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Comparison between the Serological and Molecular Methods for the Detection of *Helicobacter pylori*

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) infection is a worldwide health issue associated with chronic gastritis, stomach ulcers, and neoplasms. Management requires an accurate diagnosis, and the currently available technologies can be divided into molecular and serological approaches. Every approach has advantages and disadvantages, highlighting the importance of comparing.

Objectives: This study aimed to evaluate the diagnostic efficiency, reliability, and usefulness of serological and molecular methods for detecting *H. pylori* infection.

Methods: In 2023, a cross-sectional study was performed in Wasit Province, Iraq. Five hundred forty-one volunteers aged 15 to 65 years showing dyspeptic symptoms submitted blood samples for analysis. Serological testing utilized ELISA to identify IgG and IgM antibodies, whereas PCR was performed for the molecular detection of *H. pylori* DNA. Data were analyzed to assess sensitivity, specificity, and demographic correlations. The prevalence of seasonal swings was also evaluated.

Results: The frequency of *H. pylori* was nearly the same across males (270) and females (271). The most considerable prevalence was observed in the 26–35 age group, with seasonal peaks during the summer, especially in July. PCR had superior sensitivity and specificity relative to serology, although it required advanced resources. Serological tests offered significant epidemiological findings, although they could not differentiate between active and past infectious diseases.

Conclusion: Serological tests are useful for epidemiological investigations, but molecular methods are more accurate. A combined diagnostic strategy improves clinical and public health measures, especially in resource-limited regions. Seasonal and demographic changes require specific measures.

Introduction

Helicobacter pylori infection is a significant public health concern in developed and developing countries **[1]**. *Helicobacter pylori* is a microaerophilic, gram-negative helical bacteria that primarily targets gastrointestinal mucosa. Clinical indicators are associated with the progression of chronic

gastritis, gastric and duodenal ulcers, and cancer. A large proportion of infections are asymptomatic **[2, 3]**. The severity of clinical symptoms varies depending on various factors, including host genetic predisposition, immune response, bacterial burden, and virulence **[4,35,36]**.

H. pylori lives in the gastrointestinal systems of both humans and animals. Previous studies have recovered *H. pylori* from the milk of various animals, including cattle, camels, sheep, and pigs **[5, 6]**. Previous research has established the isolation of *H. pylori* from domestic cats **[7]**. Individuals who work with animals or produce animal-derived food (e.g., veterinarians, butchers, and slaughterhouse workers) have higher levels of *H. pylori* antibodies **[8]; t**his shows that ruminants could be the source of the infection in humans **[9]**.
Detecting *H. pylori* infection remains challenging due to the bacterium's ability to evade immune responses, its varied clinical manifestations, and its genetic diversity **[10].** Diagnostic techniques used for *H. pylori* detection may be classified into invasive and non-invasive methods, each with advantages and disadvantages **[11, 12]**. Invasive tests that include histological examination need an endoscopic biopsy, culture, or rapid urease test. Although these tests may be accurate and specific, they are resource-intensive and require multiple biosafety facilities. They may also cause patient discomfort **[13]**. Histological diagnosis brings in critical knowledge of inflammation and related conditions but is affected by biopsy location and small sample size **[14]**. Culture allows strains with various relative characteristics, including antibiotic resistance, to be studied, but success is limited by the complex or fastidious nature of the organism's growth **[15]**.
Non-invasive tests include serological assays, stool antigen tests, and urea breath tests (UBTs) **[16]**. While serological testing is cheap and widely available, it cannot indicate whether the infection is active or past, restricting its applicability to monitor treatment response. In contrast, stool antigen tests have high sensitivity and specificity in identifying active infections but require appropriate handling of the samples to maintain their precision **[17].** UBT detects urease activity in the breath and has sensitivities and specificities above 90%, thus taking precedence as a non-invasive method. However, some patient groups may find it a challenge **[18].**
Today's advances in molecular diagnostics, especially in PCR and next-generation sequencing, afford increased sensitivity, thus identifying *H. pylori* and antibiotic resistance patterns due to additional expenses and technical requirement factors in limited resource settings **[19, 20]**. Nevertheless, the latest tests still require a mix of types of diagnosis to enable the most reliable results in clinical practice **[21].**

The serological technique exploits the detection of various antibodies, i.e., IgG, IgA, and IgM, through ELISA, immunoblotting, and enzyme immunoassay (EIA) **[22]**. These methods are inexpensive, accessible, and easy to perform, making them suitable for epidemiological studies and certain clinical situations, such as gastrointestinal bleeding and gastric atrophy, which renders other tests susceptible to false negatives. However, serology cannot distinguish between active and past infections, as IgG antibodies may persist for months after treatment, so positive IgG is not a reliable proof of active infection **[23]**. Centromeres of the plates are also susceptible to subclinical false negative results from early infection when antibody levels are not yet high enough. Thus, Serology remains a useful diagnostic tool, especially where other more sophisticated methods are unavailable **[24]**.

Molecular methods such as the polymerase chain reaction (PCR) have enhanced the sensitivity and specificity (>95%) of H. samples compared to any other method **[25]**. PCR provides the means to detect *H. pylori* DNA from the stomach biopsies, sputum, saliva, and feces, allowing both invasive and non-invasive investigations **[26]**. It is especially helpful in identifying patients infected with *H. pylori* and having gastrointestinal bleeding and determining the presence of mutations resulting from

antibiotic resistance, as these mutations are essential for appropriate treatment **[27]**. It is, however, costly and skill-demanding, which limits its universal applicability, especially in resource-limited countries **[28]**. Moreover, PCR can give false positives as it can detect DNA fragments from non-viable bacteria, which calls for caution in interpreting the results **[29]**.

Given the various advantages and limitations of serological and molecular techniques, comparisons of their respective diagnostic performances are essential. Such comparisons help identify the most appropriate test for the clinical scenario, considering sensitivity, specificity, cost, and implementation ease. For zoonotic specialists, this understanding of testing modes is essential as the diagnosis of *Helicobacter pylori* moves from pure human medicine to an interface of animal reservoirs and zoonotic transmission pathways.

This work thus compares the efficacy, reliability, and practicality of serological and molecular techniques in detecting *H. pylori*. By assessing these diagnostic tools more thoroughly, we provide an evidential basis for clinical and public health decision-making. From the perspective of zoonosis, the result of this comparison may have implications for the study of *H. pylori* epidemiology in animal reservoirs and zoonotic transmission analysis.

Methods

Study Design and Setting

The present study aimed to compare serological and molecular methods for detecting *Helicobacter pylori* infection in Wasit Province, Iraq, in 2023. Blood samples were drawn from males and females of various age groups at Al-Zahraa Hospital in Wasit City.

Study Population

The study had 541 participants, of whom 271 were female and 270 were male, aged between 15 and 65. The participants were further classified into age groups (15-25 years, 26-35 years, 36-45 years, 46-55 years, and 56-65 years).

Inclusion Criteria:

- Adults aged 15 to 65 years.
- Presence of dyspeptic symptoms.
- No prior treatment for *Helicobacter pylori* infection.
- Willingness to provide informed consent.

Exclusion Criteria:

- Use of proton pump inhibitors or H2-receptor antagonists within the previous two weeks.
- Use of antibiotics or bismuth compounds within the previous four weeks.
- History of gastric surgery or known gastrointestinal malignancy.
- Pregnancy or lactation.
- Severe comorbid conditions that could interfere with study participation.

Sample Collection

A total of 541 blood samples were collected. Serum was extracted for serological analysis, and blood was used for molecular testing. Data collection occurred from January to December 2023, with monthly records detailing the serological and molecular results.

Diagnostic Methods

1) ***Serological Testing:***
   IgG and IgM Detection: We utilized Enzyme-Linked Immunosorbent Assay (ELISA) to detect IgG and IgM antibodies specific to *H. pylori* in serum samples. This approach

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differentiated acute and chronic infections using IgM antibodies that indicate recent exposure, while IgG antibodies suggest a longer-term or past infection.

Serum samples were diluted per the ELISA kit instructions and added to antigen-coated microtiter wells for incubation. After washing off unbound components, an enzyme-conjugated secondary antibody was applied, followed by substrate addition to produce a color change indicating antibody levels. The reaction was stopped, and absorbance was measured. Then, we documented the monthly IgG and IgM positivity rates, stratifying the data by gender and age groups.

2) ***Molecular Testing:***

We utilized PCR to detect *H. pylori* DNA in blood samples, offering a highly sensitive and specific method to identify the pathogen independent of antibody levels.

Blood samples were processed to extract genomic DNA using a commercial extraction kit, following the manufacturer's protocol to ensure high-quality DNA for amplification. The different laboratory processes involved targeting specific genes of *H. pylori*, 16S rRNA genes with carefully designed primers. The PCR reaction mixture contained the extracted DNA, primers, nucleotides, buffer, and DNA polymerase. The thermal cycling applied included the initial denaturation stages followed by denaturation, annealing, and extension cycles. Finally, it concluded with the final extension step to yield the target sequences.

The amplified DNA products were analyzed with ethidium bromide-stained agarose gel electrophoresis under UV light, which confirmed the presence of *H. pylori* DNA. Subsequently, the PCR results were compared with serological results to assess the concordance and sensitivity between the two methods.

PCR Protocol for Detecting *Helicobacter pylori*

Primer Design:

Primer design involves targeting specific *H. pylori* genes and ensuring their specificity to prevent non-specific amplification.

PCR Reaction Setup:

The PCR reaction setup includes extracted DNA, forward and reverse primers, dNTPs, PCR buffer, and DNA polymerase, with a total reaction volume of 25-50 µL based on assay requirements.

Thermal Cycling Conditions:

Step	Temperature (°C)	Time	Notes
Initial Denaturation	94-95	2-5 minutes	Denatures DNA to single strands.
Amplification Cycles		25-35 cycles	Repeated steps for DNA amplification.
Denaturation	94-95	30 seconds	Separates double-stranded DNA.
Annealing	50-65	30 seconds	Adjust temperature based on primer melting temp.
Extension	72	30 seconds to 1 minute	DNA synthesis by polymerase.
Final Extension	72	5-10 minutes	Ensures complete extension of amplified products.

Post-PCR Analysis:

Post-PCR analysis involves gel electrophoresis, visualization, and interpretation of *H. pylori* DNA. The process involves separating amplified products on an agarose gel, staining the gel with ethidium bromide, and comparing band sizes to a DNA ladder.

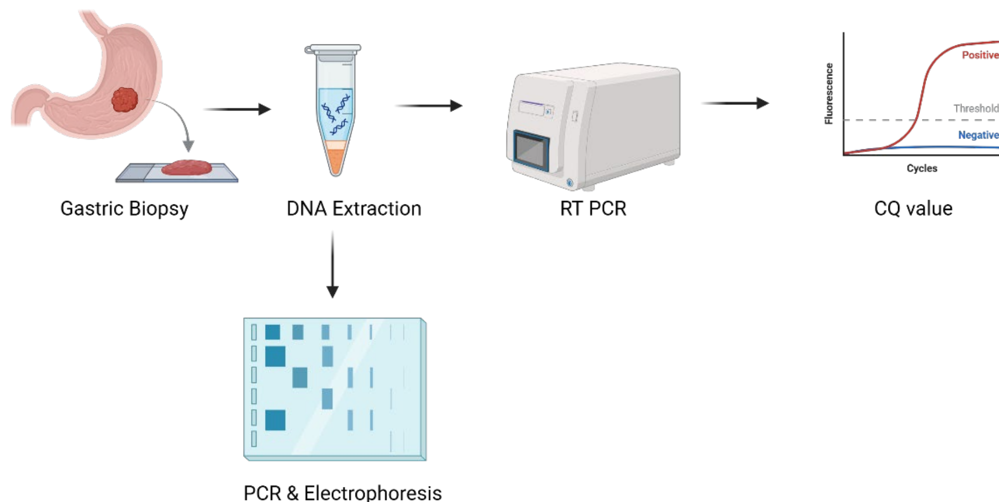


Figure 1: Diagrammatic presentation of the study concept. Abbreviations: DPO-PCR, dual priming oligonucleotide-based multiplex polymerase chain reaction; PCR, Polymerase Chain Reaction; DNA, Deoxyribonucleic acid

Data Collection and Analysis

The study's objectives were to determine the prevalence of *H. pylori* infection and compare molecular and serological diagnostic methods, so data were collected every month. Blood tests were done after ELISA methods were used to detect IgG and IgM antibodies, and PCR methods were used to detect *H. pylori* DNA.

Based on the results of IgG, IgM, and PCR tests, the infection rate was compared across gender and age groups.

We also examined how the infection rate changed with the seasons by comparing data from each month to see how it changed at different times of the year; this gave a clear picture of the diagnosis's results and how the infection spread.

Statistical Analysis

The SPSS software (version 25) was used to analyze the data to estimate the prevalence and the course of diagnosis for *H. pylori* infection. Chi-square tests were applied to test whether notable differences exist in serological and molecular results across age groups, sex, and months, thus providing a statistical framework for testing demographic and seasonal variations.

A p-value of ≤0.05 is considered significant, while ≤0.01 is more critical.

Ethical Considerations

The study followed ethical guidelines, with all participants providing informed consent. Confidentiality of data was maintained.

This methodology provided a comprehensive framework for assessing and comparing the phenomenological of *H. pylori* detection, considering demographic and seasonal factors.

Results

Prevalence of *H. pylori* by Gender and Age Group

A total of 541 samples were analyzed, 271 from females and 270 from males. The prevalence of *H. pylori* infection was stratified into five age groups: 15–25, 26–35, 36–45, 46–55, and 56–65.

As shown in **Table 1**, the highest prevalence was observed in the 26–35 age group for both females (81 cases) and males (79 cases). The lowest prevalence was reported in the 56–65 age group, with

36 cases in females and 40 cases in males. The total prevalence across all age groups was nearly equal between genders, with females accounting for 271 cases and males for 270 cases.

Table 1: Prevalence of *H. pylori* by Gender and Age Group

Age Group	Female	Male	Total
15–25 years	39	46	85
26–35 years	81	79	160
36–45 years	72	67	139
46–55 years	43	38	81
56–65 years	36	40	76
Total	271	270	541

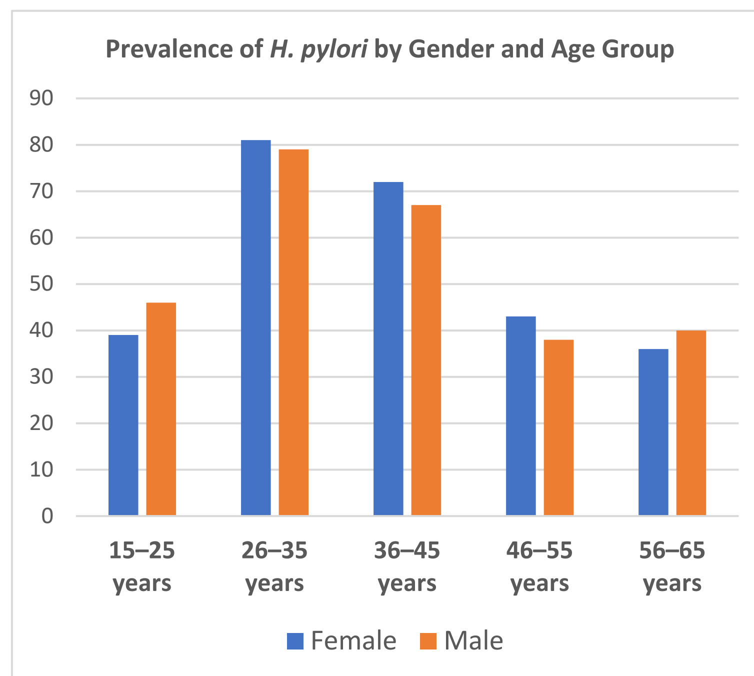


Figure 2: Prevalence of *H. pylori* by Gender and Age Group

Monthly Prevalence of *H. pylori* Infection in 2023

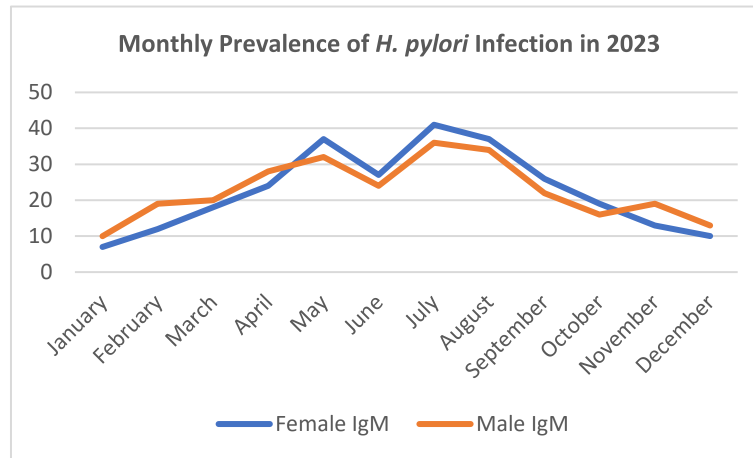
Table 2 summarizes the monthly distribution of *H. pylori* infection from January to December 2023. The highest number of cases was recorded in July, with 77, followed by May (69 cases) and August (68 cases). The lowest prevalence was observed in December, with only 23 cases reported.

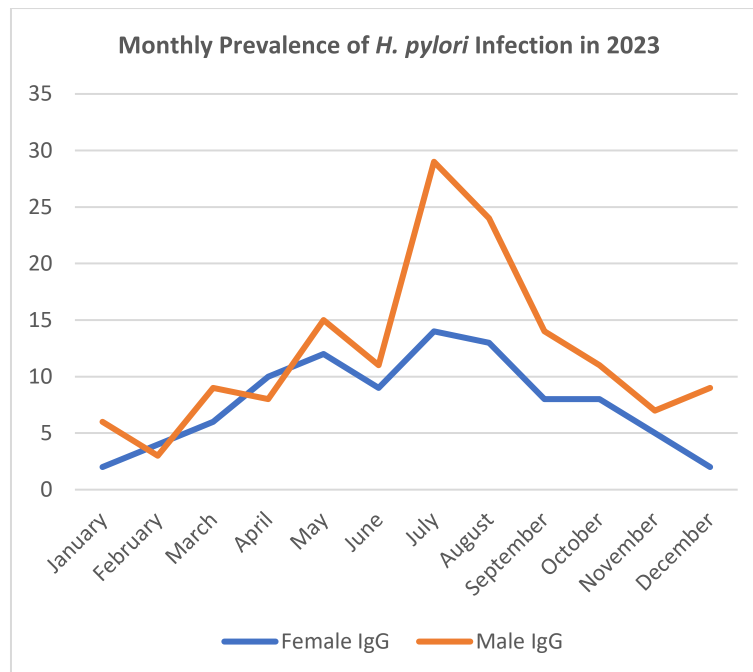
Both IgM and IgG antibodies were measured in the collected samples. The IgM positivity rates indicate recent infections, while IgG positivity reflects past or chronic infections. Female IgM positivity peaked in July (41 cases), while male IgM positivity was highest in the same month (36 cases). Similarly, female IgG positivity was highest in July (14 cases), while male IgG positivity peaked in July (29 cases).

Table 2: Monthly Prevalence of *H. pylori* Infection in 2023

Month	Female IgM	Female IgG	Male IgM	Male IgG	Total
January	7	2	10	6	17
February	12	4	19	3	31
March	18	6	20	9	38
April	24	10	28	8	52
May	37	12	32	15	69
June	27	9	24	11	51

July	41	14	36	29	77
August	37	13	34	24	68
September	26	8	22	14	48
October	19	8	16	11	35
November	13	5	19	7	32
December	10	2	13	9	23
Total	271	93	273	141	541





Seasonal Variations

The number of people infected with *H. pylori* fluctuated with the season. However, most people become infected throughout the summer (May-August). This pattern may indicate that people are more likely to be exposed to risk factors during this time, such as dietary or environmental changes.

Post-PCR Analysis

Figure 3 shows results from the gel electrophoresis for PCR-amplified *H. pylori* DNA. Bands corresponding to target sizes (~294 bp) are observed in the positive controls, indicating successful amplification. In contrast, no bands are observed in the negative control, attesting to the assay's specificity.

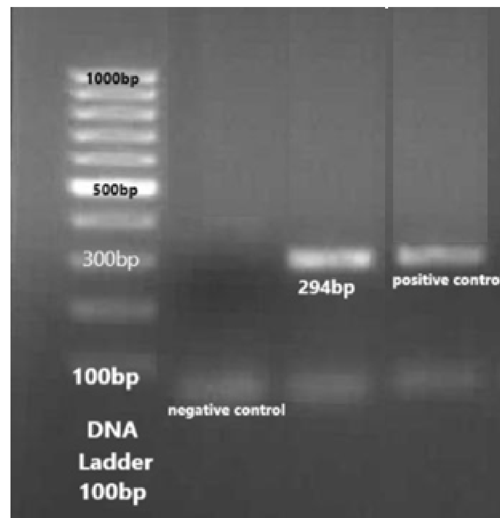


Figure 3: Gel electrophoresis image showing PCR amplification of *Helicobacter pylori* DNA. Lanes include a DNA ladder (M) for size estimation, positive controls (PC), negative controls (NC), and PCR products at different concentrations. The target amplicon of approximately 294 bp is indicated.

Discussion

This study examined 541 samples to determine how common *Helicobacter pylori* (*H. pylori*) infections were in people of various ages and genders and how they fluctuated with the seasons in 2023. The highest number of cases were seen in adults aged 26 to 35, with 81 instances in women and 79 in men. July had the highest cases (77 cases), while December had the fewest (23 cases).

Many studies have demonstrated that *H. pylori* infections peak in early adulthood, supporting our findings on 26-35-year-olds. In a 2014 systematic assessment, Peleteiro et al. found that over half of adult humans globally had *H. pylori*, and the prevalence rises with age. [30]. Similarly, a study in China reported a peak prevalence at 36 years of age, with an overall infection rate of 48.4% among 53,260 subjects [31]. These findings support our results, suggesting that young adults are at a higher risk, possibly due to increased social interactions and lifestyle factors that facilitate transmission.

Our study found nearly equal prevalence between females (271 cases) and males (270 cases); this contrasts with some studies that report higher prevalence in males. For example, a meta-analysis by de Martel et al. (2006) found that male gender was significantly associated with *H. pylori* infection, with an odds ratio of 1.16 [32]. However, other studies have reported marginal differences. For instance, a study in China found age-standardized prevalence rates of 44.5% in women and 43.5% in men, indicating minimal gender disparity [31]. These discrepancies may be attributed to regional, cultural, or socioeconomic factors influencing exposure risk.

We observed a peak in *H. pylori* infections during summer, particularly in July. This finding contrasts with studies conducted in Israel, which reported higher infection rates during winter months (December–January) compared to summer (July–September), with 60.9% vs. 42.3% positivity, respectively [33]. The variation in seasonal patterns across different regions could be due to differences in climate, dietary habits, or seasonal behaviors affecting transmission dynamics.

Our study's overall prevalence is the same as projections made worldwide. Hooi et al. (2017) found that 44.3% of people worldwide had an *H. pylori* infection, but there were significant differences between regions **[34]**. Our results help us learn more about how *H. pylori* spread in our area, which can be used to plan more effective public health measures.

Conclusion

The study highlights *H. pylori* prevalence trends across demographics and seasons, emphasizing molecular methods' diagnostic accuracy and serological methods' epidemiological value. Findings underscore the importance of combining diagnostic approaches for effective management and public

health interventions.

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