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Helicobacter pylori protein-specific antibodies and risk of colorectal cancer

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Abstract

Background—There is biological plausibility as to why infection with *Helicobacter pylori,* the leading cause of gastric cancer, may also increase the risk of colorectal cancer, but the epidemiological findings have been inconsistent. We assessed the association of *H. pylori* protein-specific infection and colorectal cancer risk in the prospective cohort, the Southern Community Cohort Study.

Methods—Multiplex serology was utilized to measure antibodies to 15 *H. pylori* proteins in pre-diagnostic blood among 188 incident colorectal cancer cases and 370 controls matched by age, race, sex, and blood collection timing. Conditional logistic regression was used to calculate ORs and 95% confidence intervals (CI).

Results—Overall *H. pylori* prevalence was not associated with colorectal cancer risk (OR, 1.03; 95% CI, 0.59–1.77). However, sero-positivity to any of five specific *H. pylori* proteins (VacA, HP231, HP305, NapA, HcpC) was associated with a significant 60 to 80% increase in odds of risk. These associations became even stronger when limited to colon cancer risk, particularly for the known *H. pylori* toxin VacA (OR, 2.24; 95% CI, 1.22–4.11), including a significant, positive dose-response association by VacA antibody levels in quartiles (P<0.05). Associations with VacA sero-positivity were especially strong for early onset and late stage cancers.

Conclusions—The findings raise the hypothesis that individuals with high levels of antibodies to specific *H. pylori* proteins may be at higher risk of colon cancer.

Impact—Further investigation of the *H. pylori* – colorectal cancer association is warranted to determine the possibility of protein-specific antibody levels as a risk biomarker.

Keywords

Helicobacter pylori; colorectal cancer; colon cancer; rectal cancer; VacA; CagA

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INTRODUCTION

Infection with *Helicobacter pylori*, which induces chronic inflammation in the gastric mucosa, is the strongest known risk factor for gastric cancer. Recent studies suggest that *H. pylori* infection may also increase the risk of colorectal cancer, and although these findings have been inconsistent, two recent meta-analyses found significant 40% to 50% increased odds for colorectal cancer among individuals with evidence of a current or past *H. pylori* infection (1, 2). A primary mechanism by which *H. pylori* infection might increase the risk of colorectal cancer is via an increase in the release of gastrin (3), a peptide hormone whose main role is to stimulate gastric acid secretion, but which also functions as a mitogen (4). Chronic gastritis that results from persistent *H. pylori* infection is associated with hypergastrinemia, as *H. pylori* infection-related gastritis reduces acid secretion, which, in a negative feedback loop, induces the production of high gastrin levels (3). *H. pylori* infection could also increase risk of colorectal cancer through mechanisms related to those secondary to chronic infection and/or alteration of the bacterial flora that comprise the gastrointestinal microenvironment (4, 5, 6). Other evidence that colorectal and gastric cancers may share some aspects of a common etiology include the facts that: colorectal cancer has consistently been found to be the most common synchronous cancer among gastric cancer patients (7, 8); second primary gastric cancers are increased following colorectal cancer diagnosis (9); and, correspondingly, second primary colorectal cancers are increased following gastric cancer diagnosis (10).

The majority of the previously published studies examining the association between *H. pylori* and colorectal cancer risk did not take into account *H. pylori* strain type. As over 50% of the world's population is infected with the bacteria, and only a small percentage of those individuals develop cancer, it is important that both host and bacterial factors are considered when assessing associations with disease. Furthermore, *H. pylori* has colonized the stomach of humans for over 50,000 years, and has evolved over time to become highly genetically diverse (11). While the authors of the most recent study of the association of *H. pylori* and colorectal cancer risk did stratify by presence of the *H. pylori* protein and known gastric cancer virulence factor cytotoxin-associated antigen (CagA), the association found with CagA-positive strains was not substantially stronger than that with *H. pylori* sero-prevalence alone (significant ORs of 1.20 and 1.18, respectively) (12). Additionally, this case-control study collected blood samples at hospitalization for cancer treatment, and thus the measurement of *H. pylori* status among cases may have been hindered by presence of the tumor and/or the initiation of cancer treatment. Finally, as the authors discuss themselves, they did not inspect the association of other *H. pylori* virulence factors, such as VacA, HcpC, and GroEL, with colorectal cancer risk. Only two other studies to our knowledge have investigated CagA status and colorectal cancer risk; in the Alpha-Tocopherol, Beta-Carotene Study cohort of Finnish male smokers, no association was found with *H. pylori* sero-positivity, and there was only a suggestion of an increase in risk for colorectal cancer among persons sero-positive to CagA (OR, 1.17; 95% CI, 0.74–1.84) (13), while in a small hospital-based case-control study in Israel comprised of 67 cases of colorectal adenocarcinoma and 45 individuals hospitalized for transesophageal echocardiography as controls, CagA sero-positivity was linked to an over 10-fold increase in risk for colorectal cancer (OR, 10.6; 95% CI, 2.7–41.3) (14).

Additionally, none of the published studies of *H. pylori* and colorectal cancer risk have included a substantial number of African Americans, the racial/ethnic group with the highest incidence of and mortality from colorectal cancer in the United States (15). We recently found exceptionally high prevalence of *H. pylori* infection among African American participants in the Southern Community Cohort Study (SCCS), comprised of individuals recruited from community health centers in the southeastern United States that serve

primarily low-income and uninsured persons (16). In fact, both African Americans and whites in the SCCS have sero-prevalences that rival developing countries (89% and 69%, respectively), and African Americans are significantly more likely than whites to be infected with eight different *H. pylori* proteins suspected to be gastric cancer-associated virulence constituents, including CagA (OR, 6.4; 95% CI, 4.5–9.1) and VacA (OR, 2.3; 95% CI, 1.5–3.5) (16).

The present study sought to assess the association of *H. pylori* and colorectal cancer risk by including *H. pylori* protein-specific analyses of the bacteria in a prospective, nested case-control design among an under-studied population with a high prevalence of *H. pylori*, the SCCS.

MATERIALS AND METHODS

Study Population

As previously described in detail (17), the SCCS is a prospective cohort study that enrolled approximately 86,000 men and women from 12 southeastern states from 2002 to 2009. Participants, aged 40 to 79, were primarily (86%) recruited from community health centers (CHCs), institutions offering basic health and preventative services mainly to the medically un- and under-insured, as well as by mail (14%). Individuals recruited from CHCs participated in in-person, comprehensive computer-assisted interviews that obtained information on demographic and lifestyle factors, as well as regular diet, personal and family medical history, and health services utilization. The SCCS was reviewed and approved by institutional review boards at Vanderbilt University and Meharry Medical College. Written informed consent was obtained from all study participants. The individuals recruited by mail completed a paper version of the same baseline questionnaire. Individuals recruited at the CHCs were also asked to donate venous blood samples (20 mL) which were then immediately refrigerated. The samples were then shipped overnight to Vanderbilt University to be centrifuged the next day, and then stored at −80°C. For the assay of *H. pylori* protein antibodies as examined in the present project, a serum sample for each study subject was aliquoted into 50 μL portions.

Case identification

Incident colorectal cancer cases (*International Classification of Diseases-Oncology* [*ICD-O-3*] codes C180-189, C199, and C209) diagnosed after entry into the SCCS were identified through linkage with state cancer registries from the 12-state study area and/or from National Death Index mortality records. A total of 188 colorectal cancer cases were identified as occurring after biospecimen collection and before the end of 2011 among those SCCS participants recruited at CHCs who donated a blood sample.

Control selection

For each case, two controls were chosen, matched on age (+/− 2 years), race (African American, white, or other), sex, menopausal status (for women), CHC site, date of sample collection (+/− 6 months) and availability of serum. Matching with these criteria to identify two controls was successful for 87% of cases. For 10% of cases, the requirement for the control being recruited from the same CHC as the case was relaxed to a CHC in the same state, and for the remaining 3% of cases, the requirement for matching on CHC location was dropped altogether. Of the 376 controls chosen in this manner, three were excluded due to missing baseline data, one was missing serum, one was a duplicate, and one was not matched, resulting in 370 controls and 188 cases for the present study.

H. pylori multiplex serology

As previously described, *H. pylori* multiplex serology uses a glutathione *S*-transferase capture immunosorbent assay combined with fluorescent bead technology (Luminex) to detect antibody levels to a constellation of IgA, IgM, and IgG values to 15 *H. pylori* proteins (UreA, Catalase, GroEL, NapA, CagA, CAgM, Cagδ, HP0231, VacA, HpaA, Cad, HyuA, Omp, HcpC, and HP0305) (18, 19). These proteins were chosen for the assay based on known surface exposure and immunogenicity in 2-dimensional immunoblots (UreA, Catalase, NapA, CagA, HP231, VacA, and HpaA), serologic association with gastric cancer (GroEl, Cad, HyuA, Omp, and HcpC) and/or gastric ulcer (HP305 and CagM), and specific recognition in *H. pylori*-positive sera (Cagδ and CagM). All sera are analyzed once within a single assay day. Antigen-specific cut-off points were calculated (mean MFI plus three times standard deviation, excluding positive outliers) in 17 *H. pylori* negative sera previously classified for *H. pylori* status run within the same experiment. Defining *H. pylori* sero-positivity as reactivity with at least 4 proteins has shown good agreement (kappa=0.70) with commercial serological assay, resulting in 89% sensitivity and 82% specificity (18). For quality control, 18 samples from one pooled sample were included in the assay. The determination of sero-positivity for all of the *H. pylori* proteins was strongly consistent, and the inter-assay coefficients of variation ranged from 10–21% (notably, the CagA coefficient of variation was 13% and the VacA coefficient of variation was 15%).

Statistical analysis

To assess the individual associations of sero-positivity to each of the 15 *H. pylori* proteins with colorectal cancer risk, conditional logistic regression was used in separate models for each protein to determine ORs and 95% confidence intervals. To determine the possibility of confounding by baseline characteristics, Pearson's chi-square test was used to assess differences between cases and controls, and between individuals sero-positive and sero-negative for *H. pylori,* and between individuals sero-positive and sero-negative for VacA. No baseline characteristic was found to be associated with both colorectal cancer risk and *H. pylori* or VacA status. Smoking status was the only baseline factor associated with colorectal cancer risk, but it was not associated with *H. pylori* or VacA status. Potential confounders associated with *H. pylori* or VacA status, including education, income, regular use of aspirin, vitamin supplement use, and colorectal cancer screening history were not associated with colorectal cancer risk in this population, and, like smoking status, inclusion of these variables in the model did not alter the main effects by 10% or more. Race was strongly associated with *H. pylori* status, but this demographic characteristic could not confound the association as controls were matched to cases by race, and conditional logistic regression ensured that the association between *H. pylori* and colorectal cancer compared risk among race-concordant sets.

To examine a potential dose-response relationship between level of antibodies and colorectal cancer risk, we created quartiles of antibody levels (MFI) for each of the 15 *H. pylori* proteins assessed based on the distribution among control subjects. Conditional logistic regression was used again to determine the association of increasing quartile of *H. pylori* protein-specific antibody level and colorectal cancer risk with indicator variables representing antibody levels, using the lowest quartile as the reference category. To test for a linear trend across antibody quartiles of each protein, a continuous variable was created with the values of 0, 1, 2, and 3 for the four quartiles. Bonferroni correction for multiple testing was considered but rejected as the 15 *H. pylori* proteins assessed are significantly correlated with one another.

To further explore the relationship between *H. pylori* status and risk of colorectal cancer, we assessed the association in separate models for colon cancer (C180-189) and rectal cancer

(C199, C209), for cancer of the right/ascending colon (C180-184) and cancer of the left/descending colon (C-185-187), for cancers diagnosed at local, regional, and distant stages, individually, and for cancers by age at diagnosis (separately, in tertiles and dichotomously comparing <60 to 60+ years old). For these models we again performed conditional logistic regression, and for analyses by stage adjusted for a marker of socio-economic status (high school graduate, yes v. no) and for a history of colorectal cancer screening (ever v. never). We examined effect modification of the association of *H. pylori* status and colon cancer risk through models stratified by race and sex, and assessment of an interaction term using the likelihood ratio test.

RESULTS

Baseline characteristics of colorectal cancer cases and controls were similar in most respects, including markers of socio-economic status and body mass index (Table 1). While the great majority of individuals in this population (87.1%) were sero-positive for *H. pylori* (i.e., were positive to at least 4 of the proteins), individuals sero-negative for *H. pylori* were significantly more likely to be white (rather than African American), have obtained a high school or greater education, have a household annual income above $15,000, be a regular aspirin user, and use vitamin supplements. The same proportion of colorectal cancer cases and controls – 87% – were sero-positive to *H. pylori* overall, leading to no difference in risk (OR, 1.03; 95% CI, 0.59–1.77).

Sero-positivity to five of the 15 *H. pylori* proteins investigated – VacA, HP231, HP305, NapA, and HcpC – was associated with a statistically significant 60 to 80% increase in odds of colorectal cancer risk (Table 2). The association of sero-positivity to 9 of the other 10 *H. pylori* proteins were also in the direction of increased risk, the one exception being CagA (OR, 0.99; 95% CI, 0.64–1.53). When examining the associations with colon cancer alone, the association of these five proteins became even stronger, resulting most significantly in an over 2-fold increase in risk for individuals sero-positive to VacA (OR, 2.24; 95% CI, 1.22–4.11). The association between CagA sero-positivity and colon cancer moved in the positive direction, although it did not reach statistical significance (OR, 1.26; 95% CI, 0.74–2.16). Overall *H. pylori* sero-positivity was associated with a non-significant increase in risk for colon cancer (OR, 1.47; 95% CI, 0.74–2.93). No associations were found between overall *H. pylori* sero-positivity or sero-positivity to specific *H. pylori* proteins and rectal cancer risk. Analyses separating cancers of the right colon from those of the left colon revealed that the associations with sero-positivity to individual proteins seemed stronger for right-sided tumors than left-sided tumors (Supplementary Table S1). The association of overall *H. pylori* sero-positivity became stronger, and close to significance, for cancers of the right colon only (OR, 2.58; 95% CI, 0.94–7.05; $P = 0.06$).

When examining the association of VacA-positive *H. pylori* sero-prevalence with colorectal cancer in separate models by stage, we found no associations with local cancer (OR, 1.06; 95% CI, 0.48–2.37)or regional cancer (OR, 2.09; 95% CI, 0.88–4.92), but significant excess risk with distant colorectal cancer (OR, 5.67; 95% CI, 1.51–21.37). Similarly, when comparing the association of VacA-positive *H. pylori* sero-prevalence by age at diagnosis, significance was found only for those at the youngest end of the spectrum (diagnosed at <55 years old) (OR, 3.48; 95% CI, 1.45–8.38), and the association diminished as age at diagnosis increased to 55–64 years old (OR, 1.42; 95% CI, 0.69–2.93) and 65 years or older (OR, 1.34; 95% CI, 0.60–2.97). Stratification by race and sex in separate models did not reveal effect modification by these factors (data not shown).

A significant positive dose-response association by quartile of antibodies for risk of colorectal cancer was found for 7 of the 15 *H. pylori* proteins assessed (VacA, HP231,

HP305, NapA, HcpC, Cad, and GroEL) (Table 3). When examining these trends with colon cancer only, the dose-response trends for each of these 7 *H. pylori* proteins were strengthened (although statistical significance was lessened, due to smaller numbers), statistical significance in trend was reached for an additional protein (UreA), and a suggestion of a dose-response association was found for CagA (*P* for trend = 0.06). No dose-response associations between antibodies to *H. pylori* proteins and risk of rectal cancer were found.

DISCUSSION

In this prospective study of a predominantly low-income population with a high prevalence of *H. pylori* infection, sero-positivity to five *H. pylori* specific-proteins, most notably VacA, was associated with a 60–80% increase in risk of colorectal cancer, and this risk increased with increasing quartile of protein-specific antibody levels. The dose-response association of VacA antibody levels was particularly strong with late stage and early onset colon cancers. There was no significant association with overall *H. pylori* sero-prevalence or for rectal cancer separately.

VacA is a multi-functional toxin that targets the mitochondria through the formation of pores in the epithelial cell membrane, entering host cells through vacuoles in the cytoplasm, where it induces apoptosis as well as blocks T-cell proliferation and induces cell-cycle arrest (20). *In vivo* experiments have found that expression of VacA plays an important role in the colonization of the host (21). While most strains of *H. pylori* possess the *vacA* gene and express the associated protein, less than half produce the most active form (22). The association of increased levels of antibodies to VacA with colorectal cancer risk may be related to gastrin, a peptide hormone that induces gastric acid secretion and stimulates protein expression of COX-2 (23). Laboratory studies have found that administration of VacA-positive strains of *H. pylori* (as well as CagA-positive *H. pylori* strains) induces an inflammatory response and increases gastrinemia (24, 25, 26), and that after *H. pylori* eradication, serum gastrin levels decrease significantly (27). An association between high gastrin levels and colorectal cancer risk has been suggested in several studies of human populations (28, 29, 30), although the association only reached significance in one (30). In the most recent study of the gastrin – colorectal cancer association, mean levels of gastrin did not differ between cases and controls, although significantly higher levels were seen among patients with lymph node metastasis compared to patients without (*P* = 0.03) (29).

With the exception of the present study, no other studies to our knowledge have examined colorectal cancer risk with *H. pylori* sub-types other than CagA-positive strains. In regards to gastric cancer, serum antibodies to both CagA and VacA have been found to be associated with increased risk of disease as well as a stronger inflammatory response (31). Some studies have even found stronger associations for gastrointestinal disease among individuals with VacA antibodies than among those with CagA antibodies (32, 33, 34). However, overall, the findings for the association of VacA antibodies and gastrointestinal pathology are inconsistent, with studies that find positive associations with disease (32, 34, 35) and those that find null associations (31, 36).

While in the current study we found a null association of CagA with colorectal cancer risk, the association of *colon* cancer risk with CagA-positive strains (OR, 1.26; 95% CI, 0.74–2.16) is similar to what has been seen in other studies, although it did not reach significance in our analysis. It is possible that differences in risk associated with CagA-positive *H. pylori* might emerge if one could differentiate CagA isoforms (i.e., phylo-geographic origin of the strain and/or EPIYA-motifs) as has been done in studies of gastric cancer (37, 38, 39), although this requires the use of gastric specimens, rather than blood samples that are

collected in most prospective studies. Additionally, the present study found no evidence of an interaction by VacA and CagA strain (data not shown), whereby a VacA-CagA interaction has been suggested in gastric cancer etiology (40).

In the present study, the strongest associations with increased level of VacA antibodies were found for colon cancer and specifically cancer of the right colon, as well as for colon cancer diagnosed at earlier ages and presenting at later stage. These results conflict with a case-control study of overall and CagA-positive *H. pylori* and colorectal cancer risk in Germany that included 1,712 cases and 1,669 controls and found stronger associations with cancer of the left colon and rectum and with early-stage tumors (12). However, VacA status was not assessed and the cases were recruited during their hospital stay or even after surgery, which could affect the measurement of *H. pylori* antibodies.

Our findings of significant, strong dose-response associations of VacA antibodies with cancer of the right colon and cancers diagnosed at late stage and early onset provoke further questions. Since our findings are new and the interactions observed not specified *a priori*, and our sample is of only moderate size, the results need replication in additional prospective studies before causal inferences could be considered. Also, as sero-positivity to the five *H. pylori* proteins associated with colorectal cancer risk was highly correlated, we did not have the power to investigate the independent effects of each, although the results suggested that the findings appears to be primarily due to the association of VacA. There are mechanistic pathways – including gastrin production, gastric atrophy, and the expression of COX-2 – that might explain causality, but the presence of high levels of VacA antibodies could also be an indicator of a different underlying factor that we have not measured, such as baseline inflammatory state of the host. However, because of the high prevalence of *H. pylori* in the population we studied, and even in the general United States population for whom it is estimated that 53% of African Americans and 22% of whites are infected with *H. pylori* (41), the potential for a causal relationship needs to be explored in depth, as should it exist, opportunity for targeted cost-effective cancer prevention through *H. pylori* eradication therapy in high-risk populations could be explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Zhao YS, Wang F, Chang D, Han B, You DY. Meta-analysis of different test indicators: *Helicobacter pylori* infection and the risk of colorectal cancer. Int J Colorectal Dis. 2008; 23:875–82. [PubMed: 18506454]
2. Zumkeller N, Brenner H, Zwahlen M, Rothenbacher D. *Helicobacter pylori* infection and colorectal cancer risk: a meta-analysis. Helicobacter. 2006; 11:75–80. [PubMed: 16579836]
3. Konturek SJ, Konturek PC, Hartwich A, Hahn EG. *Helicobacter pylori* infection and gastrin and cyclooxygenase expression in gastric and colorectal malignancies. Regul Pept. 2000; 93:13–9. [PubMed: 11033048]

4. Sobhani I, Lehy T, Laurent-Puig P, Cadiot G, Ruszniewski P, Mignon M. Chronic endogenous hypergastrinemia in humans: evidence for a mitogenic effect on the colonic mucosa. Gastroenterology. 1993; 105:22–30. [PubMed: 8514038]
5. Burnett-Hartman AN, Newcomb PA, Potter JD. Infectious agents and colorectal cancer: a review of *Helicobacter pylori*, Streptococcus bovis, JC virus, and human papillomavirus. Cancer Epidemiol Biomarkers Prev. 2008; 17:2970–9. [PubMed: 18990738]
6. Peek RM Jr. *Helicobacter pylori* strain-specific modulation of gastric mucosal cellular turnover: implications for carcinogenesis. J Gastroenterol. 2002; 37 (Suppl 13):10–6. [PubMed: 12109657]
7. Eom BW, Lee HJ, Yoo MW, Cho JJ, Kim WH, Yang HK, et al. Synchronous and metachronous cancers in patients with gastric cancer. J Surg Oncol. 2008; 98:106–10. [PubMed: 18452218]
8. Lee JH, Bae JS, Ryu KW, Lee JS, Park SR, Kim CG, et al. Gastric cancer patients at high-risk of having synchronous cancer. World J Gastroenterol. 2006; 12:2588–92. [PubMed: 16688807]
9. Yoon SN, Oh ST, Lim SB, Kim TW, Kim JH, Yu CS, et al. Clinicopathologic characteristics of colorectal cancer patients with synchronous and metachronous gastric cancer. World J Surgery. 2010; 34:2168–76.
10. Kan JY, Hsieh JS, Pan YS, Wang WM, Chen FM, Jan CM, et al. Clinical characteristics of patients with sporadic colorectal cancer and primary cancers of other organs. Kaohsiung J Med Sci. 2006; 22:547–53. [PubMed: 17110343]
11. Blaser MJ. An endangered species in the stomach. Sci Am. 2005; 292:38–45. [PubMed: 15715390]
12. Zhang Y, Hoffmeister M, Weck MN, Chang-Claude J, Brenner H. *Helicobacter pylori* infection and colorectal cancer risk: evidence from a large population-based case-control study in Germany. Am J Epidemiol. 2012; 175:441–50. [PubMed: 22294430]
13. Limburg PJ, Stolzenberg-Solomon RZ, Colbert LH, Perez-Perez GI, Blaser MJ, Taylor PR, et al. *Helicobacter pylori* seropositivity and colorectal cancer risk: a prospective study of male smokers. Cancer Epidemiol Biomarkers Prev. 2002; 11:1095–9. [PubMed: 12376513]
14. Shmuely H, Passaro D, Figer A, Niv Y, Pitlik S, Samra Z, et al. Relationship between *Helicobacter pylori* CagA status and colorectal cancer. Am J Gastroenterol. 2001; 96:3406–10. [PubMed: 11774957]
15. Edwards BK, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, et al. Annual report to the nation on the status of cancer, 1975–2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer. 2010; 116:544–73. [PubMed: 19998273]
16. Epplein M, Signorello LB, Zheng W, Peek RM Jr, Michel A, Williams SM, et al. Race, African ancestry, and *Helicobacter pylori* infection in a low-income United States population. Cancer Epidemiol Biomarkers Prev. 2011; 20:826–34. [PubMed: 21357376]
17. Signorello LB, Hargreaves MK, Steinwandel MD, Zheng W, Cai Q, Schlundt DG, et al. Southern community cohort study: establishing a cohort to investigate health disparities. J Natl Med Assoc. 2005; 97:972–9. [PubMed: 16080667]
18. Michel A, Waterboer T, Kist M, Pawlita M. *Helicobacter pylori* multiplex serology. Helicobacter. 2009; 14:525–35. [PubMed: 19889070]
19. Waterboer T, Sehr P, Michael KM, Franceschi S, Nieland JD, Joos TO, et al. Multiplex human papillomavirus serology based on in situ-purified glutathione s-transferase fusion proteins. Clin Chem. 2005; 51:1845–53. [PubMed: 16099939]
20. Palframan SL, Kwok T, Gabriel K. Vacuolating cytotoxin A (VacA), a key toxin for *Helicobacter pylori* pathogenesis. Front Cell Infect Microbiol. 2012; 2:92. [PubMed: 22919683]
21. Salama NR, Otto G, Tompkins L, Falkow S. Vacuolating cytotoxin of *Helicobacter pylori* plays a role during colonization in a mouse model of infection. Infect Immun. 2001; 69:730–6. [PubMed: 11159961]
22. Cover TL, Tummuru MK, Cao P, Thompson SA, Blaser MJ. Divergence of genetic sequences for the vacuolating cytotoxin among *Helicobacter pylori* strains. J Biol Chem. 1994; 269:10566–73. [PubMed: 8144644]
23. Kountouras J, Zavos C, Chatzopoulos D, Katsinelos P. New aspects of *Helicobacter pylori* infection involvement in gastric oncogenesis. J Surg Res. 2008; 146:149–58. [PubMed: 17720195]

24. Alvarez A, Ibiza S, Hernandez C, Alvarez-Barrientos A, Esplugues JV, Calatayud S. Gastrin induces leukocyte-endothelial cell interactions in vivo and contributes to the inflammation caused by Helicobacter pylori. FASEB J. 2006; 20:2396–8. [PubMed: 17015411]
25. Brzozowski T, Konturek PC, Mierzwa M, Drozdowicz D, Bielanski W, Kwiecien S, et al. Effect of probiotics and triple eradication therapy on the cyclooxygenase (COX)-2 expression, apoptosis, and functional gastric mucosal impairment in *Helicobacter pylori*-infected Mongolian gerbils. Helicobacter. 2006; 11:10–20. [PubMed: 16423085]
26. Konturek PC, Brzozowski T, Konturek SJ, Kwiecien S, Pajdo R, Drozdowicz D, et al. Functional and morphological aspects of *Helicobacter pylori*-induced gastric cancer in Mongolian gerbils. Eur J Gastroenterol Hepatol. 2003; 15:745–54. [PubMed: 12811305]
27. Gatta L, Di Mario F, Vaira D, Rugge M, Franze A, Plebani M, et al. Quantification of serum levels of pepsinogens and gastrin to assess eradication of *Helicobacter pylori*. Clin Gastroenterol Hepatol. 2011; 9:440–2. [PubMed: 21172454]
28. D'Onghia V, Leoncini R, Carli R, Santoro A, Giglioni S, Sorbellini F, et al. Circulating gastrin and ghrelin levels in patients with colorectal cancer: correlation with tumour stage, *Helicobacter pylori* infection and BMI. Biomed Pharmacother. 2007; 61:137–41. [PubMed: 17258885]
29. Strofilas A, Lagoudianakis EE, Seretis C, Pappas A, Koronakis N, Keramidaris D, et al. Association of *Helicobacter pylori* infection and colon cancer. J Clin Med Res. 2012; 4:172–6. [PubMed: 22719803]
30. Thorburn CM, Friedman GD, Dickinson CJ, Vogelman JH, Orentreich N, Parsonnet J. Gastrin and colorectal cancer: a prospective study. Gastroenterology. 1998; 115:275–80. [PubMed: 9679032]
31. Janulaityte-Gunther D, Kupcinskas L, Pavilonis A, Valuckas K, Wadstrom T, Andersen LP. Combined serum IgG response to *Helicobacter pylori* VacA and CagA predicts gastric cancer. FEMS Immunol Med Microbiol. 2007; 50:220–5. [PubMed: 17567283]
32. Sokic-Milutinovic A, Wex T, Todorovic V, Milosavljevic T, Malfertheiner P. Anti-CagA and anti-VacA antibodies in Helicobacter pylori-infected patients with and without peptic ulcer disease in Serbia and Montenegro. Scand J Gastroenterol. 2004; 39:222–6. [PubMed: 15074390]
33. Rudi J, Kolb C, Maiwald M, Zuna I, von Herbay A, Galle PR, et al. Serum antibodies against Helicobacter pylori proteins VacA and CagA are associated with increased risk for gastric adenocarcinoma. Dig Dis Sci. 1997; 42:1652–9. [PubMed: 9286230]
34. Epplein M, Zheng W, Xiang YB, Peek RM Jr, Li H, Correa P, et al. Prospective study of *Helicobacter pylori* biomarkers for gastric cancer risk among Chinese men. Cancer Epidemiol Biomarkers Prev. 2012; 21:2185–92. [PubMed: 23035179]
35. Sezikli M, Guliter S, Apan TZ, Aksoy A, Keles H, Ozkurt ZN. Frequencies of serum antibodies to *Helicobacter pylori* CagA and VacA in a Turkish population with various gastroduodenal diseases. Int J Clin Pract. 2006; 60:1239–43. [PubMed: 16669834]
36. Kuo CH, Wu DC, Lu CY, Su YC, Yu FJ, Lee YC, et al. Low molecular weight protein of *Helicobacter pylori* and its relation to gastroduodenal diseases. Hepato-gastroenterology. 2003; 50:897–901. [PubMed: 12845945]
37. de Sablet T, Piazuelo MB, Shaffer CL, Schneider BG, Asim M, Chaturvedi R, et al. Phylogeographic origin of *Helicobacter pylori* is a determinant of gastric cancer risk. Gut. 2011; 60:1189–95. [PubMed: 21357593]
38. Hatakeyama M. *Helicobacter pylori* and gastric carcinogenesis. J Gastroenterol. 2009; 44:239–48. [PubMed: 19271114]
39. Sicinschi LA, Correa P, Peek RM, Camargo MC, Piazuelo MB, Romero-Gallo J, et al. CagA C-terminal variations in *Helicobacter pylori* strains from Colombian patients with gastric precancerous lesions. Clin Microbiol Infect. 2010; 16:369–78. [PubMed: 19456839]
40. Argent RH, Thomas RJ, Letley DP, Rittig MG, Hardie KR, Atherton JC. Functional association between the *Helicobacter pylori* virulence factors VacA and CagA. J Med Microbiol. 2008; 57:145–50. [PubMed: 18201978]
41. Cardenas VM, Graham DY. Smoking and *Helicobacter pylori* infection in a sample of U.S adults. Epidemiology. 2005; 16:586–90. [PubMed: 15951682]

Table 1

Baseline characteristics of the nested colorectal cancer case-control study in the Southern Community Cohort Study, recruited from 12 southeastern states between 2002 and 2009

	Controls (N=370)		Colorectal cancer cases (N=188)		*H. pylori*− (N=72)		*H. pylori*+ (N=486)	
	N	%	N	%	N	%	N	%
Age, years								
40–49	96	26.0	45	23.9	22	30.6	119	24.5
50–59	152	41.1	79	42.0	27	37.5	204	42.0
60–79	122	33.0	64	34.0	23	31.9	163	33.5
Race †								
African American	288	77.8	145	77.1	35	48.6	398	81.9
White	69	18.7	36	19.2	34	47.2	71	14.6
Other	13	3.5	7	3.7	3	4.2	17	3.5
Sex								
Male	167	45.1	86	45.7	33	45.8	220	45.3
Female	203	54.9	102	54.3	39	54.2	266	54.7
Marital status								
Married	127	35.1	52	28.1	24	35.3	155	32.4
Separated/Divorced	133	36.7	64	34.6	25	36.8	172	35.9
Widowed	42	11.6	31	16.8	8	11.8	65	13.6
Single/Never married	60	16.6	38	20.5	11	16.2	87	18.2
Education †								
<High school	143	39.5	80	43.2	18	26.5	205	42.8
High school	137	37.9	67	36.2	22	32.4	182	38.0
>High school	82	22.7	38	20.5	28	41.2	92	19.2
Household income †								
<$15,000	225	62.7	121	66.5	34	50.0	312	66.0
$15,000 – <$25,000	84	23.4	38	20.9	18	26.5	104	22.0
$25,000 – <$50,000	37	10.3	18	9.9	11	16.2	44	9.3
≥$50,000	13	3.6	5	2.8	5	7.4	13	2.8
Smoking status *								

	Controls (N=370)		Colorectal cancer cases (N=188)		*H. pylori*− (N=72)		*H. pylori*+ (N=486)	
	N	%	N	%	N	%	N	%
Current smoker	150	41.4	57	30.8	24	35.3	183	38.2
Former smoker	110	30.4	60	32.4	24	35.3	146	30.5
Never smoker	102	28.2	68	36.8	20	29.4	150	31.3
Body mass index								
18.3 – 24.9	91	25.2	36	20.0	15	22.7	112	23.6
25.0 – 29.9	102	28.3	64	35.6	21	31.8	145	30.5
≥30.0	168	46.5	80	44.4	30	45.5	218	45.9
Medical conditions								
Hypertension	222	61.7	110	59.5	40	58.8	292	61.2
Diabetes	100	27.7	65	35.1	18	26.5	147	30.8
High cholesterol †	129	35.8	70	38.3	35	51.5	164	34.5
COPD	37	10.3	27	14.6	10	14.7	54	11.3
Asthma	37	10.3	23	12.4	9	13.2	51	10.7
Allergies	80	22.2	41	22.2	17	25.0	104	21.8
Colorectal polyps	21	5.8	12	6.5	6	8.8	27	5.7
Heartburn/reflux	90	25.0	44	23.8	20	29.4	114	23.9
Ulcer	61	16.9	33	17.9	11	16.2	83	17.4
Family history of:								
Colorectal cancer	21	5.6	16	8.5	3	4.2	34	7.0
Gastric cancer	12	3.2	11	5.9	3	4.2	20	4.1
Regular use of:								
Aspirin †	123	34.2	72	38.9	33	49.3	162	33.9
Acetaminophen	48	13.3	27	14.6	10	14.7	65	13.6
Have insurance								
No	141	39.2	76	41.3	26	38.8	191	40.0
Yes	219	60.8	108	58.7	41	61.2	286	60.0
Ever CRC screening[a]								
No	242	68.0	135	74.2	40	61.5	337	71.3
Yes	114	32.0	47	25.8	25	38.5	136	28.8
Fruit & veg intake, times/day								

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	Controls (N=370)		Colorectal cancer cases (N=188)		*H. pylori*− (N=72)		*H. pylori*+ (N=486)	
	N	%	N	%	N	%	N	%
0–2	151	41.9	67	36.4	30	44.8	188	39.4
3–4	144	40.0	82	44.6	27	40.3	199	41.7
5+	65	18.1	35	19.0	10	14.9	90	18.9
Meat intake, times/day								
0	22	6.1	16	8.7	7	10.5	31	6.5
1–2	256	71.1	129	70.1	51	76.1	334	70.0
3+	82	22.8	39	21.2	9	13.4	112	23.5
Alcohol, drinks per day								
None	176	49.3	98	53.6	31	46.3	243	51.4
1–2	127	35.6	62	33.9	29	43.3	160	33.8
3+	54	15.1	23	12.6	7	10.5	70	14.8
Vitamin supplement use [†]								
No	211	58.9	107	58.2	29	43.3	289	60.8
Yes	147	41.1	77	41.9	38	56.7	186	39.2

[*] P<0.05 comparing colorectal cancer cases and controls

[†] P<0.05 comparing individuals by *H. pylori* status

[a] defined as ever had a sigmoidoscopy or colonoscopy (standard screening vs. diagnostic screening not distinguishable)

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Table 2

Sero-prevalence for antibodies to *H. pylori* proteins in relation to colorectal cancer incidence in a nested case-control study within the Southern Community Cohort Study*

	Risk of colorectal cancer			Risk of colon cancer			Risk of rectal cancer		
	Controls n (%)	Cases n (%)	Conditional OR (95% CI)	Controls n (%)	Cases n (%)	Conditional OR (95% CI)	Controls n (%)	Cases n (%)	Conditional OR (95% CI)
VacA−	117 (31.6)	41 (21.8)	1.00 (Reference)	78 (30.1)	25 (18.9)	1.00 (Reference)	33 (36.3)	13 (28.3)	1.00 (Reference)
VacA+	253 (68.4)	147 (78.2)	**1.84 (1.17, 2.89)**	181 (69.9)	107 (81.1)	**2.24 (1.22, 4.11)**	58 (63.7)	33 (71.7)	1.46 (0.66, 3.22)
HP231−	129 (34.9)	46 (24.5)	1.00 (Reference)	94 (36.3)	32 (24.2)	1.00 (Reference)	30 (33.0)	12 (26.1)	1.00 (Reference)
HP231+	241 (65.1)	142 (75.5)	**1.74 (1.14, 2.66)**	165 (63.7)	100 (75.8)	**1.90 (1.15, 3.14)**	61 (67.0)	34 (73.9)	1.45 (0.61, 3.43)
HP305−	172 (46.5)	66 (35.1)	1.00 (Reference)	120 (46.3)	47 (35.6)	1.00 (Reference)	41 (45.1)	16 (34.8)	1.00 (Reference)
HP305+	198 (53.5)	122 (64.9)	**1.63 (1.13, 2.35)**	139 (53.7)	85 (64.4)	**1.61 (1.04, 2.50)**	50 (55.0)	30 (65.2)	1.52 (0.72, 3.22)
NapA−	117 (31.6)	42 (22.3)	1.00 (Reference)	81 (31.3)	29 (22.0)	1.00 (Reference)	29 (31.9)	11 (23.9)	1.00 (Reference)
NapA+	253 (68.4)	146 (77.7)	**1.67 (1.10, 2.53)**	178 (68.7)	103 (78.0)	**1.68 (1.03, 2.75)**	62 (68.1)	35 (76.1)	1.50 (0.65, 3.47)
HcpC−	229 (61.9)	96 (51.1)	1.00 (Reference)	156 (60.2)	62 (47.0)	1.00 (Reference)	60 (65.9)	28 (60.9)	1.00 (Reference)
HcpC+	141 (38.1)	92 (48.9)	**1.66 (1.13, 2.43)**	103 (39.8)	70 (53.0)	**1.89 (1.19, 2.99)**	31 (34.1)	18 (39.1)	1.23 (0.57, 2.66)
Cad−	227 (61.4)	102 (54.3)	1.00 (Reference)	157 (60.6)	71 (53.8)	1.00 (Reference)	61 (67.0)	25 (54.4)	1.00 (Reference)
Cad+	143 (38.7)	86 (45.7)	1.34 (0.94, 1.92)	102 (39.4)	61 (46.2)	1.34 (0.87, 2.07)	30 (33.0)	21 (45.7)	1.64 (0.81, 3.35)
HpaA−	188 (50.8)	83 (44.2)	1.00 (Reference)	132 (51.0)	58 (43.9)	1.00 (Reference)	49 (53.9)	22 (47.8)	1.00 (Reference)
HpaA+	182 (49.2)	105 (55.9)	1.33 (0.93, 1.92)	127 (49.0)	74 (56.1)	1.36 (0.88, 2.11)	42 (46.2)	24 (52.2)	1.26 (0.61, 2.64)
Omp−	114 (30.8)	48 (25.5)	1.00 (Reference)	76 (29.3)	30 (22.7)	1.00 (Reference)	31 (34.1)	15 (32.6)	1.00 (Reference)
Omp+	256 (69.2)	140 (74.5)	1.34 (0.88, 2.03)	183 (70.7)	102 (77.3)	1.49 (0.90, 2.49)	60 (65.9)	31 (67.4)	1.05 (0.49, 2.28)
UreA−	211 (57.0)	96 (51.1)	1.00 (Reference)	148 (57.1)	65 (49.2)	1.00 (Reference)	52 (57.1)	25 (54.4)	1.00 (Reference)
UreA+	159 (43.0)	92 (48.9)	1.28 (0.90, 1.82)	111 (42.9)	67 (50.8)	1.39 (0.92, 2.12)	39 (42.9)	21 (45.7)	1.10 (0.53, 2.26)
Cagδ−	184 (49.7)	85 (45.2)	1.00 (Reference)	132 (51.0)	57 (43.2)	1.00 (Reference)	47 (51.7)	23 (50.0)	1.00 (Reference)
Cagδ+	186 (50.3)	103 (54.8)	1.18 (0.83, 1.68)	127 (49.0)	75 (56.8)	1.35 (0.88, 2.07)	44 (48.4)	23 (50.0)	1.04 (0.52, 2.11)
CagM−	143 (38.7)	67 (35.6)	1.00 (Reference)	101 (39.0)	46 (34.9)	1.00 (Reference)	34 (37.4)	18 (39.1)	1.00 (Reference)
CagM+	227 (61.4)	121 (64.4)	1.18 (0.79, 1.74)	158 (61.0)	86 (65.2)	1.26 (0.78, 2.04)	57 (62.6)	28 (60.9)	0.93 (0.44, 1.96)
HyuA−	199 (53.8)	98 (52.1)	1.00 (Reference)	139 (53.7)	72 (54.6)	1.00 (Reference)	47 (51.7)	22 (47.8)	1.00 (Reference)
HyuA+	171 (46.2)	90 (47.9)	1.07 (0.76, 1.52)	120 (46.3)	60 (45.5)	0.97 (0.64, 1.47)	44 (48.4)	24 (52.2)	1.18 (0.56, 2.50)
GroEL−	79 (21.4)	38 (20.2)	1.00 (Reference)	52 (20.1)	25 (18.9)	1.00 (Reference)	22 (24.2)	12 (26.1)	1.00 (Reference)
GroEL+	291 (78.7)	150 (79.8)	1.08 (0.69.1.70)	207 (79.9)	107 (81.1)	1.10 (0.63.1.92)	69 (75.8)	34 (73.9)	0.89 (0.39, 2.02)

	Risk of colorectal cancer			Risk of colon cancer			Risk of rectal cancer		
	Controls n (%)	**Cases n (%)**	**Conditional OR (95% CI)**	**Controls n (%)**	**Cases n (%)**	**Conditional OR (95% CI)**	**Controls n (%)**	**Cases n (%)**	**Conditional OR (95% CI)**
Catalase−	178 (48.1)	89 (47.3)	1.00 (Reference)	133 (51.4)	57 (43.2)	1.00 (Reference)	37 (40.7)	26 (56.5)	1.00 (Reference)
Catalase+	192 (51.9)	99 (52.7)	1.05 (0.73, 1.51)	126 (48.7)	75 (56.8)	1.43 (0.93, 2.22)	54 (59.3)	20 (43.5)	0.51 (0.24, 1.10)
CagA−	115 (31.1)	59 (31.4)	1.00 (Reference)	81 (31.3)	37 (28.0)	1.00 (Reference)	28 (30.8)	20 (43.5)	1.00 (Reference)
CagA+	255 (68.9)	129 (68.6)	0.99 (0.64, 1.53)	178 (68.7)	95 (72.0)	1.26 (0.74, 2.16)	63 (69.2)	26 (56.5)	0.47 (0.20, 1.12)

*Results are from a conditional logistic regression model, among cases and controls matched on age, race, sex, menopausal status (for women), CHC site, date of sample collection, and availability of serum, assessing the association of sero-positivity to each *H. pylori* protein, in separate models, with odds of incident colorectal cancer.

NOTE: Bold indicates statistically significant at $P < 0.05$.

Table 3

Risk of colorectal cancer by antibody level of *H. pylori* proteins in the Southern Community Cohort Study*

	Risk of colorectal cancer			Risk of colon cancer			Risk of rectal cancer		
Antibody level (MFI)	**Controls n (%)**	**Cases n (%)**	**Conditional OR (95% CI)**	**Controls n (%)**	**Cases n (%)**	**Conditional OR (95% CI)**	**Controls n (%)**	**Cases n (%)**	**Conditional OR (95% CI)**
VacA									
Q1	93 (25.1)	33 (17.6)	1.00 (Reference)	61 (23.6)	19 (14.4)	1.00 (Reference)	28 (30.8)	12 (26.1)	1.00 (Reference)
Q2	92 (24.9)	49 (26.1)	1.63 (0.93, 2.85)	67 (25.9)	37 (28.0)	**2.21 (1.04, 4.70)**	22 (24.2)	9 (19.6)	0.93 (0.34, 2.55)
Q3	93 (25.1)	52 (27.7)	**1.82 (1.02, 3.25)**	66 (25.5)	39 (29.6)	**2.53 (1.17, 5.50)**	18 (19.8)	10 (21.7)	1.43 (0.50, 4.08)
Q4	92 (24.9)	54 (28.7)	**1.96 (1.07, 3.57)**	65 (25.1)	37 (28.0)	**2.53 (1.13, 5.69)**	23 (25.3)	15 (32.6)	1.74 (0.60, 5.00)
P trend			**0.04**			**0.05**			0.29
HP231									
Q1	93 (25.1)	30 (16.0)	1.00 (Reference)	68 (26.3)	20 (15.2)	1.00 (Reference)	20 (22.0)	8 (17.4)	1.00 (Reference)
Q2	92 (24.9)	39 (20.7)	1.31 (0.75, 2.30)	65 (25.1)	26 (19.7)	1.38 (0.70, 2.73)	22 (24.2)	12 (26.1)	1.32 (0.44, 3.92)
Q3	93 (25.1)	57 (30.3)	**2.02 (1.17, 3.49)**	55 (21.2)	41 (31.1)	**2.70 (1.39, 5.25)**	31 (34.1)	12 (26.1)	0.95 (0.32, 2.84)
Q4	92 (24.9)	62 (33.0)	**2.17 (1.28, 3.70)**	71 (27.4)	45 (34.1)	**2.27 (1.20, 4.27)**	18 (19.8)	14 (30.4)	1.83 (0.61, 5.45)
P trend			**0.002**			**0.005**			0.36
HP305									
Q1	93 (25.1)	32 (17.0)	1.00 (Reference)	67 (25.9)	23 (17.4)	1.00 (Reference)	21 (23.1)	9 (19.6)	1.00 (Reference)
Q2	92 (24.9)	38 (20.2)	1.20 (0.68, 2.12)	63 (24.3)	28 (21.2)	1.30 (0.67, 2.54)	21 (23.1)	7 (15.2)	0.78 (0.25, 2.47)
Q3	94 (25.4)	57 (30.3)	**1.76 (1.05, 2.94)**	66 (25.5)	36 (27.3)	1.61 (0.86, 3.00)	26 (28.6)	17 (37.0)	1.47 (0.56, 3.86)
Q4	91 (24.6)	61 (32.5)	**2.11 (1.22, 3.65)**	63 (24.3)	45 (34.1)	**2.31 (1.21, 4.41)**	23 (25.3)	13 (28.3)	1.40 (0.44, 4.45)
P trend			**0.003**			**0.009**			0.37
NapA									
Q1	96 (26.0)	39 (20.7)	1.00 (Reference)	65 (25.1)	28 (21.2)	1.00 (Reference)	24 (26.4)	10 (21.7)	1.00 (Reference)
Q2	89 (24.1)	37 (19.7)	1.07 (0.62, 1.85)	70 (27.0)	25 (18.9)	0.88 (0.46, 1.68)	16 (17.6)	9 (19.6)	1.41 (0.41, 4.89)
Q3	93 (25.1)	55 (29.3)	1.48 (0.89, 2.46)	68 (26.3)	43 (32.6)	1.51 (0.84, 2.71)	22 (24.2)	9 (19.6)	0.97 (0.32, 2.94)
Q4	92 (24.9)	57 (30.3)	1.59 (0.95, 2.64)	56 (21.6)	36 (27.3)	1.57 (0.84, 2.96)	29 (31.9)	18 (39.1)	1.47 (0.57, 3.79)
P trend			**0.03**			**0.05**			0.53
HcpC									
Q1	93 (25.1)	41 (21.8)	1.00 (Reference)	65 (25.1)	26 (19.7)	1.00 (Reference)	24 (26.4)	12 (26.1)	1.00 (Reference)

	Risk of colorectal cancer			Risk of colon cancer			Risk of rectal cancer		
Antibody level (MFI)	**Controls n (%)**	**Cases n (%)**	**Conditional OR (95% CI)**	**Controls n (%)**	**Cases n (%)**	**Conditional OR (95% CI)**	**Controls n (%)**	**Cases n (%)**	**Conditional OR (95% CI)**
Q2	92 (24.9)	36 (19.2)	0.91 (0.52, 1.58)	66 (25.5)	22 (16.7)	0.87 (0.44, 1.73)	19 (20.9)	12 (26.1)	1.24 (0.44, 3.54)
Q3	93 (25.1)	52 (27.7)	1.36 (0.80, 2.30)	61 (23.6)	40 (30.3)	1.88 (0.98, 3.61)	29 (31.9)	10 (21.7)	0.68 (0.24, 1.93)
Q4	92 (24.9)	59 (31.4)	1.52 (0.90, 2.55)	67 (25.9)	44 (33.3)	1.84 (0.97, 3.51)	19 (20.9)	12 (26.1)	1.16 (0.42, 3.21)
P trend			**0.05**			**0.02**			0.96
Cad									
Q1	95 (25.7)	41 (21.8)	1.00 (Reference)	69 (26.6)	31 (23.5)	1.00 (Reference)	23 (25.3)	8 (17.4)	1.00 (Reference)
Q2	91 (24.6)	45 (23.9)	1.12 (0.67. 1.86)	64 (24.7)	29 (22.0)	0.95 (0.51, 1.75)	24 (26.4)	13 (28.3)	1.62 (0.55, 4.78)
Q3	93 (25.1)	31 (16.5)	0.74 (0.42, 1.30)	65 (25.1)	20 (15.2)	0.66 (0.34, 1.31)	21 (23.1)	9 (19.6)	1.18 (0.38, 3.67)
Q4	91 (24.6)	71 (37.8)	**1.71 (1.07, 2.72)**	61 (23.6)	52 (39.4)	**1.77 (1.02, 3.05)**	23 (25.3)	16 (34.8)	1.99 (0.70, 5.61)
P trend			**0.04**			**0.05**			0.28
HpaA									
Q1	94 (25.4)	33 (17.6)	1.00 (Reference)	67 (25.9)	21 (15.9)	1.00 (Reference)	25 (27.5)	9 (19.6)	1.00 (Reference)
Q2	91 (24.6)	49 (26.1)	1.56 (0.92, 2.64)	63 (24.3)	37 (28.0)	**1.95 (1.02, 3.73)**	23 (25.3)	12 (26.1)	1.40 (0.50, 3.90)
Q3	93 (25.1)	54 (28.7)	1.71 (1.01, 2.90)	59 (22.8)	41 (31.1)	**2.39 (1.23, 4.64)**	24 (26.4)	11 (23.9)	1.28 (0.46, 3.53)
Q4	92 (24.9)	52 (27.7)	1.66 (0.97, 2.86)	70 (27.0)	33 (25.0)	1.62 (0.83, 3.18)	19 (20.9)	14 (30.4)	1.98 (0.69, 5.66)
P trend			0.08			0.20			0.25
Omp									
Q1	93 (25.1)	40 (21.3)	1.00 (Reference)	61 (23.6)	25 (18.9)	1.00 (Reference)	26 (28.6)	13 (28.3)	1.00 (Reference)
Q2	92 (24.9)	45 (23.9)	1.17 (0.70, 1.97)	66 (25.5)	31 (23.5)	1.20 (0.63, 2.29)	22 (24.2)	11 (23.9)	0.97 (0.38, 2.50)
Q3	93 (25.1)	47 (25.0)	1.24 (0.73, 2.10)	66 (25.5)	36 (27.3)	1.40 (0.74, 2.63)	23 (25.3)	9 (19.6)	0.78 (0.26, 2.30)
Q4	92 (24.9)	56 (29.8)	1.53 (0.89, 2.63)	66 (25.5)	40 (30.3)	1.60 (0.84, 3.06)	20 (22.0)	13 (28.3)	1.43 (0.44, 4.68)
P trend			0.13			0.13			0.75
UreA									
Q1	93 (25.1)	37 (19.7)	1.00 (Reference)	74 (28.6)	25 (18.9)	1.00 (Reference)	16 (17.6)	10 (21.7)	1.00 (Reference)
Q2	92 (24.9)	47 (25.0)	1.27 (0.75, 2.13)	56 (21.6)	32 (24.2)	1.67 (0.88, 3.17)	29 (31.9)	11 (23.9)	0.64 (0.23, 1.77)
Q3	93 (25.1)	40 (21.3)	1.06 (0.61, 1.82)	70 (27.0)	29 (22.0)	1.17 (0.61, 2.23)	20 (22.0)	10 (21.7)	0.75 (0.25, 2.33)
Q4	92 (24.9)	64 (34.0)	**1.77 (1.06, 2.95)**	59 (22.8)	46 (34.9)	**2.38 (1.29, 4.39)**	26 (28.6)	15 (32.6)	0.91 (0.32, 2.64)
P trend			0.05			**0.02**			0.89
Cagδ									

	Risk of colorectal cancer			Risk of colon cancer			Risk of rectal cancer		
Antibody level (MFI)	**Controls n (%)**	**Cases n (%)**	**Conditional OR (95% CI)**	**Controls n (%)**	**Cases n (%)**	**Conditional OR (95% CI)**	**Controls n (%)**	**Cases n (%)**	**Conditional OR (95% CI)**
Q1	94 (25.4)	47 (25.0)	1.00 (Reference)	72 (27.8)	32 (24.2)	1.00 (Reference)	20 (22.0)	12 (26.1)	1.00 (Reference)
Q2	92 (24.9)	38 (20.2)	0.80 (0.47, 1.35)	62 (23.9)	25 (18.9)	0.88 (0.48, 1.63)	27 (29.7)	11 (23.9)	0.60 (0.19, 1.85)
Q3	92 (24.9)	43 (22.9)	0.93 (0.56, 1.53)	57 (22.0)	32 (24.2)	1.27 (0.70, 2.30)	28 (30.8)	8 (17.4)	0.44 (0.15, 1.31)
Q4	92 (24.9)	60 (31.9)	1.29 (0.80, 2.08)	68 (26.3)	43 (32.6)	1.41 (0.81, 2.48)	16 (17.6)	15 (32.6)	1.63 (0.56, 4.81)
P trend			0.25			0.15			0.57
CagM									
Q1	93 (25.1)	44 (23.4)	1.00 (Reference)	69 (26.6)	30 (22.7)	1.00 (Reference)	20 (22.0)	11 (23.9)	1.00 (Reference)
Q2	92 (24.9)	40 (21.3)	0.94 (0.56, 1.58)	61 (23.6)	28 (21.2)	1.08 (0.58, 2.01)	24 (26.4)	11 (23.9)	0.79 (0.26, 2.40)
Q3	93 (25.1)	48 (25.5)	1.12 (0.66, 1.90)	66 (25.5)	36 (27.3)	1.31 (0.70, 2.45)	23 (25.3)	11 (23.9)	0.85 (0.29, 2.46)
Q4	92 (24.9)	56 (29.8)	1.32 (0.79, 2.20)	63 (24.3)	38 (28.8)	1.45 (0.78, 2.68)	24 (26.4)	13 (28.3)	0.94 (0.32, 2.71)
P trend			0.21			0.20			1.00
HyuA									
Q1	94 (25.4)	39 (20.7)	1.00 (Reference)	66 (25.5)	29 (22.0)	1.00 (Reference)	21 (23.1)	9 (19.6)	1.00 (Reference)
Q2	92 (24.9)	51 (27.1)	1.36 (0.82, 2.25)	65 (25.1)	36 (27.3)	1.28 (0.70, 2.34)	22 (24.2)	13 (28.3)	1.33 (0.46, 3.82)
Q3	92 (24.9)	46 (24.5)	1.24 (0.74, 2.08)	67 (25.9)	31 (23.5)	1.09 (0.59, 2.00)	22 (24.2)	13 (28.3)	1.34 (0.45, 3.99)
Q4	92 (24.9)	52 (27.7)	1.39 (0.83, 2.32)	61 (23.6)	36 (27.3)	1.36 (0.75, 2.47)	26 (28.6)	11 (23.9)	1.00 (0.31, 3.28)
P trend			0.30			0.46			0.93
GroEL									
Q1	93 (25.1)	44 (23.4)	1.00 (Reference)	63 (24.3)	30 (22.7)	1.00 (Reference)	25 (27.5)	13 (28.3)	1.00 (Reference)
Q2	92 (24.9)	27 (14.4)	0.64 (0.36, 1.14)	67 (25.9)	17 (12.9)	0.54 (0.26, 1.10)	19 (20.9)	7 (15.2)	0.69 (0.23, 2.11)
Q3	93 (25.1)	48 (25.5)	1.09 (0.64, 1.83)	59 (22.8)	33 (25.0)	1.17 (0.63, 2.20)	29 (31.9)	10 (21.7)	0.60 (0.21, 1.74)
Q4	92 (24.9)	69 (36.7)	**1.69 (1.02, 2.82)**	70 (27.0)	52 (39.4)	1.67 (0.92, 3.04)	18 (19.8)	16 (34.8)	1.71 (0.61, 4.74)
P trend			**0.008**			**0.01**			0.37
Catalase									
Q1	94 (25.4)	45 (23.9)	1.00 (Reference)	69 (26.6)	30 (22.7)	1.00 (Reference)	19 (20.9)	13 (28.3)	1.00 (Reference)
Q2	91 (24.6)	48 (25.5)	1.12 (0.68, 1.83)	70 (27.0)	31 (23.5)	1.04 (0.57, 1.90)	19 (20.9)	13 (28.3)	1.03 (0.40, 2.69)
Q3	93 (25.1)	45 (23.9)	1.03 (0.61, 1.73)	58 (22.4)	33 (25.0)	1.34 (0.73, 2.46)	28 (30.8)	10 (21.7)	0.49 (0.16, 1.45)
Q4	92 (24.9)	50 (26.6)	1.16 (0.70, 1.93)	62 (23.9)	38 (28.8)	1.47 (0.80, 2.69)	25 (27.5)	10 (21.7)	0.62 (0.22, 1.72)
P trend			0.64			0.15			0.20

	Risk of colorectal cancer			Risk of colon cancer			Risk of rectal cancer		
Antibody level (MFI)	**Controls n (%)**	**Cases n (%)**	**Conditional OR (95% CI)**	**Controls n (%)**	**Cases n (%)**	**Conditional OR (95% CI)**	**Controls n (%)**	**Cases n (%)**	**Conditional OR (95% CI)**
CagA									
Q1	93 (25.1)	46 (24.5)	1.00 (Reference)	68 (26.3)	28 (21.2)	1.00 (Reference)	21 (23.1)	17 (37.0)	1.00 (Reference)
Q2	92 (24.9)	40 (21.3)	0.91 (0.53, 1.58)	64 (24.7)	29 (22.0)	1.25 (0.63, 2.44)	26 (28.6)	7 (15.2)	**0.30 (0.10, 0.94)**
Q3	93 (25.1)	51 (27.1)	1.14 (0.66, 1.95)	66 (25.5)	35 (26.5)	1.46 (0.75, 2.87)	21 (23.1)	13 (28.3)	0.65 (0.23, 1.83)
Q4	92 (24.9)	51 (27.1)	1.19 (0.68, 2.07)	61 (23.6)	40 (30.3)	1.87 (0.95, 3.67)	23 (25.3)	9 (19.6)	0.42 (0.13, 1.34)
P trend			0.40			0.06			0.23

*Results are from a conditional logistic regression model, among cases and controls matched on age, race, sex, menopausal status (for women), CHC site, date of sample collection, and availability of serum, assessing the association of sero-positivity to each *H. pylori* protein, in separate models, with odds of incident colorectal cancer.

NOTE: Bold indicates statistically significant at $P < 0.05$.