Seroincidence of *Helicobacter pylori* Infection in a Cohort of Rural Bolivian Children: Acquisition and Analysis of Possible Risk Factors

M. Kathleen Glynn, Cindy R. Friedman, Benjamin D. Gold, Bhawna Khanna, Lori Hutwagner, Naomi Iihoshi, Carmen Revollo, and Robert Quick

1Division of HIV/AIDS Prevention, National Center for HIV, STD, and TB Prevention, 2Bioterrorism Preparedness and Response Program and 3Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Disease, 4National Center for Infectious Diseases, Centers for Disease Control and Prevention, and 5Division of Pediatric Gastroenterology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia; 6National Center for Tropical Diseases, Santa Cruz, and 7Community and Child Health Project, La Paz, Bolivia

High seroprevalence rates for *Helicobacter pylori* are reported in developing countries, yet few seroincidence studies exist that determine age of initial acquisition and risk factors for *H. pylori* seroconversion. Two *H. pylori* serosurveys were conducted in August 1996 and November 1997. Of 188 children aged 21 months to 6 years who were seronegative in the first survey, 44 (23%) had seroconverted at follow-up, yielding an 18% annual seroincidence. The largest increase in seroincidence occurred between children aged 2 years (10%) and children aged 3 years (32%). Use of a lidded, narrow-mouthed water vessel was protective against seroconversion (odds ratio [OR], 0.3; 95% confidence interval [CI], 0.1–0.8), and the presence of another *H. pylori*-seropositive sibling in the household was a risk factor for seroconversion (OR, 3.1; 95% CI, 1.3–8.7). Although not a randomized intervention trial, this study suggests that the use of a narrow-mouthed water vessel may prevent the transmission of *H. pylori* in households in developing countries.

*Helicobacter pylori* is a leading cause of antral gastritis in both adults and children [1]. Infection with *H. pylori*, which may be lifelong if untreated, causes gastritis, duodenal and gastric ulcers, and stomach cancer [2–4]. IgG antibodies specific for *H. pylori* indicate past or present infection [5–7]. Point prevalence and retrospective sero surveys conducted in South America have shown higher rates of *H. pylori* seroprevalence than in developing countries and have associated seropositivity with crowding, low socioeconomic status, family members who are seropositive, lack of a municipal water source, and exposure to animal feces. However, no prospective studies have been conducted to determine the age of initial acquisition of *H. pylori* infection or to evaluate risk factors for *H. pylori* seroconversion [8–15].

Although the exact routes of transmission are not definitively known, a majority of studies suggest that humans are the major reservoir for *H. pylori* [10, 15, 16]. Transmission from one person to another probably occurs via fecal-oral, oral-oral, or gastro-oral routes. Commonly mentioned specific paths include transmission from infected siblings living in the same household or from other close household contacts who are seropositive [14, 15, 17]. Studies conducted in South America have shown...
America suggest that water contaminated at the source or in
the home during water storage is a vehicle for \textit{H. pylori} trans-
mision \cite{18, 19}. Therefore, we postulated that the introduction
of a narrow-mouthed, safe--drinking water storage vessel in the
home may decrease household \textit{H. pylori} transmission.

The objectives of this study were to determine the serocon-
version rate and thereby the incidence of \textit{H. pylori} infection in
a cohort of rural Bolivian children aged 21 months through 6
years, to determine age of initial acquisition and risk factors
for \textit{H. pylori} seroconversion, and to assess the possible effec-
tiveness of a household-based safe drinking water intervention
for preventing \textit{H. pylori} seroconversion.

\section*{METHODS}

\textbf{Study population.} This 2-phase study took place in 17 rural
communities in Santa Cruz Department, Bolivia, which had
been selected in July 1996 for a safe water intervention social
marketing study. Village residents were predominantly subsis-
tence farmers, living at an extremely low socioeconomic level.

\textbf{Safe water social marketing study.} The social marketing
study was conducted to determine the acceptability and use of
a water quality intervention, as described in detail elsewhere
\cite{20, 21}, which had 3 basic elements: a standard 20-L plastic,
narrow-mouthed, lidded water vessel with a spigot (figure 1;
hereafter referred to as the “special vessel”), a dilute sodium
hypochlorite solution for water treatment, and community edu-
cation on the causes and prevention of diarrhea and use of the
special vessel and hypochlorite solution. Seventeen rural vil-
lages, all lacking a community water system, were selected for
the safe water social marketing study and were initially grouped
into 8 intervention villages and 9 geographically separated con-

A chronology of the \textit{H. pylori} and safe water intervention
studies is shown in figure 2. Special vessels and chlorine were
not distributed free of charge. Rather, in November 1996, resi-
dents of the 8 intervention communities could voluntarily par-
ticipate in a community work effort and be awarded the vessel
and hypochlorite solution. However, not all households in inter-
vention villages chose to participate. During the intervention
period (December 1996–February 1997), households in the con-

\textbf{H. pylori study design.} In August 1996, we conducted a
serosurvey (survey I) to establish baseline \textit{H. pylori} seropreva-
ence rates among children in the 17 villages as a part of a
children’s health day. All children aged 6 months through 9
years were eligible to enroll in the health day activities, including
testing for \textit{H. pylori}; participation was voluntary. To determine
\textit{H. pylori} seroprevalence, we collected capillary blood samples
by fingerstick from each child and then separated and stored
serum samples in liquid nitrogen for transport to the US Cen-
ters for Disease Control and Prevention (CDC; Atlanta, Geor-
gia). In November 1997, we returned to the same 17 villages

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Chronology of \textit{Helicobacter pylori} seroprevalence surveys and safe water intervention study, Bolivia, 1996–1997}
\end{figure}

\textbf{Figure 2.}
and conducted a second serosurvey (survey II). All participating children were offered the same tests as in survey I, including *H. pylori* determination. Because high seroprevalence rates among children aged >6 years in survey I allowed for limited opportunity for seroconversion, we restricted enrollment in survey II to children aged ≤6 years. In both surveys, we excluded children aged <6 months, because previous studies have shown that seropositivity among newborns probably represents passive transfer of maternal immunity, most of which wanes over the first months of life [22–25].

As a part of survey II, we collected epidemiological data from study participants. For each child, a questionnaire was administered to the accompanying adult, addressing dietary and hygienic practices previously implicated in *H. pylori* seroprevalence studies. Previously identified or proposed risk factors were assessed in this study questionnaire, along with age, sex, and symptoms of possible *H. pylori* infection. To address the possible association between *H. pylori* seroconversion and contaminated drinking water stored in the home, questions regarding the use of the special vessel and chlorine solution were included in the questionnaire; investigators also visited study households on the same day as the interviews to directly observe whether the special vessel and chlorine solution were being used and to test stored water for the presence of residual chlorine by means of a Hach colorimeter and the \( N,N\)-diethyl-p-phenylenediamine method.

**Laboratory methods.** Serum samples were thawed and tested for the presence of anti-*H. pylori* IgG antibody by use of a research ELISA previously well validated with serum samples obtained from children living in developing countries [5, 26]. This previously validated ELISA confers high sensitivity and specificity when *H. pylori* serostatus is being determined in this population and has shown greater accuracy in this population than has commercially available serological assays. Serum samples (10 μL) were tested by means of a standard 96-well microtiter plate ELISA spectrophotometer (Fisher Scientific) at a wavelength of 492 nm. The mean optical density (OD) reading for each patient’s serum samples then was quantitated. A mean OD range of 0.86–1.32 separated the limits of the *H. pylori*–negative and –positive serum values, respectively, and then was designated the indeterminate range. Indeterminate samples were retested in triplicate by ELISA. If the resulting value was still in the indeterminate range, the patient’s serostatus was recorded as indeterminate. Samples with discrepant values on repeat ELISA testing were confirmed by means of Western blot [5, 24].

Environmental water samples (100 mL) were collected from all common water sources in each village during survey II. Undiluted and 1:10 dilutions were tested for fecal coliforms by the membrane filtration technique. Colony counts of fecal coliforms were made according to methods described elsewhere [21].

**Statistical analyses.** Data entry and descriptive analyses were conducted with Epi-Info, version 6.04 (CDC). Children were classified as “seroconverters,” if they tested seronegative in survey I and seropositive in survey II, or as “nonseroconverters,” if they tested seronegative in both surveys. Children without reported age, who tested seropositive in survey I, or who had indeterminate serostatus in either survey were excluded from analysis.

To control for effects of age in the multivariate analysis, children were classified into 12-month age groups with the exception of the 2 youngest age groups; these 2 groups included ranges of 15 and 14 months, respectively. All children aged 6–20 months, ineligible for the seroincidence cohort, were grouped into the youngest category and are not included in seroincidence analyses. Children aged 21–23 months (\( n = 3 \)) were included in the second age group, to allow for the balance of 12-month age groups. \( \chi^2 \) analysis was used to seek differences among groups. Multivariate analyses with SAS statistical software, version 6.12 (SAS Institute), used generalized estimating equations to control for the repeated observations within families [27].

**Ethical approval.** The study protocol was approved by the CDC Human Subjects Research Institutional Review Board (IRB); the Bolivian Ministry of Health and the College of Medicine, Santa Cruz, Bolivia, agreed to accept the CDC IRB’s assessment of this proposal (assurance no. S-15070-02). Verbal consent was obtained from the parents or guardians of all study participants in accordance with requirements of the CDC IRB and the Bolivian National Secretariat of Health.

**RESULTS**

There were 1984 eligible children from 879 families living in the 17 study villages during survey I; 1392 provided serum samples for antibody testing, 1389 of whom also had a reported age. Overall, 44% were seropositive, 49% were seronegative, and 7% had indeterminate test results (table 1). Seroprevalence increased with age; 12% of children aged 6–20 months were seropositive, compared with 64% of those aged 84–108 months (7–9 years). In survey II, 1049 eligible children from 521 families living in the same 17 villages participated. Overall, 36% were seropositive, 52% were seronegative, and 12% had indeterminate *H. pylori* serostatus (table 1). As in survey I, seroprevalence increased markedly with age; only 10% of children aged 6–20 months were seropositive, compared with 56% of children aged 72–83 months (6 years). In both surveys, there was a significant trend toward increasing seroprevalence by age group (\( P < .001 \)).
Table 1. Age-specific Helicobacter pylori seroprevalence and 15-month seroincidence, Bolivia, 1996–1997.

<table>
<thead>
<tr>
<th>Age group, months</th>
<th>Present studya (n = 188)</th>
<th>1996 serosurvey (n = 1389)</th>
<th>1997 serosurvey (n = 1049)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCs</td>
<td>NCs</td>
<td></td>
</tr>
<tr>
<td>6–20</td>
<td>24 (12)</td>
<td>155 (78)</td>
<td>21 (11)</td>
</tr>
<tr>
<td>21–35</td>
<td>34 (24)</td>
<td>101 (70)</td>
<td>9 (6)</td>
</tr>
<tr>
<td>36–47</td>
<td>60 (34)</td>
<td>101 (58)</td>
<td>14 (8)</td>
</tr>
<tr>
<td>48–59</td>
<td>76 (54)</td>
<td>53 (38)</td>
<td>11 (8)</td>
</tr>
<tr>
<td>60–71</td>
<td>72 (44)</td>
<td>78 (48)</td>
<td>13 (8)</td>
</tr>
<tr>
<td>72–83</td>
<td>82 (55)</td>
<td>59 (40)</td>
<td>8 (6)</td>
</tr>
<tr>
<td>84–108</td>
<td>268 (64)</td>
<td>130 (31)</td>
<td>20 (5)</td>
</tr>
<tr>
<td>Total</td>
<td>616 (44)</td>
<td>677 (49)</td>
<td>96 (7)</td>
</tr>
</tbody>
</table>

NOTE. Percentages do not always total 100% because of rounding. IND, indeterminate; NCs, nonseroconverters; SCs, seroconverters; SN, seronegative; SP, seropositive.

* Age group based on subjects' ages at the 1997 serosurvey.

Of 361 children aged 21–83 months in 1997 who participated in both surveys, 173 (48%) met 1 of the exclusion criteria and thus were not part of the seroincidence cohort. Of the 173 excluded, 122 (71%) were seropositive in survey I, and 51 (29%) had indeterminate serostatus in one or both surveys. Sixteen children, distributed throughout the 5 age groups, were seropositive in survey I and seronegative in survey II. Of the 188 children who remained eligible for the seroincidence cohort, 44 (23%) seroconverted and 144 (77%) remained seronegative. Fifteen-month seroconversion rates for the 188-member cohort (by age group, based on age at the time of survey II) are presented in table 1. There was a significant jump in seroconversion rate between the group aged 21–35 months, in which only 5 (10%) of 50 children seroconverted, and the group aged 36–47 months, in which 15 (32%) of 47 children seroconverted (P = .007). When the 15-month seroconversion was converted into annual rates, the resulting 12-month seroconversion for the entire cohort was 18%. Although not significant, annual seroconversion rates in older 12-month age groups were slightly lower (21%–22%) than the rate in the group aged 36–47 months (26%).

Because of the association between H. pylori seroprevalence and age, we controlled for age in all remaining analyses. Assessment of individual risk factors with use of seroconversion as the outcome measure, while controlling for age group and family clustering, identified only 2 factors significantly associated with seroconversion (table 2). The presence of a previously identified seropositive sibling in the household was a risk factor (OR, 2.2; 95% CI, 1.0–4.7), and the observed use of the special vessel was a protective factor (OR, 0.4; 95% CI, 0.2–0.9). Reported use of the special vessel combined with chlorine solution was not significant, nor was observed use of chlorine solution or presence of residual chlorine in stored household water. Other factors not significantly associated with seroconversion in this study include village of residence, consumption of premasticated food, crowding within the home, number of persons sharing a bed with the child, methods of relieving teething pain, infection with intestinal parasites, documented microbial contamination of water source, or consumption of specific foods or drinks. Behaviors associated with breast-feeding, including breast-feeding from multiple women or breast-feeding from a woman who was simultaneously nursing other children, also were not significantly associated with seroconversion.

In the final multivariate model, again controlling for age and family clustering, only 2 factors had independent, statistically significant association with seroconversion. Children from households with siblings seropositive for H. pylori were more likely to seroconvert in the 15-month time period (OR, 3.1; 95% CI, 1.3–8.7); in 63% of the children, the sibling or siblings were older than the child who seroconverted. Children from households that used the special vessel (independent of use of the chlorine solution) were less likely to seroconvert (OR, 0.3; 95% CI, 0.1–0.8) than were those from households not using the special vessel. However, among participants in survey II, observed special vessel use was not more common in villages that had formerly been intervention villages. Neither intervention status of village of residence in the water study nor microbial contamination of the household water source as defined by fecal coliform counts significantly changed the final model when considered either as independent risk factors or as interaction terms with other variables.

DISCUSSION

In this study in rural Bolivian children aged ≤6 years, we found an annual seroconversion rate of 18% and found that the largest
increase in the rate of seroconversion occurred when comparing children aged 2 years with children aged 3 years. These 2 groups had the lowest and highest annual seroincidence: 8% and 26%, respectively. Changes in behaviors between these 2 age groups are characterized by attainment of important developmental milestones, including increasing independence and interactions with persons other than the primary caretaker; these interactions, however, are still mostly limited to family and household members. Among the older children, seroconversion rates decreased slightly, compared with that of children aged 3 years, but seroprevalence steadily increased.

By aged 7 years, 55% of children from the 17 study villages were seropositive for *H. pylori*. These rates are much higher than reported seroconversion rates for developed countries, which have been suggested to be <1% per year [10, 16], but are consistent with other studies among children in developing countries, where seroprevalence rates in children aged <10 years were 40%–60%, and overall population seroprevalence rates approached 100% [8, 13, 18, 28–31].

Contaminated household water sources have been suggested as a possible source for *H. pylori* infections, but the difficulty of culturing *H. pylori* from environmental samples has made determining the exact sources of infection and route or routes of transmission for *H. pylori* open to debate [18, 32–36]. Although contamination of water sources varied among villages, we found no association between fecal contamination of the source of the household water and *H. pylori* seroconversion; therefore, we believe that contamination of the rivers and wells with *H. pylori* organisms does not play a major role in transmission. Rather, our hypothesis is that stored household water, contaminated in the home, serves as a vehicle for infection. In this hypothesis, when cups or other receptacles are dipped into traditional, wide-mouthed water containers storing household water, fecally or otherwise contaminated hands occasionally touch the water, contaminating it with *H. pylori*. Findings in our study and findings reported elsewhere support this theory. The presence of seropositive siblings in the household was a risk factor for seroconversion in our study, and use of the special vessel that did not allow hands or other objects to contact the stored household water was protective. Carriage of *H. pylori* on hands and fingernails has been demonstrated in similar environments [37], and the protective effect of a narrow-mouthed water vessel has been shown with other enteric infections [20, 38].

A limitation of this study was that implementation of the safe water intervention occurred in the context of a social marketing study, so distribution of the special vessel was not random, either by village (because initially only households from intervention villages were able to obtain special vessels) or by household (because villagers voluntarily participated in the vessel-for-work program). Therefore, it is possible that some unidentified characteristics of households choosing to use the special vessel varied from households that did not. However, we did observe that residence in a former intervention or control village was not a risk factor for seroconversion. In addition, as shown in figure 2, no household in any village had the complete benefit of using the special vessel for the entire 15-month intervention period; this factor would decrease our ability to detect a real difference in seroconversion rates and suggests that the protective effect of the water vessel identified in this study could be a conservative estimate of the true effect. A prospective, randomized study is underway in another area of rural Bolivia to specifically address the efficacy of the special vessel for preventing *H. pylori* seroconversion [39].

This study concentrated on young children, because this is the age when seroconversion occurs in developing countries. At least some studies have suggested that younger children, because they have shorter intestinal transit times, are more likely to shed *H. pylori* in their feces and thus spread infection...
than are older persons [40]. The exact role that older infected persons might play in the infection of the young children in our cohort was not explored in this study. Future studies of the exact role of infected siblings and other older family members could pinpoint particular risk factors. This study was designed to identify possible risk factors for *H. pylori* seroconversion among a population at highest risk, among which households shared many characteristics that have been associated with high *H. pylori* seroprevalence, including extremely low socioeconomic status, lack of running water, overcrowding in the home, and poor diet. Therefore, we could not assess the role of these characteristics in seroconversion.

*H. pylori* infection is the most common bacterial infection worldwide. In rural Bolivian villages, most children are infected at a young age and probably remain infected for life; a proportion will ultimately develop chronic gastritis and gastric cancer. Although antimicrobial treatment is available, routine triple-agent therapy is beyond the financial reach of most developing nations, and its long-term effectiveness is unclear in settings of endemicity in which reinfection rates would be expected to be high. Vaccination against infection, proposed as a possible solution [41], is not yet available. Identifying risk factors for infection and routes of transmission remains a key to developing practical, effective prevention strategies. In this study, we have identified an age group with the highest seroconversion rates that could be a key target population for prevention efforts. We also have identified a potential prevention strategy, warranting more formal evaluation of the use of a narrow-mouthed water vessel with a lid and a spigot as an inexpensive method to prevent *H. pylori* transmission in households where drinking water is stored.

**Acknowledgments**

We thank the personnel of the National Center for Tropical Diseases and the Child and Community Health Project (Santa Cruz, Bolivia), whose invaluable assistance in the field study portion of this study made these findings possible; Cheryl Bopp (Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention) for assistance in our preparations; Joe Nalepa (Ortho Pharmaceuticals, Johnson & Johnson) and Rosemary Gaddy (Mead Johnson Nutritional) for donation of iron-fortified vitamins distributed to study participants; the health promoters of the study communities for their indispensable assistance in organizing the work in the villages; and the village residents for their warm welcome and collaborative spirit.

**References**


