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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   Cagδ. 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

2

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

3

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   Cagδ (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 IFNγ), 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 log_{10} 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, Cagδ, 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   Cagδ 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 Cagδ (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 (rho=-0.01-0.51) (Supplementary Figure S2), with the

177   strongest correlation being observed for HP0305 and HP1564 (rho=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, Cagδ, 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, Cagδ,

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 Cagδ, 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: Cagδ, 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

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

Conflict of interest

303   None

304

305

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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 Lymphotrophic virus. **B. Burden of infection**. Infection count represents the number of infections the

453 individuals tested positive for.

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466   **Tables**

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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
***H. pylori* 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 *H. pylori* 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) p-value	0.98 (0.96 - 0.99) 0.002	0.96 (0.95 - 0.98) <0.001	0.91 (0.90 - 0.93) <0.001	1.00 (0.99 - 1.01) 0.540	1.03 (1.01 - 1.04) <0.001	1.04 (1.03 - 1.06) <0.001
GroEL						
OR (95% C.I) p-value	1.08 (1.04 - 1.11) <0.001	1.06 (1.03 - 1.1) 0.001	1.10 (1.06 - 1.14) <0.001	0.98 (0.96 - 1.01) 0.153	1.04 (1.01 - 1.07) 0.019	1.11 (1.07 - 1.14) <0.001
HP1564						
OR (95% C.I) p-value	0.95 (0.92 - 0.97) <0.001	0.89 (0.87 - 0.92) <0.001	0.86 (0.84 - 0.89) <0.001	0.93 (0.91 - 0.95) <0.001	1.02 (0.99 - 1.05) 0.146	0.99 (0.97 - 1.02) 0.699
VacA						
OR (95% C.I) p-value	1.01 (0.99 - 1.03) 0.224	0.99 (0.97 - 1.01) 0.399	0.97 (0.94 - 0.99) 0.002	0.98 (0.97 - 1.00) 0.019	1.03 (1.01 - 1.05) 0.004	1 (0.98 - 1.02) 0.701
HP0305						
OR (95% C.I) p-value	0.94 (0.91 - 0.98) 0.004	0.94 (0.90 - 0.98) 0.004	0.95 (0.91 - 1.00) 0.041	0.94 (0.91 - 0.97) <0.001	1.01 (0.97 - 1.05) 0.523	1.05 (1.01 - 1.09) 0.013
HpaA						
OR (95% C.I) p-value	1.03 (0.99 - 1.06) 0.106	0.98 (0.94 - 1.02) 0.290	1.02 (0.98 - 1.06) 0.257	0.97 (0.95 - 1.00) 0.023	1.04 (1.00 - 1.07) 0.026	1.03 (0.99 - 1.06) 0.102
CagD						
OR (95% C.I) p-value	1.03 (1.01 - 1.05) 0.002	1.04 (1.02 - 1.06) 0.001	1.03 (1.01 - 1.05) 0.005	0.95 (0.93 - 0.96) <0.001	1.01 (1.00 - 1.03) 0.143	0.98 (0.97 - 1.00) 0.112
HyuA						
OR (95% C.I) p-value	1.02 (0.98 - 1.06) 0.254	1.12 (1.07 - 1.16) <0.001	1.15 (1.10 - 1.19) <0.001	0.96 (0.93 - 0.99) 0.003	1.01 (0.97 - 1.05) 0.613	1.02 (0.98 - 1.06) 0.325
CagM						
OR (95% C.I) p-value	1.01 (0.98 - 1.04) 0.408	0.99 (0.96 - 1.03) 0.746	0.99 (0.96 - 1.03) 0.686	0.98 (0.96 - 1.01) 0.151	1.01 (0.98 - 1.04) 0.475	1.03 (1.00 - 1.06) 0.022
Catalase						
OR (95% C.I) p-value	0.98 (0.96 - 1.01) 0.169	0.98 (0.95 - 1.01) 0.173	0.99 (0.96 - 1.02) 0.485	0.97 (0.95 - 0.99) 0.001	1.01 (0.99 - 1.04) 0.319	1.03 (1.01 - 1.06) 0.008
HP0231						
OR (95% C.I) p-value	0.96 (0.93 - 1.00) 0.028	0.93 (0.89 - 0.97) 0.001	1.00 (0.96 - 1.04) 0.838	0.97 (0.94 - 1.00) 0.023	1.01 (0.98 - 1.05) 0.515	1.06 (1.03 - 1.1) <0.001
HcpC						

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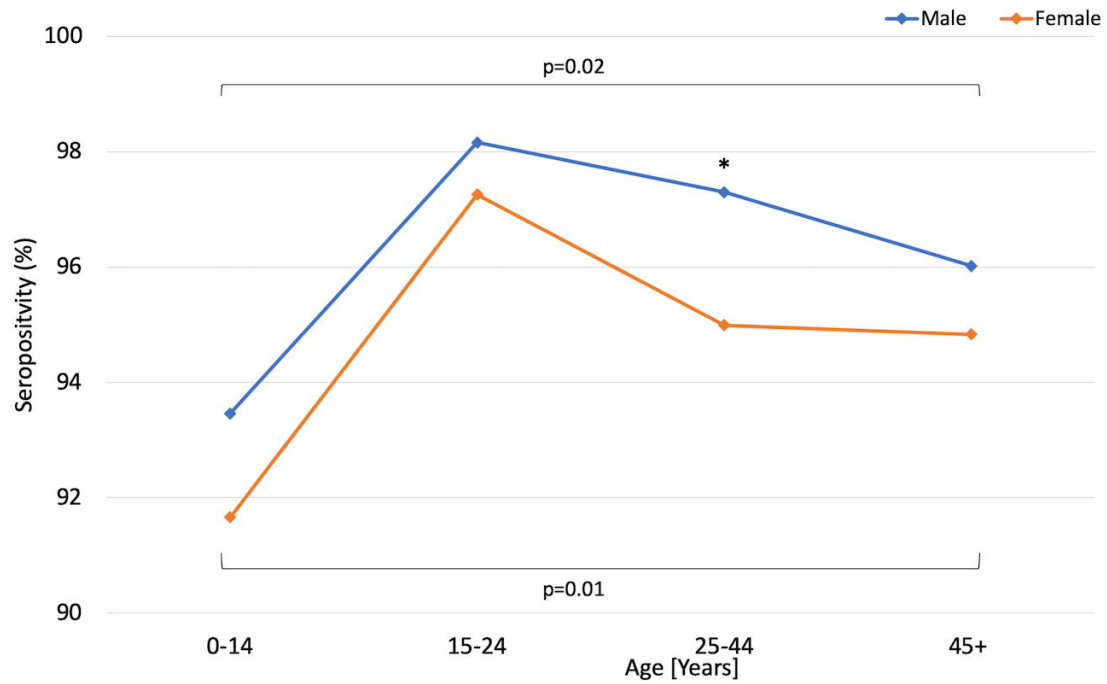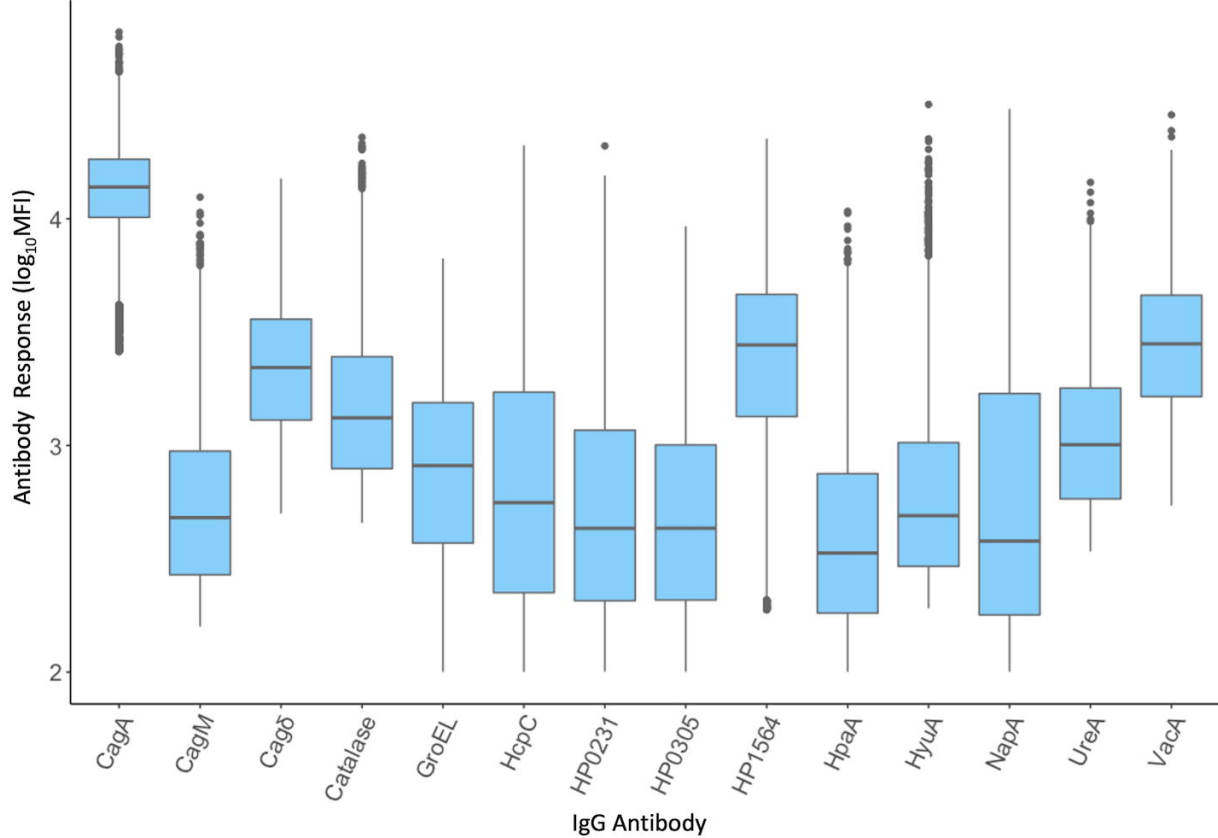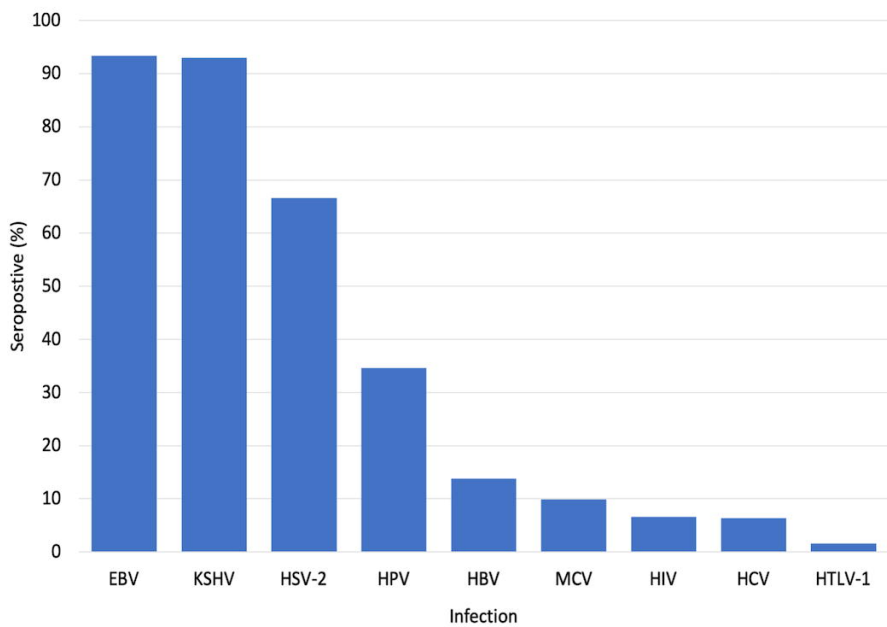|						
OR (95% C.I)	1.00 (0.95 - 1.05)	1.06 (1.00 - 1.13)	1.13 (1.07 - 1.20)	0.96 (0.92 - 1.00)	1.03 (0.98 - 1.09)	1.04 (0.99 - 1.09)
p-value	0.924	0.049	<0.001	0.044	0.219	0.137
NapA						
OR (95% C.I)	1.13 (1.07 - 1.20)	1.14 (1.07 - 1.21)	1.13 (1.06 - 1.20)	1.00 (0.95 - 1.04)	0.98 (0.92 - 1.03)	1.00 (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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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)	<0.0001
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)	<0.0001
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)	<0.0001	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)	<0.0001	0.90 (0.82 - 0.97)	0.010	0.94 (0.76 - 1.16)	0.539	0.68 (0.55 - 0.83)	<0.0001	0.75 (0.64 - 0.88)	0.001
HSV-2: Ref=Negative										
Positive	1.10 (1.05 - 1.15)	<0.0001	1.11 (1.05 - 1.16)	<0.0001	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)	<0.0001	1.30 (1.16 - 1.45)	<0.0001	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 -	0.189	1.09 (1.01 -	0.030	1.08 (0.87 -	0.492	0.89 (0.73 -	0.280	1.02 (0.87 -	0.847

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	1.13)		1.19)		1.32)		1.1)		1.19)	
HBV: Ref=Negative										
Positive	1.00 (0.93 - 1.07)	0.996	0.97 (0.9 - 1.04)	0.382	1.19 (0.98 - 1.44)	0.081	1.08 (0.89 - 1.3)	0.427	1.16 (1 - 1.34)	0.048
MCV: Ref=Negative										
Positive	0.97 (0.91 - 1.03)	0.341	1.02 (0.96 - 1.09)	0.518	1.19 (1 - 1.41)	0.046	1.04 (0.88 - 1.23)	0.664	1.03 (0.91 - 1.18)	0.618
HCV: Ref=Negative										
Positive	1.00 (0.92 - 1.09)	0.984	0.99 (0.9 - 1.09)	0.905	0.94 (0.74 - 1.2)	0.626	0.98 (0.77 - 1.25)	0.879	0.94 (0.78 - 1.14)	0.552
HTLV-1: Ref=Negative										
Positive	0.83 (0.66 - 1.03)	0.093	1.28 (1.01 - 1.62)	0.043	0.93 (0.5 - 1.7)	0.807	0.80 (0.44 - 1.46)	0.472	0.76 (0.48 - 1.22)	0.260

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