A Community-Based Seroepidemiologic Study of Helicobacter pylori Infection in Mexico

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A nationwide community-based survey for Helicobacter pylori infection had not been done. This study sought to determine the seroprevalence of infection in Mexico, and the socioeconomic and demographic variables that are risk factors for infection. The survey assessed 11,605 sera from a sample population representing persons ages 1–90 years from all socioeconomic and demographic levels and from all regions of Mexico. Antibodies against *H. pylori* were studied by ELISA using whole cell antigen. Among the findings were that 66% of the population was infected and that age was the strongest risk factor for infection. By age 1 year, 20% were infected and by age 10 years, 50% were infected. Crowding (odds ratio [OR], 1.4), low educational level (OR, 2.42), and low socioeconomic level (OR, 1.43) were risk factors for infection. Prevalence was similar in urban and in rural communities (OR, 0.95). This study is the largest community-based seroepidemiologic study of *H. pylori* to date.

*Helicobacter pylori* is one of the most prevalent human infections, and in most persons infection with the organism is asymptomatic. However, some persons develop upper gastrointestinal symptoms. The most common complication is peptic ulcer; gastric carcinoma is a less frequent but more devastating complication [1]. The Eurogast Study Group recently reported that in different international populations, high prevalence of *H. pylori* infection correlated with increased risk of gastric cancer [2]. Evidence is accumulating that early acquisition of infection is a risk factor for the development of gastric cancer [1]. People from developing countries might have a higher risk of gastric cancer because infection in these countries typically occurs in childhood [3].

In developing countries, infection is acquired early in life: By age 5 years, >20% of children are infected and by age 20 years, >80% of the population is infected [4–6]. The pattern is quite different from that in developed countries where infection is rare during childhood and prevalence steadily increases during adult life, so that even by age 60, just under 50% of the population is infected [7]. However, in both developing and developed countries, individuals usually remain infected throughout life in the absence of specific treatment [8]. Most serologic studies in developed and developing countries have been done with selected groups of subjects (e.g., blood donors, those in health programs, and patients attending medical clinics for minor ailments) [9]. Although there have been some community-based studies, most have been small and geographically limited [10, 11]. We found no reports of a community-based national serologic survey of a sample population representing all regions and ages.

Most people infected with *H. pylori* develop a chronic local inflammatory response and a systemic antibody response. Detection of serum antibodies to *H. pylori* by ELISA is a reliable method for determining the prevalence of *H. pylori* infection in large communities [12]. Most epidemiologic studies have defined *H. pylori* infection using serologic tests to detect antibodies to *H. pylori*. Several risk factors for infection with *H. pylori* have been described. The most common is increasing age. Low socioeconomic status, crowding, low level of education, and race have also been reported as factors that increase the risk of infection, mainly during childhood [13–15].

In this study we took advantage of a national survey in Mexico in which serum samples were collected from individual homes using a master-sampling frame based on data from the national population census. The survey included people from all regions and all socioeconomic levels. Our goals were to determine the prevalence of *H. pylori* infection in Mexico and to examine the variation in *H. pylori* seroprevalence according to different socioeconomic and demographic variables.

Materials and Methods

Description of the National Serologic Survey

Study design. During 1987 and 1988, the Ministry of Health of Mexico performed a national serologic survey to create the
National Serum Bank. Serum samples were collected according to a master-sampling frame based on general population census data. The survey included all 32 states of Mexico and was designed to include persons ages 1–90 years. All socioeconomic levels and all geographic zones were included [16].

Sample. In total, 32,200 households were visited and more than 70,000 serum samples were collected. The overall response rate was 78.4% of the homes surveyed. Serum samples were aliquoted and stored at −20°C in the National Serum Bank.

Questionnaire. Each individual completed a questionnaire detailing personal, socioeconomic, and other demographic data.

Indexes. To define socioeconomic level, an index was constructed that included number of persons per room, type of material on the floor of the house, availability of municipal water and waste disposal, and years of education of the head of the family [17]. An urban population was defined as those living in an area with ≥2500 inhabitants; a rural population was defined as those residing in an area with <2500 inhabitants.

Regionalization. For the purposes of this study, the country was divided into four regions that reflected the level of development. Regions were classified as low, medium-low, medium-high, and high. Development level was based on socioeconomic, demographic, and public health indexes according to the criteria of Kunz et al. [18]. Indexes in this regionalization included level of education, household characteristics, rate of infant mortality, rate of maternal mortality, rate of mortality due to transmissible and nontransmissible diseases, number of physicians in the population, proportion of the population economically active, and urban versus rural communities [18]. The distribution of regions by development level is shown in figure 1.

Study Sample

Sample size. The sample size for each region was calculated by Z test, estimating an H. pylori prevalence of 50%, with a 95% confidence interval [CI] and a relative error rate of 0.05. Thus, it was determined that 3073 serum samples were needed per region, and 12,292 serum samples were needed for the entire country. Some of the originally selected samples were not recovered because they were contaminated, had dried, or were missing. Ultimately, 2997 serum samples from region 1 (97.5%), 2944 from region 2 (95.8%), 2805 from region 3 (91.3%), and 2859 from region 4 (93.1%) were studied: 11,605 serum samples in total, corresponding to 94.1% of the calculated original sample size.

Selection of serum samples. A multistage stratified design was used to select serum samples. In stage I, the sampling unit was the region stratified by type of population (urban/rural). In stage II, the sample units were the household characteristics, and in stage III, sample units were characteristics of the individual (sex, age). In all stages, random selection was proportional to the size of the unit.

Serum storage. Aliquots (100 μL) of selected serum samples were transported to our laboratory where they were frozen at −20°C until tested. These aliquots had been frozen and thawed three or four times prior to this study. Although we did not investigate the effect of freeze/thaw on the serum antibody titers, one of us (GPP) previously tested this effect on serum samples and found that after 6 freeze-thaw cycles, the serum antibody titers remain unchanged.

Definition of H. pylori Infection and Prevalence

Serologic testing. H. pylori infection was determined by ELISA by detection of IgG antibodies to specific H. pylori antigens. This ELISA has been validated for use in children and adults in Mexico [19] as previously described [20]. In brief, a pool of whole cell antigens was obtained from sonicated preparations of 3 H. pylori isolates [12] from Mexican patients (1 child, 2 adults). Microtiter well plates (Combiplate 8; Labsystems, Helsinki) were coated with 0.5 μg/well of the antigen in carbonate buffer (pH 9.6) at 4°C overnight. Plates were blocked for 1 h with 0.1% gelatin and 0.5% gamma globulin in PBS (PBSGG) at pH 7.4.
Serum samples were diluted 1:1000, and 100-μL aliquots were plated and incubated for 1 h at 37°C. Next, monoclonal antibodies conjugated to alkaline phosphatase, anti-human IgG (Southern Biotech, Birmingham, AL) diluted 1:1000 with PBSGG were incubated for 1 h at 37°C. p-nitrophenyl phosphate, 1 mg/mL (Southern Biotech) was used as substrate, and absorbance was read at 405 nm (iEMS analyzer; Labsystems).

Definition of *H. pylori* infection. Threshold was calculated as the mean + 3 SD of the optical density (OD) values from 30 Mexican subjects not infected with *H. pylori*. During the testing of the unknown serum samples, the corresponding positive control sample was always included in quadruplicate in every microplate. The result for each serum sample was expressed as the ratio of the OD value of the sample to the threshold value and expressed as ELISA units. Accordingly, serum samples with >1.0 ELISA U were considered seropositive. Each serum sample was tested in duplicate and, if OD values were discordant in >30%, the sample was again tested in duplicate.

Magnitude of the response. The magnitude of the response was calculated in ELISA units and was expressed as the OD of the test sample divided by the threshold value.

Statistics

Age as variable. Because age was known to be a strong predictor of seroprevalence, we separately examined the change in seropositivity within a 5-year period for children <10 years old and within 10-year periods for persons >10 years of age. In addition, the annual change in seroprevalence was calculated in 5- or 10-year periods: The increment in seropositivity in the period was calculated by subtracting the nonsusceptible population (percentage seropositive in the previous period) from the total seropositive at the end of the period and dividing by the number of years in the period.

Socioeconomic and demographic factors as variables. The association between *H. pylori* seroprevalence and socioeconomic and demographic factors was examined by the χ² test and the Mantel-Haenszel test for trend when appropriate. Analysis was stratified by age in 10-year periods. Variables associated with *H. pylori* seroprevalence with *P* < .05 were then included in logistic regression models to remove confounding effects; odds ratio (OR) estimates with associated 95% CI were calculated.

Results

Role of Age

*H. pylori* seroprevalence. Of the 11,605 individuals studied, 7720 (66%) were seropositive for *H. pylori*. By age 1 year, 20% of the infants were infected. Seroprevalence increased sharply in the first decade, and by age 10 years, 50% of children were infected. More than 80% of the adults were infected by age 25, and seroprevalence remained almost unchanged for other ages (figure 2).

Magnitude of response. The magnitude of the immune response to *H. pylori* antigens (expressed in ELISA units) and age is shown in figure 2. During the first 4 years of life, the response increased ~0.55 ELISA U/year, whereas in subjects ages 5–19 years, it increased ~0.1 ELISA U/year. After age 20, the height of the response remained constant. After age 70, it decreased by ~0.33 ELISA U/year.

Association of *H. pylori* infection with age. The association of *H. pylori* seroprevalence with age is shown in table 1. The risk of becoming seropositive for *H. pylori* significantly increased with age: The OR for infection was 29.3-fold higher in people ages 60–69 years than for children ages 1–4 years. An apparent decrease in the risk for infection was observed in persons >70 years (OR, 0.70; 95% CI, 0.54–0.90) compared with risk of infection in those ages 30–69 years.

Next, the annual change in seroprevalence was estimated (figure 3). The calculated increment in seropositivity per year was >6% for children <5 years old; this rate decreased to <3% in children ages 10–14 years and to <0.5% in persons ages 30–69 years. In subjects >70 years old, the annual change in seropositivity became negative.
Table 1. Influence of age on the seroprevalence of *H. pylori* in Mexico on the basis of a multivariate logistic regression model.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>No. (% seropositive)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–4</td>
<td>527 (24.5)</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>5–9</td>
<td>1809 (42.5)</td>
<td>2.26</td>
<td>1.81–2.81</td>
</tr>
<tr>
<td>10–14</td>
<td>1854 (55.3)</td>
<td>3.92</td>
<td>3.15–4.88</td>
</tr>
<tr>
<td>15–19</td>
<td>1418 (65.1)</td>
<td>6.26</td>
<td>4.98–7.87</td>
</tr>
<tr>
<td>20–29</td>
<td>1944 (76.5)</td>
<td>11.20</td>
<td>8.93–14.06</td>
</tr>
<tr>
<td>30–39</td>
<td>1423 (81.2)</td>
<td>15.00</td>
<td>11.77–19.10</td>
</tr>
<tr>
<td>40–49</td>
<td>954 (84.4)</td>
<td>18.60</td>
<td>14.23–24.30</td>
</tr>
<tr>
<td>50–59</td>
<td>723 (86.2)</td>
<td>21.95</td>
<td>16.35–29.48</td>
</tr>
<tr>
<td>60–69</td>
<td>506 (89.1)</td>
<td>29.30</td>
<td>20.68–41.50</td>
</tr>
<tr>
<td>&gt;70</td>
<td>447 (78.7)</td>
<td>13.17</td>
<td>9.68–17.93</td>
</tr>
</tbody>
</table>

NOTE: Variables analyzed were age group, crowding, socioeconomic level, and gender. OR, odds ratio; CI, confidence interval.

Role of Socioeconomic and Demographic Variables

Univariate analysis. By univariate analysis of the influence of socioeconomic and demographic variables in the seroprevalence of *H. pylori* infection, crowding, level of education, and socioeconomic level were significantly associated with seropositivity in the first four decades of life ($P < .05$), but not after this age. Therefore, the association of these variables with *H. pylori* seroprevalence was studied using logistic regression analysis only in persons <40 years old.

Logistic regression model. As shown in table 2, crowding and socioeconomic level were significantly associated with *H. pylori* infection. Gender was minimally associated with infection, with females at a slightly increased risk. We assessed the influence of years of education in subjects ages 15–39 years (table 2) and found an inverse correlation; persons with fewer years of education had a significantly higher risk of infection. Illiterate individuals had the highest risk for seropositivity. No association was observed between the prevalence of infection and the type of population (urban vs. rural) nor with a region’s level of development (low, medium-low, medium-high, and high; table 2).

Discussion

Numerous epidemiologic serosurveys have been done in both developed and developing countries. However, few of these studies represent large communities as most used selected groups of patients. Until now, no study has attempted to determine the prevalence of *H. pylori* infection in an entire country. Our community-based study represents the largest seroepidemiologic report of *H. pylori* infection to date and one of the few that has studied persons recruited by visiting households. The sample studied represented all ages, all socioeconomic groups, and all regions of the country.

The early age of acquisition of infection in Mexico is remarkable; 20% of children at age 1 year were already infected, and by age 10, 50% of the children were infected. This seroprevalence is similar to that found in other developing countries [4, 5, 21, 22]. However, it is lower than found in Africa [6], where 80% of the children are infected by age 5 years. In contrast, seroprevalence among Mexican children was higher than observed in young children from developed countries such as Sweden [3] and France [23].

We observed an increased rate in *H. pylori* seropositivity of ~5% per year during the first 10 years of life, suggesting that in Mexico, most *H. pylori* infections occur early in childhood. A similar pattern of infection was seen in China [11]. In previous studies, a limited number of children have been studied, and the annual increase in seroprevalence during childhood has been poorly documented.

In our study, the increase in magnitude of IgG levels versus *H. pylori* was greatest during the first 5 years of life, but levels...
continued to rise constantly until they stabilized at age 20 years. These data suggest that in Mexico, most infections occur during the first years of life and that new infections are uncommon in adults. These results support the proposed birth-cohort model of infection, which suggests that most H. pylori infections occur in childhood and are not the result of constant recruitment of infection throughout life [24, 25].

Of interest, after age 60, the annual change in seropositivity became negative and the magnitude of the IgG anti–H. pylori response decreased, suggesting that seroreversion occurs preferentially in this age group. The fall in seroprevalence and in the magnitude of the response in elderly persons might be due to senescence of the immune system. Alternatively, these data could indicate that clearance of infection is common in older people. Finally, these results could be explained by the premature death of infected patients.

The influence of socioeconomic and demographic factors on H. pylori infection was also analyzed. Overcrowding, low education level, and low socioeconomic level were risk factors for infection as previously reported [10, 13, 26]. We observed the effect of these factors only in persons younger than 40 years. Perhaps overcrowding, low education level, and low socioeconomic level were not risk factors for H. pylori infection in people born in Mexico before 1950. It is also possible that the population of Mexico born before 1950 was more homogeneous with regard to living conditions and socioeconomic and education levels.

In contrast to other studies, we found that in Mexico, geography had little influence on H. pylori infection. Regions with different levels of development had similar rates of infection. Moreover, no difference in infection between urban and rural communities was found. This observation is surprising, because in Mexico, rural communities seldom have municipal water; water is usually obtained from wells or rivers. Although water supplies have been considered an important source of H. pylori infection in some countries [4, 27], this may not be true in Mexico. The lack of association of infection with geographic regions or with level of development might be due to the multiplicity of variables used to construct these indexes; influence of demographic variables should be further studied by using more subtle indexes.

An interesting finding in this study was that females were slightly but significantly more likely to be infected than were males. In most previous studies, gender has not been reported as a risk factor [15]; in the few studies in which gender was implicated as a risk factor, males were more likely to be infected [4, 28]. The difference in results might be due to the large sample that we studied.

This study is the largest community-based survey of H. pylori infection reported to date and describes the epidemiology of the infection in an entire nation. In Mexico, H. pylori infection is common, starts early in life, and is associated with low socioeconomic conditions. Perhaps most surprisingly, this study demonstrated that in Mexico, geographic and population characteristics (urban vs. rural and regional level of development) are factors that are not associated with infection. Future epidemiologic, clinical, and laboratory studies may eventually show how H. pylori is transmitted in human populations.

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References


