PROSPECTIVE STUDY OF THE INCIDENCE OF DIARRHEAL DISEASE AND HELICOBACTER PYLORI INFECTION AMONG CHILDREN IN AN ORPHANAGE IN THAILAND

DANIEL W.ISENBARGER, LADAPORN BODHIDATTA, CHARLES W. HOGE, WARAWADEE NIRDNOY, CHITTIMA PITARANGSI, UNCHALEE UMPAWASIRI, AND PETER ECHEVERRIA

Department of Bacteriology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Department of Psychiatry, Walter Reed Army Medical Center, Washington, District of Columbia; Rangsit Babies Home, Pathumthani, Thailand

Abstract. To evaluate the hypothesis that gastric infection with Helicobacter pylori increases risk for diarrheal disease in children, we conducted a yearlong prospective study among 160 orphanage children < 5 years of age in Nonthaburi, Thailand. Serum samples collected at six-month intervals were examined by ELISA for antibodies to H. pylori, and children were followed daily for the development of diarrhea. Seven percent of children were seropositive on enrollment, 59% were seronegative, and 34% were indeterminate. Among the seronegative children, seroconversion occurred at a rate of 7% per six months. Forty-six percent of children developed 214 total episodes of diarrhea. By age group, children < 18 months, 18–24 months and > 24 months of age experienced 2.6, 1.1, and 0.2 mean diarrhea episodes per six months. The incidence of diarrhea was not significantly different between children by H. pylori serostatus. We conclude that H. pylori infection was not associated with an increased risk of diarrheal disease.

In 1983, Warren and Marshall identified Helicobacter pylori in gastric biopsies from patients in Australia.1 This organism has subsequently been shown to play an important role in gastritis and peptic ulcer disease in industrialized and developing countries worldwide.2,3 Acute infection is associated with hypochlorhydria, which persists for several months after exposure.3,5 In addition, there is strong evidence that chronic H. pylori infection is associated with atrophic gastritis and gastric carcinoma.3,6

Antibodies to H. pylori are more prevalent and are acquired at a younger age among people in developing countries than among people living in industrialized countries.7,8 In a recent study in rural and urban communities in Thailand, >50% of children had serologic evidence of infection by eight years of age.7 At an orphanage in Bangkok, Thailand, where enteric infections were hyperendemic, 74% of the children between one and four years of age were seropositive for H. pylori.9 The high prevalence of H. pylori among children in developing countries and the association with hypochlorhydria have suggested that this infection may predispose to acute or persistent diarrheal disease.10 In a retrospective study from the Gambia, infants with chronic diarrhea and malnutrition were significantly more likely to have serologic evidence of H. pylori infection compared to age-matched healthy controls and malnourished children without diarrhea.11 Additionally, in a retrospective study from Bangladesh, the odds of H. pylori seropositivity among subjects with severe cholera were significantly higher than in age-matched controls.12 Prospective studies correlating the incidence of diarrhea with H. pylori infection have not been published. To determine if H. pylori infection increases the risk of diarrheal disease, we conducted a prospective study of the correlation between this infection and diarrheal disease in an orphanage in Nonthaburi, Thailand.

MATERIALS AND METHODS

This study was conducted in the Pakkred Babies Home in Nonthaburi, Thailand from November 1990 to November 1991. Approval was obtained prior to beginning the study from the Scientific Review Board and the Human Use Review Board at the Walter Reed Army Institute of Research. A review of human use was also undertaken and approved by the Thai Ministry of Health. Informed consent for the children to participate in the study was obtained from the Director of the Pakkred Babies Home.

Overall, 166 children six months to five years of age were enrolled at the beginning and another 29 children were enrolled after six months. None of the children had acquired immunodeficiency virus infection. Serum samples were collected from children at enrollment and at six-month intervals. Sera were tested in triplicate for antibodies to H. pylori on the same day and in the same plate, using a recently described ELISA.7

Initially, sera were analyzed considering a test positive when the optical density (OD) value was ≥ the mean plus three standard deviations for the OD values of sera collected from 56 Thai children 6–23 months of age who did not live in the orphanage.7 This cut-off point has been shown to have a sensitivity and specificity of 98% and 76%, respectively, when tested in Thai adults.7 Given the relatively low specificity of the test, we were concerned about associated low positive predictive values that would result in nondifferential misclassification of serostatus and bias towards the null. The purpose of this study was to compare diarrhea incidence between H. pylori-infected and noninfected subjects; thus, it was essential to isolate those groups adequately for comparison. In this regard, ELISA criteria were redefined to include three categories: positive, negative, and indeterminate. These criteria maintained the cut-off point for a negative test result, but that for a positive test result was increased to a point that improved the specificity to 97%, with a resultant decrease in sensitivity to 56%. Test results between these values were considered to be indeterminate, and it was accepted that this would be a relatively large group given the revised test specifications. Based on revised criteria, a seroconversion was defined as one of the following: 1) a change in OD from negative to positive; 2) a change from negative to indeterminate with a ≥ four-fold increase in OD; or 3) a change from indeterminate to positive with a ≥ four-fold increase in OD; or 4) a change from positive to negative with a ≥ four-fold decrease in OD; or 5) a change from indeterminate to negative with a ≥ four-fold decrease in OD.
Table 1
Incidence density of diarrheal disease in orphanage children in Thailand, by age and *Helicobacter pylori* serostatus

<table>
<thead>
<tr>
<th>Age</th>
<th>H. pylori Serostatus</th>
<th>Total no. of child-months observation</th>
<th>Number of diarrheal episodes (incidence/child-month) overall and by pathogen*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Campylobacter</td>
</tr>
<tr>
<td>&lt;18 months</td>
<td>Positive</td>
<td>12</td>
<td>4 (0.33)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>222</td>
<td>98 (0.44)</td>
</tr>
<tr>
<td></td>
<td>Equivocal</td>
<td>61</td>
<td>23 (0.38)</td>
</tr>
<tr>
<td></td>
<td>Conversion</td>
<td>22</td>
<td>12 (0.55)</td>
</tr>
<tr>
<td>18–24 months</td>
<td>Positive</td>
<td>24</td>
<td>3 (0.13)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>18</td>
<td>20 (0.17)</td>
</tr>
<tr>
<td></td>
<td>Equivocal</td>
<td>68</td>
<td>14 (0.21)</td>
</tr>
<tr>
<td></td>
<td>Conversion</td>
<td>15</td>
<td>4 (0.27)</td>
</tr>
<tr>
<td>&gt;24 months</td>
<td>Positive</td>
<td>96</td>
<td>2 (0.02)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>410</td>
<td>20 (0.05)</td>
</tr>
<tr>
<td></td>
<td>Equivocal</td>
<td>393</td>
<td>14 (0.04)</td>
</tr>
<tr>
<td></td>
<td>Conversion</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>&lt;18 months</td>
<td>317</td>
<td>137 (0.43)†</td>
</tr>
<tr>
<td>Total</td>
<td>18–24 months</td>
<td>225</td>
<td>41 (0.18)†</td>
</tr>
<tr>
<td>Total</td>
<td>&gt;24 months</td>
<td>916</td>
<td>36 (0.04)</td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td>1,458</td>
<td>214</td>
</tr>
</tbody>
</table>

* Sum of pathogen-specific episodes may be greater than total episodes due to mixed infections. ETEC = enterotoxigenic *Escherichia coli*.
† P < 0.05 compared with the next age category.

Increase. No specific antimicrobial treatment for *H. pylori* was provided during the study. No attempt was made to isolate *H. pylori* from the stool.

Children were examined daily for diarrhea throughout the year. Diarrhea was defined as three or more unformed stools associated with at least one symptom of enteric infection (e.g., fever, nausea, vomiting, or abdominal colic). A new diarrheal episode was defined as one that began at least three days after the completion of a previous episode. Children with diarrhea were treated according to the institute’s protocol and severe cases were referred to a hospital for further investigation and management. Children who could not be followed daily for at least six months were excluded from the study.

Figure 1. Diarrheal incidence by age and *Helicobacter pylori* serostatus. seroneg = seronegative; seropos = seropositive; indeterm = indeterminate; mos = months.
Stool specimens collected from children with diarrhea were examined for enteric pathogens as previously described. Briefly, stool samples were cultured for Escherichia coli, Salmonella, Shigella, Aeromonas, Plesiomonas, and Vibrio species. Campylobacter was isolated using a membrane filter method on blood agar before and after enrichment. Ten E. coli colonies per patient were tested for production of heat-labile and heat-stable enterotoxin. Enteroinvasive E. coli (EIEC) and Enteropathogenic E. coli (EPEC) were identified using radiolabeled probes. Rotavirus and adenoviruses were identified with monoclonal antibody assays. Stools were examined microscopically for Cryptosporidium and intestinal protozoa.

Analysis of diarrhea incidence was performed for each of the two six-month study intervals independently by age groups of 6–12 months, 13–18 months, 19–24 months, 25–30 months, 31–36 months, and >36 months. Incidence of diarrheal disease was calculated per child-month and per child-six months of observation. To determine the association of diarrheal disease with H. pylori infection, the incidence and etiology of diarrheal disease were compared among the four groups: 1) seropositive throughout; 2) seronegative throughout; 3) indeterminate throughout; and 4) seroconversion during the interval. Statistical analysis was performed using the asymptotic test for person-time data and the Wilcoxon two-sample test for continuous nonparametric variables.

RESULTS

Of 195 children enrolled in this study, 35 were followed for less than six months due to adoptions and were excluded from analysis. Of 160 children included in the study, 83 were followed for two six-month periods and had three serum specimens collected, and 77 were in the study for one six-month period (either the first or second period) and had two serum specimens collected. There were a total of 1,458 child-months (243 child-six-months) of observation. The mean age of the children at enrollment was 29 months (range = 6–60 months) and 63% were male. Excluded children were similar to included children in terms of mean age (26 months versus 29 months), gender (72% male versus 63% male), and baseline H. pylori serostatus (0.089 OD units versus 0.091 OD units).

Helicobacter pylori serostatus. Based on initial ELISA test criteria, among 74 children < two years of age, 13 (18%) were seropositive for H. pylori infection at the time of enrollment, compared with 35 (41%) of 86 children ≥ two years of age (P = 0.003). Given these prevalences and the ELISA test characteristics of 76% specificity and 98% sensitivity, the positive predictive values of the test for children less than or greater than two years of age were 47% and 74%, respectively. In other words, less than half of those < two years of age with a positive ELISA result were truly positive. Using these criteria, no association of H. pylori serostatus with diarrhea incidence was noted in either age group; however, assessment of diarrhea incidence between serostatus groups could be severely biased towards the null due to nondifferential misclassification (mixing of populations). Therefore, revision of the test criteria was essential to adequately isolate truly H. pylori positive and negative groups for analysis of diarrhea rates between the two. Based on the revised criteria, two (3%) of children < two years of age were seropositive at enrollment, compared with nine (11%) of children ≥ two years of age (P = 0.02). Among 95 seronegative children followed for 134 child-six months, seroconversion occurred in nine, giving a seroconversion rate of 7% per six months. Of the nine seroconversions, six involved a ≥ four-fold increase from negative to indeterminate serostatus and three involved a change from negative to positive, also with a ≥ four-fold increase in OD. No seroreversions occurred.

Diarrhea incidence. The relationship of age and H. pylori infection with the number of diarrhea episodes and the most common enteric pathogens identified is shown in Table 1 and Figure 1. Overall, 214 episodes occurred during 1,458 child-months (243 child-six months) of follow-up. No significant differences were noted between results of the first and the second six-month study periods; thus, the results were pooled. Three distinct diarrhea risk groups were identified by age. Children < 18 months of age had a mean 2.6 diarrhea episodes per six months, compared with 1.1 episodes for children 18–24 months of age (relative risk [RR] = 2.4, P < 0.0001) compared with 0.2 episodes for children > 24 months of age (RR = 4.7, P < 0.0001). These rates are comparable to the average in other developing countries of 2.6 episodes per year in children < five years of age and five episodes per year in those 6–11 months of age.14

The overall incidence of diarrhea was not significantly different between children who remained seropositive, remained seronegative, remained indeterminate, or acquired serologic evidence of H. pylori infection during the period of observation (Table 1 and Figure 1). Nevertheless, certain patterns emerged. Compared with seronegative subjects, diarrhea rates in the seropositive group were lower across age categories, and rates in the seroconversion group were highest in both younger age groups. The diarrhea rate ratios comparing seropositive to negative individuals were 0.75 (95% confidence interval [CI] = 0.27–2.09), 0.76 (95% CI = 0.26–2.23), and 0.4 (95% CI = 0.09–1.83) among children < 18 months, 18–24 months, and > 24 months old, respectively. The summary rate ratio comparing seropositive to seronegative children across age strata was 0.65 (95% CI = 0.33–1.27). The median duration of diarrhea was 3.0 (range = 1–17) days for children who remained seropositive and 4.0 (1–18) days for those who remained seronegative.

Enteric pathogens were identified in 135 (63%) of 214 specimens. The most common enteric pathogens isolated were Campylobacter spp., enterotoxigenic E. coli (ETEC), Shigella spp., nontyphoidal Salmonella, and rotavirus. Mixed infections were present in 34 specimens (16%), consistent with prior observations in Thailand.2 No significant differences were detected in the rates of isolation of specific enteric pathogens and the presence or development of H. pylori antibodies during the surveillance period (Table 1).

To summarize, despite clear differences in rates of diarrhea between age groups, no association with H. pylori infection was identified either within age groups or in summary. This was true with respect to overall diarrhea incidence and when broken down by etiologic organism.
DISCUSSION

This study is the first to prospectively evaluate the association of *H. pylori* infection with diarrhea incidence. We documented a high incidence of *H. pylori* seroconversion (7% per six months) among young children residing in an orphanage in Bangkok. Neither acute nor chronic *H. pylori* infection was associated with significantly increased rates of diarrheal disease.

Studies of *H. pylori* incidence in children are absent from the literature; however, some investigators have attempted to infer incidence from seroprevalence data based on the assumption that *H. pylori* infection, once acquired, tends to persist to old age and that seropositivity persists during infection and wanes with cure. In Western countries, seroprevalence increases steadily with age to about 50% by age 50. Thus, an annual incidence of about 1% is inferred. In lesser-developed countries, seroprevalence is 60–80% by age 20, implying annual incidence of 3–4%. The rates of seroconversion documented in the present study represent an annualized rate of 13–14%, suggesting hyperendemic transmission in this orphanage setting.

Diarrhea incidence, on the other hand, was roughly comparable with worldwide estimates of diarrheal disease in lesser-developed countries. Bern and others, in their classic systematic review of the magnitude of the global problem of diarrheal disease, estimated a global incidence of diarrhea in children < five years of age of 2.6 episodes per child per year (five episodes per child per year among infants 6–11 months old). Asia-specific rates were similar overall in this study.

The issue of gastric hypoacidity in acute *H. pylori* infection is well established and appears to persist for several months, though gastric pH was not specifically measured as part of this study. Given the absence of a significant association of diarrhea incidence with *H. pylori* seropositivity, it may be that hypochlorhydria does not last long enough to impact on diarrhea risk long-term. Interestingly, though not statistically significant, diarrhea rates were generally higher in seroconverters. The purpose of the present study was to evaluate the diarrhea risk associated with chronic rather than acute *H. pylori* infection. If seroconverters are interpreted as acute infections, adequate follow up was achieved in our study to detect moderate to strong associations with diarrhea incidence, though mild associations may have been missed. Given the approximate four-month period of gastric hypoacidity following acute *H. pylori* infection, it is this specific population that is most likely susceptible to diarrheal disease. Alternatively, seroconversion to *H. pylori* may also be a marker for poor hygiene with concomitant diarrhea risk.

Our results differ in some respects from those of prior studies evaluating the association of diarrheal risk with *H. pylori* infection. Clemens and others, in a nested case-control study conducted in Bangladesh, found significantly elevated risk (RR = 1.61, 95% CI = 1.07–2.42) of *H. pylori* infection only in the subgroup of patients with severe disease that lacked natural serum vibriocidal antibodies. No overall association was found, and there was no association with severe disease found in those with these antibodies. Thailand has little endemic cholera and our study detected only a single case of diarrhea due to *Vibrio*, precluding meaningful comparisons. Sullivan and others, in a case control seroprevalence study of *H. pylori* infection in children with chronic diarrhea and malnutrition in the Gambia, found significantly increased risk (odds ratio = 2.04, P < 0.01) of *H. pylori* seropositivity in cases relative to controls with malnutrition and to healthy age-matched controls. Gastric pH was not measured in this study, but it was postulated that *H. pylori*-induced hypochlorhydria might result in bacterial overgrowth of the upper intestine with associated small intestinal dysfunction. Additionally, it may be that the combination of hypochlorhydria and immunocompromise due to malnutrition is necessary to increase susceptibility to chronic diarrheal disease, or that a common factor may increase susceptibility to both *H. pylori* infection and chronic diarrhea etiologies. No control group with chronic diarrhea, but without malnutrition, was included, which may have helped answer these issues. Nevertheless, in the present study, too few cases of chronic diarrhea occurred (five cases ≥ 14 days duration) for reasonable analysis. The populations comprising those with acute diarrhea without malnutrition, and those with chronic diarrhea and malnutrition differ in many ways, not the least of which are the likely state of immunity and the likely etiologic agent(s) causing diarrhea.

Studies finding absence of an association invariably must address the issue of adequate sample size. The issue is a difficult one in this study. Generally, these calculations use outcomes that are dichotomous, and cumulative incidence type of data. Incidence density data using person-time as the denominator, and disease outcomes that can occur more than once in a subject, do not fit this mold. If one uses child-month as the denominator and episodes of diarrhea as the numerator, however, then the numerator in this study is less than one and conforms to a power calculation. Overall, our study had 95% power to detect a relative risk of 2.0 between seropositive and negative individuals. When broken down by age groups, power is 85–90% for those < 18 months old to detect similar differences between seropositive and negative individuals, and > 95% between seroconverters and seronegative individuals. For those 18–24 months of age, power is 51% when comparing seropositive to negative individuals, and decreases to less 50% in the oldest age group, given the very low incidence of diarrhea. Based on these calculations, the greatest confidence can be placed in the results of the youngest age group at greatest risk for diarrhea, with progressively less confidence in results from the older children. However, the relative incidence of diarrheal disease in the older groups across *H. pylori* serostatus categories was generally consistent with that seen in the younger group, suggesting that findings in the older children are reliable as well.

We conclude that chronic *H. pylori* infection and hyperendemic transmission in this orphanage in Nonthaburi was not associated with increased risk of diarrheal disease.

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Authors’ addresses: Daniel W. Isenbarger, Ladaporn Bodhidatta, Warawadee Nirdnoy, Chittima Pitarangsi, and Peter Echeverria, Armed Forces Research Institute of Medical Sciences, Department of Bacteriology, 315/6 Rajawithi Road, Bangkok, 10400, Thailand. Charles W. Hoge, Department of Psychiatry, Walter Reed Army Medical Center, Washington, DC 20307-5000. Unchalee Umpawsiri: Rangsit Babies Home, 2/40 Moo 2, Rangsit Nakorn Nayok Road, Pathumthani 12110, Thailand.

Reprint requests: Daniel W. Isenbarger, Department of Bacteriology, USAMC-AFRIMS, APO AP 96546.

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