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Sallah, Neneh, Hayes, Alexander, Osei-Tutu, Nana et al. (2021) Antibody response patterns to *Helicobacter pylori* infection in a rural Ugandan population cohort. [Preprint]

<https://doi.org/10.1101/2021.03.01.433355>

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1 **Antibody response patterns to *Helicobacter pylori* infection in a rural Ugandan population**
2 **cohort.**

3

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17

18 Keywords: *Helicobacter pylori*, antibody, bacteria, Africa, infection

19

20 Key Messages:

- 21 • Antibody responses to *H. pylori* antigens are found to be associated with risk of gastric cancer,
22 however, despite the high seroprevalence in African populations, data from Africa are scarce.
23 This is the first study of antibody response patterns and their determinants from an African
24 population.
- 25 • Our study shows a population where *H. pylori* is ubiquitous from childhood, and seroprevalence
26 of virulent antigens is distinctively high suggesting an increased of disease compared to other
27 populations.

- 28 • We observe inter-individual variation in virulent antibody responses partly influenced by co-
29 infection.
- 30 • We highlight crucial insights into antibody-based biomarkers of disease risk and reinforce the
31 need for population-based *H. pylori* screening and treatment programmes for gastric cancer
32 control.

33 Abstract word count: 248, Text word count: 2773

34

35 **Abstract**

36 Background: *Helicobacter pylori* (*H. pylori*) establishes life-long infection in humans in the absence
37 of treatment and has been associated with a variety of gastrointestinal conditions including peptic
38 ulcer and gastric cancer. Antibody responses to *H. pylori* antigens are found to be associated with
39 disease risk, however, data from Africa are scarce.

40 Methods: To assess the seroprevalence of *H. pylori* and characterise antibody response patterns, we
41 measured serum IgG antibody levels to 14 antigens among 7,211 individuals in a rural Ugandan
42 population cohort. Multivariate-adjusted linear regression models were fitted to investigate the
43 influence of age, sex, and co-infection on antibody seroreactivity levels.

44 Results: *H. pylori* seroprevalence was 95% in our study population, with 94% of individuals
45 seropositive in childhood (<15 years). In *H. pylori* positive individuals, we found a markedly high
46 seroprevalence (~99%) and antibody levels to the high-risk antigens CagA and VacA, in addition to
47 Cag δ . HSV-2 co-infection was significantly associated with higher IgG levels of CagA and VacA
48 (OR=1.10, 95% C. I.=1.05-1.16). HIV infection was associated with lowered IgG levels to CagA
49 (OR=0.86, 95% C.I.=0.80-0.93), and HPV infection was associated with increased IgG levels to
50 VacA (OR=1.16, 95% C.I.=1.11-1.21).

51 Conclusions: *H. pylori* in this population is ubiquitous from childhood, with a high prevalence and
52 high seroreactivity levels of high-risk antigens, suggesting chronic active inflammatory responses in
53 individuals that are indicative of risk of disease. Further investigation is warranted to fully understand

54 the relationship between host, immunogenicity, and clinical outcomes to better stratify by risk and
55 improve treatment.

56

57 **Introduction**

58 *Helicobacter pylori* (*H. pylori*), is a gram-negative bacterium that colonises the gastric epithelium of
59 the human host and is typically acquired in childhood via intrafamilial contact[1]. Globally, 4.4
60 billion individuals are estimated to be infected with *H. pylori* with substantial geographic variation in
61 prevalence ranging from ~20-50% in developed countries to ~80% in developing countries[2]. While
62 the majority of infections are asymptomatic, in the absence of treatment, *H. pylori* can evade the
63 host's immune responses and subsequently persist throughout a person's lifetime. Chronic *H. pylori*
64 infection is the strongest risk factor for several gastrointestinal conditions including, peptic ulcer,
65 chronic atrophic gastritis, and gastric cancers worldwide[3]. In 2018, *H. pylori* was responsible for
66 810,000 (37%) new cases of cancers caused by an infectious agent and caused ~90% of non-cardia
67 gastric cancers[3]. The relative risk of developing gastric cancer with the *H. pylori* infection is 17[4].
68 In sub-Saharan Africa, the estimated population attributable fraction (PAF) of non-cardia gastric
69 cancer is 92%[4]. Between the 1960s and 2008, Uganda experienced a 7-fold increase in gastric
70 cancer incidence[5] . In 2008, The Uganda Cancer Working Group recognised that in areas of
71 endemic *H. pylori* infection, *H. pylori* predicated the multistage process that lead to the development
72 of gastric cancer[5]. They recommended that *H. pylori* eradication be utilised as a prevention strategy
73 in areas with a high incidence of gastric cancer[5]. This recommendation is shared by the
74 International Agency for Research on Cancer (IARC) working group that population-based *H. pylori*
75 screening and treatment programmes are needed for gastric cancer control[6].

76

77 While the understanding of potential disease outcome following *H.pylori* infection is limited, it is
78 found to be mediated by the complex interplay between bacterial virulence factors, environmental
79 factors, lower socioeconomic status and host factors[7]. During the course of infection, chronic
80 inflammatory changes occur as the bacterium adapts to its host and modulates the immune system.
81 Depending on the bacterium's protein expression pattern and the host's adaptive immune response

82 distinctive antibody response patterns may result and potentially reflect virulence of the infecting
83 bacterium. As an example, antibody responses to particular *H. pylori* antigens: CagA (cytotoxin-
84 associated antigen A), VacA (vacuolating cytotoxin), GroEL (chaperonin GroEL), HP1564
85 (hypothetical protein) and HcpC (conserved hypothetical secreted protein) have been identified as
86 being associated with development of chronic atrophic gastritis and gastric cancers[8, 9]. A recent
87 study characterised the dynamics of antibody responses to 15 antigens in a healthy German
88 population-based cohort with a *H. pylori* seroprevalence of 48% and found multiple seropositivity and
89 higher antibody levels associated with increasing age, suggestive of persistent infection and lifelong
90 stimulation of immune responses[10]. Despite the high seroprevalence in African populations, there
91 is a paucity of data, particularly from the continent compared to other populations[11, 12]. To better
92 understand the relationship between host and pathogen in individuals the investigation of distinctive
93 antibody patterns and factors that could potentially lead to disease following chronic infection is
94 essential. Therefore, in this study, we assess the seroprevalence of *H. pylori* and antibody patterns
95 against 14 antigens in a rural African population cohort and explore the factors associated with inter-
96 individual variation in antibody response.

97

98 **Methods**

99 **Sample selection and ethics**

100 The General Population Cohort (GPC) is a population-based cohort in rural south-west Uganda
101 consisting of 25 neighbouring villages inhabited mainly by farmers[13, 14]. The Baganda are the
102 predominant tribal group constituting ~70% of the population, with a substantial number of migrants
103 who settled from neighbouring Rwanda. Blood samples from 7,211 GPC study participants,
104 representing 11 self-reported ethnolinguistic groups, were collected during census and medical survey
105 sampling rounds conducted in the study area in 1992, 2000 and 2008, as described previously[13].
106 Serum was tested for infections and the remainder was stored at -80 degrees Celsius in freezers in
107 Entebbe prior to further serological testing. Informed consent was obtained from all participants either
108 in conjunction with parental/guardian consent for under 18-year olds with signature or a thumbprint if
109 the individual was unable to write. The study was approved by the Uganda Virus Research Institutes,

110 Research Ethics committee (UVRI-REC) (Ref. GC/127/10/10/25), the Uganda National Council for
111 Science and Technology (UNCST) and the London School of Hygiene & Tropical Medicine
112 (LSHTM) Ethics Committee (reference number 17686).

113

114 **Helicobacter pylori multiplex serology**

115 We quantified mean fluorescent intensity (MFI) of IgG antibodies to 14 *H. pylori* antigens using
116 multiplex serology on the Luminex® platform based on glutathione-S-transferase (GST) fusion
117 capture immunosorbent assays combined with fluorescent bead technology as previously
118 described[15]. The 14 recombinant affinity purified *H. pylori* proteins that were used as antigens
119 were: CagA (cytotoxin-associated antigen A), GroEL (chaperonin GroEL), HP1564 (hypothetical
120 protein protein HP1564), VacA (vacuolating cytotoxin), HP0231 (hypothetical protein HP0231),
121 HP0305 (hypothetical protein HP0305), HpaA (neuraminyllactose-binding hemagglutinin homolog),
122 Cag δ (cag pathogenicity island protein δ), HyuA (hydantoin utilization protein A), CagM (cag
123 pathogenicity island protein M), Catalase, HcpC (conserved hypothetical secreted protein - paralogue
124 HcpA induces IFN γ), NapA (neutrophil activating protein (bacterioferritin)) and UreA (urease alpha
125 subunit). Antibody seropositivity was defined as reactivity greater than the antigen-specific cut-off.
126 *H. pylori* seropositivity was defined as seropositivity to at least 4 antigens as described previously[10,
127 15].

128

129 **Statistical analysis of variability in antibody responses**

130 Statistical significance of differences in continuous variables, i.e. antibody reactivities (MFI values)
131 and multiple seropositivity (number of antigens recognized) were analysed with the Wilcoxon two
132 sample signed rank sum test. Fisher's Exact test was used to test for differences in dichotomous
133 variables, i.e. *H. pylori* seropositivity and antibody seroprevalence. All tests were performed two-
134 sided. Analyses were performed with R software version 3.4. P-values below 0.05 were considered
135 statistically significant. Pairwise-correlation between antigens for seropositive individuals were tested
136 using Spearman's rank-based test in R. We also investigated factors influencing IgG antibody
137 response levels to *H. pylori* antigens. Antibody levels were log₁₀ transformed and multivariate linear

138 regression models were fitted to examine variables predictive of antibody levels. The variables tested
139 were: sex, age/age group (categorized as 0-14, 15-24, 25-44 or ≥ 45 years), census (sampling) year
140 (1992, 2000 or 2008) and infection status for Epstein-Barr virus (EBV), Hepatitis B virus (HBV),
141 Hepatitis C virus (HCV), Human-immunodeficiency virus (HIV), Human papillomavirus (HPV) high
142 risk types 16 and 18, Herpes simplex virus type 2 (HSV-2), Human T-lymphotropic virus (HTLV-1),
143 Kaposi's sarcoma-associated herpesvirus (KSHV), and Merkel cell polyomavirus (MCV). A multiple
144 testing p -value of <0.005 adjusted for all variables was used to determine statistical significance
145 unless specified otherwise.

146

147 **Results**

148 ***Helicobacter pylori* seroprevalence in the GPC.**

149 In this study, we analysed the sera of 7,211 individuals between the ages of 0-98 years (median=16,
150 IQR=10-35) for antibody responses to 14 different *H. pylori* antigens: CagA, GroEL, HP1564, VacA,
151 HP0231, HP0305, HpaA, Cag δ , HyuA, CagM, Catalase, HcpC, NapA, and UreA. We categorised
152 95% of individuals as seropositive for *H. pylori* infection based on reactivity to at least 4 antigens
153 (Table 1). While *H. pylori* seroprevalence was similar for both sexes (males 95.5% vs females 94%),
154 we found that in the 25-44 years age group seroprevalence was significantly higher in males (97.3%)
155 compared to females (94.9%) ($p=0.03$) (Figure 1). We also found that seroprevalence was
156 significantly higher ($>2\%$) in the oldest age group (45+) compared to the youngest age group (0-14)
157 for both males ($p=0.02$) and females ($p=0.01$) (Figure 1). Seroprevalence was also similarly high
158 across census years (94-96%) (Table1). Seroreactivity to multiple antigens was high (median=8.7,
159 IQR=7-11) with 3375 individuals (47%) testing seropositive for >9 antigens (Supplementary Figure
160 1.A). We found no significant differences in the number of antigens recognised across age groups or
161 between sexes ($P>0.05$) (Supplementary Figure 1.B). We compared the seroprevalences of all
162 antibodies to the 14 antigens in seropositive (HP+) vs seronegative (HP-) individuals and found that
163 antibody seroprevalences were significantly higher in HP+ compared to HP- sera for all antigens
164 ($p<0.0001$) (Table2). In the 6,834 *H. pylori* seropositive individuals, antibodies to CagA, VacA and

165 Cag δ were the highest with ~99% seroprevalence and antibodies to NapA and HcpC had the lowest
166 seroprevalence being less than 50% (Table2).

167

168 **Helicobacter pylori antibody response levels**

169 Using the multiplex flow immunoassay, we quantified the antibody levels of response to the 14
170 different *H. pylori* antigens. In *H. pylori* positive and antigen-specific antibody positive individuals,
171 we observed variation in antibody responses with the highest IgG levels observed for CagA (max=
172 66,614, median=13,793, IQR=10,138-18,298) followed by VacA (max=28,722, median=2,809,
173 IQR=1641-4600), HP1564 (max=22,526, median:2776, IQR=1341-4640) and Cag δ (max=15,044,
174 median:2,206, IQR=1,293-3,609), all other antibody responses had medians below 2000 with HpaA
175 having the lowest median IgG level (max= 10,783, median:335, IQR:182-751) (Figure 2.). There was
176 a modest correlation between all antigens (ρ =-0.01-0.51) (Supplementary Figure S2), with the
177 strongest correlation being observed for HP0305 and HP1564 (ρ =0.51) (Supplementary Figure S2).

178

179 We investigated the association between intrinsic factors, age, sex and sampling year to inter-
180 individual variation in antibody response levels in seropositive individuals using a multivariable linear
181 regression model for each antibody (Table 3). We found that higher age groups in comparison to the
182 youngest age group (0-14) were significantly associated with decreased antibody levels to CagA,
183 HP1564, VacA and HP0231 (ORs between 0.84-99) (Table 3). In contrast, higher age groups were
184 significantly associated with increased antibody levels to GroEL, Cag δ , HyuA, NapA, HcpC and
185 UreA (ORs between 1.01-1.21) in comparison to the youngest age group (Table 3). Being female
186 was significantly associated (p <0.0125) with lower antibody responses to HP1564, HP0305, Cag δ ,
187 HyuA and Catalase (ORs between 0.91-99) (Table 3) compared to being male.

188

189 **The influence of co-infection on Helicobacter pylori antibody responses**

190 We then investigated the seroprevalence of co-infection in 1,735 individuals (aged 1-91 years,
191 median= 31) that had a record of infection serostatus for nine additional pathogens. We found that in

192 1,679 (97%) *H.pylori* positive individuals there was a high burden of co-infection, particularly with
193 Epstein-Barr Virus (EBV) and Kaposi's Sarcoma-associated herpesvirus (KSHV) (93%) (Figure 3.A.)
194 followed by Herpes-simplex virus-2 (HSV-2) (67%), Human Papilloma Virus (HPV) (35%) and
195 Hepatitis B Virus (HBV) (13%). The remaining four pathogens were <10% with Human T
196 lymphotropic virus-1 (HTLV-1) having the lowest seroprevalence (~1%) (Figure 3.A.). Most
197 individuals (~70%) were co-infected with at least four pathogens (Figure 3.B).

198

199 We then investigated the influence of co-infection on inter-individual variation in antibody response
200 levels to the five virulent *H. pylori* antigens, CagA, VacA, GroEL, HcpC and HP1564, in *H.pylori*
201 seropositive individuals using multivariable linear regression models adjusting for age, sex, sampling
202 year and infection status. Of the infections tested (Figure 3. A), HIV co-infection resulted in
203 significantly lowered antibody responses to CagA, HcpC, and HP1564 (OR= 0.55-0.93) (Table 4).
204 HSV-2 co-infection was associated with moderately higher levels of CagA and VacA (OR= 1.05-
205 1.16) but no other antibodies (Table 4). HPV co-infection was significantly associated with higher
206 antibody levels (OR= 1.11-1.45) to VacA and GroEL antigens (Table 4). None of the other co-
207 infections had significant effects on variation for any antibody response ($p>0.004$) (Table 4).

208

209 **Discussion**

210 Data on *Helicobacter pylori* antibody response patterns and their determinants are limited, with most
211 studies having been conducted in Western and non-African populations, there is a paucity of data
212 particularly from Africa. Here, we present the first population-based study to characterise *H. pylori*
213 antibody response patterns to 14 antigens and their determinants in ~7000 individuals from an African
214 population. The presence of antibodies representing host immune response is commonly used as
215 diagnostic markers for the stage of infection [16] and for *H.pylori* they have been found to be
216 associated with disease risk[8, 9]. In the GPC, prevalence estimates of *H. pylori* of ~94% are higher
217 than previous findings in Uganda and more broadly in developing countries[17-21]. Here, we see a
218 population with a high seroprevalence of >90% from childhood, much higher than previously
219 observed in Uganda of 44%; a seroprevalence higher than in other African countries, with studies

220 reporting estimates in children ranging from 14% in Ghana to 73% in Kenya[11, 12, 17, 22]. We
221 observed slightly higher seroprevalence in the highest age group compared to the lowest age groups
222 ($p < 0.05$), consistent with previous findings in other populations. In comparison to a previous study of
223 German individuals[10] that assessed the presence of the 14 antigens tested here, we observed a 2-
224 fold increase in seropositivity to *H. pylori* (95% in the GPC vs 48% in Germans). In *H. pylori*
225 seropositive individuals, we observed >90% seropositivity to the following antibodies: CagA,
226 HP1564, VacA and Cag δ , which was much higher than reported in previous studies. The highest
227 difference in seroprevalence (2.7-fold) was observed for CagA (90% vs 33% Germans). For the
228 following antigens: Cag δ , HP0231, HpaA, CagM and Catalase we also observed a >2-fold difference
229 in seroprevalence with higher estimates in Ugandans compared to Germans. The lowest difference in
230 seroprevalence was observed for HcpC, 1.17-fold higher in Ugandans compared to Germans. Another
231 study in adults from high-risk gastric cancer areas of Latin America also reported lower antibody
232 seroprevalences compared to our study, including for CagA (74%) and VacA (71%) despite an overall
233 *H. pylori* seroprevalence of 85%[23]. Differences in seropositivity have also been reported in a low-
234 income population in the U.S.A that had a higher *H. pylori* seropositivity 89% compared to the
235 general US population (~30%), thought to be driven by lower socio-economic status[24]. They also
236 observed higher seroprevalence in and higher in African Americans 79% compared to 69% in whites
237 in addition to higher antibody seroprevalence for 12 out of the 14 antigens tested here, particularly
238 for CagA, with a 2.6-fold increase in African Americans (68%) compared to white Americans
239 (26%)[24]. This could be suggestive of higher seroprevalence in individuals with African ancestry.
240 While seroprevalence estimates of CagA in Africa are scarce, the ubiquity of CagA in the GPC
241 mirrors estimates in East Asian countries where the risk of gastric cancers due to *H. pylori* are higher
242 than the rest of the world[3, 25]. CagA and VacA are the most well studied virulence factors of *H.*
243 *pylori* and are essential for bacterial persistence in the stomach in addition to possessing oncogenic
244 potential. CagA-host interaction induce a state of chronic inflammation which worsens over time and
245 has been associated with an increased risk of gastric cancers, peptic ulcers, and other
246 complications[26-28]. Studies that have evaluated antibody response levels to CagA and VacA report

247 high antibody levels are associated with gastric mucosal inflammation, the grade of histological
248 gastritis, and gastric cancer risk[29, 30]. It is interesting to observe such high seropositivity and
249 immunogenicity to the high-risk serotypes, CagA and VacA in the GPC compared to non-African
250 populations. A recent study in individuals in Southeast USA also found significantly higher levels of
251 antibodies to CagA and VacA in African Americans compared to whites[31] that was likely driven by
252 differences in ancestry.

253

254 Differences in antibody responses compared to other populations could occur as a result of multiple
255 factors particularly, host genetic and environmental variation. In the GPC, a high burden of co-
256 infection exists, with most individuals having up to four infections (Figure 3), therefore we sought to
257 investigate whether inter-individual variation in antibody responses to the five virulent antigens
258 CagA, VacA, HP1564, HcpC and GroEL was influenced by co-infection with pathogens previously
259 reported to have oncogenic potential[3]. Out of the infections tested, only HIV, HSV-2 and HPV
260 (high risk genotypes 16 or 18) co-infection influenced antibody levels. We found that while HIV
261 infection was associated with decreased antibody responses (OR=0.53-0.99), co-infection with HSV-2
262 or HPV were significantly associated with moderate increases in antibody responses (OR=1.05-1.45)
263 (Table 4). Co-infections can cause an imbalance in host immune system, likely modulating shared
264 inflammatory pathways and thus, resulting in antibody variation[32-35]. However, as effect sizes in
265 this study are small, they only partially explain inter-individual differences in antibody patterns.

266

267 In summary, we characterised antibody response patterns to 14 *H. pylori* antigens in ~7,000
268 individuals from a rural Ugandan population cohort and identified factors associated with
269 inter-individual variation in response. A major finding of this study is that *H. pylori*
270 seropositivity is ubiquitous from childhood (94%), and higher than previous reports in
271 Uganda and other countries which could be due to lower-socioeconomic status in this cohort
272 compared to urban areas. We also found that antigen specific seropositivity to the 14 antigens
273 tested here, particularly for CagA, are higher than for non-African populations. Inter-

274 individual variation in antibody responses was partly explained by HIV, HSV-2 and HPV co-
275 infections, suggesting other factors not examined here such as host genetics might be
276 important. These insights are useful to better stratify individuals at risk of developing adverse
277 outcomes following infection, as this population sustains such high levels of *H. pylori*,
278 further investigation will be essential to inform appropriate therapeutic interventions to
279 prevent disease in those at risk. Future studies would need to assess the relationship between
280 antibody responses and clinical outcomes, and also investigate the relationship between host
281 genetic variation and antibody variation to fully understand disease risk and population
282 specific differences that are not explained by environmental variation.

283

284 **Acknowledgements**

285 We thank all study participants who contributed to this study and acknowledge the support of the
286 MRC/UVRI & LSHTM Uganda Research Unit

287

288 **Funding**

289 The GPC is jointly funded by the UK Medical Research Council (MRC) and the UK Department for
290 International Development (DFID) under the MRC/DFID Concordat agreement. Additional financial
291 support for accessing serological samples was provided by a Wellcome Trust Clinical Training
292 Fellowship awarded to Dr Katie Wakeham for work on the Kaposi's sarcoma associated herpesvirus
293 (Grant number: 090132).

294

295 **Author Contributions**

296 N.S, R.N. ,T.W. & M.L.H. designed the study. N.S & R.N wrote the manuscript. J.B & T.W
297 performed phenotype assay development and validation. W.T.J, carried out curation of the cohort data
298 and managed the database. N.S, A.H & N.O carried out all statistical analyses and data visualisation.
299 N.S, R.N. & J.B. interpreted the results. G.A was the programme leader for the GPC. All authors
300 commented on the interpretation of the results, reviewed and approved the final manuscript.

301

302 **Conflict of interest**

303 None

304

305

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307

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438 **Figure Legends**

439 **Figure 1. *H. pylori* seropositivity by gender and age.** Star indicates statistically significant
440 difference between genders in age 25-44, $p=0.03$ (Fisher's exact test). Horizontal brackets indicate
441 statistically significant differences between the youngest (0-14) and oldest (45+) age groups for both
442 genders: Males: $p=0.02$, females: $p= 0.01$ (Wilcoxon two sample signed rank sum test).

443

444 **Figure. 2. Distribution of antibody responses (\log_{10} MFI) in *H. pylori* positive and antigen
445 positive individuals.** Horizontal lines denote median and vertical lines denote interquartile range
446 (IQR).

447

448 **Figure 3. A. Seroprevalence of co-infection in *H. pylori* seropositive individuals (n=1679).** EBV:
449 Epstein Barr Virus, KSHV: Kaposi's Sarcoma-associated Herpesvirus, HSV-2: Herpes Simplex
450 Virus-2, HBV: Hepatitis B virus, HPV: Human papilloma virus, MCV: Merkel Cell polyoma virus,
451 HIV: Human Immunodeficiency virus, HCV: Hepatitis C virus and HTLV-1: Human T-cell
452 Lymphotropic virus. **B. Burden of infection.** Infection count represents the number of infections the
453 individuals tested positive for.

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466 **Tables**

467

Table.1 Characteristics of samples in the GPC (n=7211)

Variable	Category	N	%	HP+(%)
Sex	Female	3689	51	94
	Male	3522	49	96
Age [Years]	0-14	3129	43	93
	15-24	1509	21	98
	25-44	1307	18	96
	45+	1266	18	95
<i>H. pylori</i> Status	Positive	6834	95	100
	Negative	377	5	-
Census Year	1992	1920	27	94
	2000	2342	33	96
	2008	2949	41	94

^a*H. pylori* seropositivity determined as antibody reactivity with at least 4 antigens

*Fisher's exact test, comparison of Hp- and Hp+ sera.

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Table.2 Seroprevalence of antibodies to *H. pylori* antigens by HP serostatus

Seroprevalence (%)						
Antigen	Name	Cut-off [MFI]	All (n=7211)	Hp+ ^a (n=6834)	Hp- (n=377)	P*
HP0547	CagA	2592	90	100	28	<0.0001
HP0010	GroEL	100	62	69	6	<0.0001
HP1564	HP1564	188	83	93	18	<0.0001
HP0887	VacA	541	89	99	22	<0.0001
HP0305	HP0305	100	45	51	2	<0.0001
HP0410	HpaA	100	56	63	7	<0.0001
HP0522	Cagδ	500	90	99	46	<0.0001
HP0695	HyuA	191	48	54	7	<0.0001
HP0537	CagM	158.75	65	72	20	<0.0001
HP0875	Catalase	455	64	71	22	<0.0001
HP0231	HP0231	100	65	73	7	<0.0001
HP1098	HcpC	100	40	44	0	<0.0001
HP0243	NapA	100	43	49	6	<0.0001
HP0073	UreA	340	50	57	14	<0.0001

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Table 3. Predictors of antibody response levels to <i>H. pylori</i> antigens in seropositive individuals						
Variable	Age Group: Ref =0-14 years			Sex: Ref=Male	Census Year: Ref=1990	
Antigen	15-24	25-44	45+	Female	2000	2008
CagA						
OR (95% C.I)	0.98 (0.96 - 0.99)	0.96 (0.95 - 0.98)	0.91 (0.90 - 0.93)	1.00 (0.99 - 1.01)	1.03 (1.01 - 1.04)	1.04 (1.03 - 1.06)
p-value	0.002	<0.001	<0.001	0.540	<0.001	<0.001
GroEL						
OR (95% C.I)	1.08 (1.04 - 1.11)	1.06 (1.03 - 1.1)	1.10 (1.06 - 1.14)	0.98 (0.96 - 1.01)	1.04 (1.01 - 1.07)	1.11 (1.07 - 1.14)
p-value	<0.001	0.001	<0.001	0.153	0.019	<0.001
HP1564						
OR (95% C.I)	0.95 (0.92 - 0.97)	0.89 (0.87 - 0.92)	0.86 (0.84 - 0.89)	0.93 (0.91 - 0.95)	1.02 (0.99 - 1.05)	0.99 (0.97 - 1.02)
p-value	<0.001	<0.001	<0.001	<0.001	0.146	0.699
VacA						
OR (95% C.I)	1.01 (0.99 - 1.03)	0.99 (0.97 - 1.01)	0.97 (0.94 - 0.99)	0.98 (0.97 - 1.00)	1.03 (1.01 - 1.05)	1 (0.98 - 1.02)
p-value	0.224	0.399	0.002	0.019	0.004	0.701
HP0305						
OR (95% C.I)	0.94 (0.91 - 0.98)	0.94 (0.90 - 0.98)	0.95 (0.91 - 1.00)	0.94 (0.91 - 0.97)	1.01 (0.97 - 1.05)	1.05 (1.01 - 1.09)
p-value	0.004	0.004	0.041	<0.001	0.523	0.013
HpaA						
OR (95% C.I)	1.03 (0.99 - 1.06)	0.98 (0.94 - 1.02)	1.02 (0.98 - 1.06)	0.97 (0.95 - 1.00)	1.04 (1.00 - 1.07)	1.03 (0.99 - 1.06)
p-value	0.106	0.290	0.257	0.023	0.026	0.102
CagD						
OR (95% C.I)	1.03 (1.01 - 1.05)	1.04 (1.02 - 1.06)	1.03 (1.01 - 1.05)	0.95 (0.93 - 0.96)	1.01 (1.00 - 1.03)	0.98 (0.97 - 1.00)
p-value	0.002	0.001	0.005	<0.001	0.143	0.112
HyuA						
OR (95% C.I)	1.02 (0.98 - 1.06)	1.12 (1.07 - 1.16)	1.15 (1.10 - 1.19)	0.96 (0.93 - 0.99)	1.01 (0.97 - 1.05)	1.02 (0.98 - 1.06)
p-value	0.254	<0.001	<0.001	0.003	0.613	0.325
CagM						
OR (95% C.I)	1.01 (0.98 - 1.04)	0.99 (0.96 - 1.03)	0.99 (0.96 - 1.03)	0.98 (0.96 - 1.01)	1.01 (0.98 - 1.04)	1.03 (1.00 - 1.06)
p-value	0.408	0.746	0.686	0.151	0.475	0.022
Catalase						
OR (95% C.I)	0.98 (0.96 - 1.01)	0.98 (0.95 - 1.01)	0.99 (0.96 - 1.02)	0.97 (0.95 - 0.99)	1.01 (0.99 - 1.04)	1.03 (1.01 - 1.06)
p-value	0.169	0.173	0.485	0.001	0.319	0.008
HP0231						
OR (95% C.I)	0.96 (0.93 - 1.00)	0.93 (0.89 - 0.97)	1.00 (0.96 - 1.04)	0.97 (0.94 - 1.00)	1.01 (0.98 - 1.05)	1.06 (1.03 - 1.1)
p-value	0.028	0.001	0.838	0.023	0.515	<0.001
HcpC						

OR (95% C.I)	1.00 (0.95 - 1.05)	1.06 (1.00 - 1.13)	1.13 (1.07 - 1.20)	0.96 (0.92 - 1.00)	1.03 (0.98 - 1.09)	1.04 (0.99 - 1.09)
p-value	0.924	0.049	<0.001	0.044	0.219	0.137
NapA						
OR (95% C.I)	1.13 (1.07 - 1.20)	1.14 (1.07 - 1.21)	1.13 (1.06 - 1.20)	1.00 (0.95 - 1.04)	0.98 (0.92 - 1.03)	1.00 (0.95 - 1.06)
p-value	<0.001	<0.001	<0.001	0.813	0.389	0.879
UreA						
OR (95% C.I)	1.01 (0.98 - 1.04)	1.02 (0.99 - 1.05)	1.07 (1.04 - 1.10)	0.97 (0.95 - 0.99)	1.00 (0.97 - 1.03)	1.02 (0.99 - 1.05)
p-value	0.395	0.159	<0.001	0.012	0.864	0.120

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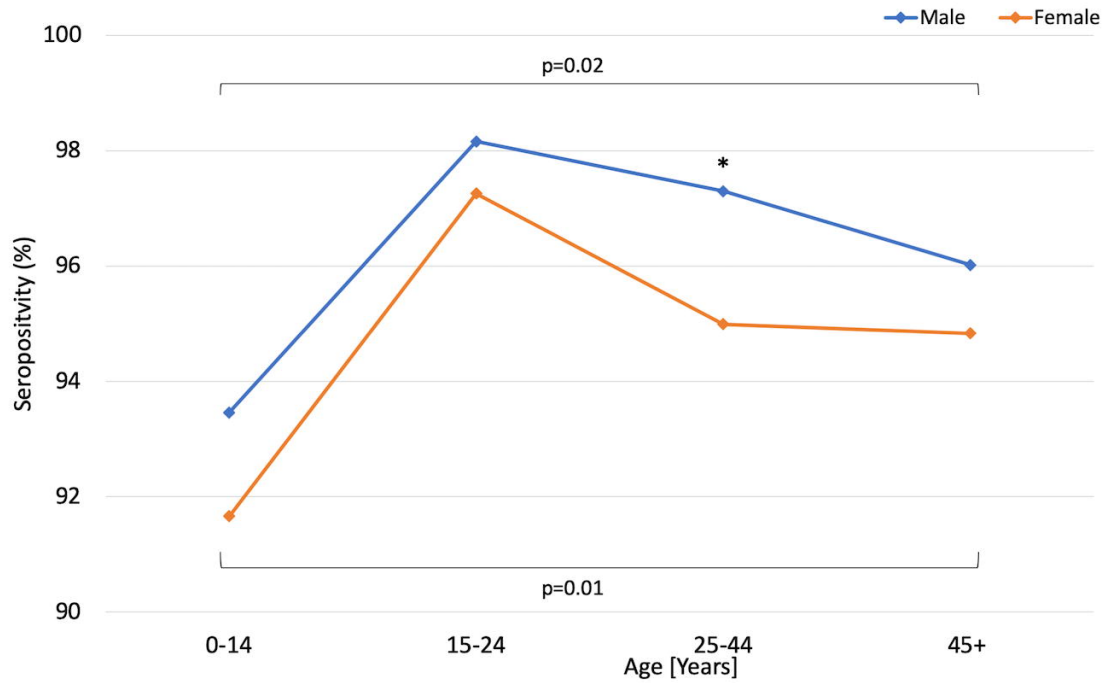
471

Table 4. The influence of co-infection on antibody response levels to virulent *H. pylori* antigens (N=1679)

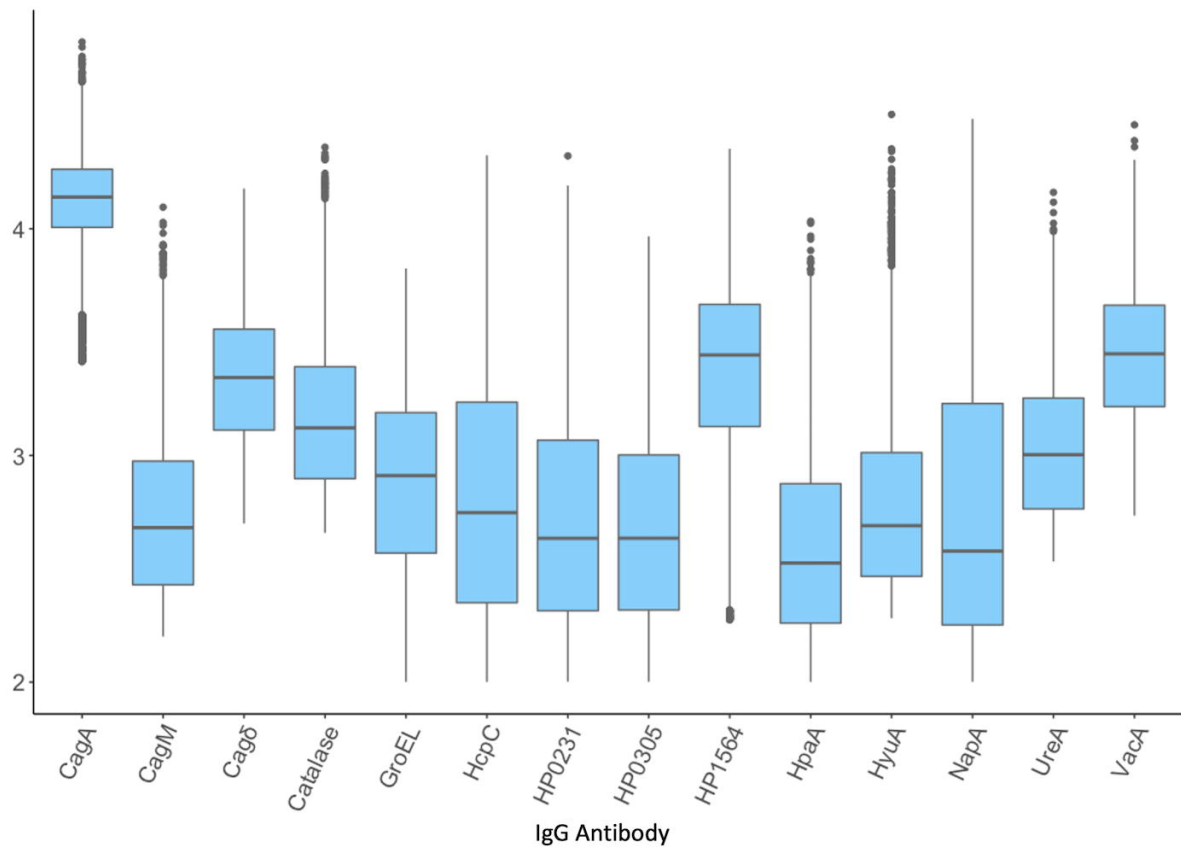
Antigen	CagA		VacA		GroEL		HcpC		HP1564	
Variable	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	0.999 (0.997 - 1)	0.007	0.998 (0.997 - 0.999)	0.001	1.001 (0.998 - 1.004)	0.330	0.996 (0.993 - 0.999)	0.003	0.995 (0.992 - 0.997)	<0.001
Sex: Ref=Male										
Female	0.98 (0.94 - 1.02)	0.282	0.98 (0.94 - 1.02)	0.414	1.00 (0.9 - 1.1)	0.923	0.90 (0.81 - 1)	0.050	0.84 (0.77 - 0.91)	<0.001
Year: Ref=1992										
2000	1.03 (0.96 - 1.12)	0.389	1.07 (0.98 - 1.16)	0.111	1.2 (0.98 - 1.48)	0.083	1.00 (0.82 - 1.23)	0.980	1.06 (0.9 - 1.25)	0.459
2008	1.08 (1.01 - 1.16)	0.034	1.05 (0.97 - 1.13)	0.239	1.47 (1.21 - 1.78)	<0.001	1.06 (0.88 - 1.28)	0.535	1.03 (0.89 - 1.19)	0.707
HIV: Ref=Negative										
Positive	0.86 (0.8 - 0.93)	<0.001	0.90 (0.82 - 0.97)	0.010	0.94 (0.76 - 1.16)	0.539	0.68 (0.55 - 0.83)	<0.001	0.75 (0.64 - 0.88)	0.001
HSV-2: Ref=Negative										
Positive	1.10 (1.05 - 1.15)	<0.001	1.11 (1.05 - 1.16)	<0.001	1.18 (1.04 - 1.34)	0.011	1.02 (0.9 - 1.15)	0.782	1.06 (0.96 - 1.17)	0.246
HPV: Ref=Negative										
Positive	1.04 (1 - 1.09)	0.032	1.16 (1.11 - 1.21)	<0.001	1.30 (1.16 - 1.45)	<0.001	1.07 (0.96 - 1.19)	0.231	1.12 (1.03 - 1.22)	0.007
EBV: Ref=Negative										
Positive	0.94 (0.87 - 1.01)	0.099	1.09 (1 - 1.18)	0.050	0.87 (0.7 - 1.08)	0.208	1.08 (0.88 - 1.34)	0.454	0.96 (0.81 - 1.13)	0.608
KSHV: Ref=Negative										
Positive	1.05 (0.98 - 1.12)	0.189	1.09 (1.01 - 1.18)	0.030	1.08 (0.87 - 1.34)	0.492	0.89 (0.73 - 1.08)	0.280	1.02 (0.87 - 1.19)	0.847

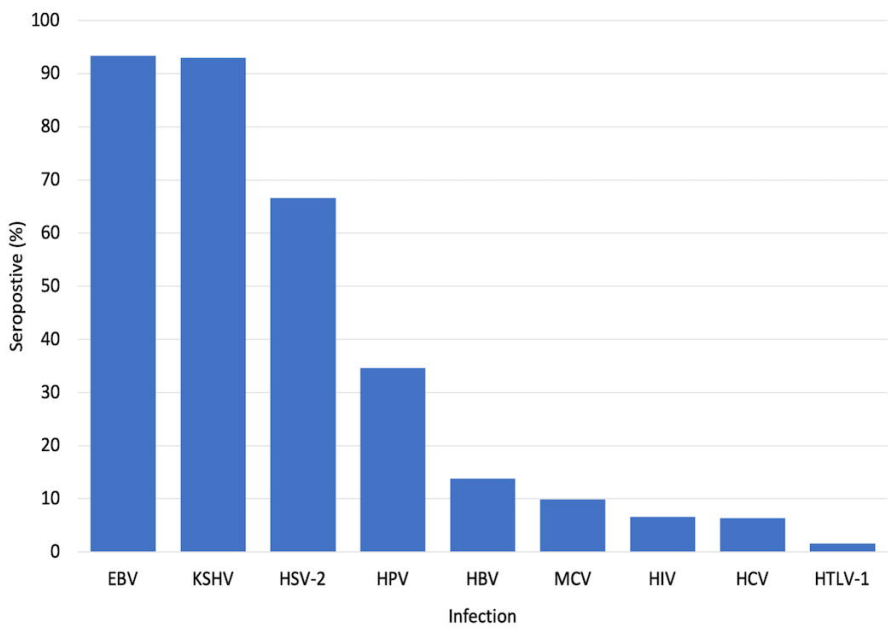
	1.13)		1.19)		1.32)		1.1)		1.19)	
HBV: Ref=Negative										
Positive	1.00 (0.93 - 1.07)	0.99 6	0.97 (0.9 - 1.04)	0.38 2	1.19 (0.98 - 1.44)	0.081	1.08 (0.89 - 1.3)	0.42 7	1.16 (1 - 1.34)	0.04 8
MCV: Ref=Negative										
Positive	0.97 (0.91 - 1.03)	0.34 1	1.02 (0.96 - 1.09)	0.51 8	1.19 (1 - 1.41)	0.046	1.04 (0.88 - 1.23)	0.66 4	1.03 (0.91 - 1.18)	0.61 8
HCV: Ref=Negative										
Positive	1.00 (0.92 - 1.09)	0.98 4	0.99 (0.9 - 1.09)	0.90 5	0.94 (0.74 - 1.2)	0.626	0.98 (0.77 - 1.25)	0.87 9	0.94 (0.78 - 1.14)	0.55 2
HTLV-1: Ref=Negative										
Positive	0.83 (0.66 - 1.03)	0.09 3	1.28 (1.01 - 1.62)	0.04 3	0.93 (0.5 - 1.7)	0.807	0.80 (0.44 - 1.46)	0.47 2	0.76 (0.48 - 1.22)	0.26 0

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Antibody Response (\log_{10} MFI)



A**B**