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 1968, 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 analysis for antibodies to *H. pylori*.

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

| Parameter          | 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)     |

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...
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-
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 coun-

tries, while recent studies by us and others have shown that using local strains led to a significantly improved sensitivity (6, 14) and specificity (6).

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 \textit{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 \textit{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 \textit{H. pylori} infection in rural and urban Vietnam. The \textit{H. pylori} strains used for antigen were sonicated Vietnamese strains, and cross-reacting antibodies were absorbed by sonicated \textit{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

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**TABLE 3. Multivariate logistic regression model**

| Variable     | OR | 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 |

*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.

*Odds ratio.*
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 causes 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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