Mixed infection with cagA positive and cagA negative strains of Helicobacter pylori lowers disease burden in the Gambia

Ousman Secka  
*Medical Research Council Unit - Fajara*

Martin Antonio  
*Medical Research Council Unit - Fajara*

Douglas E. Berg  
*Washington University School of Medicine in St. Louis*

Mary Tapgun  
*Medical Research Council Unit - Fajara*

Christian Bottomley  
*London School of Hygiene and Tropical Medicine*

See next page for additional authors

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Introduction

*Helicobacter pylori* is a genetically diverse microaerophilic gram negative bacterial species that chronically infects the human gastric mucosa, often starting in infancy and lasting for life [1]. About 50% of the world’s adult population is colonized, with prevalences of over 80% in many developing countries including The Gambia [2–4]. Earlier reports indicated high prevalence of *H. pylori* infection, but a low frequency of *H. pylori*-associated disease in Africa [2,5,6], a phenomenon that was called the “African enigma” [2]. DNA sequencing of housekeeping and virulence genes has shown that different sets of genotypes predominate in different human populations [7]. Of particular interest has been *H. pylori’s* cagA oncogene and toxigenic s1 and m1 alleles of its vacA gene, which have been implicated in gastroduodenal diseases caused by this pathogen both in epidemiologic [8,9], experimental animal and cell culture infection [10] studies. This said, several studies from different world regions have not detected such an association [8,11,12], an outcome suggesting the possibility of other virulence-modulating factors.

Individuals can be colonized by either a single or multiple strains of *H. pylori*, and even colonization by what is initially a single strain can, over time, lead to the emergence of multiple *H. pylori* subpopulations, due variously to mutation or to genetic recombination either between duplicate sequences in the single strain’s genome or with DNAs from other transiently colonizing strains [8]. The prevalence of such mixed infections has been reported to vary (5–68%) [13–17] depending on geographical region, whether in a developed or developing country (low and high overall infection risk, respectively), and probably also methods of analysis. The *H. pylori* virulence-associated vacuolating cytotoxin (vacA) and cag pathogenicity island (cag PAI) genes, and...
also the \textit{cag} empty site in strains lacking the \textit{cag} PAI, are typically found in only one copy per genome [17–20]. Accordingly, detection of both the \textit{cagA} gene and the \textit{cag} empty site, or of both \textit{s1} and \textit{s2} (signal sequence; at 5’ end of gene) or both \textit{m1} and \textit{m2} (middle region) alleles of \textit{vacA} in a biopsy or in pool of \textit{H. pylori} from a person indicates mixed infection.

We wondered if having mixed infection might influence the risk of gastric disease; for example, if strains of different genotypes might occupy a broader range of niches in the stomach as has been seen during experimental infection [21] and thereby impact on clinical outcome. In this study, we investigated the genotypes of \textit{H. pylori} in The Gambia and the relation of apparently single versus mixed infections to gastrroduodenal diseases.

\section*{Materials and Methods}

\textbf{Ethics statement}

Ethical approval of this study was obtained from the joint Medical Research Council (MRC) Unit, The Gambia/Gambia Government Ethics Committee and Division of Microbiology Infectious Diseases (DMID) International Review Board.

\textbf{Patients}

Clinical data from the MRC Unit in The Gambia revealed that of 428 patients with gastric complaints investigated by gastric endoscopy between 2003–2008, 8 (1.9\%) had gastric carcinoma, 20 (4.7\%) and 15 (3.5\%) had gastric and duodenal ulcers respectively, and that the others (89.9\%) did not have such overt disease (diagnosed as non-ulcer dyspepsia; NUD (data not shown). All patients referred for endoscopy to the MRC Unit during the years 2003 to 2008 were initially considered eligible for inclusion: All 169 subjects who agreed to join this study provided written informed consent; in addition, for children less than 18 years, all 169 subjects was 35, ranging from 9 to 80 years. All the subjects were Gambians and most of them (75) came from the Greater Banjul Area, 38 from the West Coast region, 5 from Lower River region and 3 from North Bank region of The Gambia.

\begin{table}[h]
\centering
\caption{Primers used in this study.}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Region & Primer & Nucleotide sequence & bp & reference \\
\hline
\textit{cagA} & \textit{cagA}-F & \textit{cagA} & gat aac agg caa gct ttt gag g & 349 & [23] \\
& \textit{cagA}-R & & ctg caa aag att gtt tgg cag a & & \\
\textit{cag} empty site & Luni-1 & & aga ttt tgg cta aat aaa cgc tg & 535 & [23] \\
& RS280 & & ggt tgc acg cat ttt ccc tta atc & & \\
\textit{vacA}s1 & \textit{vacA}s1-F & \textit{vacA}s1 & atg gaa ata caa caa aca cac & 290 & [23] \\
& \textit{vacA}s1-R & & ctt gta gac gcc gaa a c & & \\
\textit{vacA}m1 & \textit{vacA}m1-F & \textit{vacA}m1 & ggt caa aat gcc gtc atg g & 352 & [23] \\
& \textit{vacA}m1-R & & ctc cta gta gtc ctt gca c & & \\
\textit{vacA}m2 & \textit{vacA}m2-F & \textit{vacA}m2 & gga gcc cca gga aac att g & & \\
& \textit{vacA}m2-R & & cat aac tag cgc ctt gca c & & \\
\hline
\end{tabular}
\end{table}

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\textbf{Gastroscopy results}

Endoscopic examination showed that of the 121 study subjects, 11 had gastric ulcer, 7 had duodenal ulcer, 1 had both gastric and duodenal ulcers, 7 had gastric erosions, 1 had gastric carcinoma and all other subjects (94) who presented with either abdominal pain or dyspepsia had no evidence or history of gastric or duodenal ulcers.

The biopsies collected from patients were stored in Brain Heart Infusion (BHI) broth containing 20\% glycerol and transported on ice to the laboratory for processing or stored at −70°C until used. Our previous data demonstrated that in The Gambia detection of mixed \textit{H. pylori} strains in individual biopsies was best undertaken by PCR amplification directly from biopsy material rather than by bacterial culture [22].

\textbf{Genomic DNA extraction directly from biopsies}

Total genomic DNA was extracted directly from the biopsy material by using a combination of bead-beater and the QIAamp DNA isolation kit (Qiagen, UK) as previously described [22].

\textbf{PCR amplification}

PCR was performed to detect the \textit{cagA} gene and \textit{cag} empty site, and the signal sequence (\textit{s1} and \textit{s2}) and middle region (\textit{m1} and \textit{m2}) alleles of the \textit{vacA} gene, as previously described [23] using the primers listed in table 1 and the following cycling conditions: 30 cycles of 94°C for 1 min, 55°C or 60°C for 1 min and 72°C for 1 min. The amplified genes were detected by electrophoresis in a 1.5\% gel with ethidium bromide (500 ng/ml) and bands were visualized using Gel Doc 2000 (Bio-Rad laboratories, Milan, Italy). The presence of a particular gene or allele was inferred when a product of the expected size (table 1) was obtained using appropriate primers.

\textbf{Statistical analysis}

We assessed the prevalences of infection with single vs. multiple strains. For the \textit{cagA} gene, for example, we noted the occurrence of \textit{cagA} positive, \textit{cag} empty site and mixed (\textit{cagA} positive and \textit{cag} PAI negative) infections. Prevalences were compared between disease groups and p-values were determined using Fisher’s exact test.

\begin{table}[h]
\centering
\caption{Prevalence of \textit{Helicobacter pylori} genotypes.}
\begin{tabular}{|l|c|c|}
\hline
\textit{H. pylori} genotypes & n & \% \\
\hline
\textit{cagA} & 74 & 61.2 \\
\textit{cagA} & 21 & 17.4 \\
\textit{cagA} & 23 & 19.0 \\
\hline
No amplification of \textit{cagA} or \textit{cag} empty site & 3 & 2.5 \\
\textit{s1} & 93 & 76.9 \\
\textit{s2} & 23 & 19.0 \\
\textit{s1} & 1 & 0.8 \\
\textit{s1} & 4 & 3.3 \\
\textit{m1} & 55 & 45.5 \\
\textit{m2} & 36 & 29.8 \\
\text{m1} & 22 & 18.2 \\
\text{No amplification of m1 or m2} & 8 & 6.6 \\
\hline
\end{tabular}
\end{table}

doi:10.1371/journal.pone.0027954.t002

\section*{Discussion}

According to the World Health Organization [24], the eradication rates for \textit{H. pylori} in the world are variable and in low-income countries are often 50\% or less. The 2008/2009 guidelines from the Infectious Diseases Society of America [25] and the American College of Gastroenterology [26] are evidence-based for \textit{H. pylori} eradication and provide advice on how to achieve better results. Some guidelines have addressed the role of mixed infection in \textit{H. pylori} eradication with an emphasis on a single \textit{cagA} gene or \textit{cag} empty site and no PAI to determine the highest cure rate; however, we found that in The Gambia, mixed infection of \textit{H. pylori} strains is frequent and is associated with a higher prevalence of \textit{cagA} and \textit{cag} empty site. Additionally, we confirmed that mixed infection of \textit{H. pylori} strains is associated with more severe gastritis than single infection. Our results agree with those of previous reports showing that mixed infection of \textit{H. pylori} strains is associated with more severe disease than single infection and that mixed infection strains might have a higher virulence than single infection strains [27].
Table 3. Association of vacA with cagA Helicobacter pylori genotypes.

<table>
<thead>
<tr>
<th>H. pylori genotypes</th>
<th>cagA&lt;sup&gt;+&lt;/sup&gt;</th>
<th>cagA&lt;sup&gt;−&lt;/sup&gt;</th>
<th>cagA&lt;sup&gt;+&lt;/sup&gt; &amp; cagA&lt;sup&gt;−&lt;/sup&gt;</th>
<th>Incomplete cagA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>s1m1</td>
<td>49 (92.5)</td>
<td>1 (1.9)</td>
<td>3 (5.7)</td>
<td>0 (0)</td>
<td>53</td>
</tr>
<tr>
<td>s1m2</td>
<td>9 (50)</td>
<td>3 (16.7)</td>
<td>5 (27.8)</td>
<td>1 (5.6)</td>
<td>18</td>
</tr>
<tr>
<td>s2m1</td>
<td>0 (0)</td>
<td>16 (88.9)</td>
<td>2 (11.1)</td>
<td>0 (0)</td>
<td>18</td>
</tr>
<tr>
<td>s2m1&lt;sup&gt;−&lt;/sup&gt;</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>s1m1m2</td>
<td>12 (66.7)</td>
<td>0 (0)</td>
<td>6 (33.3)</td>
<td>0 (0)</td>
<td>18</td>
</tr>
<tr>
<td>s1m2m2</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (100)</td>
<td>0 (0)</td>
<td>3</td>
</tr>
<tr>
<td>Incomplete vacA</td>
<td>4 (40)</td>
<td>1 (10)</td>
<td>3 (30)</td>
<td>2 (20)</td>
<td>10</td>
</tr>
</tbody>
</table>

Incomplete cagA = cagA and cag empty site were not detected.
Incomplete vacA = either vacAs or vacA m regions were not detected (4/10 vacAs1 was detected & vacAm was missing, 2/10 vacAs2 detected and vacAm missing, 2/10 vacAm1 detected and vacAs missing and for 2/10 both vacAs and vacAm were missing).

doi:10.1371/journal.pone.0027954.t003

Results

Prevalence of H. pylori genotypes

One hundred and twenty one patients of the 169 study participants were inferred to be infected with H. pylori when DNAs extracted from their biopsies were tested by PCR for the presence of H. pylori cagA gene and cag empty site. Seventy four biopsies (61.2%) were positive for the cagA gene only, 21 (17.4%) were positive for the cag empty site only and 23 (19%) were positive for both. In parallel we also tested for the vacA gene presence and allele types. In all, 93 of 121 (76.9%) were positive only for the vacAs1 allele, 23 (19.0%) were positive only for the vacAs2 allele and 1 (0.8%) was positive for both. Only m1 or only m2 alleles of vacA were detected in 55 (45.5%) and 36 (29.8%) of biopsies tested respectively; both m1 and m2 (mixed infections) were found in 22 (18.2%) biopsies and up to 6.6% of biopsy DNAs failed to amplify for individual alleles (table 2).

Association between H. pylori genotypes

Of the 93 H. pylori strains that were positive only for vacAs1, 72 (77.4%) were cagA positive compared with only 1 (4.3%) cagA positive among the 23 strains that were positive only for vacAs2; most (16) of them contained the cag empty site only (table 3). Similarly, nearly all s1m1 positive biopsies (92.5%) contained cagA genes, whereas none of those containing only vacA s2m2 allele were cagA positive (table 3).

Table 4. Association between cagA genotypes and disease type.

<table>
<thead>
<tr>
<th>cagA status</th>
<th>DU</th>
<th>GC</th>
<th>GE</th>
<th>GU</th>
<th>GUDU</th>
<th>NUD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>cagA&lt;sup&gt;+&lt;/sup&gt;</td>
<td>6 (85.7)</td>
<td>1 (100)</td>
<td>5 (71.4)</td>
<td>8 (72.7)</td>
<td>1 (100)</td>
<td>53 (56.4)</td>
<td>74 (61.2)</td>
</tr>
<tr>
<td>cagA&lt;sup&gt;−&lt;/sup&gt;</td>
<td>1 (14.3)</td>
<td>0 (0)</td>
<td>2 (28.6)</td>
<td>3 (27.3)</td>
<td>0 (0)</td>
<td>15 (16.0)</td>
<td>21 (17.4)</td>
</tr>
<tr>
<td>cagA&lt;sup&gt;+&lt;/sup&gt; &amp; cagA&lt;sup&gt;−&lt;/sup&gt;</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>23 (24.5)</td>
<td>23 (19.0)</td>
</tr>
<tr>
<td>No amplification</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (3.2)</td>
<td>3 (2.5)</td>
</tr>
<tr>
<td>Total</td>
<td>7 (58)</td>
<td>1 (8.8)</td>
<td>7 (58)</td>
<td>11 (91)</td>
<td>1 (8.8)</td>
<td>94 (77.7)</td>
<td>121 (100)</td>
</tr>
</tbody>
</table>

DU = duodenal ulcer, GC = gastric carcinoma, GE = gastric erosion, GU = gastric ulcer, GUDU = gastric ulcer and duodenal ulcer, NUD = Non-ulcerative disease.

doi:10.1371/journal.pone.0027954.t004

Association of H. pylori virulence genes with upper gastric diseases

cagA positive H. pylori strains were found more frequently among study participants with gastroduodenal diseases than those with NUD: duodenal ulcers (6/7; 85.7%), gastric erosions (5/7; 71.4%), gastric ulcers (8/11; 72.7%); no overt gastric disease (53/94, 56.4%) (table 4). In the 27 patients with overt gastric disease, 77.8% were cagA positive compared to 56.4% of those with NUD (p-value = 0.05, table 5). Toxigenic s1m1 alleles were found in 6 of the 11 (54.5%) patients diagnosed with gastric ulcer, 42.9%, 42.9% and 42.6% in those with duodenal ulcers, gastric erosions and NUD, respectively (table 6). The prevalence of vacA alleles were similar in the two groups of patients; overt disease vs. NUD. That is, no association was found between vacA alleles and clinical outcome (p = 0.94, table 7).

All 27 subjects with overt gastric diseases were of uniform cagA status (that is, uniquely cagA gene positive or cag empty site positive), whereas only 72.3% (68/94) of NUD were of uniform status; the other 23 contained mixed (cagA positive, cag empty site positive) infections. Three other biopsy samples did not give cagA gene or cag empty site amplification (table 5). This association between uniform cagA status and overt disease was statistically significant (p = 0.002).

In terms of age distribution, no association was found between age and overt gastric disease (24.5%<30 years, 12.5% 30–40 years and 27.8%>40 years; p-value = 0.26, table 8), or frequency of...
mixed infection (15.1% < 30 years, 18.8% 30–40 years, 25% > 40 years; p-value = 0.46, table 9).

**Discussion**

*H. pylori* infection is common in dyspeptic adults in The Gambia [4,22], as is typical of developing countries. The range of *H. pylori* genotypes implicated in overt gastroduodenal disease as opposed to benign colonization or possibly even beneficial carriage [9,24] had not been extensively investigated in Sub-Saharan Africa. Here we studied the distribution of *H. pylori’s* main virulence genes, *cagA* and toxigenic alleles of *vacA*, in The Gambia, and their possible associations with disease outcome.

The prevalence of gastroduodenal disease (10%) that we detected endoscopically is similar to that reported elsewhere in Sub-Saharan Africa [25] and may be lower than that in Europe and North America. However, these estimated prevalences should be interpreted with caution as they may not be representative of the general population, but instead indicate the prevalence of disease among people with gastric complaints of sufficient severity to prompt diagnostic endoscopy. We found that just over half of Gambian isolates (61.2%) carried the *cagA* gene (table 2) and that mixed infections (both *cagA* positive and *cag PAI* negative) were common. We also found toxigenic *vacA*1 and *m1* alleles to be abundant, but not universal (76.9% and 45.5%). In contrast, most *H. pylori* strains from Egypt carried *vacA*2 and *m2* alleles, (57.1% and 85.7%, respectively) [9], whereas some 90% or more of strains in Japan, coastal China, and India carried *vacA*1 alleles [23,26–28]. *vacA*1 type alleles were also nearly universal in Japanese main island strains, whereas *vacAm2* strains were predominant in coastal China [28,29]. In this study, most of the *vacA*1 strains (77.4%) were *cagA* positive similar to what was observed in a study in South Africa [30]. Up to 6.6% of samples in this study failed to amplify for individual genes consistent with other findings [22,30], perhaps due to PCR inhibitors or potent nucleases in some gastric biopsies as also suggested previously [22,30,31].

Thus, our data reinforce conclusions that different *H. pylori* genotypes, especially types of genes or alleles implicated in virulent vs. benign infections, predominate in different human populations. Strains of the *vacA* *s1m2* type were most common in coastal China, and also seemed to predominate in Southern Nigeria [32]; and both *s1m1* and *s2m2* allele types were abundant in South Africa [30]. This contrasts with the predominance of *vacA* *s1m1* strains found in our Gambian study participants. This apparent difference between Nigerian and Gambian strain genotypes could have several explanations, including the distance separating these two West African countries (>3000 km), or climatic differences (high rainfall and humidity in Southern Nigeria vs aridity for much of the year in The Gambia). Such explanations would entail genetic divergence by random genetic drift and selection for adaptation to local conditions respectively.

**Table 6. Association between *vacA* genotypes and disease type.**

<table>
<thead>
<tr>
<th>vacA genotype</th>
<th>DU*</th>
<th>GC*</th>
<th>GE*</th>
<th>GU*</th>
<th>GUDU*</th>
<th>NUD*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>s1m1</em></td>
<td>3 (42.9)</td>
<td>1 (100)</td>
<td>3 (42.9)</td>
<td>6 (54.5)</td>
<td>0 (0)</td>
<td>40 (42.6)</td>
<td>53 (43.8)</td>
</tr>
<tr>
<td><em>s1m2</em></td>
<td>1 (14.3)</td>
<td>0 (0)</td>
<td>2 (28.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>15 (16.0)</td>
<td>18 (14.9)</td>
</tr>
<tr>
<td><em>s2m2</em></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (28.6)</td>
<td>3 (37.3)</td>
<td>0 (0)</td>
<td>13 (13.8)</td>
<td>18 (14.9)</td>
</tr>
<tr>
<td><em>s1m1m2</em></td>
<td>3 (42.9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (9.1)</td>
<td>0 (0)</td>
<td>14 (14.9)</td>
<td>18 (14.9)</td>
</tr>
<tr>
<td><em>s2m1m2</em></td>
<td>1 (14.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (1.1)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td><em>s2m1m2</em></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (3.2)</td>
<td>3 (2.5)</td>
</tr>
<tr>
<td>Uncomplete</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>8 (8.5)</td>
<td>10 (8.3)</td>
</tr>
<tr>
<td>vacA</td>
<td>7</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>11</td>
<td>94</td>
<td>121</td>
</tr>
</tbody>
</table>

*Du = Duodenal ulcer; GC = gastric cancer; GE = gastric erosion; GU = gastric ulcer; GUDU = gastric and duodenal ulcers; NUD = non-ulcerative diseases.*

**Table 7. Association between *vacA* genotypes and clinical outcome.**

<table>
<thead>
<tr>
<th>vacA genotypes</th>
<th>Overt gastric disease</th>
<th>NUD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>s1m1</em></td>
<td>13 (48.1)</td>
<td>40 (42.5)</td>
</tr>
<tr>
<td><em>s1m2</em></td>
<td>3 (11.1)</td>
<td>15 (16.0)</td>
</tr>
<tr>
<td><em>s2m2</em></td>
<td>5 (18.5)</td>
<td>13 (13.8)</td>
</tr>
<tr>
<td><em>s1m1m2</em></td>
<td>4 (14.8)</td>
<td>14 (14.9)</td>
</tr>
<tr>
<td><em>s2m1m2</em></td>
<td>0 (0)</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td><em>s2m1m2</em></td>
<td>0 (0)</td>
<td>3 (3.2)</td>
</tr>
<tr>
<td>Uncomplete</td>
<td>2 (7.4)</td>
<td>8 (8.5)</td>
</tr>
<tr>
<td>Total</td>
<td>27 (100)</td>
<td>94 (100)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>H. pylori genotypes</th>
<th>Overt gastric disease</th>
<th>NUD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>s1m1</em></td>
<td>13 (48.1)</td>
<td>40 (42.5)</td>
</tr>
<tr>
<td><em>s1m2</em></td>
<td>3 (11.1)</td>
<td>15 (16.0)</td>
</tr>
<tr>
<td><em>s2m2</em></td>
<td>5 (18.5)</td>
<td>13 (13.8)</td>
</tr>
<tr>
<td><em>s1m1m2</em></td>
<td>4 (14.8)</td>
<td>14 (14.9)</td>
</tr>
<tr>
<td><em>s2m1m2</em></td>
<td>0 (0)</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td><em>s2m1m2</em></td>
<td>0 (0)</td>
<td>3 (3.2)</td>
</tr>
<tr>
<td>Uncomplete</td>
<td>2 (7.4)</td>
<td>8 (8.5)</td>
</tr>
<tr>
<td>Total</td>
<td>27 (100)</td>
<td>94 (100)</td>
</tr>
</tbody>
</table>

**Table 8. Association between age and disease.**

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Overt disease</th>
<th>NUD</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30 years</td>
<td>13 (24.5)</td>
<td>40 (74.5)</td>
</tr>
<tr>
<td>30–40 years</td>
<td>4 (12.5)</td>
<td>28 (87.5)</td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>10 (27.8)</td>
<td>26 (72.2)</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>96</td>
</tr>
</tbody>
</table>

p-value = 0.26.

doi:10.1371/journal.pone.0027954.t008
Table 9. Association between age and mixed infection.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Mixed cagA (cagA* &amp; cagA*) n (%)</th>
<th>Uniform cagA status (cagA* or cagA-) n (%)</th>
<th>No amplification for cagA gene n (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30 years</td>
<td>8 (15.1)</td>
<td>44 (83.0)</td>
<td>1 (1.9)</td>
<td>53</td>
</tr>
<tr>
<td>30–40 years</td>
<td>6 (18.8)</td>
<td>25 (78.1)</td>
<td>1 (3.1)</td>
<td>32</td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>9 (25.0)</td>
<td>26 (72.2)</td>
<td>1 (2.8)</td>
<td>36</td>
</tr>
</tbody>
</table>

p-value = 0.46.

Previous reports of a lower than expected prevalence of *H. pylori* associated disease in Africa [2,5,6] might in principle reflect the influence of bacterial and/or human genotypes, environment including other infections (e.g. parasitic infections that affect host response to *H. pylori*), normal gut flora, diet (including antioxidants, salt, spices) or likelihood of seriously ill persons being diagnosed and their cases entered in registries. In accord with this last explanation, have been suggestions that gastroduodenal disease is actually common in Africa, that there is no African enigma [11,12]. The cagA gene and vacA s1 and m1 alleles are often linked to severe disease, and the vacA s2 and m2 alleles with more benign infections (gastritis only) in other populations. This is partially reflected in our results: with respect to cagA we did not find a “Gambian-*H. pylori* virulence gene” enigma; disease associations with vacA were less clear cut. The possible effects of bacterial or human genetic and physiologic differences, food, history of other infections and other environmental and lifestyle factors, on outcomes of chronic *H. pylori* infections in sub-Saharan Africa merit further more detailed analyses.

Most important, was our finding that co-existence of cagA positive and cagA negative strains was significantly more common amongst patients with NUD than among those with overt disease, which suggests that mixed colonization is protective. In principle, protection against development of overt gastric disease might stem from simple competition – whereby carriage of an avirulent (cagA-negative) strain diminishes the vigour of growth of a coexisting virulent strain, thereby reducing its impact on host tissues. It is also possible that factors in cagA negative strains that diminish the impact of virulence proteins such as CagA might predominate during cagA-positive and cagA-negative mixed infections [33]. On, more generally, an increased complexity of immune responses during chronic infection by multiple divergent *H. pylori* strains might effectively diminish the inflammatory action of an individual virulent strain, and thereby resultant pathology in host tissues, as noted with other infections [34–37]. In accord with this idea, the risk of developing overt disease seemed higher in subjects apparently colonized only with cagA negative *H. pylori*, than in those with mixed cagA positive and negative strains. Conversely, however, the presence of mixed infections might also stem from increased intrinsic host susceptibility to *H. pylori* infection and equally the development of a more severe clinical outcome [38,39].

This study has revealed frequent gastro-duodenal disease among Gambians with gastric complaints. Many strains carried cagA* and s1, m1 alleles of vacA, which are disease associated in many European and North American populations. Although cagA status was associated with disease in The Gambia, alleles of vacA were not. Comparison of our data with those from southern Nigeria pointed to a potentially significant difference in linkage of signal sequence (s1 vs. s2) and middle region (m1 vs. m2) alleles, which control the potency and tissue specificity of toxin action respectively (s1m1 most common in The Gambia, vs. s1m2 most common in Nigeria). The possibility that such differences reflect selection for optimal genotypes or random genetic drift in these well-separated West African nations merit further study. We suggest that our most interesting finding is the significantly lower disease burden in Gambians infected with a mixture of cagA-positive and cagA-negative strains, relative to those containing only cagA-positive or only cagA-negative strains. The possibility that repeated exposure would be beneficial in Sub-Saharan Africa and in developing countries more generally needs to be considered when developing more effective treatment strategies for treating *H. pylori* infection and risks of gastro-duodenal disease [40].

Author Contributions

Conceived and designed the experiments: RAA JET DEB. Performed the experiments: OS. Analyzed the data: OS MA CB DEB JET. Contributed reagents/materials/analysis tools: MT TG RW JET VT. Wrote the paper: OS MA CB DEB JET RAA.

References

Effect of Mixed Infection of H. pylori on Disease


