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Seroprevalence of *Helicobacter pylori* Infection in Urban and Rural Vietnam

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***Helicobacter pylori*-associated diseases, such as peptic ulcer and gastric cancer, are common in Vietnam, but the prevalence of the infection is largely unknown. A validated enzyme-linked immunosorbent assay was used for seroepidemiology with 971 samples from the general population, ages 0 to 88 years, with 546 samples from an urban population (Hanoi), and with 425 samples from a poor, rural province (Hatay). The overall seroprevalence of the infection was 746 per 1,000, with a prevalence of 788 per 1,000 in Hanoi and 692 per 1,000 in Hatay ($P = 0.0007$). The risk for infection in the rural area of Hatay was 40% lower than in the urban population of Hanoi, with the odds ratio being 0.59 (95% confidence interval, 0.43 to 0.81). The study shows that the prevalence of *H. pylori* infection is high in Vietnam and especially high in a large urban area, such as the city of Hanoi.**

Helicobacter pylori infection causes gastritis and peptic ulcer disease and is a cofactor in the development of gastric cancer (16). The prevalence of *H. pylori* infection is decreasing in developed countries but remains high in many developing countries (11). Data on the epidemiology of *H. pylori* infection in Vietnam are scarce, but peptic ulcer disease and gastric cancer represent major health problems. In a large survey, conducted at the Hanoi Military Hospital from 1963 to 1983, peptic ulcer was found by endoscopy in 7.8% of 300,000 volunteers, ages 18 to 60 years (19). Official statistics for the year 2001 indicate an age-standardized incidence of 77.26 per 100,000 person-years for gastric and duodenal ulcer disease (4). Gastric cancer is the second-most-common cancer form in men and the third most common in women, with an age-standardized incidence of 23.7 and 10.8 per 100,000 person-years in the year 2000, respectively (12)

Seroepidemiological investigations represent the most rapid and convenient way of obtaining a picture of the prevalence of *H. pylori* infection in a population, but the assays used need to be validated in the population studied (6, 10, 14). Enzyme-linked immunosorbent assay (ELISA) for immunoglobulin G (IgG) detection can be based either on whole-cell sonicate antigen or on one or several purified components of the bacterium as the antigen. A majority of serological studies are now conducted with commercial kits that have been evaluated in developed countries. These commercial kits are often too expensive for developing countries, and use of a validated in-house ELISA assay based on sonicate antigens would seem preferable.

We have previously evaluated with both Swedish and Vietnamese populations an in-house ELISA based on sonicated *H. pylori* antigen, supplemented with an absorption step with sonicated *Campylobacter jejuni* antigen to remove cross-reacting antibodies (2, 6, 15, 17). The studies showed that the local strains used for the *H. pylori* antigen give a better diagnostic performance and also that the cutoff level used for serodiagnosis in the general population needs to be adjusted (6, 17).

The aim of the present study was to apply the best-performing assay and cutoff level to an investigation of the seroprevalence of *H. pylori* infection in Vietnam, represented by an urban population (Hanoi) and a poor, rural area (Hatay).

MATERIALS AND METHODS

Subjects. Healthy individuals 1 to 88 years of age in Hanoi ($n = 546$) and in Hatay ($n = 425$) were asked to volunteer a blood sample for the study. Both the Hanoi (urban) and Hatay (rural) areas are located in the Red River region of northeast Vietnam. The majority of the blood samples were drawn at routine health controls that are granted by the Vietnamese health insurance system. The subjects fill in a form on their health status before taking the medical examination. Individuals who reported good health and no peptic disease were enrolled in our study. Information on age and gender was recorded for each volunteer. Some samples were drawn from healthy individuals as prevaccination samples in the context of a cholera vaccine trial. Samples from children were also drawn at routine checkups or at day care centers. In Hanoi, the samples were collected at the Bach Mai Hospital or at the Friendship Hospital, and in Hatay, they were collected at clinics in Sontay, a large district in the province. Demographic characteristics of the samples are shown in Table 1. Approximately 5 ml of blood was drawn, and the serum aliquoted and immediately stored at −20°C until analyzed for antibodies to *H. pylori*.

Serological assays. (i) Enzyme-linked immunosorbent assay. The *H. pylori* antigen used was based on five clinical isolates from Vietnamese patients with peptic ulcer diseases and the NCTC 11638 strain. The bacteria were sonicated and used to coat 96-well microplates at a concentration of 5 μg/ml, as previously described (2, 15). Sera were diluted 1:1,000, first 1:100 in phosphate-buffered saline and then 1:10 in phosphate-buffered saline containing 70 mg of *C. jejuni* antigen (four clinical isolates)/ml to remove cross-reacting antibodies. Alkaline phosphatase-conjugated antihuman IgG (Euro-Diagnostica, Malmö, Sweden) was used to detect bound antibodies.

The upper limit of normal values for the healthy population was found in a previous study to need adjustment compared to cutoff levels used for peptic ulcer

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TABLE 1. Characteristics of participating subjects in urban Hanoi and rural Hatay

Parameter	Value for subjects in:		
	Hanoi	Hatay	Both regions
Total no. of subjects	546	425	971
No. (%) male	231 (42.3)	197 (46.4)	428 (44.1)
Mean (SEM) age, yr	39.3 (0.9)	38.7 (1.1)	39.1 (0.7)
Median age, yr	42	37	40
No. (%) in age group (yr):			
0–4	14 (2.6)	16 (3.8)	30 (3.1)
5–9	32 (5.9)	27 (6.4)	59 (6.1)
10–14	44 (8.1)	8 (1.9)	52 (5.4)
15–19	52 (9.6)	31 (7.4)	83 (8.6)
20–24	47 (8.7)	91 (21.6)	138 (14.4)
25–29	18 (3.3)	6 (1.4)	24 (2.5)
30–34	24 (4.4)	19 (4.5)	43 (4.5)
35–39	18 (3.3)	26 (6.2)	44 (4.6)
40–44	55 (10.2)	34 (8.1)	89 (9.3)
45–49	43 (8.0)	13 (3.1)	56 (5.8)
50–54	40 (7.4)	25 (5.9)	65 (6.8)
55–59	33 (6.1)	23 (5.5)	56 (5.8)
60–64	35 (6.5)	35 (8.3)	70 (7.3)
65–69	26 (4.8)	9 (2.1)	35 (3.6)
70–74	34 (6.3)	43 (10.2)	77 (8.0)
75–79	16 (3.0)	11 (2.6)	27 (2.8)
≥80	10 (1.9)	4 (1.0)	14 (1.5)

patients (6). The establishment of the cutoff levels for the present study was therefore based on levels for 431 healthy population controls, with immunoblotting as reference method.

(ii) Immunoblot. All sera were tested by immunoblotting, using the commercial available HelicoBlot 2.1 (Genelabs Diagnostics, Singapore) for detection of antibodies against *H. pylori*-specific antigens. The kit consists of Western blot membrane strips, made with a surface antigen-enriched preparation of *H. pylori*, including CagA (116 kDa), VacA (89 kDa), and the urease A subunit (30 kDa). All buffer and reagents used were supplied with the kit and used according to the manufacturer's recommendations. The assay was performed with an automated Western blot system (Autoblot system 36; Genelabs Diagnostics). The blots were evaluated as positive or negative according to the criteria supplied by the manufacturer.

(iii) Pyloriset EIA-G kit. The kit from Orion Diagnostica (Espoo, Finland) was used according to the instructions of the manufacturer. Values of ≥20 U/ml were considered positive, and values of <20 U/ml were considered negative.

(iv) HM-cap kit. The kit from Enteric Products Inc.(E-Z-EM Inc., Westbury, N.Y.) was used according to the instructions of the manufacturer. ELISA values of <2.2 were considered positive, values of 1.8 to 2.2 were considered indeterminate, and values of <1.8 were considered negative.

Statistical methods. The optical density cutoff point that maximized sensitivity and specificity was selected, and the prevalence (number of subjects with an antibody titer above the selected cut point divided by the total number of tested subjects in the studied stratum) was calculated overall and in strata of age, gender, and region of residence. We computed 95% confidence intervals of prevalence, using the Wilson method (1). A trend line was drawn for age-specific prevalence (both genders and both regions combined), using a fourth-order polynomial. Differences between proportions were tested with the chi-square test. The independent effects of age (categorized in 5-year age groups), gender, and region of residence (Hanoi versus Hatay) on the probability of being infected with *H. pylori* were estimated with a multivariate logistic regression model with mutual adjustments for the variables included. Trend tests were done with the exposure transformed into a semiquantitative variable.

Ethical clearance. The project was approved by the Karolinska Hospital ethics committee in Sweden. Approval for the project was also granted in Vietnam by the relevant authorities.

RESULTS

Assay validation and performance. Calculation of sensitivity for the ELISA was based on 376 immunoblot-positive individuals out of 431 individuals of a Vietnamese normal population. Calculation of assay specificity was based on the 55 normal population controls, found to be negative by immunoblotting, and further tested by using two commercial serological kits (Pyloriset and HM-cap). This precautionary step was taken in order to ensure that no samples seronegative by immunoblotting were false negatives. Three samples were found to be positive both by Pyloriset and HM-cap, while three were positive and three were indeterminate by HM-cap. These nine sera were excluded from the analysis of specificity, with this analysis being based on the remaining 46 samples. A cutoff level of optical density 0.22 was found to give the highest sensitivity, 94.1% (354 of 376), and the highest specificity, 97.8% (45 of 46).

***H. pylori* infection seroprevalence.** Table 2 shows the crude seroprevalence (defined as optical density of ≥0.22) by gender, region of residence, and 5-year age groups. The overall seroprevalence was high, 746 (95% confidence interval [CI], 717 to 772) per 1,000 individuals, slightly higher among women (766 per 1,000) than among men (720 per 1,000) ($P = 0.099$, chi-square test). The female predominance was not particularly consistent in age-specific analyses (Table 2). The seroprevalence already was substantial among children; below the age of 5, one-third of them were infected, and this proportion rose to three-quarters in late adolescence. The increase leveled off at around 80% from age 30 (Fig. 1). In Table 2, it is obvious that the overall seroprevalence was higher in urban Hanoi (788 per 1,000; 95% CI, 751 to 820) than in rural Hatay (692 per 1,000; 95% CI, 646 to 734) ($P = 0.0007$, chi-square test). The seroprevalence by 5-year age groups in the two regions is shown in Fig. 2. However, since the samples from Hanoi and Hatay differed with regard to age distributions ($P < 0.0001$), we had to disentangle the independent effects of region, age, and gender in a multivariate logistic regression model (Table 3).

Table 3 shows that after taking age (in 5-year categories) and gender into consideration, the odds of being seropositive was 0.59 (95% CI, 0.43 to 0.81) among residents of Hatay relative to residents of Hanoi, i.e., the odds of being infected was 41% lower (95% CI, 19 to 57%) in rural Hatay than in urban Hanoi ($P = 0.0012$). After adjustments for gender and region of residence, the effect of age was statistically highly significant (P value for trend of <0.0001), but significant deviations from the reference category (20 to 24 years old) were seen only among the youngest children; the odds ratios for being infected among those aged 0 to 4 and 5 to 9 years were 0.18 (95% CI, 0.08 to 0.43) and 0.33 (95% CI, 0.18 to 0.64), respectively. In the multivariate analysis, the slightly increased risk of being infected seen among women (odds ratio, 1.15) compared to that among men was statistically nonsignificant (95% CI, 0.84 to 1.57).

DISCUSSION

The present study, using a validated ELISA assay, showed a high overall seroprevalence of *H. pylori* infection of 74.6% in a Vietnamese population sample of 971 individuals aged 1 to 88 years. In addition, the infection prevalence was significantly higher in the urban population of Hanoi (78.8%) than in the rural population of Hatay province (69.2%).

Similar, high infection rates have been reported from other developing countries, e.g., Thailand (3), Mexico (9), India (14), Japan and Korea (20), or parts of countries, e.g., Siberia (13). Also, a similarly high prevalence has been reported from an

TABLE 2. Seroprevalence in percentage and 95% CI by age, gender, and region (univariate analyses)

Variable	Seroprevalence[a]					
	Both sexes		Men		Women	
	No. positive/no. studied (%)	95% CI	No. positive/no. studied (%)	95% CI	No. positive/no. studied (%)	95% CI
All ages						
Hanoi	430/546 (78.8)	75.1–82.0	180/231 (77.9)	72.1–82.8	250/315 (79.4)	74.6–83.5
Hatay	294/425 (69.2)	64.6–73.4	128/197 (65.0)	58.1–71.3	166/228 (72.8)	66.7–78.2
Both regions	724/971 (74.6)	71.7–77.2	308/428 (72.0)	67.5–76.0	416/543 (76.6)	72.9–80.0
Age group (yr)						
0–4	10/30 (33.3)	19.2–51.2	7/20 (35.0)	18.1–56.7	3/10 (30.0)	10.8–60.3
5–9	29/59 (49.2)	36.8–61.6	13/30 (43.3)	27.4–60.8	16/29 (55.2)	37.5–71.6
10–14	36/52 (69.2)	55.7–80.1	22/26 (84.6)	66.5–93.9	14/26 (53.8)	35.5–71.2
15–19	65/83 (78.3)	68.3–85.8	38/49 (77.6)	64.1–87.0	27/34 (79.4)	63.2–89.7
20–24	100/138 (72.5)	64.5–79.2	29/47 (61.7)	47.4–74.2	71/91 (78.0)	68.5–85.3
25–29	15/24 (62.5)	42.7–78.8	3/8 (37.5)	13.7–64.9	12/16 (75.0)	50.5–89.8
30–34	37/43 (86.0)	72.7–93.4	11/14 (78.6)	52.4–92.4	26/29 (89.7)	73.6–96.4
35–39	35/44 (79.5)	65.5–88.8	6/6 (100)	61.0–100.0	29/38 (76.3)	60.8–87.0
40–44	73/89 (82.0)	72.8–88.6	34/42 (81.0)	66.7–90.0	39/47 (83.0)	69.9–91.1
45–49	43/56 (76.8)	64.2–85.9	21/26 (80.8)	62.1–91.5	22/30 (73.3)	55.6–85.8
50–54	53/65 (81.5)	70.4–89.1	19/22 (86.4)	66.7–95.3	34/43 (79.1)	64.8–88.6
55–59	41/56 (73.2)	60.4–83.0	18/27 (66.7)	47.8–81.4	23/29 (79.3)	61.6–90.2
60–64	55/70 (78.6)	67.6–86.6	29/35 (82.9)	67.3–91.9	26/35 (74.3)	57.9–85.8
65–69	33/35 (94.3)	81.4–98.4	15/16 (93.8)	71.7–98.9	18/19 (94.7)	75.4–99.1
70–74	58/77 (75.3)	64.6–83.6	30/43 (69.8)	54.9–81.4	28/34 (82.4)	66.5–91.7
75–79	21/27 (77.8)	59.2–89.4	8/10 (80.0)	49.0–94.3	13/17 (76.5)	52.7–90.4
≥80	12/14 (85.7)	60.1–96.0	3/5 (60.0)	23.1–88.2	9/9 (100.0)	70.1–100.0

[a] Proportion of infected individuals in a population, as determined by serology.

early, smaller study investigating the seroprevalence of *H. pylori* infection in Vietnam and other countries (11). Somewhat lower prevalence rates, 40 to 60%, have been reported from Mexico (18), Korea (8) and from parts of a multiracial population in Malaysia (5). Seroprevalence rates around or below 40% have been reported for ethnic Malay (5) and Seoul (9) populations.

The variability of *H. pylori* seropositivity in different popu-

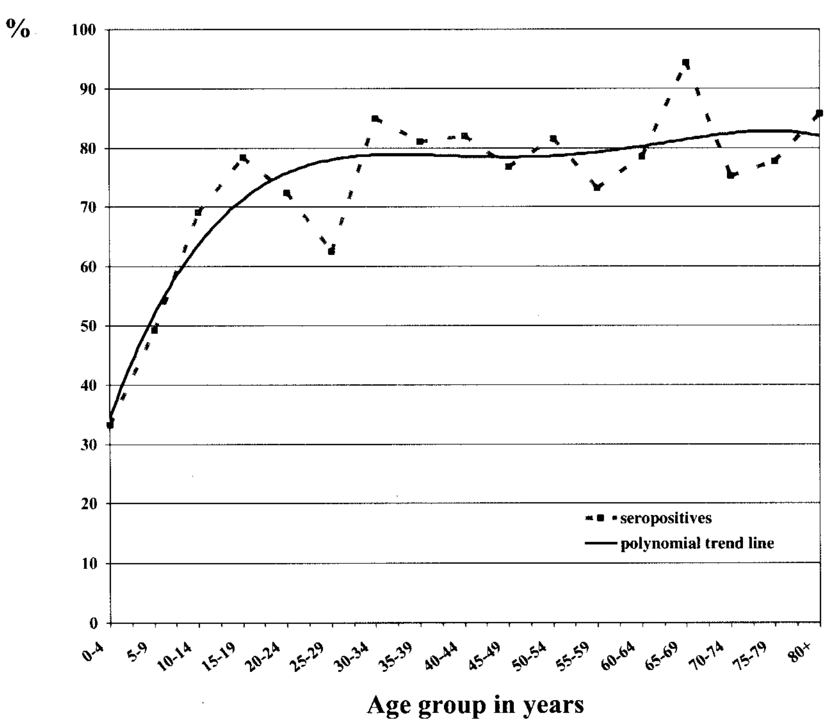

FIG. 1. Seroprevalence of *H. pylori* infection in Vietnam. The dotted line gives the exact percentage of seropositives in each age group, and the smooth line represents the polynomial trend line of the third order.

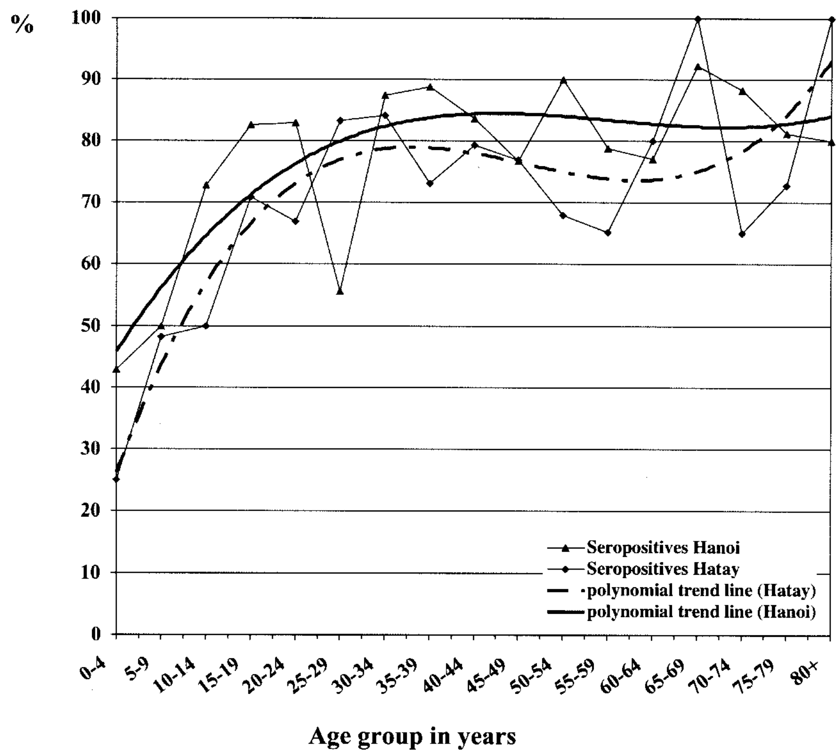

FIG. 2. Seroprevalences of *H. pylori* infection in urban (Hanoi) and rural (Hatay) regions of Vietnam. Exact percentages and polynomial trend lines of the third order are shown.

lations is likely partly explained by technical factors. Many of the studies used commercial kits that have been validated in developed countries but not in the populations investigated. These kits are usually based on strains from developed countries, while recent studies by us and others have shown that using local strains led to a significantly improved sensitivity (6, 14) and specificity (6).

TABLE 3. Multivariate logistic regression model[a]

Variable	OR[b]	95% CI
Region		
Hanoi	1	Reference
Hatay	0.59	0.43–0.81
Gender		
Male	1.0	Reference
Female	1.15	0.84–1.57
Age group (yr)		
0–4	0.18	0.08–0.43
5–9	0.33	0.18–0.64
10–14	0.67	0.32–1.38
15–19	1.24	0.64–2.39
20–24	1.0	Reference
25–29	0.51	0.20–1.27
30–34	2.10	0.82–5.41
35–39	1.39	0.61–3.19
40–44	1.55	0.79–3.02
45–49	1.02	0.49–2.15
50–54	1.47	0.70–3.07
55–59	0.93	0.46–1.90
60–64	1.32	0.66–2.64
65–69	5.24	1.19–23.11
70–74	1.14	0.60–2.18
75–79	1.18	0.44–3.17
≥80	1.90	0.40–9.00

[a] The model disentangles the independent effects of region, age (in 5-year strata), and gender on probability of being seropositive. The variables in the model are mutually adjusted for each other.
[b] Odds ratio.

In addition, even if validated with a local population, some of the assays used in seroepidemiological studies had been validated with patients undergoing endoscopy for gastrointestinal diseases. This approach allows determination of the sensitivity of the assay against the "gold standard," i.e., culture of *H. pylori*. It entails, however, the risk of setting an erroneous cutoff level if antibody concentrations differ significantly between symptomatic and asymptomatic individuals. We found significantly higher IgG antibody concentrations in Vietnamese peptic ulcer patients (immunoblot and *H. pylori* culture positives) than in an immunoblot-positive Vietnamese healthy control population, indicating the need of a lower cutoff level in seroepidemiological studies (6).

For the present study, we have therefore given close attention to the parameters of the ELISA used for the study of seroprevalence of *H. pylori* infection in rural and urban Vietnam. The *H. pylori* strains used for antigen were sonicated Vietnamese strains, and cross-reacting antibodies were absorbed by sonicated *C. jejuni* antigen. Sensitivity and specificity were established both with Vietnamese peptic ulcer patients and with an immunoblot-positive control population (6). Since none of the commercial serological assays on the market had been validated with a Vietnamese population, specificity in the present study was established by using three different assays in order to exclude false positives in the population investigated.

Findings similar to those in our study, showing a higher overall infection rate in an urban population (78.8%) than in a rural population (69.2%), have previously been reported from Nepal, with an infection rate of 67.2% in a suburban village of Kathmandu compared to 41.5% in an isolated, rural village (7). Also, in a study from Mexico, persons living in the region with the highest socioeconomic development had the highest risk of seropositivity (9), consistent with our finding in Vietnam. Interestingly, a previous large community-based seroepidemiological study from Mexico (18) had not found a difference by regional development, but the authors of this last study speculate that this could be due to the multiplicity of variables used to construct their indexes.

The situation in Vietnam resembles that of many developing countries, with a growing population and rapid changes in socioeconomic and demographic variables. The total population of Vietnam was estimated in 2001 to be 78.7 million, up from 66 million in 1990, and projected to further increase to between 85.6 million and 87.1 million by year 2010 (4). The Red River delta region, where both Hanoi and Hatay are located, had an estimated population of 17.2 million in 2001, with 2.8 million in Hanoi and 2.4 million in Hatay. The proportion of urban population in the two areas was estimated to be 57.8 and 8.2%, respectively.

The level of economic development in Hanoi is higher than that in the Hatay province by any official measure, e.g., income per capita, rate of malnutrition in children <5 years of age, and access to medical care (4). These official data do not, however, include known risk factors for *H. pylori* infection, e.g., crowding in households. The level of crowding in the city of Hanoi is largely unknown but may be even more extensive than could be calculated, since a large number of people work and live in Hanoi although they are officially registered in their home province. In addition, the influx of people from other parts of the country was accelerated when the official ban on moving into the city from other areas was lifted in 1990.

In conclusion, our study has shown a high seroprevalence of *H. pylori* infection in Vietnam and in particular in the urban area of Hanoi. The high infection prevalence in children and adolescents indicates that the major public health problems of peptic ulcer disease and gastric cancer, one of the leading forms of cancer in Vietnam, will not be eliminated in the near future. The exact causes of the high prevalence of infection in urban Vietnam remain unknown, and although it may be speculated to be due to crowding, further studies are needed as a basis for future prevention and treatment strategies.

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