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The association of *Helicobacter pylori* with adverse pregnancy outcomes in three European birth cohorts

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Abstract

Background *Helicobacter pylori* is a prevalent infection that may complicate pregnancy, but evidence remains limited, controversial and may not apply to all pregnant women.

Objective This study aims to evaluate whether *Helicobacter pylori* is a risk factor for adverse pregnancy outcomes and to identify vulnerable subpopulations.

Study design Multiplex serology was utilized to measure blood levels of immunoglobulin G against eight *Helicobacter pylori* antigens in 1372 pregnant women from three European birth cohorts: BiB (United Kingdom), Rhea (Greece) and INMA (Spain). Outcomes of interest included gestational diabetes mellitus, gestational hypertension, preeclampsia, preterm birth and small for gestational age neonates, as well as prenatal anxiety and depression. Adjusted logistic regression models were used to evaluate the association between *Helicobacter pylori* seropositivity (overall and by antigen) and antigen specific antibody levels with the outcomes. We examined effect modification of the associations by ethnicity.

Results *Helicobacter pylori* seropositivity was detected in 18.8% (258/1372) of pregnant women. Preeclampsia was the least common outcome (26/830). *Helicobacter pylori* seropositivity was associated with the development of two or more adverse pregnancy outcomes (gestational hypertension, gestational diabetes, preterm birth, small gestational age and preeclampsia) [OR:1.32 (95% CI: 1.06–1.65), p-value: 0.01], especially in women with high antibody levels to OMP antigen [OR: 2.12 (95% CI: 1.62–2.76), p-value: 0.001]. Women with high antibody levels to *Helicobacter pylori* antigens GroEL and NapA were more likely to develop preeclampsia [OR: 2.34 (95% CI: 1.10–8.82), p-value: 0.03; OR: 4.09 (95% CI: 1.4-11.93), p-value 0.01)]. *Helicobacter pylori* seropositivity increased the odds of developing any hypertensive disorder during pregnancy among women of western ethnicity (948/1372) [OR:3.35 (95% CI: 1.29–8.74), p-value 0.03].

Conclusion Our study suggests that *Helicobacter pylori* seropositivity is a risk factor for multiple adverse pregnancy outcomes and particularly in women of western origin for hypertensive disorders during pregnancy. Moreover,

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pathogen specific characteristics reflected in the antibody responses against OMP, GroEL and NapA seem to determine disease associations.

Keywords *Helicobacter pylori*, Pregnancy outcomes, Preeclampsia, Hypertension, Ethnicity, Europe, Cohort

Background

Pregnancy-related complications continue to be a major public health problem and can have lifelong consequences for both mother and offspring [1]. Smoking, alcohol, maternal weight and age among others, are well defined risk factors for pregnancy-related complications, but if we want to develop better treatment and prevention strategies we should rethink beyond these conventional risk factors [2, 3]. A systematic review and meta-analysis, showed that *Helicobacter pylori (H.pylori)* may have a role in the pathogenesis of pregnancy complications including preeclampsia, gestational diabetes mellitus, fetal growth restriction, spontaneous abortion and birth defects [4].

H.pylori is a bacterium that colonizes the gastric environment of 44% of the world population [5]. The majority of *H.pylori* infected people are asymptomatic. *H.pylori* is related to the development of gastroduodenal disturbances such as chronic gastritis, peptic ulcer disease and gastric cancer [6], however, growing evidence indicates that *H.pylori* is implicated in several extragastric manifestations [7, 8]. Previous studies on the association between *H.pylori* and pregnancy disorders are hampered by small sample size, their focus on symptomatic *H.pylori* infections and limitations on *H.pylori* detection methods. For example, *H.pylori* induces complex antibody responses that cannot be captured by assays targeting only one antigen e.g. Cag pathogenicity island protein A (CagA). Different mechanisms are suggested about how the pathogen complicates pregnancies, including chronic inflammation (in gestational diabetes), suboptimal placental development (in preeclampsia) and micronutrient depletion (in fetal growth restriction) [9]. Moreover, during pregnancy, physiological immunological adaptations could activate a latent *H.pylori* infection and accelerate disease processes related to pregnancy disorders [10]. The association of *H.pylori* with perinatal mental disorders (depression and anxiety) has not been studied. Our interest in such associations is founded on previous studies linking *H.pylor*i with neuro-psychiatric diseases [11, 12] and symptoms of depression and anxiety in the general population [13, 14].

The aim of this study is to evaluate the association between *H.pylori* and adverse pregnancy outcomes pooling data from three European birth cohorts. We identified *H.pylori* seropositive women during pregnancy by performing multiplex serology in the same lab for all samples limiting inter-assay variability and used harmonized data between cohorts on outcomes of interest. We examined antigen-specific associations based on seropositivity and magnitude of antibody response. We hypothesize that *H.pylori* seropositivity is associated with adverse pregnancy outcomes and adverse perinatal mental disorders, and that in both cases the association is antigen specific and stronger in women with higher antibody levels. Moreover, the effect of *H.pylori* seropositivity on pregnancy outcomes does not uniformly impact the pregnant women and that susceptible individuals exist.

Methods

This is a cross-sectional analysis using data from three population-based birth cohort studies in Europe: *Born in Bradford* (BiB) in the UK, *Rhea Mother Child Cohort* study in Greece and *INfancia y Medio Ambiente* (INMA) in Spain (Sabadell and Valencia). These studies were selected because they could provide existing blood samples and they represent different populations in Europe (north, south, west and east). Overall, inclusion criteria for BiB were: to give birth at the Bradford Royal Infirmary and to be resident in Bradford before delivery [15]. Inclusion criteria for Rhea were: to be resident in the study area, to be at least 16 years old, to have the first antenatal visit in any hospitals or private clinic in Heraklion district and to not have any communication handicap [16]. Inclusion criteria for INMA were: to be resident in one of the study areas, to be at least 16 years old, to have a singleton pregnancy, to not have followed any programme of assisted reproduction, to wish to deliver in the reference hospital and to have no communication problems [17]. In this analysis we only considered singleton pregnancies. National and international guidelines (*Declaration of Helsinki and Ethical code*) were adhered to, and data was managed according to the Spanish Law on Data Confidentiality (*Ley Orgánica 15/1999 de 13 de Diciembre de Protección de Datos de carácter personal [LOPD]*) eliminating personal identifiers. The study objectives were explained to each participant, followed by the signing of an informed consent.

Exposure

Blood samples were collected at the end of the first trimester in the INMA cohort, at the end of the second trimester in the BiB cohort) and at birth (cord blood) in the Rhea cohort processed following standard procedures and stored at −80 °C until analysis. Serum aliquots of 100μL masked as regard individual identity and characteristics were shipped on dry ice to the immunology

lab in ISGlobal for serological analysis. This small blood volume required, minimizes selection bias. Immunoglobulin G (IgG) seroreactivity was measured against eight *H.pylori* antigens by fluorescent bead-based multiplex serology (1:1000 serum dilution). *H.pylori* antigens included were: Chaperonin GroEL (GroEL), Urease alpha subunit (UreaA), HP0231, Neutrophil-activating protein (NapA), *H.pylori* adhesion A (HpaA), Cag pathogenicity island protein A (CagA), catalase, vacuolating cytotoxin A (VacA), *Helicobacter* cysteine-rich protein C (HcpC), and outer membrane protein (OMP). Antibody reactivities were simultaneously quantified as median fluorescence intensity (MFI) values. Seropositivity for a given protein was defined as seroreactivity greater than the protein specific cut-off. Cut-offs were based on the reactivity of negative controls and were calculated as 10 $^{\text{mean+3 sd}}$ of $\log_{10}$-transformed MFIs of the negative controls for each antigen. *H.pylori* overall seropositivity was defined as being seropositive to at least three out of the eight antigens.

We should note that IgG levels in cord blood reflect the mother's IgG levels at delivery, which are strongly correlated with maternal levels during pregnancy according to correlations between pregnancy and cord blood samples in a group of 21 participants from the Rhea study [18].

Pregnancy outcomes

We considered the following outcomes based on a recent systematic review and meta-analysis [4]: *gestational hypertension*, *preeclampsia* (missing from INMA), *gestational diabetes mellitus (GDM)*, *preterm birth*, *small for gestational age (SGA)*. These variables were harmonized between cohorts during the EC-H2020 *LifeCycle* Project 19 and the *Helix* project [19, 20]. We also considered clustering of the aforementioned outcomes (gestational hypertension, GDM, preterm birth and SGA) and created a multimorbidity variable with and without considering preeclampsia (due to missing information from INMA). We also considered mental health outcomes particularly *prenatal depression* and *anxiety*. Outcomes definitions can be found in *additional file1*.

Statistical analysis

Descriptive statistics were used to examine differences between demographic characteristics and *H.pylori* serological results. Logistic regression models were used to study the association of: (i) *H.pylori* status (seropositive vs. seronegative), (ii) antigen-specific *H.pylori* status (seropositive vs. seronegative) and (iii) antigen-specific antibody levels [high levels (≥median of seropositive), low levels (<median of seropositives) or seronegative], with the outcomes of interest. Associations with VacA seropositivity were not examined because only two women were seropositive. Multinomial logistic regression was used to evaluate the association between *H.pylori* status and multimorbidity variables. Possible confounders were identified with directed acyclic graphs [21] based on background knowledge on determinants of the exposure and outcome variables (*additional files2* to *4*) and include: any smoking during pregnancy (yes, no); ethnicity [western (EU, Andorra, Australia, Canada, Iceland, Liechtenstein, Monaco, New Zealand, Norway, San Marino, Switzerland, USA, UK and Vatican City), non-western (all other countries) or mixed]; educational level (high: short cycle tertiary, Bachelor, Masters, Doctoral or equivalent; medium: upper secondary, post-secondary non-tertiary; low: no education, early childhood, pre-primary, primary, lower secondary or second stage of basic education); pre-pregnancy body mass index (BMI) (kg/m^2 before pregnancy); and maternal age. These variables were harmonized between cohorts during the EC-H2020 LifeCycle Project 19 and the Helix project 19,20. Statistical power was assessed previous to the analysis. For an expected Odds Ratio (OR) of 2.51 for the association between *H.pylori* and preeclampsia (the rarest outcome) [4], a statistical power of 0.8 with a 95% confidence interval could be achieved with our sample size (n=830 for preeclampsia).

We tested for effect modification of the associations by ethnicity, and stratified the analyses accordingly. This was based on heterogeneity in results from previous studies in western and non-western populations, indicating that we should take into account ethnicity to be able to identify vulnerable populations [22]. All analyses were performed with *RStudio Software 1.4.1106.*

Results

Population characteristics

A total of 1372 singleton pregnancies were included in this analysis, 571 from BiB, 322 from Rhea and 479 from the INMA cohort. The mean age among all mothers was 29.7 years old, 70.7% were of western ethnicity, 49.5% had a normal pre-pregnancy BMI, 20.8% smoked during pregnancy and 39.2% had a low educational level (Table 1).

Out of all pregnant women, 18.8% were *H.pylori* seropositive, with the most prevalent strains among seropositives being GroEL (81.8%) and Cag A (74.8%) (*additional file5*). Seroprevalence rates in each cohort were 16.6% (BiB), 16.8% (Rhea) and 22.8% (INMA) (p-value: 0.021). *H.pylori* seropositive women from BiB were more likely to be CagA seropositive (82.1%), whereas seropositive women from Rhea and INMA, were more likely to be GroEL seropositive (83.3% and 82.6% respectively) (*additional file6*).

Table 1 Population characteristics among all participants ($n = 1372$) (%) and % *H.pylori* positive (HP+(%))

	Total population ($n = 1372$)		Missings	By cohort		
				BiB ($n = 571$)	Rhea ($n = 322$)	INMA ($n = 479$)
	(%)	HP+(%)		(%)	(%)	(%)
Smoking in pregnancy (any)	20.8	17.7	39	10.9	26.6	29.1
p-value		0.65[a]		< 0.001[b]		
Ethnicity						
Western	70.7	17.4	31	32.6	100	98.2
Non-western	28.3	22.4		65	0	1.9
Mixed	1	15.4		2.3	0	0
p-value		0.09			< 0.001	
Education level						
High	27.7	19.3	64	24.4	28.8	30.6
Medium	33.1	18.7		14.7	52.7	41.1
Low	39.2	19.1		60.9	18.5	28.3
p-value		0.96			< 0.001	
Pre-pregnancy BMI[c]						
< 18.5 kg/m^2	3	23.1	68	0.4	4.1	5.2
18.5–24.9 kg/m^2	49.5	20.5		31.5	59.3	63.3
25–29,9 kg/m^2	28.1	14.7		35.9	25.8	21.5
≥ 30 kg/m^2	19.4	20.2		32.3	11.5	10.0
p-value		0.11			< 0.001	
Maternal age (mean, SD)	29.7, 5.8		38	28.4, 5.6	30.0, 5.6	31.0, 6.0
p-value					< 0.001	

p-values indicate differences in characteristics by H.pylori status in the overall population [a] and differences in characteristics by cohort [b]. BMI [c] (body mass index)

Table 2 Pregnancy related complications and their association with *H.pylori* seropositivity

Outcomes		(%)	H.pylori seropositive (%)	Unadjusted OR(95% CI) *p*-value	Adjusted [a] OR (95% CI) *p*- value	Missings data on outcomes
Gestational hypertension		6.0	5.2	0.84 (0.43–1.05) 0.58	1.29 (0.64–2.44) 0.44	53
Preeclampsia		3.1	4.4	1.54 (0.55–3.69) 0.36	2.37 (0.81–6.23) 0.09	542
Gestational diabetes		8.8	8.5	0.96 (0.56–1.79) 0.89	1.13 (0.63–1.94) 0.68	219
Preterm birth		13.2	11.8	0.85 (0.55–1.27) 0.46	1.04 (0.65–1.49) 0.86	22
Birth weight for gestational age	SGA	9.1	10.6	1.22 (0.78–1.91) 0.29	1.16 (0.71–1.89) 0.55	16
	AGA	86.5	86.3	1	1	
	LGA	4.4	3.1	0.67 (0.31–1.42) 0.39	0.74 (0.34–1.61) 0.46	
Prenatal depression		15.7	17.9	1.02 (0.97-1,08) 0.29	1.04 (0.98–1.09) 0.18	211
Prenatal anxiety		20.0	19.7	0.99 (0.93–1.05) 0.81	0.99 (0.93–1.05) 0.81	207

[a]Adjusted by cohort, ethnicity, educational level, maternal age, pre-pregnancy BMI (body mass index) and smoking in pregnancy. SGA (small for gestational age), AGA (adequate for gestational age), LGA (large for gestational age)

Helicobacter pylori and adverse pregnancy outcomes

The prevalence of pregnancy outcomes overall and in *H.pylori* seropositive women, as well as the unadjusted and adjusted estimates of the association between *H.pylori* and each outcome are shown in Table 2. The rarest outcome was preeclampsia (3.1%) and preterm birth was the most common (13.2%). Also 15.7% and 20.0% of women had prenatal depression and anxiety respectively (Table 2). Gestational hypertension, and GDM, were more prevalent in women from the BiB cohort. Preterm birth was more prevalent in the Rhea cohort (*additional file7*). No statistically significant associations were

Table 3 Presence of multiple pregnancy-related outcomes and their association with *H.pylori* seropositivity (HP+) ($n = 1372$)

	nº outcomes	*n*(%)	Adjusted [a] OR in HP+, (95% CI) *p*- value	Missings data on outcomes
Gestational hypertension, gestational diabetes, preterm birth and SGA	0	768 (68.1)	1	244
	1	303 (26.9)	1.01 (0.71–1.44), 0.96	
	≥ 2	57 (5.1)	1.52 (1.44–1.57), < 0.001	
Gestational hypertension, gestational diabetes, preterm birth, SGA and preeclampsia	0	372 (58.1)	1	732
	1	205 (32.0)	0.95 (0.60–1.5), 0.83	
	≥ 2	63 (9.8)	1.32 (1.06–1.65), 0.01	

[a]Adjusted by cohort, smoking during pregnancy, ethnicity, educational level, maternal age, pre-pregnancy body mass index and smoking in pregnancy educational level. SGA (small for gestational age)

Table 4 Adjusted odds ratio [OR, (95% CI)] between *H.pylori* seropositivity, gestational hypertension, preeclampsia and any hypetensive disorder of pregnancy stratified by ethnicity (western / non-western)

Outcomes	Adjusted [a] OR, 95% CI; *p*-value		*p*-value for interaction
	Western	Non-western	
Gestational hypertension	1.73 (0.77–3.92) 0.15	0.77 (0.25–2.38) 0.64	0.07
Preeclampsia	2.96 (0.86–10.23) 0.11	1.77 (0.31–9.99) 0.34	0.07
Any hypertensive disorder of pregnancy	3.35 (1.29–8.74); 0.03	0.97 (0.34–2.76) 0.82	< 0.001

[a] Adjusted by cohort, smoking during pregnancy, maternal age, pre-pregnancy body mass index and educational level

revealed between *H.pylori* seropositivity and each outcome of interest (Table 2). However, there was weak evidence (p-value 0.09) for an association between *H.pylori* seropositivity and preeclampsia (OR: 2.37 95% CI: 0.81–6.23) (Table 2). Results by cohort are shown in *additional file7*.

H.pylori seropositive versus seronegative women were more likely to present ≥ 2 adverse outcomes (OR: 1.52 95%CI: 1.44–1.57, p-value: <0.001 for the score without considering preeclampsia due to missing info in INMA cohort, and OR: 1.32 95%CI: 1.06–1.65, p-value: 0.01 when considering preeclampsia in the score) (Table 3).

Helicobacter pylori antigen-specific antibody responses and adverse pregnancy outcomes

No significant associations were revealed between antigen-specific seropositivity and single outcomes (*additional file8*). When we distinguished seropositives according to the magnitude of antibody response, we identified higher odds of developing preeclampsia in those with higher antibody levels (above the median) to GroEL (OR: 3.12 95%CI:1.10–8.82, p-value: 0.03, p-value for trend: 0.04) and NapA (OR:4.09 95%CI: 1.40-11.93, p-value: 0.01, p-value for trend: 0.03) compared to seronegative women to the corresponding antigens (*additional file9*). Regarding presentation of multiple complications, seropositivity to OMP was associated with higher odds of developing two or more complications including preeclampsia (OR: 2.12 95%CI:1.62–2.76, p-value: <0.001), whereas inverse association was observed for seropositivity to HpaA (OR: 0.84 95%CI: 1.38–2.43) (*additional file10*). Women with low levels of NapA were more likely to present multiple complications including preeclampsia (OR:1.14 95%IC: 1.01–1.96, p-value:0.04), and both high and low levels of GroEL were associated with the development of ≥2 complications (OR:2.34 95%CI: 1.71–3.19, p-value < 0.001 and OR: 1.85 (1.38–2.49),<0.001 respectively) *(additional file11)*.

Results by ethnicity

Ethnicity was found to modify the association between *H.pylori* and any hypertensive disorder of pregnancy (Table 4). When the analysis was stratified, *H.pylori* seropositivity women of western origin had higher odds of developing any hypertensive disorder (OR: 3.35 95%CI: 1.29–8.74, p-value: 0.03). Of note, women of western versus of non-western origin were less likely to be CagA, HpaA, NapA and UreaA seropositive (*additional file12*).

Discussion

In this analysis across three birth-cohorts in Europe, we observed that *H.pylori* seropositivity was associated with development of more than 2 adverse pregnancy outcomes. High responders to GroEL and NapA *H.pylori* specific antigens were more likely to develop preeclampsia, highlighting the role of pathogen-specific characteristics in these associations. Moreover, women of western origin *H.pylori* seropositive were more likely to develop any hypertensive disorder of pregnancy including preeclampsia, indicating a population at risk.

*H.pylor*i has emerged as a risk factor for a number of adverse pregnancy outcomes [4]. However, the development of more than one adverse outcome has been largely neglected in previous studies although it represents an important challenge of health systems and may help

identify common risk factors. For example, one previous study showed that *H.pylori* seropositive women were at increased risk of complicated pregnancies by both preeclampsia and SGA [23]. We showed that *H.pylori* seropositive women were more likely to present≥2 adverse pregnancy outcomes including preeclampsia, gestational hypertension, GDM, preterm birth and SGA. We also observed that OMP seropositivity was particularly associated with co-occurence of multiple adverse outcomes. Existing literature suggests OMP's influence on the virulence of *H.pylori* infection, particularly through its involvement in adhesion to human epithelial cells, bacterial penetration through defense barriers leading to colonization, and its role in evading the immune system to establish persistent infection [24].

According to a meta-analysis, *H.pylori* infection doubles the risk of developing preeclampsia [25]. We observed a similar effect estimate although at a p-value of 0.09. Given the fact that most epidemiological studies, including ours, are assessing populations with mixing ethnic backgrounds, we investigated whether ethnicity could modify the association between *H.pylori* and preeclampsia and/or hypertensive disorders of pregnancy. The effect estimates were stronger among women of western origin. This heterogeneity in associations by ethnicity could reflect differences in the age of infection, childbearing and sanitation practices, *H.pylori* variants dominant in western and non-western countries as well as genetic differences of the hosts [26]. Moreover, previous studies have concluded that *H.pylori* is likely more harmful in Asians (increasing the risk of 15 types of diseases) and in Europeans [27]. The role of ethnicity warrants further investigation.

Our antigen-specific analysis included 8 strain-specific antigens making it more informative than previous analyses that are largely focused on CagA responses. In our study, high antibody responses against GroEL and NapA were associated with the presentation of preeclampsia. Previous studies have demonstrated that NapA activates neutrophils, antagonizes oxidative stress and mediates the *H.pylori* binding to host cells and stomach mucus [28]. GroEL belongs to the family of molecular chaperones and is associated with the *H.pylori* adhesion to human gastric epithelial cells and induces inflammation responses [29]. Further, these antigen-specific associations might reflect immune mediated mechanisms implicated in the pathogenesis of hypertensive disorders during pregnancy; for example, a possible cross-reaction between specific *H.pylori* antibodies and antigens localized in placental tissue and endothelial cells (molecular mimicry) [30]. An in vivo study has demonstrated the presence of cross-reactivity between anti-CagA antibodies and β-actin of trophoblast cells [31].

No significant link between *H.pylori* and prenatal depression and anxiety was observed in our study. We should acknowledge though that the three cohorts used different methods to diagnose depression and anxiety during pregnancy. Nevertheless, prevalence of the disorders seems to be similar to previous estimates in each country [32]. It's worth noting that we are the first to examine the association with pregnancy related mental health disorders, and additional research is warranted.

In 2017, the American College of Gastroenterology expanded indications for *H.pylori* testing and recommended treating all *H.pylori* infections regardless of symptomatic or pathologic burden [33]. The most common regimens are the prescription of two antibiotics, mostly amoxicillin and clarithromycin combined for two weeks [34].

Further research is needed to provide robust evidence on the role of *H.pylori* on pregnancy related outcomes, particularly hypertensive disorders of pregnancy, taking into account pathogen specific factors and host characteristics. Moreover, we expect further studies investigating the potential association of the infection with prenatal mental disorders, since no previous literature exists on this topic.

Our study is strengthened by its study design, size and the pooling of data from three European birth cohort studies. Particularly, we performed the same serological testing in all samples in the same lab under identical conditions, to minimize heterogeneity on methods of assessment of exposure and limiting inter-assay variability and batch effects. Compared to the less extensive serological testing of previous studies, our multiplex approach allowed us to identify antigen-specific associations. Moreover we were able to assess associations related to the magnitude of antibody response. Another strength is the availability of harmonized data on outcomes (apart from depression and anxiety) and confounders [35]. These data are considered reliable and they have been used before in other studies [36, 37].

There are also limitations to consider. First, due to ethical and practical considerations, we had to rely on serology as a proxy for *H.pylori* exposure instead of the gold standard of histology. We used the presence of IgG antibodies to evaluate the exposure, which is widely used to estimate prior exposure to a particular pathogen It is likely that most cases of maternal *H.pylori* seropositivity correspond to a primary infection acquired before pregnancy because *H.pylori* is usually acquired during childhood. We also speculate that most cases of seropositivity in this study represent a primary untreated infection because largely *H.pylori* infection remains asymptomatic and undiagnosed, while re-infections are unusual. Secondly, the Rhea cohort provided cord blood samples instead of maternal samples. However, IgG levels

in cord blood reflect the mother's IgG levels at delivery, which are strongly correlated with maternal levels during pregnancy [18]. Lastly, missing data on preeclampsia might have limited the power of the specific analyses, although our sample size ($n=830$) was sufficient for a statistical power of 0.8, and the missing information was for all participants of a specific cohort (e.g. information for preeclampsia was not available in INMA cohort). Chance findings are always of concern when multiple comparisons are performed but the analyses have followed a priori hypothesis and the pattern of results does not point to chance findings. Nonetheless, after accounting for multiple testing of all statistical tests between exposures and outcomes by controlling the false discovery rate at 0.25 based on the method by Benjamini and Hochberg, we still observed statistically significant associations for *H.pylori* seropositivity, seropositivity and levels of CagA, GroEL, HcpC, HpaA, NapA and OMP and seropositivity to UreaA with the development of multiple adverse outcomes; and for high NapA level with preeclampsia.

Conclusion

Among women from three European birth cohorts, *H.pylori* seropositivity in pregnancy was associated with development of two or more adverse pregnancy outcomes. We also provided support for heterogeneous associations between ethnic groups for *H.pylori* and hypertensive disorders of pregnancy, with women of western origin being at increased risk. Further research is needed to accumulate the necessary evidence required for the development of long-awaited opportunities to prevent pregnancy-related disorders by screening and treating *H.pylori*.

Abbreviations

H.pylori	*Helicobacter pylori*
IgG	Immunoglobulin G
BiB	Born in Bradford
Rhea	Rhea Mother Child Cohort
INMA	INfancia y Medio Ambiente
GroEL	Chaperonin GroEL
UreaA	Urease alpha subunit
NapA	Neutrophil-activating protein
HpaA	*H.pylori* adhesion A
CagA	Cag pathogenicity island protein A
VacA	Catalase, vacuolating cytotoxin A
HcpC	*Helicobacter* cysteine-rich protein C
OMP	Outer membrane protein
GDM	Gestational diabetes mellitus
SGA	Small for gestational age
OR	Odds Ratio

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12884-024-06901-5.

Additional file 1: Outcomes definition. Harmonized through either EC-H2020 LifeCycle or Helix project (.docx). This file provides the definition used for each of the outcomes evaluated in this study. Because we worked with three different cohorts, the definitions were harmonized through the LifeCycle or the Helix project.

Additional file 2: Pregnancy outcomes directed acyclic graph (pdf). Green circles indicate exposures, blue circles with a black lines indicate outcome, solid blue circles indicate intermediate variables, solid red circles indicate confounding variables. Red arrows indicate a confounded variable path and green arrows indicate a causal path.

Additional file 3: Birth outcomes acyclic graph and legend (pdf).

Additional file 4: Perinatal mental disorders outcomes directed acyclic graph (pdf).

Additional file 5: *Helicobacter pylori* seroprevalence overall and to 8 specific antigens in pregnant women (.docx). This table provides the *Helicobacter pylori* seroprevalence overall (defined as being positive to at least three antigens) and the seroprevalence for each of the antigens analyzed.

Additional file 6: Overall *Helicobaceter pylori* seropositivity and seropositivity rates for each antigen among all participants and by cohort (.docx). This table provides the *Helicobacter pylori* seroprevalence overall (defined as being positive to at least three antigens) and the seroprevalence for each of the antigen analyzed for each cohort, and the p-value for de difference in seroprevalence between them.

Additional file 7: Pregnancy outcomes and their association with *Helicobacter pylori* seropositivity by cohort. This table provides the prevalence for each outcome in the overall population and by cohort, as well as the adjusted odds ratio for the relationship between *Helicobacer pylori* seropositivity and pregnancy outcomes. It also shows the p-values for the differences by cohort in outcomes prevalence.

Additional file 8: Adjusted Odds Ratio [(95% CI), p-value] between *Helicobacter pylori* antigen-specific seropositivity and pregnancy-related complications ($n=1372$). This table provides the adjusted odds ratios for the relationship between the *Helicobacter pylori* antigens seropositivity analyzed and pregnancy outcomes in the overall population.

Additional file 9: Adjusted Odds Ratio [95% CI, p-value] between *Helicobacter pylori* antigen-specific antibody levels and pregnancy-related complications ($n=1372$).This table provides the adjusted odds ratios for the relationship between the *Helicobacter pylori* antigens level (low if the antibody level is below the median seropositivity level, and high if the level is above the median) analyzed and pregnancy outcomes in the overall population.

Additional file 10: Adjusted Odds Ratio [(95%CI), p-value] between *Helicobacter pylori* antigen-specific seropositivity and multimorbidity outcomes ($n=1372$). This table shows the adjusted odds ratio for the association between *Helicobacter pylori* antigens seropositivity and multimorbidity (gestational hypertension, gestational diabetes, preterm birth and small gestational age and preeclampsia). Likelihood of developing 0, 1 or 2 or more outcomes at the same time when participants were seropositive to the antigens.

Additional file 11: Adjusted Odds Ratio [(95%CI), p-value] between *Helicobacter pylori* antigen-specific antibody levels and presence of multiple pregnancy-related outcomes.This table shows the adjusted odds ratio for the association between *Helicobacer pylori* antigens serological level (low if the antibody level is below the median seropositivity level, and high if the level is above the median) and multimorbidity (gestational hypertension, gestational diabetes, preterm birth and small gestational age and preeclampsia). Likelihood of developing 0, 1 or 2 or more outcomes at the same time depending on the serological level to the antigens.

Additional file 12: Protein specific seropositivity rates by ethnicity among all ($n=1372$). This table shows the *Helicobacter pylori* specific antigens seroprevalence according to ethnicity (seroprevalence among western and non-western women).

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Data availability
Data used for this study are not publicly available.

Declarations

Ethics approval and consent to participate
All studies received approval from the ethics committees of the centers involved and written informed consent was obtained from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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