Evidence that close contact, crowding, and poor sanitation (8, 9) have been associated with person-to-person spread, although shared exposure to environmental risk factors may also play a role. Intrafamilial clustering was first proposed by Drumm et al. in 1990 (6) and was later confirmed by Oderda et al. (28) and by Malaty et al. (20), who also found clustering of infection between spouses.

With the failure to identify another reservoir for \textit{H. pylori}, the theory of person-to-person spread is now generally accepted, although far from proven. However, the route of infection remains open to conjecture. The oral cavity supports many ecological niches, some of which may provide the microaerophilic environment necessary for \textit{H. pylori} survival and multiplication. Periodontal pocketing is the consequence of bacterially induced loss of attachment between the tooth and supporting bone, with the resultant formation of a soft tissue-lined pocket surrounding the tooth. This creates a unique environment for colonization by some 200 to 300 bacterial species, of which \textit{H. pylori} may be a transient or permanent member. \textit{H. pylori} has been cultured from dental plaque and saliva by other groups, but recovery has been infrequent (3, 16). PCR has proved more successful for the detection of oral \textit{H. pylori}, and results of studies using this method suggest that the prevalence of oral \textit{H. pylori} is between 0 and 90%, as reviewed by Mudnii et al. (19), with a tendency for patients with upper abdominal complaints to have a higher prevalence of \textit{H. pylori} colonization than those without. The demonstration of even transient oral carriage of \textit{H. pylori} could have implications for the prevention of person-to-person transmission. It has been suggested that fecal transmission also plays a role in the spread of \textit{H. pylori} (14, 15, 19, 33, 34).

Since most evidence indicates that the prevalence of \textit{H. pylori} infection is higher in nonindustrialized nations, the aim of this study was to identify possible routes of infection in an isolated rural community in Central America. The village of San Juan La Laguna is situated in the central highlands of

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\textbf{Helicobacter pylori} Infection in Indigenous Families of Central America: Serostatus and Oral and Fingernail Carriage

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\textit{Helicobacter pylori} infection remains one of the most common in humans, but the route of transmission of the bacterium is still uncertain. This study was designed to elucidate possible sources of infection in an isolated, rural population in Guatemala. A total of 242 subjects in family units participated in the study. A medical history, including a history of dyspepsia, was taken by a physician and immunoglobulin G antibodies to \textit{H. pylori} were detected with the QuickVue (Quidel, San Diego, Calif.) onsite serology test. Overall, 58% of subjects were seropositive, with a positive relationship between mother and child (\(P = 0.02\)) and a positive correlation between the serostatuses of siblings (intraclass correlation coefficient = 0.63). There was no association between serostatus and gastric symptoms. Oral \textit{H. pylori} was detected from periodontal pockets of various depths and the dorsum of the tongue by nested PCR. Eighty-seven percent of subjects had at least one oral site positive for \textit{H. pylori}, with the majority of subjects having multiple positive sites. There was no association between periodontal pocket depth and the detection of \textit{H. pylori}. Nested PCR was also used to detect \textit{H. pylori} from beneath the nail of the index finger of each subject’s dominant hand. Overall, 58% of subjects had a positive fingernail result, with a significant positive relationship between fingernail and tongue positivity (\(P = 0.002\)). In conclusion, the results of this study suggest that oral carriage of \textit{H. pylori} may play a role in the transmission of infection and that the hand may be instrumental in transmission.

Since \textit{Helicobacter pylori} was first reported by Marshall and Warren in 1983 (23), its importance in the field of gastroenterology has been significant. As well as causing type B gastritis, \textit{H. pylori} has a well-documented role in the development of gastric ulcers. Furthermore, it is now designated a type I carcinogen by the World Health Organization due to its association with gastric adenocarcinoma.

Despite the considerable impact of \textit{H. pylori} infection on health worldwide, there is still little understanding of its mode of transmission, hindering the successful implementation of preventive measures. The proceedings of the 1997 International Update Conference on \textit{H. pylori} (30) reported that well-designed studies assessing oral-oral and fecal-oral transmission, as well as other potential mechanisms of transmission, are necessary and will be key in eliminating \textit{H. pylori} and its associated diseases. As a result, such studies should be given high priority in future \textit{H. pylori} research.

Most evidence suggests that transmission occurs from person to person since, with the exception of the rhesus monkey, there are no other identified natural reservoirs for \textit{H. pylori} (1). Evidence that close contact, crowding, and poor sanitation (8, 24, 26) are risk factors for infection supports the theory of person-to-person transmission, exemplified by the clustering of \textit{H. pylori} infection in institutionalized patients (35) and submarine crews (10) and by the high prevalence of infection reported in the populations of nonindustrialized countries.

Clustering of infection within families has also been commonly observed and reinforces the importance of person-to-person spread, although shared exposure to environmental risk factors may also play a role. Intrafamilial clustering was first proposed by Drumm et al. in 1990 (6) and was later confirmed by Oderda et al. (28) and by Malaty et al. (20), who also found clustering of infection between spouses.

With the failure to identify another reservoir for \textit{H. pylori}, the theory of person-to-person spread is now generally accepted, although far from proven. However, the route of infection remains open to conjecture. The oral cavity supports many ecological niches, some of which may provide the microaerophilic environment necessary for \textit{H. pylori} survival and multiplication. Periodontal pocketing is the consequence of bacterially induced loss of attachment between the tooth and supporting bone, with the resultant formation of a soft tissue-lined pocket surrounding the tooth. This creates a unique environment for colonization by some 200 to 300 bacterial species, of which \textit{H. pylori} may be a transient or permanent member. \textit{H. pylori} has been cultured from dental plaque and saliva by other groups, but recovery has been infrequent (3, 16). PCR has proved more successful for the detection of oral \textit{H. pylori}, and results of studies using this method suggest that the prevalence of oral \textit{H. pylori} is between 0 and 90%, as reviewed by Mudnii et al. (19), with a tendency for patients with upper abdominal complaints to have a higher prevalence of \textit{H. pylori} colonization than those without. The demonstration of even transient oral carriage of \textit{H. pylori} could have implications for the prevention of person-to-person transmission. It has been suggested that fecal transmission also plays a role in the spread of \textit{H. pylori} infection (14, 15, 19, 33, 34).

Since most evidence indicates that the prevalence of \textit{H. pylori} infection is higher in nonindustrialized nations, the aim of this study was to identify possible routes of infection in an isolated rural community in Central America. The village of San Juan La Laguna is situated in the central highlands of
Guatemala and comprises some 2,500 indigenous, non-Hispanic people living in close proximity within large family units and with limited sanitation facilities. This community presented the possibility of conducting an extensive study in a noninstitutional setting, in contrast to the majority of previous studies, which have tended to focus on the oral carriage of \textit{H. pylori} in individual gastric patients.

**MATERIALS AND METHODS**

**Study subjects.** Permission to solicit participation in the study was obtained from the office of the mayor of the village. Subjects were recruited in defined family units (i.e., parents and their children \(\geq 12\) years of age) with the assistance of church and village officials. Written informed consent was obtained from all prospective participants and approved by the institutional review boards of the University of Texas Health Science Center at San Antonio and Universidad Mariano Galvez, Guatemala City, Guatemala.

**Medical history.** A detailed medical history was taken by a physician from all volunteers in Spanish but recorded in English. A number of questions concerned the presence or absence of dyspeptic symptoms, including epigastric pain or discomfort, bloating, belching, flatulence, burning, fullness, nausea, and vomiting. In those cases where subjects spoke only the local Indian language (\textit{tz\'utujil}) a \textit{tz\'utujil}-Spanish translator from the village was employed.

**Periodontal examination.** A standard full-mouth periodontal examination was preceded by an oral soft tissue examination to detect any significant oral pathology. Six sites (mesiobuccal, distobuccal, distolingual, midlingual, and mesiolingual) on all existing teeth were examined. Periodontal pocket probing depths were measured to the nearest millimeter with a UNC 15 probe (Hu-Friedy, Zurich, Switzerland). Each parameter was measured by a single trained examiner, with the subject in a portable dental chair and under uniform dental lighting powered by a generator.

**Blood grouping and serotyping.** Standard ABO blood grouping was assayed from a finger-prick blood sample with a Glucolet lancing device and disposable Fingerstix lancets (Organon Teknika, Malvern, Pa.). \textit{H. pylori} antibody status was determined from the same sample with an onsite serology kit, QuickVue (Quidel, San Diego, Calif.). Nuclear family relationships were recorded in order to look for familial clustering of \textit{H. pylori} infection diagnosed by serology.

**Sample collection.** All bacterial samples were collected with sterile absorbent paper points (Dentsply, York, Pa.). Both healthy sites and periodontal pockets were sampled individually by placing a single paper point into each site for 5 s. A sample was also taken from within the fissures of the dorsum of the tongue. A further sample was collected from beneath the nail of the index finger of the dominant hand. All samples were placed in separate sterile bags, which were sealed, transported to the laboratory, and stored at room temperature until processed.

**Sample processing.** Samples were processed within 3 months of collection. The absorbent points were placed in Eppendorf tubes and 100 \(\mu\)l of distilled sterile water was added. Samples were incubated in a water bath at 92°C for 10 min, placed on ice for 5 min, and then centrifuged at 850 \(\times\) g for 5 min. The supernatant was used for the amplification of a highly conserved region of the 16S rRNA gene of \textit{H. pylori}.

**Primers.** DNA amplification was performed according to the method employed by Saiki et al. (29), with primer sequences previously described and tested (Quidel, San Diego, Calif.). Where it was subjected to an initial denaturation step at 95°C for 1 min, followed by 25 cycles of amplification as follows: denaturation at 92°C for 1 min, annealing at 50°C for 2 min, and elongation at 72°C for 2 min, a final cycle was performed that was identical to the previous 25 cycles, except that elongation was increased to 4 min. One microoliter of the primary amplification product was used in a 30-\(\mu\)l reaction mixture with primers H1p and H2p under the conditions described above. The product of the nested PCR amplification reaction (expect- ed size, 109 bp) was resolved on a 1.5% agarose electrophoresis gel, run for 40 min at 70 V (35 mA). DNA, extracted from \textit{H. pylori} (ATCC 43504) by the TRIzol reagent method of Chomczynski and Sacchi (4), was also employed as a positive control and amplified as described above. As a negative control, a reaction mixture without DNA was included and subjected to the steps described above.

**RESULTS**

A total of 242 subjects (112 males and 130 females) participated in the study, with ages ranging from 12 to 75 years (mean, 31.4 years). Table 1 shows the distribution of subjects by age. Subjects were in defined family units, which included a mother, a father, and a number of their children. With regard to dyspepsia, 70 of 242 (29%) subjects reported past or present gastric symptoms. Blood grouping revealed that 208 of 242 (86%) had type O blood; the majority of the remainder had type A. Tobacco smoking was rare, with seven individuals reporting only infrequent use (<1 to 7 cigarettes per week).

**Serostatus.** Testing with the QuickVue serology kit showed that 141 (58%) subjects were seropositive for \textit{H. pylori}. No obvious age trend was seen, with the exception of the youngest and oldest age groups, which appeared to have lower rates of seropositivity (Table 1). No statistically significant relationship between serostatus and gastric symptoms was found, and as far as could be determined, there was no association of serostatus and ABO blood group.

For family data analysis, 129 mother-child, 108 father-child, and 95 husband-wife relationships were analyzed (Table 2). Univariate analysis by generalized-estimating-equation methods applied to logistic regression revealed that a mother’s serostatus was predictive of her child’s serostatus (\(P = 0.02\)). However, this was not the case for a father and his child; multivariate analysis revealed that a seronegative mother and a seropositive father tended to have a seronegative child (in 29

**TABLE 1. \textit{H. pylori} serostatus of subjects by age**

<table>
<thead>
<tr>
<th>Age range (yr)</th>
<th>(n)</th>
<th>No. (%) seropositive</th>
</tr>
</thead>
<tbody>
<tr>
<td>12–17</td>
<td>56</td>
<td>23 (41)</td>
</tr>
<tr>
<td>18–34</td>
<td>98</td>
<td>63 (64)</td>
</tr>
<tr>
<td>35–44</td>
<td>49</td>
<td>32 (65)</td>
</tr>
<tr>
<td>45–54</td>
<td>20</td>
<td>13 (65)</td>
</tr>
<tr>
<td>55–64</td>
<td>10</td>
<td>9 (90)</td>
</tr>
<tr>
<td>(\geq 65)</td>
<td>9</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Total</td>
<td>242</td>
<td>141 (58)</td>
</tr>
</tbody>
</table>

**Statistical analysis.** Results were analyzed with the Statistical Analysis System package, PC SAS, version 6.12 (SAS Institute, Inc., Cary, N.C.). For some of the analyses, subjects were arbitrarily categorized into age group cohorts.

<table>
<thead>
<tr>
<th>Family member and status</th>
<th>No. of relationships</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child(^a)</td>
<td>Negative</td>
</tr>
<tr>
<td>Mother</td>
<td>42</td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
</tr>
<tr>
<td>Father</td>
<td>11</td>
</tr>
<tr>
<td>Child(^b)</td>
<td>Negative</td>
</tr>
<tr>
<td>Father</td>
<td>24</td>
</tr>
<tr>
<td>Positive</td>
<td>41</td>
</tr>
<tr>
<td>Wife(^c)</td>
<td>Negative</td>
</tr>
<tr>
<td>Husband</td>
<td>17</td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
</tr>
</tbody>
</table>

\(^a\) \(P = 0.02\).  
\(^b\) \(P = 0.01\).  
\(^c\) \(P = 0.15\).
of 41 cases). There was no statistically significant relationship between the serostatuses of spouses (Table 2).

The intraclass correlation coefficient was used to determine intrafamilial serostatus correlation. This was calculated as 0.63, showing that seropositivity between siblings was significantly correlated.

**Oral and fingernail samples.** Up to 13 oral sites (mean = 6) were sampled for each subject. Of a total of 242 subjects, 209 (87%) had at least one positive oral sample as analyzed by nested PCR. On the basis of results for tongue samples alone, 130 of 232 (56%) subjects were positive. Table 3 shows that, in the majority of subjects, multiple sites were found to be positive for *H. pylori*.

Regarding periodontal status, there was no statistically significant relationship between *H. pylori* status and periodontal pocket probing depth. Table 4 shows the number and percentage of sites which tested positive by nested PCR in relation to pocket depth.

In the case of fingernail samples, 136 of 233 (58%) subjects were positive for *H. pylori*, with a significant correlation between these results and those for tongue samples ($P = 0.002$). There was also a weak positive relationship between a positive fingernail sample and seropositivity ($P = 0.075$).

**DISCUSSION**

In the context of the current controversy regarding possible modes of transmission of *H. pylori*, the purposes of this study were primarily the following: firstly, to confirm previous studies showing familial clustering of *H. pylori* infection (6, 20, 21, 28); secondly, to determine whether the oral cavity is a significant reservoir for *H. pylori* in subjects from a rural community of a nonindustrialized country; thirdly, to elucidate the possible relationship between oral carriage and serostatus; and finally, by examining fingernail samples, to test the hypothesis that the hand may be instrumental in transmission.

Previous investigations have tended to use study subjects who were gastric patients or blood donors; few studies have used subjects randomly selected from within a community. In this study, we examined 242 subjects from nuclear families within a rural community of Central America. Past or present gastric symptoms were reported by 29% of subjects, which is comparable to other reports reviewed by Talley and Noack (31, 32). Blood grouping demonstrated that the great majority of subjects (86%) had type O blood, a finding consistent with other studies of the indigenous populations of Central and South America (25).

Serological testing for *H. pylori* infection was performed with the onsite QuickVue test, which detects anti-*H. pylori* immunoglobulin G (IgG) antibodies in an enzyme immunoassay. The test has been shown to have a sensitivity of more than 90% (36) and a specificity of 73 to 89% (9) and is a suitable screening tool for epidemiological studies in remote sites where use of the urea breath test would be logistically difficult. Not unexpectedly, more than 50% of subjects in this sample population had a positive serological result; in such a population where no treatment is available, this is likely to indicate current, rather than recent past, infection. Studies conducted in other developing countries have reported comparable infection rates of 41 to 96%, depending on subjects' age group, as reviewed by Goodman and Correa (7) and Talley and Noack (31). Suggested reasons for these high infection rates include close contact, crowding and poor sanitation (8, 24, 26), lack of hot water (24), lack of an external water supply (15), and coffee drinking (2), which is common from infancy in this population.

There was no relationship between seropositivity and symptoms of dyspepsia, although the reliability of subject histories may be questionable. As was expected, the prevalence of infection was low in the youngest age group (41% in subjects 12 to 17 years old), but there was no significant increase in prevalence in each of the subsequent age cohorts up to 54 years. Beyond this age, sample sizes were too small for further inference. In the ≥65-year age group, only one of nine subjects was seropositive, which again may reflect the small sample size. It may also be the result of a reduced antibody response in the elderly (27) or progressive gastric atrophy, which mitigates against colonization by *H. pylori* (5).

Crowding and close contact, both risk factors for infection as discussed above, are also relevant when considering infection within families; intrafamilial clustering of infection has been commonly observed. Drummond et al. (6) demonstrated intrafamilial clustering of *H. pylori* infection, diagnosed by serology, in Canadian families with children reporting upper gastrointestinal tract symptoms; these results were later confirmed by Oderda et al. (28) in Italy with a similar study design. Family clustering of *H. pylori* in healthy volunteers has also been reported by Malaty et al. (21), who showed that the frequency of infection, identified by the urea breath test and serology, was strikingly higher among children with a seropositive parent and among spouses of seropositive subjects, the latter suggesting environmental, rather than genetic, factors. In the present study, serological testing was performed for all subjects without preselection, other than that they be members of defined nuclear families. The results confirmed those of other studies, demonstrating a positive correlation in serostatus between siblings and that a mother's serostatus was predictive of her children's. Interestingly, a father's serostatus was not a positive predictor. This may not be surprising when we consider the absence of the men from the households throughout the daylight hours, six days a week, while they work in the fields.

Although intrafamilial clustering of infection has been widely reported, the mode of transmission of *H. pylori* remains controversial. In this study, we attempted to confirm the findings of other investigations that suggest oral carriage of *H. pylori*, but with improved experimental design over other pub-

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**TABLE 3.** Subjects with oral sites testing positive for *H. pylori* by nested PCR

<table>
<thead>
<tr>
<th>No. (%) of subjects</th>
<th>% Positive oral sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>32 (13)</td>
<td></td>
</tr>
<tr>
<td>42 (17)</td>
<td></td>
</tr>
<tr>
<td>62 (26)</td>
<td></td>
</tr>
<tr>
<td>57 (24)</td>
<td></td>
</tr>
<tr>
<td>25 (10)</td>
<td></td>
</tr>
<tr>
<td>23 (10)</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 4.** Periodontal sites testing positive for *H. pylori* by nested PCR

<table>
<thead>
<tr>
<th>No. (%) of positive sites</th>
<th>Probing pocket depth (mm) of site</th>
</tr>
</thead>
<tbody>
<tr>
<td>359 (45)</td>
<td>1</td>
</tr>
<tr>
<td>387 (40)</td>
<td>2</td>
</tr>
<tr>
<td>209 (44)</td>
<td>3</td>
</tr>
<tr>
<td>19 (53)</td>
<td>4</td>
</tr>
<tr>
<td>399 (43)</td>
<td>5</td>
</tr>
<tr>
<td>79 (48)</td>
<td>6</td>
</tr>
<tr>
<td>29 (48)</td>
<td>7</td>
</tr>
<tr>
<td>4 (50)</td>
<td>8</td>
</tr>
<tr>
<td>12 (67)</td>
<td>9</td>
</tr>
</tbody>
</table>
lished studies. The objective was to sample a large population which had little access to standard medical and dental care. Moreover, the subjects were in family units; oral sampling was performed on a site-specific basis to determine any possible association with periodontal disease.

In this sample population of 242 people, 87% of subjects had at least one oral site positive for *H. pylori*, detected by nested PCR. Furthermore, 70% of subjects had more than 25% positive sampled sites (Table 3). A high rate of oral carriage was found irrespective of periodontal status, showing no association with pocket depth. This high prevalence of oral *H. pylori* was also reflected by the positive tongue samples from 56% of subjects. Others have demonstrated similarly high detection rates in the oral cavities of patients with upper abdominal complaints, reviewed by Madinier et al. (19). However, population-based investigations comparable to the present study are few and demonstrate only a 10 to 10% rate of detection of oral *H. pylori* by PCR (19). The high prevalence of oral *H. pylori* in the present study may be a characteristic of the population investigated. The specificity of PCR is also an issue, but the primers employed in this study have previously been validated and employed in other studies (11, 12, 18, 22).

In addressing the question of route of transmission, PCR was also used for the detection of *H. pylori* beneath the fingernail in a high proportion of subjects at sites where it has been detected by PCR. Our finding of *H. pylori* warrants further investigation to gain additional insight into the routes of transmission.

ACKNOWLEDGMENTS

We are grateful to Barry Katz and George Eckert, Department of Biostatistics, Indiana University School of Medicine, for their additional help and advice; the staff of Luis Archila, Guatemala City, Guatemala; and, most importantly, the villagers of San Juan La Laguna, Guatemala.

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