Prevalence of Cytotoxin-associated gene A (CagA) positive *Helicobacter pylori* strains in asymptomatic *H. pylori*-infected children attending Kenyatta National Hospital, Nairobi and correlation with risk factors associated with infection acquisition

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ABSTRACT

**Background:** *Helicobacter pylori* bacteria colonize the gastric mucosa of 20-80% of humans worldwide. Approximately 60-70% of *H. pylori* strains possess the Cytotoxin-associated gene A (CagA gene) and express the CagA protein, an oncoprotein and a highly immunogenic virulence factor that has been linked to gastric disease.

**Objective:** To determine the prevalence of CagA positive *H. pylori* among asymptomatic children attending Kenyatta National Hospital (KNH), Nairobi.

**Design:** Cross-sectional descriptive study at KNH paediatric outpatient clinic and paediatric wards and at the immunology laboratory, University of Nairobi.

**Subjects:** Children aged 2-13 years attending KNH, presenting with non-gastro-intestinal tract conditions.

**Methods:** Socio-demographic information was collected by direct interview of the participants’ parents/guardians (respondents) and entered into a study questionnaire. Serum samples from the subjects were tested by ELISA for the anti-*H. pylori* IgG antibody. Those that tested positive were tested for anti-CagA IgG antibody.

**Results:** A total of 175 children with a mean age of 7.5 years were enrolled, 57.3% being males. The overall prevalence of *H. pylori* was 50.3%. CagA positive *H. pylori* prevalence among those who tested positive for *H. pylori* was 64.8%. There was significant positive correlation of *H. pylori* seropositivity with age (p<0.001), education level of the parent/guardian (p = 0.030) and human waste disposal practices (p=0.031). Participants in the rural areas had a significantly higher seroprevalence of *H. pylori* than the urban and semi-urban dwellers (p=0.043). Males had a higher prevalence (53.5%) compared to females (45.9%), however this was not statistically significant (p=0.326). There was no significant correlation between socio-demographic characteristics of the study participants and CagA status.

**Conclusion:** Prevalence of *H. pylori* infection in the study population was 50.3% and 64.8% of the infections were from CagA positive *H. pylori* strains. Seropositivity increases with age and low social economic factors play a key role in contributing to the risk of *H. pylori* infection.

INTRODUCTION

*Helicobacter pylori* is a Gram negative, spiral bacterium first isolated in inflamed gastric tissues in 1982 by Warren and Marshal. Diagnostic approaches to infection include direct identification of the bacterium by means of microscopy and/or culture from biopsy specimens of gastric mucosa and, indirectly, by serology and the urea breathe test. Serodiagnostic evaluation of *H. pylori* infection, though less sensitive, is most commonly used in epidemiological studies1. *H. pylori* particularly inhabit the gastric antrum and pylorus and cause a chronic low-level inflammation of the mucosal lining, and are strongly linked to the development of gastric and duodenal ulcers and gastric malignancies. It is one of the commonest causes of chronic bacterial infections in humans; at least 50% of the world’s population is infected by the bacterium2,3 and about 20% eventually develop severe disease. Infection is usually acquired in early childhood and is usually life-long unless eradicated2. Moreover, the bacterium has been associated with several extra gastric diseases4-6. *H. pylori* bacteria consists of a large diversity of strains with large genetic differences7-9. The genome of the 26,695 strain consists of a circular chromosome of 1,667,867 base pairs. A region at
the end of the sequence has been shown to contain a single contiguous 40kb-long cag Pathogenicity Island (PAI), a common gene sequence believed to be responsible for *H. pylori* pathogenesis and that is usually absent from *H. pylori* strains isolated from asymptomatic human carriers. The CagA gene codes for one of the major *H. pylori* virulence proteins, CagA protein. Bacterial strains that have the gene have been associated with an ability to cause ulcers. (The gene codes for a relatively long 1186 amino acid protein, CagA, an oncprotein incriminated for the associated pathology.

Actual infection rates vary from country to country, being highest in the developing countries, most likely due to poor socio-economic factors. Person to person transmission by either oral-oral or feco-oral route is the most likely mode of transmission. Risk factors significantly associated with *H. pylori* infection include: lack of clean water supply, poor sanitation, overcrowding, low maternal education level and low socio-economic status. Study objectives: The objective of the study was to determine the prevalence of CagA positive *H. pylori* among asymptomatic children attending Kenyatta National Hospital. We specifically aimed to determine prevalence of *H. pylori* among the children and to determine prevalence of CagA positive strains in those infected with *H. pylori*. We also sought to correlate seropositivity with risk factors associated with infection including age, crowding, low education level, unemployment and low monthly income, living in rural areas, housing type, lack of clean water supply, poor sanitation, domestic animals and human waste disposal facilities as well as correlate CagA positivity with the same risk factors associated with *H. pylori* infection.

**MATERIALS AND METHODS**

**Study design:** This was a cross-sectional descriptive study conducted between October 2012 and May 2013.

**Study area:** The study was conducted at Kenyatta National Hospital (KNH), a tertiary, referral and teaching hospital in Nairobi, Kenya. Study participants were recruited from the paediatric outpatient clinic and paediatric wards at KNH. All blood specimens collected were processed and analyzed at the immunology laboratory, University of Nairobi.

**Study population:** Out-/in-patient children aged 2-13 years at KNH and presenting with non-gastro-intestinal disease.

**Selection criteria**

*Inclusion criteria:* Children aged 2-13 years on follow-up or admitted at KNH for non-GIT diseases and whose parents/guardians gave informed consent for their participation in the study were included in the study.

*Exclusion criteria:* Children on treatment for chronic gastrointestinal disorders, that is, patients presenting with chronic symptoms (over two weeks) including nausea, vomiting, abdominal pain/discomfort, indigestion, diarrhoea or diagnosed gastric diseases including chronic gastritis and peptic ulcer disease. Those previously treated for *H. pylori*-associated disorders and those whose parents/guardians declined to consent to participate in the study were excluded as well.

**Recruitment and screening**

This was done at the general Paediatric Out-Patient Clinic (POPC) and paediatric wards by the PI assisted by a trained research assistant (registered clinical officer). All patients who met the inclusion criteria were recruited into the study until the desired sample size was achieved. The study participants were further screened by direct interview of their parents/guardians using a screening questionnaire. Informed consent was sought from the parents/guardians and assent was sought from the older children.

**Data collection procedures**

Socio-demographic data was obtained by direct interview as well as review of the patient’s file and captured in a pre-designed structured study questionnaire. Venous whole blood was then collected for the laboratory tests.

**Anti-*H. pylori* IgG**

Frozen specimens were tested in batches. Before testing, frozen specimens were thawed on the bench or in a water bath at room temperature, and then inverted several times to ensure homogeneity before testing using human *Helicobacter pylori* IgG ELISA (Human Gesellschaft für Biochemica und Diagnostica mbH, Germany). The ELISA has a sensitivity and specificity of 94.3% and 98.9%, respectively and has shown no cross reactivity with RSV IgG, Adenovirus IgG and Yersinia IgG. Measurement of the absorbance of controls and specimens at 450nm wavelength using the automated HUMAREADER plate reader was promptly done.

**Anti-*H. pylori* CagA IgG**

This was performed sequentially on the anti-*H. pylori* positive samples using anti-*H. pylori* CagA ELISA (IgG) (EUROIMMUN Medizinische Labordiagnostika AG, Germany). The test uses the same principle and procedure as the anti-*H. pylori* IgG ELISA, has a sensitivity and specificity of 98% and 100%, respectively and has shown no cross reactivity. Absorbance of calibrators, controls and test samples were read at 450nm wavelength using the Humareader plate reader. The results were evaluated semi-quantitatively by calculating the ratio of the absorbance of the control or patient sample over the absorbance of the standard and interpreted accordingly.
Data management

Data analysis: Variables were categorized into independent and dependent variables. Independent variables included demographic characteristics; age, gender and socio-economic characteristics; number of children, educational level, marital status, employment, housing, child care, water source and treatment, pets and waste disposal. Dependent variables included laboratory characteristics – anti-\textit{H. pylori} IgG and anti-CagA IgG antibodies.

Socio-demographic data and test results were entered into MS Excel computer database. Where indicated, the data was grouped and then imported into SPSS (v.18) statistical software for analysis. Descriptive statistics on socio-demographic characteristics was presented using percentages and frequencies. Continuous data was presented using means and medians. Tables and graphs were used to display the results.

Pearson’s chi-square tests for independence were used to assess association between two nominal or categorical variables. The level of significance was set at 5% with p-values of ≤0.05 being considered statistically significant. Correlation analysis to assess for any linear association was done using Pearson correlation coefficient for the continuous variables, and considered significant at 95% confidence level.

RESULTS

Socio-demographic characteristics of the study population: A total of 175 subjects participated in the study out of which 57.3% were males. The age groups were evenly distributed among the study participants, 2-5 years = 61 (34.9%), 6 – 10 years = 60 (34.3%) and 11 – 13 years = 54 (30.9%). Seventy seven point nine percent of the study participants were under the care of their parents/legal guardians with 22.3% being under the care of house helps, siblings, aunts, uncles or grandparents. Sixty five percent lived in permanent houses and 66.9% were urban and semi-urban dwellers.

Seventy point nine percent used tap water as their source of water and 70.3% boiled their drinking water irrespective of the source. Thirty two point six percent reported keeping domestic animals as pets with 50.9% of them (29/57) mainly keeping cats. Thirty eight point three percent reported using individual toilets and only 1.1% disposed their human waste in the bush.

The average number of siblings in the family was three with 74.9% having not more than two siblings and 19.4% having three or more. Most of the study participants’ parents/guardians had post-primary education (65.7%) while 5.1% had no formal education. Eighty one point seven percent were from families in marriages, 45.1% of the parents/guardians were in formal employment with 48.1% (38/79) earning a maximum monthly income of Ksh.10, 000 (US$100). Twenty two point three percent of those in employment were the sole bread winners in the family.

Prevalence of \textit{H. pylori} in asymptomatic participants: Serum from 175 participants was analyzed and we found a \textit{H. pylori} seroprevalence of 50.3%. Prevalence increased with increasing age with a p-value of <0.001.

Association of key socio-demographic factors and prevalence of \textit{H. pylori}

<table>
<thead>
<tr>
<th>Socio-demographic characteristics</th>
<th>H. pylori</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Negative n (%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>2 – 5</td>
<td>42(68.3)</td>
</tr>
<tr>
<td>6 – 10</td>
<td>29(48.3)</td>
</tr>
<tr>
<td>11 – 13</td>
<td>16(29.6)</td>
</tr>
<tr>
<td>Education level</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>3(33.3)</td>
</tr>
<tr>
<td>Primary</td>
<td>18(35.3)</td>
</tr>
<tr>
<td>Secondary</td>
<td>44(54.3)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>22(64.7)</td>
</tr>
<tr>
<td>Human waste disposal</td>
<td></td>
</tr>
<tr>
<td>Community pit latrine</td>
<td>18(50.0)</td>
</tr>
<tr>
<td>Community toilet</td>
<td>13(34.2)</td>
</tr>
<tr>
<td>Individual toilet</td>
<td>42(62.7)</td>
</tr>
<tr>
<td>Individual pit latrine</td>
<td>14(43.8)</td>
</tr>
<tr>
<td>Other - Bush</td>
<td>0(0.0)</td>
</tr>
</tbody>
</table>
Prevalence of *H. pylori* with socio-demographic factors contributing to infection acquisition was tabulated. Table 1 shows significant positive correlation existed between age of the patient and *H. pylori* infection (p<0.001), as well as education level of parent/guardian and *H. pylori* infection (p = 0.030). Males had a higher seroprevalence (53.5%) compared with females, (45.9%), however this was not statistically significant (p=0.326). Seropositivity increased with increase in the number of children per household (p=0.130). Single parenthood, unemployment and low income level were associated with a higher prevalence; however, there was no statistical significance.

There was significant association between place of domicile of the study participants and human waste disposal facilities with *H. pylori* infection (p = 0.043 and 0.031, respectively). Children who lived in permanent houses as well as in homes where drinking water was treated were found to have a lower prevalence of *H. pylori*; however this was not statistically significant (p-values 0.227 and 0.108, respectively). There was no difference in prevalence between those who kept pets at home and those who did not.

**Prevalence of CagA in *H. pylori* positive participants:** Serum of 88 participants who tested positive for *H. pylori* antibodies was analyzed for CagA IgG antibodies and out of these, 64.8% tested positive. Figure 2 shows prevalence of CagA IgG Ab in different age groups. The highest prevalence (77.4%) was found in the 6-10 year age group while the least (47.4%) was in the 2-5 year age group. We however found that age was not a significant determinant of CagA positivity (p=0.094).

**Figure 1:** CagA IgG test results by age group, (n=88).

We cross tabulated CagA positivity with socio-demographic factors associated with *H. pylori* infection. As shown in Table 2, there was no association between age, gender, housing type and keeping of pets with CagA status (p-values 0.094, 0.654 and 0.760 respectively). Urban and semi-urban dwellers and those who treated their drinking water had a higher prevalence of CagA positive *H. pylori*. However, there was no statistical significance in these associations (p-values 0.222 and 0.370, respectively).

**Association of key socio-demographic determinants with CagA positivity**

**Table 2: Correlation between some socio-demographic characteristics and CagA positivity**

<table>
<thead>
<tr>
<th>Socio-demographic characteristics</th>
<th>CagA</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative n (%)</td>
<td>Positive n (%)</td>
<td>Total (n)</td>
<td>OR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 - 5</td>
<td>10 (52.6)</td>
<td>9 (47.4)</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 - 10</td>
<td>7 (22.6)</td>
<td>24 (77.4)</td>
<td>31</td>
<td>2.9</td>
<td>0.094</td>
</tr>
<tr>
<td>11 - 13</td>
<td>14 (36.8)</td>
<td>24 (63.2)</td>
<td>38</td>
<td>(0.9-9.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Housing type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permanent</td>
<td>18 (34.0)</td>
<td>35 (66.0)</td>
<td>53</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Semi-permanent</td>
<td>13 (37.1)</td>
<td>22 (62.9)</td>
<td>35</td>
<td>(0.4-2.1)</td>
<td>0.760</td>
</tr>
<tr>
<td><strong>Domicile</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>15 (42.9)</td>
<td>20 (57.1)</td>
<td>35</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Urban &amp; semi-urban</td>
<td>16 (30.2)</td>
<td>37 (69.8)</td>
<td>53</td>
<td>(0.2-1.4)</td>
<td>0.223</td>
</tr>
</tbody>
</table>

**DISCUSSION**

This study evaluated 175 subjects, most of who were males (57.7%). The age of the children ranged from two to thirteen years with a mean age of 7.5 years (±3.68). Several studies done in many developing countries have demonstrated a seroprevalence of *H. pylori* of 50-70%[3]. The overall *H. pylori* prevalence in the study population was 50.3%, which compares well with similar studies in Nairobi, Kenya[16] and Kampala, Uganda[17]. As observed in other studies, there was higher prevalence of *H. pylori* infection among males however the difference was not statistically significant (p=0.326)[17]. A similar finding was observed in So East Iran, (p<0.05)[18], however, there has been inference made from these findings.

Infection with *H. pylori* is usually acquired in early childhood and the prevalence increases with age. This study shows significant correlation (p<0.0) between age and *H. pylori* infection, children v
infected early in life with slightly less than a third, 31%, of children aged two to five years being seropositive. This compares with a study in South Germany and is due to increased exposure time to risk factors of infection as the child grows. Three quarters of the study population had up to three children in the family which is relatively a small family size. Seropositivity increased with increased family size; however, there was no significant correlation between the number of children per family and H. pylori prevalence. The increasing infection rates with increase in the family size as established in this study may be attributable to crowding within the family. Large families may also be economically strained, thus predisposing the children to risk of infections. Overcrowding in homes and schools has been established as a risk factor for infection.

The respondents were fairly educated, two thirds of whom had acquired post-primary education. There was significant association between the level of education and H. pylori prevalence (p=0.030), this being highest in children of respondents who had not attained formal education (67%) compared with 35% in children of those who had attained tertiary education. Langat et al. found significant correlation between family income and H. pylori infection. From this study, the population’s economic status is relatively average, other than education level, nearly half of the participants are in employment or in marriages where the spouses are in a position to provide basic needs, good hygienic standards, clean water and a clean environment to their children.

In comparison with the Ugandan study, 46.9% of those living in permanent houses were seropositive compared with 56.5% seropositivity in those living in semi-permanent houses (p=0.227). The type of housing has a bearing on the socio-economic status of the family and relates with availability of clean, safe water and human waste disposal facilities. Infection rates were significantly higher in the rural population than in the urban and semi-urban population, a similar finding made by Lindkvist et al. This may be attributed to lack of clean, safe water, poor hygienic standards and lack of proper human waste disposal facilities in the rural areas. This study however contrasts a similar study by Vivatvakin et al. in Thailand who found a higher prevalence in the urban areas compared to the rural areas. The finding was attributed to the local water supply. In Kenya, tap water treatment by local authorities has been in practice and this may explain the lower prevalence of H. pylori in the urban and semi-urban areas supplied with treated tap water.

Studies have established that person to person transmission of H. pylori by either oral-oral or feco-oral route is the most likely route, thus infection rates are likely to be high in areas which lack human waste disposal facilities or where individuals are sharing these facilities. Significant correlation between H. pylori infection and human waste disposal facilities explains the association between high prevalence and poor sanitation. However, since these children are schooling, confounders like sharing meals, latrines and toilets and drinking untreated water both in the neighborhood and schools may have impacted on the outcome of this study. There was a higher prevalence, 59.6%, in those respondents that drank untreated water regardless of its source. However this finding was not statistically significant. Although the principal reservoir for H. pylori infection appears to be humans, it has been isolated from domestic animals, especially cats and thus these could act as reservoirs of the bacteria. In this study there was no difference in prevalence between those who kept pets at home and those who did not.

About 50 – 70% of H. pylori strains carry the gene that codes for the CagA protein. In this study, 65% of those infected with H. pylori were infected with the CagA positive strains. These findings compare with the global prevalence, however, other studies in the Middle East and Asia found higher prevalence, suggestive of regional differences. The prevalence of CagA positive H. pylori was lowest in the 2–5 year age group (47.4%) and highest in the 6–10 year age group (77.4%). Though the differences were not statistically significant (p=0.094), the younger children had a much lower seropositivity. Jafarzadeh et al. observed that seroprevalence of CagA positive strains increased significantly with age. In this study, CagA positive H. pylori prevalence was only marginally higher in females (67.6% versus 63%, p=0.654). This contrasts with the Iranian study where it was observed males had a significantly higher prevalence. There was insignificant correlation between the prevalence of CagA positive H. pylori and socio-demographic characteristics of the study subjects.

CONCLUSION

Prevalence of H. pylori was 50.3% and among the positive patients, that of virulent strains was 64.8%. Seropositivity increases with age and low social economic factors play a key role in contributing to the risk of infection. There was no significant correlation between socio-demographic characteristics of the study participants and H. pylori CagA status.

RECOMMENDATION

H. pylori testing currently practiced in children should focus on the virulent strains only since half of the young population is infected by both virulent and non-virulent strains while only two thirds of infections are caused by the clinically significant virulent strains. Improvement of the socio-economic status of the population will reduce risk of infection acquisition.
REFERENCES