis; to Angela Manns for support; and to Emad El-Omar, Charles Rabkin, James Goedert, and Elizabeth Maloney for thorough reviews of the manuscript.

REFERENCES

1. **Dixon, M. F., R. M. Genta, J. H. Yardley, and P. Correa.** 1996. Classification and grading of gastritis. The updated Sydney system. International Workshop on the Histopathology of Gastritis, Houston, 1994. Am. J. Surg. Pathol. **20:**1161–1181.
2. **Edwards, C. N., C. P. Douglin, P. R. Prussia, S. A. Garriques, and P. N. Levett.** 1997. Epidemiology of *Helicobacter pylori* infection in Barbados. West Indian Med. J. **46:**3–7.
3. **El-Omar, E. M., K. Oien, A. El-Nujumi, D. Gillen, A. Wirz, S. Dahill, C. Williams, J. E. S. Ardill, and K. E. L. McColl.** 1997. *Helicobacter pylori* infection and chronic gastric acid hyposecretion. Gastroenterology **113:**15–24.
4. **Figueiredo, C., W. G. Quint, R. Sanna, E. Sablon, J. P. Donahue, Q. Xu, G. G. Miller, R. M. Peek, Jr., M. J. Blaser, and L.-J. van Doorn.** 2000. Genetic organization and heterogeneity of the *iceA* locus of *Helicobacter pylori*. Gene **246:**59–68.
5. **Gold, B. D., B. Khanna, L. M. Huang, C.-Y. Lee, and N. Banatvala.** 1997. *Helicobacter pylori* acquisition in infancy after decline of maternal passive immunity. Pediatr. Res. **41:**641–646.
6. **International Agency for Research on Cancer.** 1994. Infection with *Helicobacter pylori*. IARC Monogr. Eval. Carcinog. Risks Hum. **61:**177–240.
7. **Khanna, B., A. Cutler, N. R. Israel, M. Perry, A. Lastovica, P. I. Fields, and B. D. Gold.** 1998. Use caution with serologic testing for *Helicobacter pylori* infection in children. J. Infect. Dis. **178:**460–465.
8. **Lee, M. G., M. Arthurs, S. I. Terry, E. Donaldson, P. Scott, F. Bennett, B. Hanchard, and P. N. Levett.** 1994. *Helicobacter pylori* in patients undergoing upper endoscopy in Jamaica. West Indian Med. J. **43:**84–86.
9. **Lindo, J. F., A. E. Lye-Sun, C. J. Palmer, M. G. Lee, P. Vogel, and R. D. Robinson.** 1999. Seroepidemiology of *Helicobacter pylori* infection in a Jamaican community. Trop. Med. Int. Health **4:**862–866.
10. **Malaty, H. M., D. Y. Graham, W. A. Wattigney, S. R. Srinivasan, M. Osato, and G. S. Berenson.** 1999. Natural history of *Helicobacter pylori* infection in childhood: 12-year follow-up cohort study in a biracial community. Clin. Infect. Dis. **28:**279–282.
11. **Miehlke, S., M. F. Go, J. G. Kim, D. Y. Graham, and N. Figura.** 1998. Serologic detection of *Helicobacter pylori* infection with *cagA*-positive strains in duodenal ulcer, gastric cancer, and asymptomatic gastritis. J. Gastroenterol. **33**(Suppl. 10)**:**18–21.
12. **NIH Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Disease.** 1994. *Helicobacter pylori* in peptic ulcer disease. JAMA **272:**65–69.
13. **Parsonnet, J., S. Hansen, L. Rodriguez, A. B. Gelb, R. A. Warnke, E. Jellum, N. Orentreich, J. H. Vogelman, and G. Friedman.** 1998. *Helicobacter pylori*

infection and gastric lymphoma. N. Engl. J. Med. **330:**1267–1271.
14. **Peek, R. M., Jr., M. F. Vaezi, G. W. Falk, J. R. Goldblum, G. I. Perez-Perez, J. E. Richter, and M. J. Blaser.** 1999. Role of *Helicobacter pylori cagA*$^+$ strains and specific host immune responses in the development of premalignant and malignant lesions of the gastric cardia. Int. J. Cancer **82:**520–524.
15. **Pei, Z., R. T. Ellison, and M. J. Blaser.** 1991. Identification, purification, and characterization of major antigenic proteins of *Campylobacter jejuni*. J. Biol. Chem. **266:**16363–16369.
16. **Pérez-Pérez, G. I., W. R. Brown, T. L. Cover, B. E. Dunn, P. Cao, and M. J. Blaser.** 1994. Correlation between serological and mucosal inflammation responses to *Helicobacter pylori*. Clin. Diagn. Lab. Immunol. **1:**325–329.
17. **Pérez-Pérez, G. I., B. M. Dwoskin, J. E. Chadon, and M. J. Blaser.** 1998. *Campylobacter pylori* antibodies in humans. Ann. Intern. Med. **109:**11–17.
18. **Sambrook, J., E. F. Fritch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Harbor Spring Laboratory Press, Cold Spring Harbor, N.Y.
19. **Stuyver, L., R. Rossau, A. Wyseur, M. Duhamel, B. Vanderborght, H. van Heuverswyn, and G. Maertens.** 1993. Typing of hepatitis C virus isolates and characterization of new subtypes using a line probe assay. J. Gen. Virol. **74:**1093–1102.
20. **van Doorn, L.-J., Y. Henskens, N. Nouhan, A. Verschuuren, R. Vreede, P. Herbink, G. Ponjee, K. van Krimpen, R. Blankenburg, J. Scherpenisse, and W. Quint.** 2000. The efficacy of laboratory diagnosis of *Helicobacter pylori* infections in gastric biopsy specimens is related to bacterial density and *vacA*, *cagA*, and *iceA* genotypes. J. Clin. Microbiol. **38:**13–17.
21. **van Doorn, L. J., C. Figueiredo, R. Rossau, G. Jannes, M. van Asbroeck, J. C. Sousa, F. Carneiro, and W. G. V. Quint.** 1998. Typing of *Helicobacter pylori vacA* gene and detection of *cagA* gene by PCR and reverse hybridization. J. Clin. Microbiol. **36:**1271–1276.
22. **van Doorn, L. J., C. Figueiredo, R. Sanna, A. Plaisier, P. Scheneeberger, W. de Boer, and W. Quint.** 1998. Clinical relevance of the *cagA*, *vacA*, and *iceA* status of *Helicobacter pylori*. Gastroenterology **115:**58–66.
23. **van Doorn, L. J., C. Figueiredo, F. Megraud, S. Pena, P. Midolo, D. M. Queiroz, F. Carneiro, B. Vanderborght, M. D. Pegado, R. Sanna, W. de Boer, P. M. Schneeberger, P. Correa, E. K. Ng, J. Atherton, M. J. Blaser, and W. G. Quint.** 1999. Geographic distribution of *vacA* allelic types of *Helicobacter pylori*. Gastroenterology **116:**823–830.
24. **van Doorn, N. E., F. Namavar, J. G. Kusters, E. P. van Rees, E. J. Kuipers, and J. de Graaff.** 1998. Genomic DNA fingerprinting of clinical isolates of *Helicobacter pylori* by REP-PCR and restriction fragment end-labeling. FEMS Microbiol. Lett. **160:**145–150.
25. **Yamaoka, Y., T. Kodama, O. Gutierrez, J. G. Kim, K. Kashima, and D. Y. Graham.** 1999. Relationship between *Helicobacter pylori iceA*, *cagA*, and *vacA* status and clinical outcome: studies in four different countries. J. Clin. Microbiol. **37:**2274–2279.
26. **Yip, R., P. J. Limburg, D. A. Ahlquist, H. A. Carpenter, A. O'Neill, D. Kruse, S. Stitham, B. D. Gold, E. W. Gunter, A. C. Looker, A. J. Parkinson, E. D. Nobmann, K. M. Petersen, M. Ellefson, and S. Schwartz.** 1997. Pervasive occult gastrointestinal bleeding in an Alaska native population with prevalent iron deficiency. Role of *Helicobacter pylori* gastritis. JAMA **277:**1135–1139.