# Seroprevalence of *Helicobacter pylori* among children in Sana'a, Yemen

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**BACKGROUND:** *Helicobacter pylori* infection is primarily acquired in early childhood. Its transmission routes are debated. The aims of this study were to determine the seroprevalence of anti-*H. pylori* immuno-globulin G (IgG) in Yemeni children under 10 years of age, the potential risk factors for contracting *H. pylori* infection and co-infection of *H. pylori* lori with intestinal parasites.

**METHODS:** Enzyme-labeled immunosorbent assay was used to determine the *H. pylori* prevalence rate among 572 healthy volunteers aged less than 10 years. Formalin ether concentration methods were used to test the prevalence of intestinal parasites (intestinal roundworms and tapeworms). In addition, we interviewed participants regarding potential risk factors for contracting *H. pylori* infection.

**RESULTS:** The seroprevalence of *H. pylori* antibodies was 9%. The prevalence according to age varied from 0% in children under 2 years to 12.5% in age group 9-10 years. There was a correlation between the amounts of positive antibodies and increasing age. The prevalence rate of *H. pylori* antibodies was also significantly associated with the practice of drinking water from reused plastic jerry cans, with poor mouth hygiene and with co-infection by intestinal parasites.

**CONCLUSION:** The prevalence of *H. pylori* antibodies in Yemen among children under 10 years of age is higher than that reported from other regions for the same age groups. Yemen shares some but not all potential risk factors for *H. pylori* infection with countries in which similar socioeconomic conditions are found. A possible way of eliminating *H. pylori* from the population would be via public health measures, i.e. preventing the reuse of plastic jerry cans, and improving sanitation and the standard of living.

Infection with *Helicobacter pylori* (*H. pylori*) is now claimed to be one of the most common bacterial infections that affect humans. The prevalence of current infection is estimated to be between 25% and 90% of the total world population.<sup>1-4</sup> *H. pylori* infection is associated with a greatly increased risk of duodenal and gastric ulceration.<sup>5-9</sup> In addition, several reports have demonstrated pre-existing *H. pylori* infections in 70-90% of cases of gastric cancer including gastric lymphoma.<sup>8-10</sup>

Vast differences exist in the sero-prevalence of *H. pylori* antibodies among different countries and age groups.<sup>3,4,11-14</sup> Two patterns of *H. pylori* infection can be identified. The first is characteristic of developing countries where a large proportion of children are infected in early life and almost all adults in different age groups have chronic *H. pylori* infection.<sup>3,4,11-14</sup> The second pattern is typical of developed From the University of Sana'a, Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Sana'a, Yemen

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countries, where the prevalence of *H. pylori* infections increases from ages 20 years onwards.<sup>3,4,11-14</sup>

The natural history of childhood H. pylori infection is inadequately understood. Moreover, rational approaches to the prevention and control of childhood H. pylori infection are critically needed, requiring characterization of the determinants for acquisition and persistence and disease outcomes following eradication. Infection with H. pylori usually occurs during childhood and lasts for a lifetime while the prevalence curve rises with increasing age.<sup>15-19</sup> The exact mode of H. pylori transmission is not completely understood.<sup>20,21</sup> However, high prevalence rates of H. pylori infection are associated with unhygienic sources of drinking water, low socioeconomic status, poor housing hygiene, poor personal hygiene and overcrowding.<sup>22,21,23</sup> In Yemen, no previous studies are known to have been conducted on the seroprevalence of H. pylori, either among children or adults. On a wider level, there is a continuing need for numerical data on disease distribution and its determinants in order to identify priorities for the health services.

This study was undertaken to determine the seroprevalence of *H. pylori* IgG antibodies among children aged 10 and younger in Sana'a city and to compare this prevalence with potential risk factors of contracting *H. pylori*.

### Methods

The study sample was selected by stratified sampling according to age, during the summer of 2002. A two-stage sampling method was used. Initial sample units of the child population were selected from nurseries, antenatal care clinics and schools. A systematic random sampling of every 5th child on the list was then performed.

Based on an expected *H. pylori* IgG antibody prevalence of 5% and a worst acceptable value of 1%, the minimum sample size was determined to be 449 randomly selected subjects. Given a total estimated population of 500 000 children less than 10 years of age in Sana'a governorate, this sample gave us a confidence level of 99.9%. The selected sample size was increased to 572 individuals to ensure that the sample was representative of the whole of Sana'a Governorate, to facilitate stratification by ecological settings, and to reduce the rate of homogeneity. Information on volunteers was supplemented with data obtained through direct communication with the study subjects or their parents by structured questionnaire. The pre-designed questionnaire included demographic data questions on socio-economic status as well as questions on potential risk factors of *H. pylori* infections. Grades were allotted according to the information supplied by the volunteers on socioeconomic and personal conditions, such as mouth hygiene, housing hygiene, presence of houseflies, and number of residents in the house. A score of one was given for each positive answer and zero for each negative answer; the scores were then totaled to give an indication of low, moderate or high risk of exposure. All the subjects provided the above information in a single interview. These data were analyzed using the Mantel Haensezel chi-square and Fisher's exact test (1-tailed) for small cell numbers. A *P* value less than 0.05 was considered significant.

Venous or capillary blood specimens and stool specimens were collected. The sera were separated and stored at -20°C until examination was performed. Stools were examined by microcopy for parasitic infections (intestinal roundworms and tapeworms) using the formalin ether concentration method. In addition, all serum samples were screened using an enzyme linked immunosorbent assay (ELISA) for *H. pylori* IgG antibodies, which was purchased in the form of commercial kits (Biokit, SA. 08186 Lliçà d'Amunt, Barcelona, Spain).

#### Results

Five hundred seventy-two volunteers completed the study questionnaire, and then donated blood and stools, which were tested for H. pylori specific IgG and intestinal parasites. The age of subjects ranged from 6 months to 10 years. All volunteers were Yemenis living in Sana'a governorate. The socioeconomic status of the subjects' families was high (5%), moderate (63%) and low (32%). The main sources of drinking water were government-provided tap water (n=441), commercial filtered water supplied in reused plastic jerry-cans (n=99) and other sources (n=62). The seroprevalence of *H. pylori* antibodies was 9% (51 positive out of 572). The prevalence according to age varied from 0% in the age group 0-2 years to 12.5% in the age group 9-10 years (Table 1). There was a positive correlation between antibody and age: older children had a higher prevalence of H. pylori IgG antibodies. The study revealed relative risk factors of 2.66 associated with poor mouth hygiene, 2.8 associated with drinking water from reused plastic jerry-cans, and 2.9 with co-parasitic infections (Table 1). Other potential risk factors were not associated with an increased likelihood of contracting the infection.

| Sex and age group                   | Positive IgG |      | Relative risk | Confidence     | P value  |
|-------------------------------------|--------------|------|---------------|----------------|----------|
|                                     | No.          | %    |               | interval (95%) |          |
| Male (n=302)                        | 29           | 9.6  | 1.18          | 0.69-2         | 0.54     |
| Female (n=270)                      | 22           | 8.1  | 0.85          | 0.5-1.44       | 0.54     |
| Age (years)                         |              |      |               |                |          |
| <2 (n=18)                           | 0            | 0    | 0             | 0.0-2.85       | 0.39     |
| 3-4 (n=51)                          | 1            | 1.9  | 0.2           | 0.03-1.4       | 0.07     |
| 5-6 (n=79)                          | 5            | 5.6  | 0.68          | 0.28-1.65      | 0.38     |
| 7-8 (n=192)                         | 16           | 8.3  | 0.9           | 0.5-1.59       | 0.72     |
| 9-10 (n=232)                        | 29           | 12.5 | 1.93          | 1.4-3.28       | 0.013*   |
| Socioeconomic<br>status             |              |      |               |                |          |
| Low (n=182)                         | 21           | 11.5 | 1.5           | 0.88-2.5       | 0.13     |
| Hygeine                             |              |      |               |                |          |
| Poor personal<br>(n=185)            | 1            | 9.1  | 1.05          | 0.6-1.82       | 0.87     |
| Poor mouth<br>(n=222)               | 32           | 14.4 | 2.66          | 1.5-4.6        | 0.0002*  |
| Poor house<br>(n=250)               | 27           | 10.8 | 1.45          | 0.8-2.24       | 0.16     |
| Presence of house flies             |              |      |               |                |          |
| Heavy (n=95)                        | 13           | 13.6 | 1.72          | 0.95-3.1       | 0.07     |
| Moderate (n=109)                    | 6            | 5.5  | 0.57          | 0.25-1.29      | 0.16     |
| Light/absent<br>(n=368)             | 31           | 8.4  | 0.86          | 0.5-1.47       | 0.57     |
| Overcrowding<br>(n=231)             | 22           | 9.5  | 1.12          | 0.6-1.9        | 0.67     |
| Usual drinking<br>water source      |              |      |               |                |          |
| Government tap<br>(n=411)           | 27           | 6.5  | 0.4           | 0.26-0.74      | 0.001*   |
| Reused plastic<br>jerry cans (n=99) | 19           | 19   | 2.8           | 1.68-4.8       | 0.00008* |
| Other sources<br>(n=62)             | 6            | 9.7  | 1.1           | 0.49-2.7       | 0.82     |
| Positive parasitic infection (n=78) | 16           | 20   | 2.9           | 1.7-4.97       | 0.0008   |

 Table 1. Potential risk factors for *H.pylori* infection among children younger than 10 years of age in Sana'a, Yemen

\* Statistically significant. Overcrowding=more than 4 persons/room; parasitic infections=intestinal roundworms and tapeworms.

## Discussion

Serological surveys of health population groups have been of major importance in mapping the epidemiology of *H. pylori* and documenting differences in its behavior of among specific age groups in various parts of the world.<sup>24-29</sup> These surveys provide information on age, exposure time, potential sources of infection, and possible routes of transmission, which might help in predicting the rate of clinical out comes of this slow-acting infection and identifying the age of acquisition. The main clinical outcomes of *H. pylori* infections are chronic gastritis and peptic ulcers, and infection also plays a major role in gastric cancer and gastric lymphoma.<sup>5-9</sup>

Great variation exists in the prevalence of H. pylori infection among different countries and age groups.<sup>1-4,30-33</sup> Our seroprevalence survey was carried out among children in whom infection with H. pylori usually occurs early and might last for a lifetime.18 The seroprevalence of H. pylori antibodies among children in our study showed a meaningful correlation between antibodies and age where older children had a higher prevalence of *H. pylori* IgG antibodies, i.e. the highest prevalence was among the 9-10 years age group (12.5%) (P=0.013). On the other hand, prevalence rates in age groups 3 to 4 years or younger were very low or zero. This indicates that the prevalence curve of acquisition of H. pylori infections rises with age, and more rapidly after the age of 5 years. In addition, this indicates that children are more at risk of infection with H. pylori after the cessation of breast-feeding. The high rate of acquisition in this age group also might be due to outdoor activities and exposure to potential external sources.

Our data also showed a higher rate of prevalence in Yemen (9%) compared with that reported for other developing countries where the rate of acquisition of H. pylori infection in children is between 2% and 5%.<sup>3,13</sup> The prevalence in Yemen is also higher than that found in developed countries where the rate ranges from 0.5% to 1%.4 This high prevalence rate among children in Yemen might be explained by three factors. The first factor could be race, since previous epidemiological studies found that Asians (including Arabs) Latin Americans and Africans are more susceptible to H. pylori infections than Caucasians.<sup>19,35,36</sup> The second factor might be genetic and related to the type of blood group; several epidemiological studies showed a high prevalence of H. pylori infections among members of O blood group.<sup>26,36</sup> In Yemen, the predominant blood group is the O type.<sup>37</sup> Finally, the third factor could

be the high level of environmental contamination with *H. pylori* and the custom of sharing food and drink utensils with others in Yemen.

Our study suggests that some, but not all, of the predisposition for H. pylori infection identified in other societies is also important in Yemen. Little is known about the environmental source of H. pylori. However, a high prevalence of *H. pylori* infection was found to be associated with drinking water contaminated by human excreta<sup>22</sup> and viable *H. pylori* was detected in drinking water.<sup>22</sup> In our study, drinking from reused plastic jerry cans was the main significant risk factor, but there is no significant association with drinking government tap water or with other sources of drinking water, even with open sources. This significant risk factor of drinking from reused plastic jerry cans can be explained by the high level of contamination of these cans during circulation by contact with infected vomit, saliva and other excreta of users, in addition to the improper sterilization of these cans before refilling or in some cases the total absence of sterilization. No significant difference in seroprevalence between genders was noted in our study and this was similar what is reported elsewhere.<sup>20</sup>

Domestic houseflies may be an extra-gastric source of *H. pylori* infection. Our study revealed a relative risk factor of 1.72 (95% CI, 0.95-3.1) if a heavy population of domestic houseflies was present in the residence. This result might be explained by the ability of *H. pylori* to colonize the midgut of houseflies, which has been confirmed by several studies.<sup>21,38</sup> In addition, these studies related the colonization of *H. pylori* in the midgut of *Musca domestica* to the low pH of the midgut, which serves as an ecological niche for *H. pylori*. There was a correlation between the amount of positive antibodies of *H. pylori* and infections with intestinal parasites, and this finding suggested that both infections share in part the same route of transmission, which might be the fecal oral-route.<sup>34</sup>

Several studies have shown variations in the prevalence rate of *H. pylori* antibodies according to socioeconomic status, overcrowding, personal and housing hygiene.<sup>4,13,15</sup> However, our data showed that *H. pylori* IgG antibody prevalence rates did not correlate significantly with low socioeconomic conditions, poor personal hygiene, and poor housing hygiene or with overcrowded accommodation. Only in the case of poor mouth hygiene did a highly significant association occur.

This study identified specific factors that may explain variations in *H. pylori* prevalence among children in Yemen. It also highlighted the early acquisition of the infection in Yemen and the high rate of co-infection with parasitic infections. A possible way of eliminating *H. pylori* from the population would be via public health measures, i.e. preventing the reuse of plastic jerry cans, and improving sanitation and the standard of living.

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