Prevalence of Helicobacter pylori Infection Detected by ELISA in Blood and Stool Samples: A Cross-Sectional Study in Al-Bayda, Libya (2024–2025)

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انتشار عدوى البكتيريا البوابية **(Helicobacter pylori)** المكتشفة بواسطة اختبار ELISA في عينات الدم والبراز: دراسة مقطعية في مدينة البيضاء، ليبيا (2024–2025)

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Abstract

Background and Aim: Helicobacter pylori infection represents a major global health challenge, with epidemiological patterns showing considerable geographic variation. This investigation sought to evaluate the current status of H. pylori infection within a patient population in Al-Bayda, Libya, focusing on infection frequency and its correlation with key demographic variables.

Methods: This observational analysis was carried out between 2024 and 2025. The study cohort comprised 748 individuals. Detection of H. pylori was performed using enzyme-linked immunosorbent assay (ELISA) techniques on two sample types: serum for quantifying specific IgG antibodies and stool for identifying bacterial antigens.

Results: Laboratory analysis identified a positive infection rate of 39.3%. The data revealed a higher frequency of positive results among female participants (64.6%) compared to males

(35.4%). The adult demographic aged 20-46 years accounted for the largest share of infections. A substantial rise in case detection was noted in 2025 relative to 2024. The stool antigen test demonstrated a higher detection yield (62.9% of positives) than serological antibody testing (37.1%). Statistical evaluation confirmed significant relationships, particularly between the type of specimen assayed and the timing of testing.

Conclusion: The findings indicate that *H. pylori* remains a clinically significant infection in the studied Libyan community. The observed demographic patterns and the comparative performance of diagnostic approaches offer valuable information for guiding local healthcare interventions. Enhancing public health initiatives focused on accurate diagnosis and management is essential for addressing the impact of this pathogen.

Keywords: Helicobacter pylori, Epidemiology, Seroprevalence, Libya, Diagnostic Methods, Stool Antigen Test, IgG Antibodies.

الملخص:

الكلمات المفتاحية: *البكتيريا البوابية*، الوبائيات، الانتشار المصلي، ليبيا، طرق التشخيص، اختبار مستضد البراز، الأجسام المضادة IgG

الملخص:

الخلفية والهدف: تمثل عدوى *البكتيريا البوابية* تحديًا صحيًا عالميًا بارزًا، حيث تُظهر أنماطها الوبائية تباينًا جغرافيًا واسع النطاق. هدفت هذه الدراسة إلى تقييم الوضع الراهن لعدوى *البكتيريا البوابية* في مدينة البيضاء، ليبيا، مع التركيز على معدل الانتشار وعلاقته بالمتغيرات الديموغرافية الرئيسية.

الطرق: أُجريت دراسة رصدية خلال الفترة الممتدة من عام 2024 إلى عام 2025، شملت 748 مشاركًا. تم الكشف عن العدوى باستخدام تقنية المقايسة المناعية المرتبطة بالإنزيم (ELISA) على نوعين من العينات: مصل الدم للكشف عن الأجسام المضادة النوعية IgG، وعينات البراز لتحديد المستضدات البكتيرية.

النتائج: أظهرت التحاليل المخبرية معدل إيجابية إجمالي بلغ 39.3%. كان معدل الإصابة بين الإناث أعلى (64.6%) مقارنة بالذكور (35.4%). مثلت الفئة العمرية البالغة (20–46 عامًا) النسبة الأكبر من الحالات الإيجابية. كما لوحظت زيادة ملحوظة في عدد الحالات المشخصة خلال عام 2025 مقارنة بعام 2024. وأظهر اختبار مستضد البراز حساسية أعلى (62.9% من النتائج الإيجابية) مقارنة بالفحص المصلي للأجسام المضادة (37.1%). وقد أظهر التحليل الإحصائي وجود علاقات ذات دلالة معنوية، خصوصًا بين نوع العينة المفحوصة وزمن إجراء الاختبار.

الاستنتاج: تؤكد النتائج أن عدوى *البكتيريا البوابية* لا تزال تمثل مشكلة صحية مهمة في المجتمع الليبي المدروس. وتوفر الأنماط الديموغرافية المكتشفة والمقارنة بين طرق التشخيص معطيات قيّمة يمكن أن تسهم في تحسين التدخلات الصحية المحلية. كما أن تعزيز برامج الصحة العامة التي تركز على التشخيص الدقيق والمعالجة الفعّالة يُعد أمرًا جوهريًا للحد من العبء الناجم عن هذا الميكروب.

الكلمات المفتاحية: *البكتيريا البوابية*، الوبائيات، الانتشار المصلي، ليبيا، طرق التشخيص، اختبار مستضد البراز، الأجسام المضادة IgG.

1. Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative, spiral-shaped, microaerophilic bacterium that colonizes the human gastric mucosa. It is a major etiological agent in chronic gastritis, peptic ulcer disease, and gastric carcinoma. Globally, infection rates approach 50% of the population, with a higher prevalence in developing nations (Hooi *et al.*, 2023). Transmission primarily occurs through oral-oral or fecal-oral routes, most often during childhood. Diagnostic methods range from invasive techniques like gastric biopsy to non-invasive options such as serology, stool antigen tests, and the urea breath test. The enzyme-linked immunosorbent assay (ELISA) is a widely used, practical,

and cost-effective tool for detecting H. pylori antigens or antibodies in various samples, including blood and stool (Malfertheiner *et al.*, 2022; Wang *et al.*, 2024).

This cross-sectional study aimed to determine the prevalence of *Helicobacter pylori* infection among patients attending a diagnostic laboratory in Al-Bayda, Libya, from 2024 to 2025. The investigation specifically assessed infection rates across different demographic groups, including age and gender, using ELISA on blood and stool samples. By analyzing the demographic distribution and diagnostic outcomes, this study provides local epidemiological data to inform improved diagnostic and preventive strategies for *H. pylori* in the region.

2. Materials and Methods

A cross-sectional study was conducted at a private diagnostic laboratory in Al-Bayda, Libya, between 2024 and 2025.

2.1 Study Population: The study included 748 patients of both sexes and all age groups. Among these participants, 294 (39.3%) tested positive for *H. pylori*, while 454 (60.7%) were negative.

2.2 Sample Collection and Processing: Two specimen types were collected: blood samples (serum) for detecting *H. pylori*-specific IgG antibodies and stool samples for detecting *H. pylori* antigen. All samples were processed according to standard laboratory protocols.

2.3 Laboratory Methods: Specimens were analyzed using commercially available ELISA kits, strictly following the manufacturer's instructions. Results were interpreted based on the following reference ranges:

2.3.1 Blood IgG: 0–0.075 IU/ml (values above the cutoff were considered positive).

2.3.2 Stool Antigen: 0–20 IU/ml (values above the cutoff were considered positive).

2.4 Statistical Analysis: Demographic data, including age, sex, and year of testing, were recorded. Statistical analyses were performed using SPSS version 25. The Chi-square test was employed to assess associations, and a p-value of less than 0.05 was deemed statistically significant.

3. Results

Out of 748 total samples analyzed, 294 (39.3%) were positive for *H. pylori*, and 454 (60.7%) were negative.

3.1 Distribution of *Helicobacter pylori* Cases by Gender.

Gender Distribution: Among the positive cases, 190 (64.6%) were female and 104 (35.4%) were male, indicating a higher infection frequency in females. The odds of a positive H. pylori test were significantly higher for females compared to males (OR = 2.15, 95% CI: 1.55–2.98).

Table. 1 Distribution of *Helicobacter pylori* Cases by Gender.

Gender		Frequency	Percent
Valid	**male**	104	35.4
	female	190	64.6
	Total	294	100.0

3.2 Distribution of *Helicobacter pylori* Cases by Age Group.

Age Distribution: The highest proportion of positive cases was observed in the 20–46 years age group. The lowest frequency was recorded among elderly patients (≥83 years). When compared to the youngest age group (2-10 years) as a reference, the 20-28 year age group had the highest odds of infection (OR = 3.21, 95% CI: 1.45–7.10).

Table. 2 Distribution of *Helicobacter pylori* Cases by Age Group.

Age		Frequency	Percent
Valid	**2-10**	20	6.8
	11-19	36	12.2
	20-28	57	19.4
	29-37	52	17.7
	38-46	46	15.6
	47-55	43	14.6
	56-64	21	7.1
	65-73	10	3.4
	74-82	7	2.4
	83-91	2	.7
	Total	294	100.0

3.3 Distribution of Helicobacter pylori Cases by Month.

This table displays the monthly distribution of the 294 confirmed *H. pylori* cases. The data reveals a non-random pattern, with a significant concentration of positives in certain periods. The analysis indicates a pronounced peak during period 2, which accounted for 21.4% of all detections. Periods 1 and 5 also showed notably high frequencies. Conversely, periods 7 and 8 registered the lowest incidence rates. This uneven distribution strongly supports the previously reported significant association between testing month and positive results. The clustering suggests that temporal factors, such as seasonal variations or shifts in healthcare-seeking behavior, likely influenced the detection rate of *H. pylori* infections within the studied population**.**

Table. 3 Distribution of *Helicobacter pylori* Cases by Month.

	Month	Frequency	Percent
Valid	**1**	44	15.0
	10	9	3.1
	11	7	2.4
	12	5	1.7
	2	63	21.4
	3	35	11.9
	4	42	14.3
	5	46	15.6
	6	26	8.8
	7	4	1.4
	8	6	2.0
	9	7	2.4
	Total	294	100.0

3.4 Distribution of *Helicobacter pylori* Cases by Year (2024–2025).

Year of Testing: In 2024, 72 positive cases (24.5%) were detected, compared to 222 (75.5%) in 2025, suggesting a notable increase in detection during the latter year.

Table. 4 Distribution of *Helicobacter pylori* Cases by Year (2024–2025).

	Year	Frequency	Percent
Valid	**2024**	72	24.5
	2025	222	75.5
	Total	294	100.0

3.5 Distribution of *Helicobacter pylori* Cases by Sample Type (Blood vs. Stool).

Specimen Type: Stool antigen testing identified 185 positive cases (62.9%), while blood IgG serology detected 109 cases (37.1%). Stool antigen testing was associated with 2.85 times higher odds of a positive result compared to blood serology (OR = 2.85, 95% CI: 2.10–3.87).

Table. 5 Distribution of *Helicobacter pylori* Cases by Sample Type (Blood vs. Stool).

Specimens	Frequency	Percent

Valid	**blood**	109	37.1
	stool	185	62.9
	Total	294	100.0

3.6 Crosstabulation of Gender and Year.

The data reveal a significant association between gender and study year (p = 0.016). Female participants had consistently higher detection rates than males in both years, with a greater rise observed in 2025. This finding indicates a possible gender-related difference in infection prevalence.

Table. 6. Crosstabulation of Gender and Year.

Gender	year		Total
	2024	**2025**	
Male	34	70	104
Female	38	152	190
Total	72	222	294

3.7 Crosstabulation of Age Group and Year.

Most positive cases were reported among adults aged 20–46 years. Although the chi-square test did not reach strong statistical significance (p = 0.092), the trend analysis showed a significant relationship (p = 0.001). This suggests that specific age groups may be more vulnerable to infection.

Table. 7 Crosstabulation of Age Group and Year.

Age	year		Total
	2024	**2025**	
2-10	1	19	20
11-19	7	29	36
20-28	9	48	57
29-37	12	40	52
38-46	13	33	46
47-55	17	26	43
56-64	6	15	21
65-73	3	7	10
74-82	3	4	7
83-91	1	1	2

Total	72	222	294

3.8 Crosstabulation of Month and Year.

The results indicate variation across months, with the highest number of cases during February, April, and May of 2025 ($p < 0.001$). Such fluctuations may reflect seasonal or lifestyle factors that influence infection risk.

Table. 8 Crosstabulation of Month and Year.

Month	year		Total
	2024	**2025**	
1	3	41	44
10	9	0	9
11	7	0	7
12	5	0	5
2	4	59	63
3	6	29	35
4	5	37	42
5	6	40	46
6	10	16	26
7	4	0	4
8	6	0	6
9	7	0	7
Total	72	222	294

9.9 Crosstabulation of Month and Sample Type (Blood vs. Stool).

A marked difference was observed between stool antigen and blood antibody detection across months ($p < 0.001$). Stool testing consistently identified more positive cases, highlighting its superior value for diagnosing active H. pylori infection.

Table. 9 Crosstabulation of Month and Sample Type (Blood vs. Stool).

months	Specimens		Total
	blood	**stool**	
1	8	36	44
10	9	0	9

11	7	0	7
12	5	0	5
2	14	49	63
3	11	24	35
4	13	29	42
5	13	33	46
6	12	14	26
7	4	0	4
8	6	0	6
9	7	0	7
Total	109	185	294

Statistical Associations: A significant correlation was found between sex and year of testing (χ^2 = 5.855, p = 0.016). The association between age and year was of borderline significance (p = 0.092). A strong association was observed between month and year (χ^2 = 146.431, p < 0.001). Furthermore, a significant association was found between the specimen type and the month of testing (p < 0.001).

4. Discussion

The diagnostic findings of this study are also notable. Stool antigen ELISA identified 62.9% of cases compared to 37.1% detected by serum IgG testing, and was associated with 2.85 times higher odds of detection. This result strongly supports the argument for using stool antigen assays as the preferred non-invasive diagnostic method when the goal is to identify current, active infections. Previous studies in Libya and internationally highlight that serological tests, while useful for seroprevalence studies, can remain positive even after successful eradication, thus overestimating the prevalence of active disease (Sugano *et al.,* 2023; Wang *et al*., 2024). This aligns with international consensus guidelines that recommend antigen-based tests over serology for initial diagnosis and confirmation of eradication (Malfertheiner *et al*., 2022). While we used commercially available kits, future local studies should include validation against a gold standard (e.g., histology or urea breath test) to report precise sensitivity and specificity for the Libyan population, further optimizing test selection and clinical applicability in our settings.

The present study identified a prevalence of 39.3% for *Helicobacter pylori* infection among patients tested in Al-Bayda between 2024 and 2025. This level of infection is broadly comparable to findings reported from other Libyan cities. For example, Ahmed *et al*., (2022) recorded a seroprevalence of approximately

42% in Benghazi, while Ibrahim and Mohamed (2021) reported a rate of 41.7% among dyspeptic patients in Tripoli. More recently, Elhaddad *et al.*, (2023) found prevalence levels of around 38% in multiple Libyan healthcare centers. These figures suggest that *H. pylori* is consistently widespread across urban regions of Libya, and not confined to a specific locality.

The higher rate of infection observed among females in Al-Bayda aligns with similar observations from Benghazi and other Libyan studies (Ahmed *et al.*, 2022; Elhaddad *et al.*, 2023). While biological factors cannot be fully excluded, this trend is more likely to reflect sociocultural influences and healthcare-seeking behaviors, as women are generally more likely to undergo medical testing. Furthermore, the age distribution in our study, with the majority of cases occurring in individuals aged 20–46 years, reflects the persistence of infection from early adulthood into middle age. Such patterns support the hypothesis that infections are often acquired earlier in life and continue unless treated.

When comparing Libyan data with studies from neighboring countries, the prevalence rates appear consistent. Research from Egypt found infection rates above 40% among dyspeptic patients (Saad & El-Tayeb, 2022), while studies from Morocco documented similarly high prevalence levels, also exceeding 40% in many groups (Benajah *et al.*, 2021). These parallels point to shared risk factors across North Africa, such as family clustering, sanitation conditions, and dietary habits. Together, the regional evidence emphasizes the need for integrated control strategies, not only at the national but also at the regional level.

Seasonal clustering of cases in early 2025, as seen in this study, may reflect changes in dietary habits, seasonal gatherings, or local awareness campaigns that increased testing uptake. Further research is required to confirm whether these patterns reflect true seasonal variation in transmission or simply differences in healthcare-seeking behavior.

4.1 Conclusion

In conclusion, H. pylori remains a common infection in Al-Bayda, with prevalence levels similar to those in other Libyan cities and comparable to neighboring North African countries. Females and adults aged 20–46 years were most frequently affected. Stool antigen ELISA demonstrated superior diagnostic value for detecting active infection compared to serology, highlighting the importance of prioritizing non-invasive antigen-based methods in clinical practice. Future population-based studies employing multivariable regression analysis are needed to control for potential confounders and identify independent risk factors. Coordinated national and regional strategies that focus on early detection using optimal tests, patient education, and standardized eradication protocols are essential to reduce the burden of H. pylori infection.

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