

Research Article



Molecular Identification of *Helicobacter Pylori* in Local Goats from Selected Regions in Central Iraq

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Abstract | The aim of the current study was to detect and examine *Helicobacter pylori* in goats from several districts in Iraq. A total of 60 goats from the central regions of Iraq were sampled between October 2021 and March 2022. Milk and fecal samples from all animals were analyzed for *H. pylori* using bacteriological culture techniques and PCR for the identification of the *glmM* gene (320 bp) and *16S rRNA* (500 bp). *H. pylori* was not isolated by bacteriological culture. Of the 60 samples that were subjected to PCR analysis, 20% (12/60) and 16.6% (10/60) were positive for *glmM*, and *16S rRNA*, respectively. The *16S rRNA* gene was detected in all goats, which were also positive for the *glmM* gene. There was no statistically significant difference in *H. pylori* infection based on sex, but a significant difference was observed between age groups. Partial genetic analysis of the *glmM* and *16S rRNA* genes from two positive samples (accession Nos. OM935719.1 and ON567364.1) showed a strong correlation with global isolates recorded in NCBI. A genetic tree was used to distinguish the *glmM* and *16S rRNA* genes between two *H. pylori* strains identified from different regions in Iraq and to assess their association with global isolates. Overall, the results showed that the percentage of *H. pylori* infection in some governorates of the Middle Euphrates in Iraq is lower than in neighboring countries.

Keywords | *glmM* gene, *H. pylori*, Goats, PCR, Iraq

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INTRODUCTION

Helicobacter pylori is an aerobic, gram-negative bacterium commonly found in the human stomach, and it is also associated with gastroenteritis. The genus name *Helicobacter* is derived from its spiral shape, which allows it to penetrate the stomach's mucous layer and cause infection. In 1982, Australian researchers Robin W. and Barry M. identified *H. pylori* for the first time. *H. pylori* is linked to the development of B-cell lymphoma in the lymphoid tissues surrounding the mucosa (Alzahrani, 2024). While many people infected with *H. pylori* do not show symp-

tom, the bacterium can lead to conditions such as obstruction in the small intestine, stomach ulcers, or gastritis (Majumdar and Looi, 2024). Long-term *H. pylori* infection can also increase the risk of certain cancers due to its prolonged presence in the stomach lining. Researchers have established that *H. pylori* is influenced by, or may protect against, a variety of stomach conditions, though many legal precedents regarding its role are still debated (Gravina *et al.*, 2018). *H. pylori* living in the stomach environment plays a significant role in influencing nearby bacteria and improving their function, as well as regulating appetite and stomach acid levels (Fiorani *et al.*, 2023). Approximately

90% of people infected with *H. pylori* do not exhibit clinical symptoms or long-term consequences. However, 10% to 20% of individuals may develop a peptic ulcer if the infection persists, with clinical symptoms including occasional vomiting, bloating, belching, nausea, and stomach pain. These symptoms can progress to chronic gastritis, causing pain, particularly between meals or when the stomach is empty, often in the morning. Loss of appetite, vomiting, and nausea are less frequent but possible symptoms (Hooi *et al.*, 2017). Because it is more difficult to maintain cleanliness in animals and their surroundings, particularly in large farms, developed and densely populated countries are more vulnerable to *H. pylori* infection, which facilitates the spread of bacteria to humans (Khalifa *et al.*, 2010). The increase in infection rates is linked to the rising consumption of animal-derived foods, driven by population growth and inadequate quality control during production, which enhances bacterial spread. Slaughterhouse workers, butchers, and employees often show elevated levels of antibodies against *H. pylori* due to their frequent contact with animals (cattle, sheep, goats, and buffaloes), as well as animal products (Talaie *et al.*, 2015). Ruminants play a significant role in the transmission of infection to humans, as *H. pylori* has been isolated from the milk and feces of various animals (Miranda *et al.*, 2023). To our knowledge, there is a lack of sufficient studies on *H. pylori* in Iraq. Therefore, the aim of the current study was to detect and examine *Helicobacter pylori* in goats from several districts in Iraq.

MATERIALS AND METHODS

ETHICAL APPROVAL

The Central Scientific Committee of the College of Veterinary Medicine, Al-Qasim Green University (Number: 2021-87) approved and provided funding for this work.

STUDY ANIMALS

A total of 60 Iraqi local goats (6 males and 54 females), aged from 1 year to over 3 years, were selected from Babylon and Baghdad in central Iraq during the period from October 2021 to March 2022. Information for each animal was recorded in a specialized chart designed specifically for this purpose.

SAMPLE COLLECTION

The blood samples were carefully collected using sterile vacutainer tubes and analyzed using two different diagnostic methods. The first method was the use of bacterial culture technique. The second method was the use of conventional polymerase chain reaction, depending on two types of main genes (*glmM* 320bp and *16S rRNA* 500bp). Two milliliters of milk and 2 grams of stool were placed in a sterile container labeled with a number, then frozen until DNA extraction and PCR testing were performed.

NUCLEIC ACID EXTRACTION

The genomic DNA was extracted from milk and fecal samples using the Genomic DNA Extraction Kit (GeneAid, USA), following the manufacturer's instructions. The extracted DNA was evaluated using a NanoDrop spectrophotometer, which measures DNA concentration in nanograms per microliter and assesses DNA purity by reading absorbance at wavelengths of 260/280 nm (Shahreza *et al.*, 2024).

PRIMER DESIGNING

The primers of current study include a 500-bp of the *16S rRNA* gene of the *H. pylori* bacteria, F: (5'-TTGGAG-GGCTTAGTCTCT-3') and R: (5'-AAGATTGGCTC-CACTTCACA-3') and 320-bp of the *glmM* gene F:(5'-GCTTACTTTCTAAGACTAACGCGC-3') and R:(5'-GGATAAGCTTTTAGGGGTGTTAGGGG-3' that were designed using Primer3 (<http://frodo.wi.mit.edu/~primer3/>).

PCR ASSAY

The *16S rRNA* and *glmM* genes were detected using a PCR technique. A total of 1 µL of each primer (10 pmol) and 12.5 µL master mix, 5.5 µL nuclease-free water, and 5 µL template DNA were included in the PCR tube (Mosa *et al.*, 2022). A thermocycler was used to perform the same PCR cycling conditions for each gene. The PCR products were analyzed using a 1.2% agarose gel (Bio Basic, Canada), and the results were visualized with a UV transilluminator.

SEQUENCING AND PHYLOGENETIC ANALYSES

Using forward primers (Macrogen, Korea), two amplicons of the products were sequenced in Korea. The results were compared by using BLAST to the sequencing data for the *glmM* and *16S rRNA* genes of the *H. pylori* bacteria that were accessible on the NCBI website. Several sequencing analyses and phylogenetic trees were produced using the MEGA 6.0 program.

RESULTS AND DISCUSSION

PCR RESULTS

By using two primers (*glmM* and *16S rRNA*), PCR analysis of the 54 milk and 6 fecal samples from goats from Babylon and Baghdad indicated total infection rates of 12/60 (20%) and 10/60 (16.6%), as show in (Table 1).

Table 1: Shows the PCR results for *H. pylori* infection rates.

Animals type	Total No.	PCR results	
		glmM	16S rRNA
Local goat	60	12 (20%)	10 (16.6%)

A total of 60 samples were analyzed, and endpoint PCR results were detected in 12 samples. These samples produced bands of the expected sizes: 500 bp (Figure 1) and 320 bp (Figure 2), corresponding to the universal ladder ranging from 100 to 1500 bp, and were considered positive. The presence of one or both genes was confirmed in all positive samples.

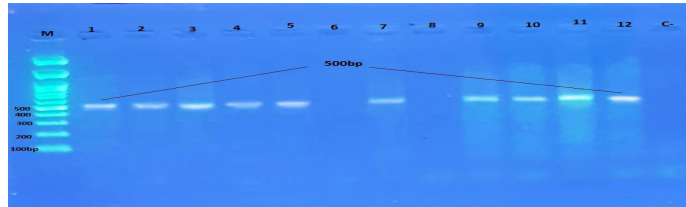


Figure 1: Agarose gel showing PCR amplicon (500 bp) of 16S rRNA gene of *H. pylori*.

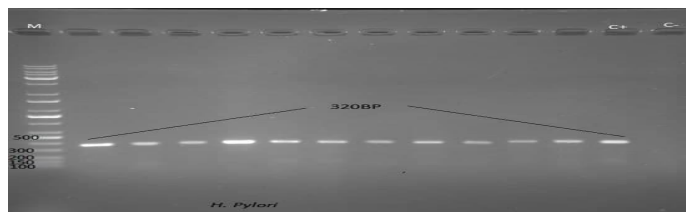


Figure 2: Agarose gel showing PCR amplicon (320 bp) of *glmM* gene of *H. pylori*.

INFECTION RATE ACCORDING TO SEX

There were no significant differences between male and female animals in their susceptibility to *H. pylori* (Table 2). The infection rate in males was 16.66%, while in females it was 20.37%.

Table 2: Sex-wise Prevalence rate of *H. pylori* in goats as assessed by polymerase chain reaction.

Sex	No. of tested animals	Positive (%)
Males	6	1 (16.66)
Female	54	11 (20.37)
Total	60	12 (20)
X ²	0.577(NS)	
P value	0.447	

NS: insignificant difference (P>0.05).

Table 3: Age-wise Prevalence rate of *H. pylori* in goats as assessed by polymerase chain reaction.

Age/ year	No. of animals	Positive (%)
>1-3	14	1 (7.14) ^a
>3-5	25	4 (16) ^b
>5	21	7 (33.33) ^c
Total	60	27 (45)
X ²	1.78 (S)	
P value	0.039	

S: significant difference (P<0.05).

INFECTION RATE ACCORDING TO AGE GROUPS

The study showed that all age groups were susceptible to *H. pylori* infection, with varying rates. A significant difference (P<0.05) was observed between age groups. The highest infection rate was recorded in animals older than five years (33.33%, 7/21), followed by animals aged 3 to 5 years (16%, 4/25). The lowest infection rate (7.14%, 1/14) was detected in animals aged 1 to 3 years (Table 3).

SEQUENCING AND PHYLOGENETIC ANALYSIS OF THE PARTIAL 16S rRNA GENE OF *H. PYLORI*

Sequence alignment analysis was performed on the partial 16S rRNA gene sequence from local *H. pylori* isolates in milk, comparing them with *H. pylori* species from NCBI GenBank. The local *H. pylori* isolate Mosa-1 (OM935719.1) was analyzed and compared with other *H. pylori* isolates from GenBank. In the phylogenetic tree, the Mosa-1 isolate was closely related to the *H. pylori* strain LVRN-53 (MT477178.1) based on the 16S ribosomal RNA gene (Table 4 and Figure 3).

Table 4: The percentage of homology between locally isolated *H. pylori* bacteria (16S ribosomal RNA gene) from goats and isolates from ribosomal RNA genes submitted to NCBI-BLAST.

H. pylori isolate No.	NCBI-BLAST Homology Sequence identity (%)			
	Identical H. pylori bacteria	Genbank Accession number	Country	Identity %
Uncultured Helicobacter sp. clone Mosa-1 16S ribosomal RNA gene, partial sequence OM935719.1	Helicobacter pylori strain LVRN-53	MT477178.1	Chile	98.60 %
	Helicobacter pylori strain LVM-53	MT477177.1	Chile	98.60 %
	Uncultured bacterium clone nck-119f06c1	KF089439.1	Spain	98.40 %
	Helicobacter pylori strain Gj49	DQ202374.1	Bangladesh	98.40 %
	Helicobacter pylori JCM 12097	LC507450.1	Japan	98.20 %
	Helicobacter pylori JCM 12096	LC507449.1	Japan	98.20 %
	Helicobacter pylori JCM 12095	LC507448.1	Japan	98.20 %
	Helicobacter pylori JCM 12093	LC507447.1	Japan	98.20 %

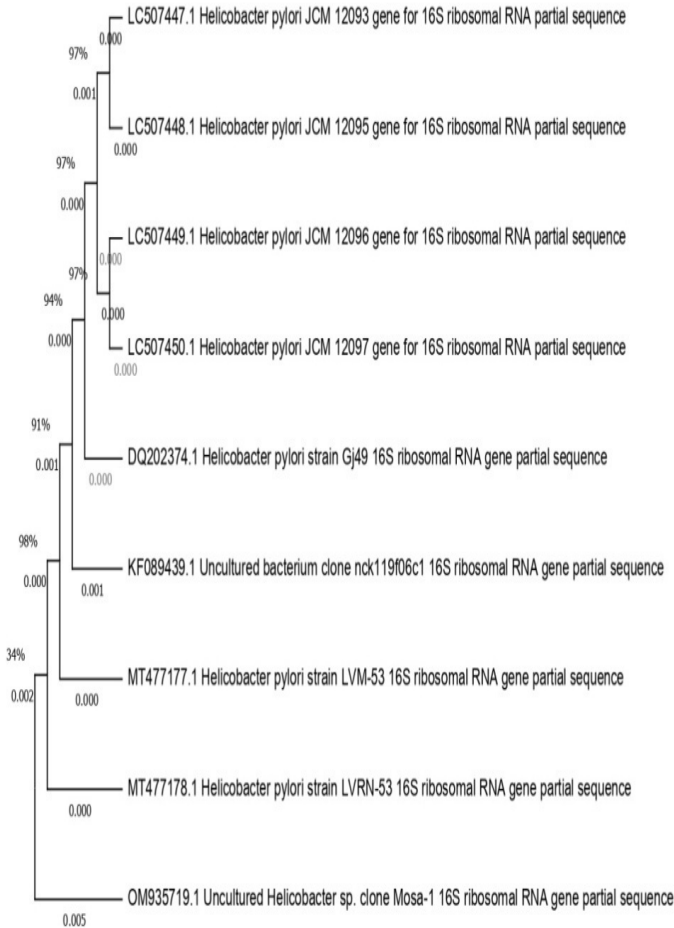


Figure 3: Phylogenetic tree analysis of the partial 16S rRNA gene sequence from a local goat carrying an Iraqi *H. pylori* sample revealed a total genetic variation of 0.010%.

Table 5: The percentage of homology between locally isolated *H. pylori* bacteria (*glmM* gene) from goats and isolates *glmM* gene submitted to NCBI-BLAST.

H. pylori isolate No.	NCBI-BLAST Homology Sequence identity %			
	Identical H. pylori bacteria	Genbank Accession number	Country	Identity %
<i>Helicobacter Pylori</i> strain Mosa-2	<i>Helicobacter pylori</i> strain J182	CP024947.1	USA	99.35 %
<i>GlmM</i> (glmM) gene, partial cds	<i>Helicobacter pylori</i> J166	CP007603.1	USA	98.71 %
ON567364	<i>Helicobacter pylori</i> strain SHIM-010	CP051505.1	USA	98.38 %
	<i>Helicobacter pylori</i> DNA, nearly complete genome, strain: F211	AP017335.1	Japan	98.09 %
	<i>Helicobacter pylori</i> phosphoglucosamine mutase (glmM) gene.	AF405553	USA	98.06 %

SEQUENCING AND PHYLOGENETIC ANALYSIS OF THE PARTIAL GLMM GENE OF H. PYLORI

Sequence alignment analysis was conducted on the partial

glmM gene sequence from local *H. pylori* isolates in milk, comparing them with *H. pylori* species from NCBI GenBank. The local *H. pylori* isolate Mosa-2 (ON567364) was analyzed and compared with other *H. pylori* isolates from GenBank. In the phylogenetic tree, the Mosa-2 isolate was closely related to the *H. pylori* strain J182 (complete genome, CP024947.1) (Table 5 and Figure 4).

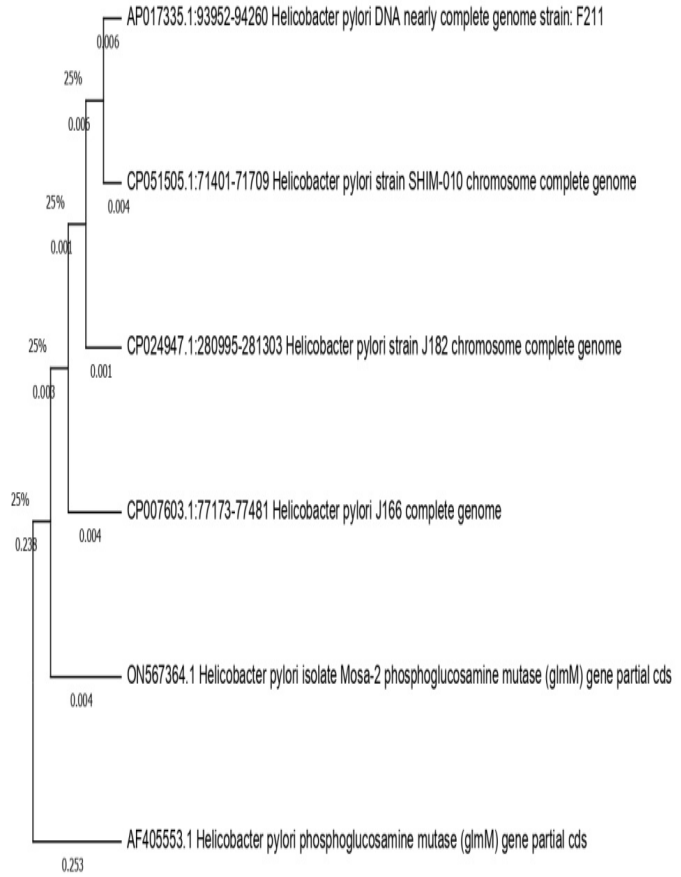


Figure 4: Phylogenetic tree analysis of the partial *glmM* gene sequence from a local goat carrying an Iraqi *H. pylori* sample revealed a total genetic variation of 0.010%.

According to Garcia-Ferres *et al.* (2022), *Helicobacter* is a microorganism with significant social impacts. In the current study, domestic goats were evaluated for *H. pylori* infection. Numerous studies conducted in various locations have yielded varying results, due to differences in animal type and conditions (Guessoum *et al.*, 2018). This study is the first to confirm the presence of *H. pylori* in local goats from some regions of Babylon and Baghdad, Iraq. The failure to isolate *H. pylori* from samples using bacterial culture in this study suggests that this method may not be the most effective for detecting *H. pylori* in animals (Azevedo *et al.*, 2007; Tabatabaei, 2012). Some studies have reported isolating the bacteria in very low proportions, with weak growth (Azevedo *et al.*, 2007). Therefore, advanced methods should be employed to detect the bacteria and determine its characteristics. To our knowledge, there are few studies focusing on detecting *H. pylori* in foods, particularly raw milk, which is a primary source of human infection (Talaie

et al., 2015). Interestingly, the prevalence of *H. pylori* infection in goat milk varies significantly between studies and dairy herds. Similarly, El Gohary *et al.* (2015) found that the prevalence of *H. pylori* infection differs across regions in Egypt. This can be explained by the various hygienic problems that occur and vary from one region to another. In the current study, the *glmM* gene was especially worked on for PCR confirmation of bacteria in raw milk and feces. *GlmM* is highly specific in detection of bacteria and appears to be important for the multiple of the organism (Osman *et al.*, 2015). In the current study, the *glmM* gene was found in 20% of the goats used in this study. Rahimi and Khairabad (2012), believed that the *glmM* gene was found in 4.1% of Iranian goat milk. It is interesting to note that *H. pylori* was prevalent in milk from countries with high production, including Japan, where it was found in 72.2% (Fujimura *et al.*, 2002) and Czech Republic, 58% in raw goats' milk (Furmancikova *et al.*, 2022). Since the immune system is less effective in goats with high milk production due to metabolic stress and because there are many people working in large herds, it is challenging to monitor and maintain hygiene conditions, the presence of bacteria in dairy farms is associated with health conditions, stress, and a high rate of milk production (Bertoni *et al.*, 2015). The purpose of this study is to detect and confirm the presence of bacteria by using conventional PCR to find the *glmM* gene of *H. pylori* in the central governorates of Iraq, the prevalence rate for fresh raw goat milk was 20%. However, 40% 31/77 of the raw cows' milk were positive for *H. pylori* (Furmancikova *et al.*, 2022). *H. pylori* was discovered in 72.2% of samples of raw cow milk in the other study, which was carried out in Japan (Fujimura *et al.*, 2002). According to an Italian study by using nested PCR on 400 milk samples, this bacteria was present in 25.6%, 50% and 33% of the sheep and goat and cow populations respectively (Quaglia *et al.*, 2008). Another study conducted in Iran using the polymerase chain reaction method showed that *H. pylori* was present in cow, sheep and goats with 14.1%, 12.2% and 8.7% respectively (Rahimi and Khairabad, 2012). In addition, samples of fresh stool samples were collected from cows, sheep, buffaloes, cats and dogs in Egypt, According to Shaaban *et al.* (2023), *H. pylori* prevalence was 4.7%, 10%, 12%, 28% and 24% respectively. Additionally, *H. pylori* was discovered in 20% of bulk cow's milk by Angelidis *et al.* (2011) using the fluorescence in situ hybridization technique. However, according to Mousavi *et al.* (2014), nested PCR testing, antimicrobial susceptibility testing and cultural techniques revealed a prevalence of *H. pylori* isolates of 19.2% from dairy product samples and 16.66%, 35%, 28%, 15%, and 13.33% from cow, sheep, caprine, buffalo, and camel milk samples, respectively. In contrast, Bianchini *et al.* (2015) used culture techniques to successfully isolate bacteria from cow milk, and nested PCR was used to confirm their results, yielding a 1.8% prevalence (3/163 samples). The

glmM gene by nested PCR is very sensitive and specific for the detection of *H. pylori* from milk samples of cows, goats, sheep, camels, and buffaloes, according to studies by (Quaglia *et al.* 2008; Rahimi and Khairabadi, 2012; Dore *et al.* 2020). It is possible for *H. pylori* to spread to humans through these animals and their products. To the best of our knowledge, this study is the first to demonstrate the presence of *H. pylori* DNA in goats in Iraq. This finding opens new avenues for research on the potential for human *H. pylori* infection through the consumption of raw milk and highlights the possibility of goats serving as a natural reservoir for the bacterium.

CONCLUSIONS AND RECOMMENDATIONS

The current study indicates that the percentage of *H. pylori* infection in some governorates of the Middle Euphrates in Iraq is lower than in neighboring countries. Additionally, culture techniques were unable to detect *H. pylori*-infected goats, and the use of multiple primer combinations is necessary to improve *H. pylori* diagnosis.

ACKNOWLEDGEMENTS

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NOVELTY STATEMENTS

The novelty of this research is the detection of *H. pylori* in goat milk and feces, which appears in most humans, objective of this research was to identify *H. pylori* by using the polymerase chain reaction technique

AUTHOR'S CONTRIBUTIONS

Contributors Ahmed Mosa and Ali Rabeea designed and organized the study, interpreted the results, and wrote the manuscript. Lina W. and Al-Jabouri collected, cultured the samples and use the polymerase chain reaction technique, and designed two gene expression profiles, and all critically reviewed the manuscript.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby Declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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