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ORIGINAL ARTICLE

Seroprevalence of *Helicobacter pylori* in Bosnia and Herzegovina: a single-centre cross-sectional study

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ABSTRACT

Aim Many studies have demonstrated that mor than half of the world's population is infected with *Helicobacter pylori (H. pylori)*. To evaluate the current *H. pylori* seroprevalence in Bosnia and Herzegovina (B&H), *H. pylori* antibodies (immunoglobulin G, IgG) from patients with suspected presence were analysed.
Methods In total, 201/471 (42.7%) males and 270/471 (57.3%) females were enrolled between June 2024 and July 2024. They were tested using the enzyme-linked immunosorbent assay (ELISA) method.
Results The overall seroprevalence of *H. pylori* infection was 214 (out of 471; 45.4%) and did not differ in relation to sex. The seroprevalence rate of *H. pylori* was highest in the 50–69 age group, 81 (out of 137; 59.1%; 95% CI: 2.2–5.6), followed by the ≥70 age group, 17 (out of 31; 54.8%; CI: 2.0–6.7), and the 30–49 age group, 101 (out of 219; 46.1%; 95% CI: 1.7–4.3). The lowest seroprevalence rate was in the younger age group (≤29) with 15 (out of 84; 17.8%). Older age groups were more likely to be *H. pylori* positive and equivocal, while younger age groups were negative for *H. pylori* infection.
Conclusion This single-canter study is the first study providing information on the *H. pylori* seroprevalence in the B&H population and investigating its association with age and sex. Further research is needed to explore other risk factors and to develop effective ways to reduce the burden of this infection.
Keywords: age distribution, cross-sectional studies, enzyme-linked immunosorbent assay, immunoglobulin G, prevalence, ELISA, immunoglobulin G, infection, population

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is one of the most prevalent bacterial infections worldwide (1), with nearly half of the global population estimated to be infected (2,3). The infection is particularly common in low- and middle-income countries (4).

Recent global meta-analyses showed that the prevalence of *H. pylori* infection declined from 52.6% before 1990 to 43.9% between 2015 and 2022, mainly due to decreases in the Western Pacific, Southeast Asian, and African regions. However, infection rates remain high in other parts of the world, especially among children and adolescents (5).

In addition to these differences in prevalence, the growing problem of antibiotic resistance, particularly to clarithromycin (6), has become a major obstacle to successful *H. pylori* eradication (7). Studies report that resistance rates frequently exceed 15% in the Balkans region, reaching around 24% in Serbia (9), 28% in B&H (8), and 34.6% in Croatia (10). In Slovenia, however, the resistance rate has declined from 16% during 2013–2018 to 13.5% in 2019–2023 (11,12). Given these resistance rates, current international guidelines emphasize the need to adapt eradication therapy to local resistance patterns. According to the Maastricht VI guidelines, when clarithromycin resistance exceeds 15% in a population, standard triple therapy is no longer considered adequate (13). In such circumstances, bismuth quadruple therapy (BQT) is recommended because it avoids clarithromycin resistance and effectively overcomes metronidazole resistance, maintaining eradication rates typically above 90% (14,15). This makes BQT the preferred first-line regimen in areas with elevated antibiotic resistance (13,16).

In this context, there is a clear need for current, locally generated data on *H. pylori* seroprevalence to support evidence-based management and prevention efforts.

Therefore, the aim of this study was to conduct a preliminary study on *H. pylori* seroprevalence in B&H and explore its variation across different age groups and between sexes to establish a basis for future population-based studies.

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PATIENTS AND METHODS

Study design and patients

This single-canter cross-sectional study was conducted in the Eurofarm Polyclinic in Sarajevo. A consecutive sampling method was employed, including all patients who came to the laboratory for *H. pylori* testing and consented to participate between 8 June and 25 July 2024. Out of 500 patients, 471 (201 males and 270 females) were included in the study. The remaining 29 were excluded because of missing data or insufficient sample quality (Figure 1). No further exclusion and inclusion criteria were applied. Since the study was anonymous, the only data of recruited patients available for statistical analyses were age and sex.

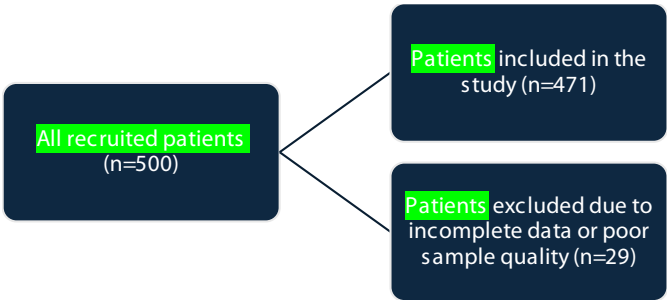

Figure 1. Patient flow diagram

The study was approved by the Ethical Committee of the Faculty of Engineering and Natural Sciences, International Burch University, Sarajevo, B&H. All patients older than 18 signed a written informed consent prior to the study, whereas parents or legal guardians provided consent for patients who were less than 18 years old.

Methods

Blood samples (3 mL) were obtained from a peripheral vein using standard venipuncture technique. Samples were kept frozen at -30°C until analysis, and each was subjected to a single freeze-thaw cycle prior to testing. *H. pylori* immunoglobulin G (IgG) was detected using commercial kits for enzyme-linked immunosorbent assay (ELISA, Euroimmun, Medizinische Laboratordiagnostika AG, Germany).

According to Euroimmun protocol (17), intra-assay coefficients of variation, determined from 20 replicates, ranged from 3.1% to 3.2%. Inter-assay coefficients of variation, based on four replicates across six runs, ranged from 3.4% to 4.6%. Sensitivity and specificity of 100% were reported based on 59 clinically characterized samples, excluding borderline results. No sensitivity or specificity data specific to the Balkan population are available.

The laboratory did not participate in external quality assessment programs during the study. Testing was performed according to the manufacturer's instructions following sample incubation, washing, conjugate incubation, washing, substrate incubation, and stopping the reaction. Afterwards, the colour intensity of samples was measured at a wavelength of 450 nm on ELISA plate reader.

Patients' *H. pylori* status was classified according to Euroimmun guidelines (17) as positive (ratio ≥1.1), equivocal (ratio ≥0.8 to <1.1), or negative (ratio <0.8). Equivocal results were not retested and were considered negative.

A post-hoc precision calculation for observed prevalence of 45.4% with a 95% confidence level (α=0.05) and sample size (n=471) yielded a confidence interval of (40.9%–49.9%), corresponding to a precision of ±4.5%.

Statistical analysis

To summarize the data, descriptive statistics were employed. The study employed the $\chi 2$ test to assess the association between categorical variables and Cramer's V to measure the strength of the association. The association for Cramer's V value was considered weak (<0.1), moderate (0.11–0.31), or strong (>0.31).

Log-binomial regression was conducted to estimate the prevalence ratio (PR) with 95% confidence intervals (CI). A $p<0.05$ was considered statistically significant.

RESULTS

In total, serum samples from 471 patients aged 3–88 years were collected. Of included patients, 214 tested positive for *H. pylori*, resulting in a seroprevalence of *H. pylori* infection of 45.4% in the study population. A higher number of samples was obtained from females, 471 (57.3%), than from males, 201 (42.7%) (Table 1).

The seroprevalence rates of anti-*H. pylori* IgG in females and males were similar, 126 (46.7%) and 88 (43.8%), respectively, without significant association between sex and *H. pylori* seroprevalence (p=0.500; Cramer's V=0.032). Log-binomial regression analysis showed that males had approximately 6% lower prevalence compared to females (PR=0.94; 95% CI: 0.77–1.15), but this difference was not statistically significant (p=0.536) (Table 1).

Most of tested patients were middle-aged, between 30–49 years, 219 (46.5%) (Table 2).

The seroprevalence rate of anti-*H. pylori* IgG was lowest in the youngest patients (≤29 years), 84 (17.8%), serving as the reference group. Prevalence increased substantially in older age groups: 46.1% among those aged 30–49 (PR=2.7; 95% CI: 1.7–4.3), 59.1% in the 50–69 group (PR=3.5; 95% CI: 2.2–5.6), and 54.8% in those aged ≥70 (PR=3.6; 95% CI: 2.0–6.7)

Table 1. Association between sex and *H. pylori* seroprevalence

Sex (No of patients)	No (%) of patients				PR (95% CI)*	p (regression)†	Cramer's V‡	χ^2 (p)§
	Positive	Equivocal	Negative	Total				
Female (270)	126 (46.7)	17 (6.3)	127 (47.0)	471 (57.3)	1 (Reference)	—	0.032	0.455 (0.500)
Male (201)	88 (43.8)	12 (6.0)	101 (50.2)	201 (42.7)	0.94 (0.7–1.1)	0.502		
Total (471)	214 (45.4)	29 (6.2)	228 (48.4)	471 (100)	—	—	—	—

*Prevalence Ratio (PR), ratio of prevalence between males and females (females as reference group); †p, statistical significance of log-binomial regression with 'Positive' as outcome and Female' as reference group; ‡Cramer's V test (ranges from 0 to 1; 0.032 indicates a weak association); §χ^2 test, association between sex and *H. pylori* status

Table 2. Association between age and *H. pylori* seroprevalence

Age group (No of patients) (years)	No (%) of patients				PR (95% CI)*	p (regression)†	Cramer's V‡	χ² (df) pδ
	Positive	Equivocal	Negative	Total				
≤29 (84)	15 (17.9)	1 (1.2)	68 (81.0)	84 (17.8)	1 (Reference)	—		
30–49 (219)	101 (46.1)	14 (6.4)	104 (47.5)	219 (46.5)	2.7 (1.7–4.3)	<0.001	0.319	44.982 (<0.0001)
50–69 (137)	81 (59.1)	9 (6.6)	47 (34.3)	137 (29.1)	3.5 (2.2–5.6)	<0.001		
≥70 (31)	17 (54.8)	5 (16.1)	9 (29.0)	31 (6.6)	3.6 (2.0–6.7)	<0.001		
Total	214 (45.4)	29 (6.2)	228 (48.4)	471 (100)	—	—	—	—

*Prevalence Ratio (PR), ratio of prevalence between males and females (females as reference group); †p, statistical significance of log-binomial regression with 'Positive' as outcome and '≤29 years' as reference group); ‡Cramer's V test (ranges from 0 to 1; 0.032 indicates a weak association); δχ^2 test, association between sex and *H. pylori* status)

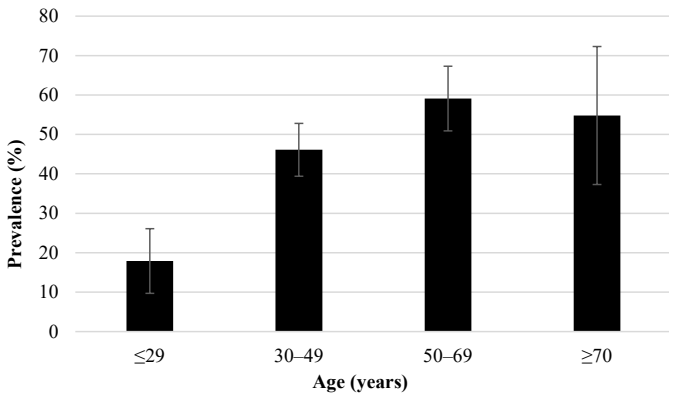

Figure 2. Age-stratified bar plot (with 95% confidence interval)

(Figure 2). A significant association was found between age group and infection status (p<0.0001), with the strength of this relationship assessed by Cramer's V at 0.319, indicating a moderate association (Table 2).

DISCUSSION

In this single-center cross-sectional study conducted in B&H, 45.4% of all tested patients were serologically positive to *H. pylori*, 6.2% were equivocal, and 48.4% were negative. These results indicate a substantial presence of *H. pylori* infection within the sampled population. When analysing demographic factors, no significant association was found between sex and *H. pylori* infection status. However, age was strongly associated with infection status, with older patients showing substantially higher prevalence rates compared to younger patients. The lowest prevalence was seen in the youngest age group (≤29 years), while infection rates increased progressively with age. This trend likely reflects cumulative lifetime exposure, variations in living conditions, and differences in health behaviours across age groups.

A recent global seroprevalence study confirmed the trend of high seroprevalence rates in the Balkans region with 13.3% in Croatia, 38.5% in Serbia and even 63.0% in Albania (5). When compared with these data from neighbouring countries, our findings indicate a higher seroprevalence in B&H compared to Croatia and Serbia, but still lower than the prevalence reported in Albania. This suggests that B&H fits within the regional pattern of a higher seroprevalence rate of *H. pylori*, although differences in socioeconomic status, sanitation, and healthcare access may influence variations between the countries.

Both the high seroprevalence rate of *H. pylori*, particularly among older populations, and the high clarithromycin resistance earlier reported to be 28% (8) underscore the need for targeted public health interventions and localized treatment strategies in B&H. According to GLOBOCAN 2022 data, B&H reported 687 new cases of stomach cancer in 2022, representing roughly 4.8% of all cancer diagnoses in the country (18). Effective *H. pylori* eradication is also a key strategy for gastric cancer prevention, given that persistent *H. pylori* infection is a major risk factor for malignancy (19).

Although this study represents an important first step in defining the seroepidemiology of *H. pylori* in B&H, it had several limitations that need to be considered. First, as the study was conducted at a single laboratory with the relatively small number of samples, the results may be affected by referral bias, potentially leading to an overestimation of the true seroprevalence in the general population and may affect the generalizability of our findings. Consequently, the findings should be interpreted as preliminary, providing a basis for further, larger-scale population studies in B&H. Second, the other relevant factors known to influence the risk of *H. pylori* infection, such as educational level, living conditions and sanitation, the type of residence (urban or rural), smoking, dietary habits, body mass index, were not included in our analysis. Inclusion of these variables could provide deeper insights into the epidemiological patterns and risk factors associated with *H. pylori* infection. Third, we did not collect data on patients' symptomatic status, clinical indications for testing (e.g., dyspepsia or post-therapy evaluation), prior *Helicobacter pylori* eradication treatments, recent systemic antibiotic use, or current proton pump inhibitor therapy (13). The absence of these data limits the ability to stratify prevalence accordingly and may have introduced the bias affecting the accuracy of our seroprevalence estimates. Fourth, the enrolment period in our study was relatively short, which may have resulted in seasonal or temporal biases. A longer enrolment period would allow for the capture of more comprehensive data, reducing potential variability and providing more accurate reflection of the seroprevalence rates over time.

Even though serological analysis is one of the most accessible and inexpensive methods for detecting *H. pylori* infection, it has important limitations that need to be acknowledged. IgG antibodies cannot distinguish active from past infection and remain detectable for months or even years after eradication, meaning that serology cannot be used to confirm cure (20). Moreover, because antibody levels decline slowly after successful treatment, studies based on serological diagnostic methods may lead to overestimation of prevalence, particularly in studies based on symptomatic populations (21).

In our study, age and sex were analysed as key demographic factors in relation to *H. pylori* seroprevalence. Although the influence on infection appears limited, both may affect host susceptibility and immune response. Age can modulate the

gastric inflammatory pattern during infection, while hormonal and behavioural differences between sexes may partly explain variations in infection rates (22).

A probability-based, multicentric follow-up study considering the relevant determinants mentioned in the limitations of the study is therefore recommended to provide a more comprehensive understanding of *H. pylori* epidemiology and to improve future public health strategies.

In conclusion, this is the first study to provide data on *H. pylori* seroprevalence in B&H. While limited by its single-canter cross-sectional design, the findings highlight the presence of a substantial burden of infection in the population. Age and sex, as key demographic factors with a negligible role in *H. pylori* infection, were included to assess their association within the studied population and to provide a basis for future research exploring additional risk factors and guiding public health strategies to reduce infection rates.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare

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