Helicobacter pylori Prevalence among Indigenous Peoples of South America

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The seroprevalence of Helicobacter pylori among secluded Indian populations of South America was determined to gain insight into the evolutionary history and possible transmission patterns of the organism. Serum samples obtained from 1024 donors in 22 different villages were tested by enzyme-linked immunosorbent assay for immunoglobulin G antibodies, and the results were confirmed by Western blot. The overall seroprevalence was 92%; >80% of children tested positive by 3 years of age, the highest prevalence in populations studied to date. Comparison of H. pylori prevalence with that of herpes simplex virus type 1, which is known to be transmitted orally, demonstrated a linear correlation in their prevalence rates, suggesting that these pathogens share risk factors. However, H. pylori seroprevalence was consistently higher, indicating that additional routes of transmission exist and/or that the organism is more transmissible. Seroprevalence did not correlate with the length of contact with the outside world. These results suggest that H. pylori was indigenous to the South American Indians and was not introduced by contact with outsiders.

Helicobacter pylori was first discovered in 1982 by a prolonged culture of human gastric specimens [1]. Definitive studies indicate that H. pylori causes gastritis and duodenal ulcers and has a strong association with certain forms of gastric cancer [2–4]. Seroepidemiologic investigations have indicated that infection with this organism is very common throughout the world [5–7]. Antibody prevalence is significantly higher in developing countries, compared with industrialized nations [5–7]. H. pylori is commonly acquired during childhood, with transmission occurring primarily within the family, as demonstrated by DNA sequencing of infecting strains [7–9]. Current evidence supports an oral-oral, gastric-oral, or oral-fecal pattern, but the routes of transmission have not been fully elucidated [10, 11].

H. pylori has been successfully infecting humans for a long time. H. pylori antigens have been found in fecal specimens from 3000-year-old Andean mummies [12]. Genotyping based on alleles within the cag pathogenicity island or the vacA sequence has provided a powerful tool for epidemiologic studies of H. pylori [13]. Analysis of specimens obtained from different regions of the world has demonstrated clustering of specific H. pylori strains in association with host geographic and ethnic origins. Such divergence suggests that the bacteria has coevolved with the host population over the years.

Seroanthropological investigations have been useful adjuncts in improving our understanding of the coevolution of humans and infecting microorganisms [14–19]. Using specific IgG antibodies as biomarkers, several previous studies evaluated the prevalence of a variety of infectious agents among secluded South American tribes with various years of initial peaceful contact with outsiders [14–19]. The more recent the contact, the less likely the population would be to have high seroprevalence rates of microorganisms brought in by outsiders. Measles and smallpox are examples of pathogens brought in by outsiders [14]. Most recently, Biggar et al. [19] reported the highest published prevalence of human herpes virus 8 among these secluded populations, suggesting that this virus has an indigenous origin.

Here, we present findings on the seroprevalence of H. pylori in 22 geographically divergent South American villages, determined with a well-established ELISA, as well as a complementary immunoblot technique. In addition, the seroprevalence of H. pylori among children was compared with that of herpes simplex virus type 1 (HSV-1), a common virus that is predominantly transmitted via the oral-salivary route [20], to evaluate possible similarities or differences in mode of transmission.
Subjects, Materials, and Methods

Study population. One thousand twenty-four serum samples (1 each from 1024 donors) obtained from 22 South American Indian villages were included in our study population. Samples from the following villages—Apalai, Arara, Arawete, Asurini Trocarra, Asurini Koatinema, Cinta Larga, Ewarhoyana, Jamamadi, Karrara-o, Karitiana, Mekranoti, Mundurucu, Parakanca C, Parakana Novo, Parakana Velho, Surui of Rondonia, Tiriyo, Urubu Kaapor, Waiampi, Warao, and Xikrin—were collected by F.L.B. in the 1970s [16-19]. Serum samples from the Yanomami village were obtained by A.O.S. as clinical samples during a tuberculosis epidemic in 1992 and had been tested for antibodies against various infectious agents to study the immune competence of the population [21], as approved by the Brazilian Indian Health Services. Serum samples were stored at −20°C until they were used. Tribal origin, sex, and age of each study participant, the date of initial peaceful contact of the tribe with outsiders, and the date of specimen collection were recorded for each serum specimen. All individuals <14 years old were tested for the presence of IgG antibodies specific to HSV-1, a well-known oral-salivary–transmitted agent. This age group was chosen because previous data suggest that there is early acquisition of this virus in populations of low socioeconomic status [20]. The younger age also decreased the likelihood that sexually acquired HSV-1 would be a confounding variable.

H. pylori serological assay: ELISA. The presence of *H. pylori* IgG antibodies was determined with a well-validated research ELISA that has been used extensively in diverse populations, including those from South America [22–24]. More specifically, the research ELISA has been validated and used for pediatric-specific study populations for which commercial assays have been fraught with inaccuracy and for which there is a more variable host immune response [25–29]. In all the different populations in which the ELISA has been tested, when possible, pilot validation studies were performed, as described by Hisada et al. [29]. The specific *H. pylori* strain and its antigens (i.e., proteins) chosen for the assay were originally tested under rigorous conditions using serum samples obtained from individuals 6 months through 93 years old who had undergone diagnostic upper endoscopy [30]. Antigen selection and the sensitivity, specificity, and predictive value of the antigens used in our research ELISA are described in detail elsewhere [30]. In brief, the wells were coated by overnight incubation at 4°C with antigens of sonicated *H. pylori* organisms (10 mg/mL). The serum samples were tested in triplicate at a dilution of 1:1000 and were incubated at 37°C for 2 h. Antibodies attached to the solid-phase antigen were probed with a rabbit anti–human IgG conjugated with horseradish peroxidase at a dilution of 1:1000 for 2 h at 37°C. The reaction was amplified with the substrate hydrogen peroxide and chromogen o-phenylenediamine. After the enzyme action was stopped with 1 N H2SO4, the optical density was measured at a wavelength of 492 nm. As defined in earlier studies [23, 24], optical density values >1.3 were considered to indicate *H. pylori* seropositivity; values <0.8 were considered to indicate *H. pylori* seronegativity, and values 0.8–1.3 were considered to be indeterminate.

*H. pylori* serological assay: Western blot. At the time of the writing of the present report, other noninvasive assays, such as the urea breath test and stool antigen test, had been neither definitively validated nor approved by the US Food and Drug Administration for use in children [31]. These assays were also not available at the time of serum collection from the study cohort. In addition, upper endoscopy and biopsy were not performed for the subjects who participated in this study; thus, an alternative method of providing complementary analyses of the serum samples for *H. pylori* infection presence was chosen. Serum samples from 4–5 ELISA antibody–positive individuals from each tribe and 15 randomly chosen, antibody-negative individuals from the present study were tested by Western blot (Helicoblot 2.1; Genelabs) [32]. This assay detects antibodies to specific individual *H. pylori* proteins, including the antigens encoded by cagA (116 kDa), vacA cytotoxin (89 kDa), urease A (30 kDa), and flagella (19.5 kDa), among others. The antigen was a protein lysate of *H. pylori* strain ATCC 49503.2. The sensitivity and specificity of the blot were determined using predetermined sets of panels of human serum samples selected for positive and negative results for 3 reference standard tests: urea-breath test, rapid urea test, and histologic test. These serum samples were selected from several multicenter trials, including trials in Hong Kong, Italy, France, and the United States. The bands were determined using polyclonal antibodies raised against the specific proteins. The manufacturer included a positive and negative control in the Helicoblot kit; its specificity and sensitivity have been noted to be 95% and 96%, respectively. In brief, individual strips were incubated for 1 h with serum samples diluted 1:1000. After washing to remove unbound protein, the strips were probed for 1 h with goat anti–human IgG conjugated with alkaline phosphatase. Those antigens binding to serum IgG antibodies were visualized by reacting with the enzyme substrate, 5-bromo-4-chloro-2-indolyl-phosphate, and nitroblue tetrazolium. A sample was determined to be “positive” if it fulfilled 1 of the 3 manufacturer-recommended criteria: (1) 116 kDa (CagA) positive, where CagA has to be present with ≥1 of the marker bands (i.e., 89-kDa [VacA], 37-kDa, 35-kDa, 30-kDa [UreA], and 19.5-kDa) together or with the current infection marker; (2) the presence of any 1 band at 89 kDa, 37 kDa, or 35 kDa, or with without the current infection marker; and (3) the presence of both the 30-kDa and 19.5-kDa bands, with or without the current infection marker. A positive and negative control from the manufacturer’s kit was included in each assay. In addition, for further validation of the Western blot assay used in this study and characterization of infected versus uninfected patients, serum samples from children (n = 10) and adults (n = 10) with *H. pylori* infection diagnosed at the time of endoscopy via histologic testing, rapid urease test, and culture were used.

*HSV-1* antibody assay. Type-specific *HSV-1* antibodies were detected by the immunodot method, using immunoaffinity-purified glycoprotein IgG1 as antigen, as described in detail elsewhere [33].

Statistical analysis. Data were recorded and analyzed using Epi Info (version 6.04; CDC) [34] and Systat statistical software (Systat). Categorical data were analyzed using the χ2 test, and continuous data were analyzed using Student’s t test. Seroprevalence rates were compared by sex, age, tribe, and duration of contact with outsiders.

Results

In total, 1024 specimens were available from the 22 villages (figure 1). Forty-six samples (4.5%) repeatedly produced in-
Seroprevalence determined by ELISA. \textit{H. pylori}–specific IgG antibodies were detected in 904 of the 978 specimens with definite ELISA results, for an overall prevalence of 92% among the study population. With the exception of one village (Parakana Velho) with a prevalence of 45%, all the populations tested exhibited a seroprevalence >79% (table 1). Ten of the villages, with a total study population of 275 individuals among them, had 100% seropositive rates. There was no statistically significant difference in the frequency of \textit{H. pylori} infection between male (93.3%) and female (90.3%) groups ($P = .10$).

Western blot analysis. Western blot analysis for antibodies to \textit{H. pylori} proteins was performed on 105 ELISA-positive samples selected from all villages, as well as on 15 ELISA-negative serum samples that served as negative controls. Reactive serum samples produced various band patterns, depending on reactivity to individual \textit{H. pylori} proteins. On the basis of the criteria recommended by the manufacturer of the immunoblot kit used (Helicoblot 2.1; Genelabs), 93% (98/105) of the ELISA-positive samples tested were positive by Western blot: 93% were reactive with the \textit{cagA}-encoded (116-kDa) protein, 77% were reactive with the urease A–encoded (30-kDa) protein, and 74% were reactive with the vacA-encoded (89-kDa) protein—the 3 major \textit{H. pylori} protein antigens. In addition, 51% were reactive with the flagella (19.5-kDa) protein, and 36% and 26% were reactive for the 37-kDa and 35-kDa proteins (currently undefined outer membrane proteins), respectively. No band patterns were detected that were unique to any individual village. However, reactivity to the 35-kDa protein was found to be significantly lower in the samples collected from the villages in the southeastern region, an area south of the Amazon River and east of its tributary Tapajaz (figure 1, tribes 2, 3, 4, 5, 9, 11, 12, 13, 14, 15, and 21). The positive rate for the 35-kDa protein in this region was 13% (7/51), compared with 37% (20/54) for the rest of the territory ($P = .006$).

Of the 15 ELISA-negative samples, 53% (8/15) reacted with the 116-kDa protein, 20% (3/15) reacted with the 19.5-kDa protein, and 13% (2/15) reacted with the current infection marker. None of the 15 ELISA-negative samples reacted with the 89-kDa, 30-kDa, 35-kDa, or 37-kDa proteins. Only 13% (2/15) met the criteria for a positive Western blot result; 87% (13/15) were determined negative to be by Western blot, demonstrating that the test had reasonable sensitivity and better specificity.

Influence of outside contact. To examine whether \textit{H. pylori} was introduced into these secluded South American Indian populations by outside visitors, we analyzed for correlation between seroprevalence rates of different villages and their length of peaceful contact with the outside world. The resulting correlation coefficient of 0.050 (95% confidence interval, 0.38–0.46) indicated that these 2 parameters were unrelated.

Age at acquisition of infection. Of the study subjects, 201 were children <14 years old. The seroprevalence in this population was examined in greater detail to better understand the epidemiology of this infection. \textit{H. pylori} infection appeared to be acquired very early in life, with >80% of the children already seropositive at age 3 years. By age 6 years, the seroprevalence had reached that of the adult level (93%). The pattern was similar in all 7 villages from which pediatric samples were available (table 2).

Comparison of \textit{H. pylori} and \textit{HSV}-1 seroprevalence among children. We determined the correlation between the seroprevalence of \textit{H. pylori} and that of \textit{HSV}-1, an infectious agent
with well-established oral-salivary transmission, among the South American Indian children. Figure 2 represents the regression curve based on data obtained from the 7 villages with available pediatric samples. A linear correlation was detected ($r^2 = 0.80$), suggesting that there were shared risk factors. However, the regression line intercepted the X-axis (HSV-1) at 0%, indicating there are additional risks for $H. pylori$ infection in these populations. The age-dependent seroprevalence rates of $H. pylori$ and HSV-1 in these pediatric samples were then compared. It was demonstrated (figure 3) that the majority of HSV-1 infection was acquired at age 3–6 years, whereas the $H. pylori$ infection rate was already >80% in the first 3 years of life.

Discussion

Our study demonstrates that $H. pylori$ infection is highly prevalent among South American Indian tribes that migrated to the Americas ≥10,000 years ago from East Asia [35]. The $H. pylori$ seroprevalence of 92% among adult South American Indians is one of the highest reported in any population studied to date [5–7]. The seroprevalence of $H. pylori$ in children (<11 years old) worldwide ranges from 3% to 74%, with the higher levels in developing countries [36–46]. The South American Indian children in this study have the highest prevalence of all populations studied, exceeding 90% by age 6 years.

The validity of the results from our US-based ELISA must be addressed for these sequestered populations. De Arruda and Passaro [47] demonstrated that a Peruvian-based ELISA was more accurate and sensitive than a US-based ELISA for testing Peruvian samples. Validation with a urea breath test, gastric biopsy specimen analysis, or stool sample $H. pylori$ antigen detection would have been ideal. However, the present study used historical blood samples, and the aforementioned data thus would be impossible to obtain. In an attempt to provide some validation of our ELISA results, the Western blot analysis was performed on a randomly selected number of ELISA-positive and -negative specimens. There was some discordance in the results of the 2 assays, which may be explained by subtle differences in antigen-antibody binding from South American and manufacturer strains. In accordance with the study by De Arruda and Passaro [47], the discordance favored sensitivity over specificity, suggesting that our ELISA cutoffs may have underestimated the number of infected individuals.

We compared the prevalence of $H. pylori$ in children to that of HSV-1, a virus that is well known to be transmitted by

<table>
<thead>
<tr>
<th>Tribe</th>
<th>No. of donors tested (% of tribal population)</th>
<th>Age, median years (range)</th>
<th>Year of initial contact with outsiders</th>
<th>Duration of contact with outsiders at the time of sampling, years</th>
<th>$H. pylori$ seroprevalence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parakana Velho</td>
<td>34 (28)</td>
<td>20 (20–48)</td>
<td>1974</td>
<td>7</td>
<td>45</td>
</tr>
<tr>
<td>Cinta Larga</td>
<td>37 (21)</td>
<td>12 (19–65)</td>
<td>1920</td>
<td>67</td>
<td>79</td>
</tr>
<tr>
<td>Jamamadi</td>
<td>19 (29)</td>
<td>37 (20–65)</td>
<td>1786</td>
<td>200</td>
<td>79</td>
</tr>
<tr>
<td>Arara</td>
<td>65 (93)</td>
<td>23 (2–71)</td>
<td>1981</td>
<td>4</td>
<td>81</td>
</tr>
<tr>
<td>Parakana Novo</td>
<td>32 (100)</td>
<td>32 (2–45)</td>
<td>1977</td>
<td>0.2</td>
<td>87</td>
</tr>
<tr>
<td>Karitiana</td>
<td>31 (24)</td>
<td>35 (20–55)</td>
<td>1961</td>
<td>26</td>
<td>89</td>
</tr>
<tr>
<td>Surui of Rondonia</td>
<td>35 (12)</td>
<td>32 (20–50)</td>
<td>1969</td>
<td>18</td>
<td>89</td>
</tr>
<tr>
<td>Apalai</td>
<td>154 (51)</td>
<td>32 (1–81)</td>
<td>1800</td>
<td>177</td>
<td>92</td>
</tr>
<tr>
<td>Meckranoti</td>
<td>34 (14)</td>
<td>41 (21–68)</td>
<td>1968</td>
<td>10</td>
<td>94</td>
</tr>
<tr>
<td>Tiryo</td>
<td>190 (19)</td>
<td>32 (1–74)</td>
<td>1961</td>
<td>9</td>
<td>94</td>
</tr>
<tr>
<td>Asurini Koatinema</td>
<td>30 (60)</td>
<td>23 (20–55)</td>
<td>1958</td>
<td>26</td>
<td>97</td>
</tr>
<tr>
<td>Parakana C</td>
<td>40 (29)</td>
<td>30 (1–60)</td>
<td>1984</td>
<td>0.2</td>
<td>98</td>
</tr>
<tr>
<td>Asurini Trocara</td>
<td>35 (25)</td>
<td>19 (20–80)</td>
<td>1953</td>
<td>31</td>
<td>100</td>
</tr>
<tr>
<td>Arwate</td>
<td>28 (16)</td>
<td>15 (22–60)</td>
<td>1975</td>
<td>11</td>
<td>100</td>
</tr>
<tr>
<td>Evarhoyana</td>
<td>11 (79)</td>
<td>31 (4–41)</td>
<td>1970</td>
<td>0.1</td>
<td>100</td>
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<tr>
<td>Karara-o</td>
<td>9 (25)</td>
<td>31 (22–60)</td>
<td>1969</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>Mundurucu</td>
<td>33 (1)</td>
<td>26 (22–74)</td>
<td>1700</td>
<td>285</td>
<td>100</td>
</tr>
<tr>
<td>UruBu Kaapor</td>
<td>31 (6)</td>
<td>43 (20–65)</td>
<td>1700</td>
<td>284</td>
<td>100</td>
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<tr>
<td>Waiampi</td>
<td>30 (8)</td>
<td>31 (20–57)</td>
<td>1700</td>
<td>287</td>
<td>100</td>
</tr>
<tr>
<td>Warao</td>
<td>24 (&lt;1)</td>
<td>32 (6–72)</td>
<td>1800</td>
<td>186</td>
<td>100</td>
</tr>
<tr>
<td>Xikrin</td>
<td>34 (23)</td>
<td>30 (18–66)</td>
<td>1955</td>
<td>26</td>
<td>100</td>
</tr>
<tr>
<td>Yanomami</td>
<td>88 (2)</td>
<td>17 (1–63)</td>
<td>1980</td>
<td>12</td>
<td>100</td>
</tr>
</tbody>
</table>

* a No. of years from known initial peaceful contact to date of serum specimen.
H. pylori seroreactivity to proteins encoded by the fecal-oral routes of transmission. are partly done in the local rivers, which may be a source for that bathing, washing of garments, and passing of human waste simulations are not known to have similar habits. It was observed the organism. However, the other South American Indian pop-

ities that include the chewing of tobacco leaves. The leaves are passed around from person to person, and the almost-dissolved leaf is given to the children (including infants). This practice play an important role in the epidemiology of H. pylori. On the other hand, the same linear regression analyses showed that, at 0% seroprevalence for HSV-1, we would still expect 70% of the population to be infected with H. pylori. This may suggest either that H. pylori is a much more contagious agent or that there are additional routes of transmission for H. pylori (e.g., fecal-oral) not described for HSV-1. It has been speculated that contaminated food or water may be vehicles for spread of these bacteria [48]. The Yanomami participate in recreational activities that include the chewing of tobacco leaves. The leaves are passed around from person to person, and the almost-dissolved leaf is given to the children (including infants). This practice lends support to the oral-oral route and early acquisition of the organism. However, the other South American Indian pop-

ulations are not known to have similar habits. It was observed that bathing, washing of garments, and passing of human waste are partly done in the local rivers, which may be a source for fecal-oral routes of transmission.

Results of our Western blot analysis indicated very common seroreactivity to proteins encoded by the H. pylori genes cagA (93%) and vacA (74%). These genes are generally associated with increased gastric disease severity, although it has been demonstrated that some asymptomatic individuals are infected with these putatively virulent strains [49–52]. Unfortunately, no clinical data related to the gastrointestinal tract in the different South American Indian tribes are available. The Western blot analysis of the 105 positive serum samples also produced a variety of band patterns that reflected the heterogeneity of antibody responses in the hosts, which has been reported by other researchers [32, 52].

Observations suggesting that H. pylori has been evolving for a significant period of time have been made by van Doorn et al. [13], who reported on the differences in genotype of H. pylori and their association with different ethnic/geographic origins. Kersulyte et al. [53] identified 5 types of genotypic motifs and found the H. pylori strains from Peruvians, primarily of Amerindian ancestry, to be most similar to strain genotypes isolated from the Spaniards in Europe. Similar observations were reported by van Doorn et al. [13]. Their interpretation was that H. pylori might have been brought to South America by European conquerors some 500 years ago and did not migrate with the Asian ancestors of these tribes across the Behring Strait and down to South America >10,000 years ago. However, such a model is not consistent with the detection of H. pylori antigens in the feces of Peruvian mummies buried for 3000 years [12]. Our findings support the premise that H. pylori is indigenous to the South American Indians. We recognize that we are making indirect comparisons, because no H. pylori isolates were available for genetic analysis in the present study. However, these data, in conjunction with the mummy findings, support the hypothesis that the organism is indigenous to these peoples. The South American villages in the present study are modern examples of ancient populations, because they have been secluded from contemporary society and continue to perpetuate ancient practices. The >90% H. pylori seroprevalence in these populations, some of which (e.g., Parakana C and Parakana...
Novo) had no peaceful contact with outsiders until the month of serum sampling, strongly implies that the infection predated European contact. Moreover, there was no significant difference in the villages with lower prevalence rates, compared with the others, with regard to length of known peaceful contact with the outside world. We do acknowledge that earlier undocumented contacts may have occurred with introductions of microorganisms into a then-naive population.

Kersulyte et al. [53] offer 2 alternate explanations for their observations: (1) the unlikely but possible similarity between the putative ancestral H. pylori strains of Central Asia and those of Spain and (2) the inability of indigenous Amerindian strains to compete with newly introduced European strains. We believe that a third possibility should be entertained: that the currently prevalent Spanish strains may have originated from the South American Indians, brought back by the conquerors to Europe.

In summary, we report a very high seroprevalence of H. pylori in secluded South American Indian populations. The presence of these antibodies in the indigenous peoples gives support to the view that H. pylori is a very old organism and migrated with these populations >10,000 years ago. Comparisons of the prevalence rates of H. pylori and HSV-1 suggest that they share similar modes of transmission, but the consistently higher H. pylori rates imply that this pathogen has additional routes and/or is a more infectious agent. Future studies that include clinical and environmental data and possible genotyping of organisms in these populations may shed light, not only on their evolution and transmission, but also on the development of virulent strains and the occurrence of clinical disease.

References

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