

## COUNTRY-SPECIFIC CONSTANCY BY AGE IN *cagA*<sup>+</sup> PROPORTION OF *Helicobacter pylori* INFECTIONS

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*Helicobacter pylori* strains may be either *cagA*<sup>+</sup> or *cagA*<sup>-</sup>, and in longitudinal studies, infection with a *cagA*<sup>+</sup> strain has been associated with increased risk for the development of atrophic gastritis and cancer of the distal stomach. We sought to determine the relative proportion of strains producing CagA in different geographic locales, and the extent to which CagA seroprevalence varied in countries with different gastric and esophageal cancer rates. Using an enzyme-linked immunosorbent assay (ELISA) to detect serum IgG to CagA, we examined sera from 468 asymptomatic *H. pylori*-infected adults from Canada, Peru, China, Thailand, The Netherlands and 3 different ethnic groups in New Zealand. The CagA seroprevalence in Peru and Thailand (82.2% and 78.8%, respectively) were each substantially higher than for the Chinese (37.9%), Canadian (41.9%), Dutch (39.0%) and New Zealand (28.2%) subjects, but within each population, rates were relatively constant across gender and age groups. Reported gastric but not esophageal cancer rates for the 8 studied populations were significantly associated with *H. pylori* seroprevalence. Variation in CagA positivity rates was not significantly associated with variation in either gastric or esophageal cancer rates. Our data suggest that CagA seroprevalence is not the major factor influencing gastric cancer rates. *Int. J. Cancer* 72:453–456, 1997.

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Infection with *Helicobacter pylori* has been found to be common throughout the world, with prevalences in adult populations ranging from 30% in developed countries, to more than 60% in developing countries (Taylor and Parsonnet, 1995). *H. pylori* usually establishes a persistent infection, which is characterized by chronic gastritis (Karttunen *et al.*, 1991). This chronic condition can be maintained in balance for many years, but may progress to ulceration, atrophic gastritis, intestinal metaplasia or cancer of the distal stomach (Sipponen, 1994; Blaser *et al.*, 1995a).

Several virulence factors, including expression of a vacuolating cytotoxin and the associated immunodominant (CagA) antigen, have been proposed to explain the different outcomes of *H. pylori* infection (Peek *et al.*, 1995b). The high m.w. (120–140 kDa) CagA protein encoded by *cagA* (Covacci *et al.*, 1993; Tummuru *et al.*, 1993) is recognized by serum antibodies from infected persons (Cover *et al.*, 1990; Crabtree *et al.*, 1992). Among Western populations, *cagA* expression *in vivo* and the presence of antibodies to CagA in either serum or mucosal secretions are significantly more prevalent among patients with peptic ulceration than among those with gastritis alone (Crabtree *et al.*, 1993; Peek *et al.*, 1995a), and *cagA*<sup>+</sup> strains induce higher grades of gastric inflammation (Peek *et al.*, 1995b), which may account for this increased risk.

*H. pylori* infection also has been associated with higher risk of adenocarcinoma of the distal stomach (Forman *et al.*, 1991; Nomura *et al.*, 1991; Parsonnet *et al.*, 1991), and current models of pathogenesis indicate multifactorial causation (Sipponen and Sepälä, 1992). In longitudinal studies, *cagA* positivity was associated with increased risk for development of atrophic gastritis (Kuipers *et al.*, 1995) and of gastric cancer (Blaser *et al.*, 1995b). The

purpose of the present study was to investigate the extent to which CagA seroprevalence among *H. pylori*-infected persons in populations from different geographic areas varies, and to determine whether this variation correlates with risk of either gastric or esophageal cancer. To accomplish these goals, we screened serum samples from asymptomatic adults from 8 different populations in 6 countries on 5 continents.

### MATERIAL AND METHODS

#### Populations

Serum samples were collected from healthy persons (who were not endoscopy subjects) in 8 populations (representing 5 continents) and were selected from stored sera previously collected. The 8 populations (and numbers of subjects) were from: Soongnern District, Thailand (n = 130) (Pérez-Pérez *et al.*, 1990); Lima, Peru (n = 178); Shandong province, China (n = 177) (Zhang *et al.*, 1996); Manitoba, Canada (n = 469) (Pérez-Pérez *et al.*, 1992); Amsterdam, The Netherlands (n = 105) (Kuipers *et al.*, 1995); and New Zealand (n = 579) (Fraser *et al.*, 1996). From New Zealand, 3 different ethnic groups were examined: Caucasian (n = 190), Pacific Islander (n = 194) and Maori (n = 195). The Canadian, Dutch and New Zealand Caucasian populations were almost or completely Caucasian and thus represented populations of European ancestry.

#### Diagnosis of *H. pylori* infection

Serologic assay for *H. pylori*-specific immunoglobulin (Ig) G was performed in all samples excluding those from New Zealand, using an enzyme-linked immunosorbent assay (ELISA), as previously described (Pérez-Pérez *et al.*, 1988). An optical density ratio (ODR) value of >1.0 was considered seropositive, while a value of <1.0 was considered negative. To correct for plate-to-plate variation, we expressed the results as ODR in relation to standard sera as previously described (Pérez-Pérez *et al.*, 1991, 1992). For the New Zealand population, the diagnosis of *H. pylori* infection was established using the Roche COBAS assay, as reported elsewhere (Fraser *et al.*, 1996). We compared the ELISA results obtained by the Cobas assay and our regular assay by examining a randomly selected 38 serum samples from a pool of 321 persons known to be

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TABLE I – *H. pylori* SEROPREVALENCE AMONG ADULTS IN 6 COUNTRIES

Country/ population	Total population <sup>1</sup>		Age 35–65 <sup>2</sup>	
	Number of samples studied	% <i>H. pylori</i> seropositive	Number of samples studied	% <i>H. pylori</i> seropositive
Thailand	130	50.8	97	49.5
Peru	178	67.4	97	72.2
China	177	62.7	177	62.7
Canada	469	35.2	265	46.0
Netherlands	105	50.4	105	50.4
New Zealand	579	55.4	579	55.4
Caucasians	190	35.8	190	35.8
Maori	195	57.4	195	57.4
Pacific Islanders	194	73.2	194	73.2

<sup>1</sup>Subjects were healthy adults over 20 years of age; age distribution of the sample varied between populations. <sup>2</sup>Subjects represent the 35–65-year-old subset of the total population.

*H. pylori*-infected. All 38 were positive in both assays, and linear regression analysis showed that results of the assays matched closely ( $r = 0.86$ ,  $p < 0.001$ ). All serological assays were performed for each sample on at least 2 different days.

#### CagA ELISA

Enzyme-linked immunosorbent assay to detect anti-CagA IgG was performed using purified recombinant CagA antigen, as previously described (Blaser *et al.*, 1995b). The assays were performed on sera from *H. pylori*-seropositive persons only; from populations in which we had large numbers of samples, we selected a subset that yielded an equal proportion of males and females, and included persons ranging from age 20–65 years. ODR values were calculated for each unknown serum in relation to reference sera. An ODR of  $\geq 0.300$  was considered seropositive, and a value of  $< 0.300$  was considered seronegative.

#### Cancer rates in the populations studied

The reported rates for stomach cancer and esophageal cancer for the 8 populations studied were obtained from the World Health Organization data sets (Parkin and Muir, 1992). The rates used in this study are calculated averages based on age-adjusted data according to an accepted standard (world-standardized ASR) (Parkin and Muir, 1992).

#### Data analysis

The chi-squared statistic was used to compare prevalence of *H. pylori* infection and CagA seroprevalence within the communities studied. We calculated the correlations between seroprevalence of *H. pylori* and CagA in the 8 populations with reported incidences of gastric and esophageal cancers by linear regression analysis using the Abstat statistical programs. To maximize comparability of data, we only examined *H. pylori* and CagA positivity rates in healthy persons of the same age (35–65 years) in each population.

## RESULTS

#### *H. pylori* seroprevalence among healthy persons

The *H. pylori* prevalence among healthy adults from 8 populations from 5 continents ranged from 35.8–73.2% (Table I). The lowest seroprevalences were observed in Canadians and Caucasians from New Zealand. The highest rates were among Pacific Islanders and Peruvians, and rates were intermediate among persons from The Netherlands, Thailand and China. Major differences observed between ethnic groups in New Zealand parallel heterogeneity reported in other countries between ethnic groups (Malaty *et al.*, 1992, 1996). Rates for the subsets from age 35–65 years parallel those for the larger groups (Table I).

#### Seroprevalence of CagA-positive *H. pylori*-infected persons

We then examined specimens from all *H. pylori*-seropositive individuals from the 8 populations to determine the prevalence of CagA positivity. Two populations (Thailand and Peru) had a substantially ( $p < 0.05$  in each case) higher CagA seroprevalence than that observed in the other 6 populations. The proportion of *H. pylori* infections due to CagA-positive strains were highly similar in the 3 Caucasian populations (Canada, The Netherlands and New Zealand). The Pacific Islander and Maori populations had the lowest CagA seroprevalence. For each of the populations, the CagA seroprevalence was relatively constant across the age groups studied (Table II). Thus, in each population sampled, both CagA<sup>+</sup> and CagA<sup>-</sup> *H. pylori* strains were in wide circulation.

#### Correlations between *H. pylori* seroprevalence, CagA seroprevalence and gastric and esophageal cancer rates

We then examined the association of reported gastric and esophageal cancer rates with seroprevalence rates of *H. pylori* and CagA from the standardized sample (healthy persons from age 35–65 years) from each of the populations studied. We found a strong association between *H. pylori* seroprevalence and gastric cancer rates (Table III). However, although positive trends were found, no significant associations were observed between gastric cancer rates and either proportion of *H. pylori*-infected persons who were CagA-seropositive or CagA seroprevalence in the total populations. Similarly, there was no significant association with infection with a CagA-negative strain. For esophageal cancer, we found no significant associations with *H. pylori* infection rates or with rates of infection with either CagA-positive or CagA-negative strains. Interestingly, although not statistically significant ( $p = 0.5$ ), there was a negative correlation between infection with CagA<sup>+</sup> strains and rates of esophageal cancers.

## DISCUSSION

In this *H. pylori* seroprevalence study, we selected populations representing different parts of the world, and differing levels of economic development. We also were able to study 3 populations of differing ethnicity in the same locale, New Zealand. Healthy adults were selected for this study, rather than dyspeptic patients presenting for endoscopy, as this would best reflect the total population and not be subject to biases related to over- (or under-) representation of *H. pylori* infection or that with *cagA*-positive strains in particular groups of symptomatic persons.

Using such a study design, we found worldwide differences in *H. pylori* seroprevalence from 35–73%, about as expected (Pérez-Pérez *et al.*, 1990; Mitchell *et al.*, 1992; Veldhuyzen van Zanten *et al.*, 1994; Malaty *et al.*, 1996) for this sample. In general, persons from developed countries had lower rates than those from developing countries, but as reported in the US and elsewhere (Polish *et al.*, 1991; Pérez-Pérez *et al.*, 1991; Malaty *et al.*, 1992), even within a country, substantial differences between majority and minority ethnic populations can occur.

Among the 8 populations, there was a nearly 4-fold difference in the proportion of strains that were *cagA*-positive, at least as reflected by CagA seropositivity (20.6–82.2%). CagA serology using recombinant antigens has been well-validated (Xiang *et al.*, 1993; Blaser *et al.*, 1995b). Thus, inaccuracy of the assay is unlikely to have any significant impact on the findings. The differences observed were not associated with variation in the level of economic development, and within each population there were no major age-related differences in seroprevalence. Assuming that most *H. pylori* infections are acquired in childhood (Mendall *et al.*, 1992; Banatvala *et al.*, 1993), the current data suggest that the proportion of *cagA*<sup>+</sup> strains as a fraction of all strains circulating in these communities has changed little over a 30+ year period. These data also suggest that the proportion of strains that are *cagA*<sup>+</sup> are intrinsic to a community, and may not be related to changes in

**TABLE II** – CagA<sup>+</sup> SEROPREVALENCE AMONG HEALTHY *H. pylori*-INFECTED PERSONS, BY POPULATION AND BY AGE GROUP

Age group (years)	Number of persons <sup>1</sup> studied (and % positive) by population							
	Thailand	Peru	China	Canada	Netherlands	New Zealand		
						Caucasian	Pacific Islander	Maori
20–34	20 (80.0)	42 (78.6)	ND <sup>2</sup>	33 (39.4)	ND	ND	ND	ND
35–49	16 (81.3)	37 (89.2)	40 (37.5)	34 (47.1)	16 (38.0)	24 (41.7)	64 (25.0)	50 (36.0)
50–65	30 (76.7)	11 (72.7)	26 (57.7)	38 (36.8)	42 (40.0)	43 (39.5)	77 (23.4)	60 (20.0)
Total	66 (78.8)	90 (82.2)	66 (45.5)	105 (40.9)	58 (39.0)	67 (40.3)	141 (24.1)	110 (27.3)

<sup>1</sup>*H. pylori*-infected individuals only. –<sup>2</sup>ND, not determined.

**TABLE III** – CORRELATION BETWEEN AGE-STANDARDIZED INCIDENCE CANCER RATES (ASR)<sup>1</sup> AND SELECTED SEROPREVALENCE RATES BY REGRESSION ANALYSIS

Category	Thailand	Peru	China	Canada	Netherlands	New Zealand			Correlations <sup>2</sup>			
						Caucasian	Maori	Pacific Islander	Gastric		Esophageal	
									<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Gastric cancer ASR	8.8	27.7	32.0	8.3	12.9	8.7	22.8	34.3				
Esophageal cancer ASR	3.4	0.8	12.0	2.5	2.2	3.8	4.0	3.5				
<i>H. pylori</i> seroprevalence <sup>3</sup>	49.5	72.2	62.7	46.0	50.4	35.8	57.4	73.2	0.91	0.002	0.08	NS <sup>8</sup>
CagA <sup>+</sup> seroprevalence in <i>H. pylori</i> ⊕ population (%) <sup>4</sup>	78.3	85.4	45.5	41.6	39.0	40.3	27.3	24.1	0.15	NS	0.22	NS
CagA <sup>+</sup> seroprevalence in total population (%) <sup>5</sup>	38.7	61.7	28.5	19.1	19.6	14.4	15.7	17.6	0.22	NS	0.19	NS
CagA <sup>-</sup> prevalence in <i>H. pylori</i> ⊕ population (%) <sup>6</sup>	21.7	14.6	54.5	58.2	61.0	59.7	72.7	75.9	0.15	NS	0.22	NS
CagA <sup>-</sup> prevalence in total population (%) <sup>7</sup>	10.7	10.5	34.2	26.8	30.7	21.4	41.7	55.6	0.54	NS	0.27	NS

<sup>1</sup>Data obtained as all-age world standardized ASR; from Parkin and Muir (1992). –<sup>2</sup>By linear regression analysis. –<sup>3</sup>In healthy persons 35–65 years old; from Table I. –<sup>4</sup>Among *H. pylori*-seropositive persons of those ages; from Table II. –<sup>5</sup>Derived as the product of 3 and 4. –<sup>6</sup>Derived as 100%–4. –<sup>7</sup>Derived as the product of 3 and 6. –<sup>8</sup>NS, not significant (*p* > 0.05).

economic development, which in general affects the aggregate incidence of (and prevalence of) *H. pylori* infection. Stated another way, these data suggest that as the incidence of *H. pylori* infection has declined in various countries with advances in economic development, this decline has affected *cagA*<sup>+</sup> and *cagA*<sup>-</sup> strains roughly proportionately. It will be important to confirm this observation in other studies.

In agreement with previous international comparisons (EURO-GAST Study Group, 1993) and with data from nested case-control studies (Forman *et al.*, 1991; Nomura *et al.*, 1991; Parsonnet *et al.*, 1991), the prevalence of *H. pylori* infection in healthy persons was associated with gastric cancer risk. However, although there was a positive correlation between CagA positivity and gastric cancer risk, we found no significant association. In a preliminary report, Webb *et al.* (1996) showed that CagA positivity was associated with gastric cancer risk in another international comparison. The reasons for our failure to identify the expected association may include the relatively small number of populations sampled, and that the individual locales within a country that we sampled were not representative of the total population. Alternatively, since the risk for gastric cancer may be acquired relatively early in life, and since age-related acquisition of atrophic gastritis may confound serologic analyses, use of persons aged 35–65 for our analysis may not have been adequate to test the hypothesis.

Esophageal cancers are usually due to squamous-cell tumors, but in developed countries, the incidence of adenocarcinoma of the

distal esophagus (and proximal stomach) has been rising (Blot *et al.*, 1991). One hypothesis is that *H. pylori* colonization may protect in some way against these tumors; the increasing incidence of these adenocarcinomas during a period when *H. pylori* incidence is falling (Parsonnet, 1995) is consistent with this hypothesis. In our analysis, there was no stratification for type or location of esophageal cancer; thus, confounding by the squamous-cell tumors would be expected.

Although not statistically significant, the negative association of infection with *cagA*<sup>+</sup> strains and esophageal cancers in this study, in contrast to other associations in which *cagA*<sup>+</sup> infections are positively associated with cancer of the distal stomach (Blaser *et al.*, 1995b), is consistent with other recent observations in the same direction (Schnell *et al.*, personal communication). Further determination of whether infection with *cagA*<sup>+</sup> strains protects against adenocarcinomas of the esophagus (and proximal stomach) will be important.

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