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Investigation of *Helicobacter pylori* contamination in broiler chickens in Golestan Province, Iran

Saeed Shateri¹, Elham Esmaili¹, Saeed Seifi^{2*}

¹Department of Veterinary Medicine, Bob.C, Islamic Azad University, Babol, Iran.

²Department of Clinical Sciences, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran.

(* Corresponding Author: s.seifi@ausmt.ac.ir)

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Abstract

This study aimed to investigate the presence of *Helicobacter pylori* (*H. pylori*) in broiler chickens processed in Golestan Province, Iran, and to evaluate its potential public health significance. A total of 240 samples were collected from the subcutaneous tissue of the neck and thigh regions of slaughtered broiler chickens (10 samples from each of 24 farms). Samples were cultured on Brucella agar supplemented with 7% defibrinated sheep blood and selective antibiotics under microaerophilic conditions. Colonies with morphological and biochemical characteristics of *H. pylori* were further confirmed by polymerase chain reaction (PCR) amplification of the 16S rRNA gene using species-specific primers. Statistical analysis was performed using the Chi-square test with a significance level set at $p < 0.05$. *H. pylori* was detected in 8.3% (2/24) of the broiler flocks sampled. The presence of *H. pylori* was confirmed by PCR assay, showing a 133 bp amplicon corresponding to the 16S rRNA gene. The detection of *H. pylori* highlights poultry as a potential source of zoonotic transmission, likely linked to poor hygienic conditions during slaughter and processing. These findings underscore the need for stricter biosecurity measures and monitoring of poultry products to mitigate the risk of *H. pylori* contamination and subsequent human infection.

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Introduction

The genus *Helicobacter* represents a diverse group of gram-negative, spiral-shaped, and microaerophilic bacteria that colonize various sites in humans and animals (Zamani et al., 2018). Based on their ecological niches, *Helicobacter* species are generally divided into gastric and enterohepatic groups. The gastric group, notably *Helicobacter pylori* (*H. pylori*), colonizes the stomach mucosa and plays a major role in chronic gastritis, peptic ulcers, and gastric carcinoma (Hooi et al., 2017). Enterohepatic species, including *H. hepaticus* and *H. bilis*, inhabit the intestinal and hepatobiliary systems, being linked to hepatic and inflammatory bowel diseases (Camilo et al., 2017).

Increasing evidence highlights that *Helicobacter* species have zoonotic potential. Several *Helicobacter* strains, such as *H. pullorum* and *H. suis*, have been detected in poultry and other domestic animals, suggesting possible foodborne or environmental transmission to humans (Kroib et al., 2024). Poultry, including chicken and turkey, constitutes one of the most consumed sources of animal protein worldwide due to its accessibility and low cost (Rapp et al., 2023). Yet, hygienic weaknesses during slaughter and processing may facilitate fecal contamination and bacterial spread across carcasses (Wyink et al., 2025). Under such conditions, *Helicobacter* species may persist in raw poultry, representing a potential link in zoonotic transmission chains. The emergence of antibiotic-resistant *H. pylori* strains constitutes a major global public health challenge (Savoldi et al., 2018). Resistance to clarithromycin, metronidazole, and fluoroquinolones has significantly increased, reducing the efficacy of commonly used eradication therapies (Salahi-Niri et al., 2024). The pathogenicity of *H. pylori* depends largely on the expression of virulence determinants, such as *cagA*, *vacA*, *babA2*, *oipA*, and *iceA*, which mediate adhesion, cytotoxicity, and inflammatory responses (Sharndama and Mba, 2022). Molecular genotyping of these virulence genes provides valuable insight into strain diversity, epidemiology, and potential cross-species transmission routes (Mendoza-Elizalde et al., 2015).

In Iran, the prevalence of *H. pylori* infection remains high; with recent nationwide meta-analyses reporting infection rates exceeding 50%

(Sayehmiri et al., 2024). Factors, such as poor hygiene, low socioeconomic status, and unsafe food or water consumption contribute to transmission (Schuppler, 2025). Therefore, this study aimed to assess the prevalence of *H. pylori* contamination in broiler chickens processed in Golestan province, to better assess their zoonotic significance and implications for public health.

Materials and Methods

Samples collection

In this study, 240 samples were collected from the subcutaneous tissue of the neck and thigh regions of chickens (10 samples from each of 24 broiler farms). Samples were obtained immediately after the slaughter line, prior to the packaging stage. The sterile swabs were immediately placed into Cary-Blair transport medium (Mirmedia, Iran) and transferred to the laboratory, being kept on ice throughout transportation. In the laboratory, the collected samples were pooled in sets of five.

Isolation of *H. pylori*

Homogenized samples were cultured on Brucella agar (Mirmedia, Iran) supplemented with 7% defibrinated sheep blood and the antibiotics vancomycin, trimethoprim, and polymyxin. Following inoculation, the plates were incubated for 7-10 days in an incubator maintained at 10% CO₂ under microaerophilic conditions. Subsequently, smears were prepared from the developed colonies, followed by Gram staining and morphological observation. Biochemical tests, including urease, oxidase, and catalase assays, were then performed. Colonies suspected of being *H. pylori* were isolated and purified for subsequent molecular diagnostic procedures (Elrais et al., 2022).

Molecular confirmation of *H. pylori*

Detection of *Helicobacter* species in suspected colonies was performed using a polymerase chain reaction (PCR) assay. Genomic DNA was extracted from the colonies employing a DNA extraction kit (CinnaGen, Iran) following the manufacturer's instructions. Species-specific primers targeting 16S rRNA were utilized for amplification in the PCR reactions. The primer sequences were listed in Table 1. Genomic DNA from the *H. pylori*

obtained from the Pasteur Institute of Iran (ATCC 43504), served as the positive control, whereas sterile distilled water was used as the negative control. PCR conditions were as follows: an initial denaturation (94°C, 5 minutes), followed by 35 cycles of denaturation (95°C, 30 seconds), annealing (60°C, 1 minute), and extension (72°C, 1 minute), with a final extension (72°C, 10 minutes).

Table 1. Primers used in this study.

Target gene	Primers sequences (5'-3')	Product size (bp)
<i>Helicobacter pylori</i> -specific 16S rRNA	5'-AGGGGTAAAATCCGTAGAGAT-3' 5'-CGTTTATAGGGCGTGGACTA-3'	133

Statistical analysis

The data obtained were analyzed using SPSS software (version 16), and the Chi-Square test was applied. Significance was determined at $p < 0.05$.

Results

In the current study, *H. pylori* was isolated from 8.3% of broiler flocks (2/24 flocks). These results were confirmed in the detection of the bacterium using the PCR method. Fig. 1 showed the electrophoresis results of the PCR products of the *H. pylori* isolated from chicken swab samples. The sequence alignment of the one product extracted from the PCR reading product of *H. pylori* with the sequences recorded was shown in Fig. 2.

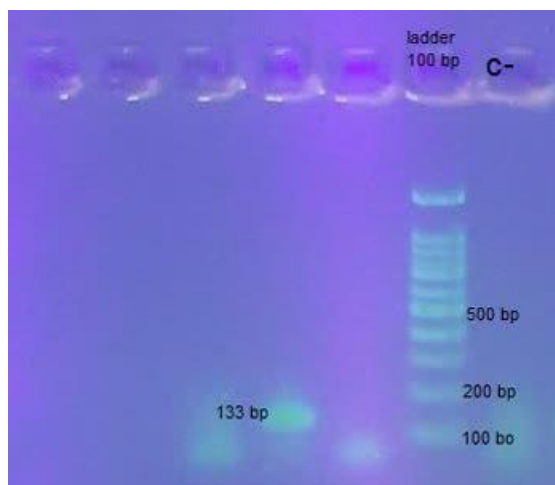


Fig. 1. Agarose gel electrophoresis of PCR of the 16S rRNA gene (133 bp) for the characterization of the *H. pylori*.

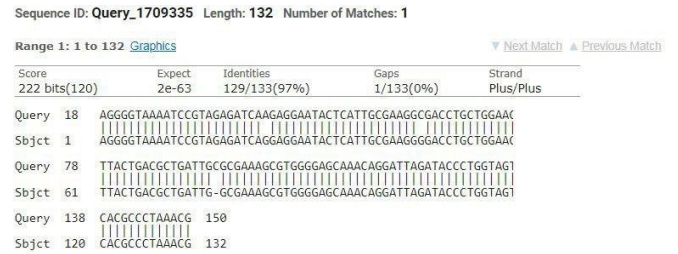


Fig. 2. Alignment of the sequence extracted from the PCR product reading of *H. pylori* with thesequences registered in the gene bank.

Discussion

The present results are consistent with the findings of several previous studies. Asadi *et al.* (2023) reported that this bacterium was found in 20 of 320 (6.25 %) raw chicken meat samples in Shahrekord, Iran. In another study, out of 340 samples, 40 (11.76%) harbored *H. pylori* and prevalence of *H. pylori* in meat, milk, and vegetable samples were 7.33%, 16%, and 12.50%, respectively (Talimkhani and Mashak, 2017). Of the 260 raw chicken meat samples analyzed in a study in Yemen, 36 (13.8%) tested positive for *H. pylori* (Almashhadany *et al.*, 2022). Furthermore, Hemmatinezhad *et al.* (2016) reported a contamination rate of 13.45% in ready-to-eat food samples in Iran. Elrais *et al.* (2022), in a study conducted in Egypt, reported that *H. pylori* was detected in 4% of chicken breast meat samples. Moreover, their study showed an overall prevalence of *H. pylori* of 5.33% among 300 broiler chicken samples (including meat and giblets). Also, hamada *et al.* (2018) rivaled that 7 of the 90 chicken samples were positive for *H. pylori* (7.78%). Similarly, Eldairouty *et al.* (2016) stated that 5% of raw poultry meat samples tested positive for *H. pylori*. Interestingly, Meng *et al.* (2008) reported a significantly higher prevalence of 36% in raw chicken meat, suggesting geographical and methodological differences in the results. This variation in prevalence rates across different studies could be due to a number of factors, including environmental conditions, slaughterhouse practices, sample sizes, or differences in the molecular techniques used for pathogen detection. The higher prevalence observed in their study might also reflect more intense contamination levels in specific regions with inadequate biosecurity and hygiene measures.

According to the findings of Ghanbari *et al.* (2020), the overall prevalence of *H. pylori* in water, vegetables, and animal-derived food products in Iran was reported to be 11.4%. This prevalence may be associated with inadequate hygienic practices during food production and processing, as well as contamination of drinking water sources. Iran is recognized as a country with a high prevalence of *H. pylori* infection in clinical specimens, ranging from 40% to 90% (Khademi *et al.*, 2017). Moreover, compared to global estimates, the prevalence of *H. pylori* resistance to commonly used therapeutic drug regimens is also considerably elevated in Iran (Khademi *et al.*, 2015).

The isolation of *H. pylori* from the subcutaneous tissue of the neck and thigh regions, sampled immediately post-slaughter and pre-packaging, points strongly towards contamination occurring during the slaughtering process itself, rather than originating solely from the initial gut flora of the live bird. This aligns with the hypothesis that poor hygiene during processing facilitates the transfer of bacteria across the carcass surface. The contamination of *H. pylori* in the chicken meat samples tested in the present study can be attributed to the contaminated hands of butchers, veterinarians, and slaughterhouse workers during the handling and preparation of the chickens (Elrais *et al.* 2022). Furthermore, the use of contaminated water during the washing of chicken carcasses may represent another potential reason for the presence of *H. pylori* in chicken meat (Hamada *et al.*, 2018). Moreover, the occurrence of *H. pylori* in chicken samples might also be due to cross-contamination from knives or other slaughterhouse equipment.

From a public health perspective, it is crucial that the meat inspection and slaughter practices undergo strict enforcement of hygiene protocols. Furthermore, consumer awareness campaigns should educate the public on the potential risks associated with raw or undercooked poultry consumption, as well as the proper handling of poultry at home to mitigate zoonotic risks. The role of biosecurity in poultry production cannot be overstated, and future studies should explore the environmental and socio-economic factors that influence the prevalence of *H. pylori* contamination in poultry.

In conclusion, the detection of *H. pylori* in broiler chickens underscores the need for

improved safety and hygiene standards in poultry farming, processing, and handling to prevent zoonotic transmission. As *H. pylori* infection remains a significant public health issue worldwide, controlling its spread in the food supply chain, particularly in poultry, is vital in reducing the global burden of this infection.

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Conflict of Interest

The authors do not have any potential conflict of interest to declare.

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