Molecular detection of Campylobacter species from broiler flocks in Kerman

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Abstract

In recent years, the increase in poultry meat consumption and food safety concerns have made the control of foodborne illnesses essential. *Campylobacter* is one of the most important causes of bacterial gastroenteritis in humans, and poultry, especially broiler chickens, are considered the main source of its transmission. *Campylobacter jejuni* and *coli* species are transmitted through contaminated poultry meat, water, or direct contact and pose a threat to public health. Given the lack of effective measures to reduce contamination in the poultry production chain, identifying and monitoring this bacterium is necessary. Therefore, this study was designed and conducted to molecularly isolate *Campylobacter* species from broiler flocks in Kerman city. In this study, samples were collected from the liver and spleen of 20 broiler flocks in the industrial slaughterhouse of Kerman during the winter of 2024. DNA extraction was performed using the SinaClon kit

according to the standard protocol. For the detection of *Campylobacter* and its species, a PCR reaction was performed with three specific primers, including the 16s rRNA gene for the *Campylobacter* genus, the mapA gene for C. jejuni, and the ceuE gene for C. coli. PCR products were electrophoresed on a 1.5% agarose gel and examined at 75 volts for 1.5 hours. In this study, out of 200 swab samples examined, 16 samples (8%) were evaluated as positive for Campylobacter genus DNA in the PCR test. Among the 16 positive samples, 8 samples (50%) contained Campylobacter jejuni and 10 samples (62.5%) contained *Campylobacter coli*. Also, 2 samples were simultaneously infected with both *Campylobacter jejuni* and *coli* species. Overall, the findings of this study indicate a relatively low prevalence of *Campylobacter* in broiler flocks in Kerman city. This lower contamination rate could be due to better management conditions on farms, differences in sampling methods, or other environmental and technical factors. Further investigations considering larger sample sizes, diverse geographical areas, and complementary diagnostic methods can contribute to a more comprehensive understanding of Campylobacter epidemiology in the poultry population.

Keywords: *Campylobacter*, *Campylobacter jejuni*, *Campylobacter coli*, Broiler Chickens, Kerman

1. Introduction

In recent years, the global surge in chicken meat consumption has precipitated escalating concerns regarding food safety and the proliferation of foodborne illnesses. Bacteria belonging to the genus *Campylobacter*, recognized as a principal etiological agent of bacterial gastroenteritis in humans, assume a significant role in this context. Poultry is acknowledged as the primary vehicle for the transmission of *Campylobacter* species to the human population (1). The elevated prevalence of *Campylobacter* contamination in broiler chickens, identified by the World Health Organization as a major contributor to diarrheal diseases worldwide, is instrumental in this transmission dynamic (1). These bacteria are commonly isolated in poultry farm environments and their surrounding areas (2). *C. jejuni* and *C. coli*, the predominant species implicated in human infections, are transmitted through the consumption of contaminated chicken meat, exposure to polluted water sources, or direct contact with infected animals (3).

Chicken meat, constituting the second most prevalent source of animal protein in urban households, has experienced a notable increase in per capita consumption in Iran, rising from 21.83 kg in 2008 to 32 kg in 2019, and thus holds a position of considerable importance in the human diet (4). This upward trend in consumption patterns accentuates concerns pertaining to the contamination of this essential food commodity with *Campylobacter*.

Campylobacter bacteria, characterized as Gram-negative, curved or spiral-shaped microorganisms, are typically isolated within the gastrointestinal tract of avian species, with infections frequently presenting asymptomatically (5). Nevertheless, these bacteria may be transmitted to humans through the consumption of contaminated chicken meat, thereby inducing gastrointestinal pathologies. Scholarly investigations

indicate that *Campylobacter*, in addition to its presence in the gastrointestinal system, can also disseminate to other internal tissues of poultry, giving rise to potential public health issues (6).

The high prevalence of *Campylobacter* in poultry flocks, particularly among broiler chickens, and its positive correlation with advancing avian age, underscores the importance of implementing control measures for this bacterium throughout the chicken meat production continuum (7). A multitude of studies have documented the elevated prevalence of *Campylobacter* contamination in broiler chickens across diverse regions within Iran (8). For instance, a study conducted by Ansari revealed that white meat, and particularly chicken meat, exhibits the highest rate of *Campylobacter* contamination in Iran (8).

The transmission of *Campylobacter* to poultry rearing facilities can occur through a variety of sources, including aged litter, contaminated drinking water, farm animals, insects, and equipment (9). Effective control of this bacterium in poultry farming operations presents a considerable challenge, necessitating the meticulous implementation of biosecurity and hygienic protocols (10).

Diagnostic methodologies for *Campylobacter* encompass microbial culture, immunological assays, and molecular techniques. Molecular methods, and particularly polymerase chain reaction (PCR), due to their enhanced speed and accuracy, are of paramount importance in the detection and identification of diverse *Campylobacter* species (11).

Considering the importance of public health and the role of chicken meat in transmitting *Campylobacter* to humans, this study aims to isolate Campylobacter species from broiler flocks in Kerman at the molecular level. It seeks to detect *Campylobacter* molecularly in broiler chickens within this region and to identify *C. jejuni* and *C. coli* among the confirmed isolates. The results are expected to enhance understanding of the contamination levels of broiler flocks with *Campylobacter* in this region and support the development of effective strategies to control and reduce the risks posed by this bacterium.

2. Materials and Methods

2.1. Sampling

Sampling was conducted on 20 broiler flocks of the Ross breed referred to an industrial slaughterhouse in Kerman during the winter season (February to March 2024). From each flock, 10 birds were sampled, and tissue samples from both the liver and spleen of each bird were collected. The microtubes containing the samples were placed in ice-filled containers and transported to the laboratory as quickly as possible to prevent DNA degradation.

2.2. DNA Extraction

DNA extraction from the tissues was performed using the Sina Clone kit (Tehran, Iran) following the manufacturer's protocol. The kit is specifically designed to extract DNA from animal tissues and offers high yield and purity. Briefly, 400 µL of lysis buffer was added to the microtubes to disrupt the cells and release

DNA. Subsequently, 30 µL of carrier RNA was added to enhance extraction yield, followed by 200 µL of the tissue sample. After vortexing and short spin centrifugation, 20 µL of proteinase K was added to the samples (excluding nasopharyngeal and serum samples) and incubated at 72°C for 10 minutes. Proteinase K digests proteins bound to DNA, facilitating DNA extraction. Incubation at 72°C increases the activity of this enzyme. Then, 300 µL of binding buffer was added and gently inverted. In the following steps, Wash I and Wash II buffers were used for washing, and several centrifugations were performed to isolate the DNA. Finally, the extracted DNA was collected with 50 µL of elution buffer.

2.3. PCR Assay

PCR assay was performed using three primers designed to identify *Campylobacter* genus (16S rRNA gene), *C. jejuni* (mapA), *C. coli* (ceuE). The PCR protocol consisted of initial denaturation (95°C, 10 min), 35 cycles of 95°C for 30 sec, primer-specific annealing (see Table 1) for 1.5 min, and 72°C for 1 min, followed by final extension (72°C, 10 min).

Table 1. Primer sequences and PCR conditions for detection of Campylobacter species.

Primer	Sequence (5'–3')	Annealing Temperature	Product Size (bp)
Campylobacter spp.	F: ATCTAATGGCTTAACCATTAAAC R: GGACGGTAACTAGTTTAGTATT	56°C	857
Campylobacter jejuni	F: CTATTTTATTTTGAGTGCTTGTG R: CTTTATTTGCCATTTGTTTTATTA	**59°C** (corrected from 89°C)	589
Campylobacter coli	F: AATTGAAAATTGCTCCAACTATG R: TGATTTTATTATTTGTAGCAGCG	58°C	462

2.4. PCR Product Analysis

A T.B.E. solution was prepared with 10.8 g of Tris base, 5.5 g of boric acid, and 0.75 g of EDTA in one liter of distilled water. 100 mL of T.B.E. was heated, 10 µL of Green Viewer was added to the dissolved agarose, and the mixture was poured into a mold. The mold was incubated at room temperature for 15–20 minutes, and then at 4°C for 15–20 minutes. The gel was separated from the mold, and electrophoresis was performed.

3. Results

3.1. Identification of *Campylobacter* Genus

Of the 200 swab samples obtained from the liver and spleen of the meat samples examined, 16 samples (8%) were positive for *Campylobacter* genus DNA using the PCR method (Figure 1).

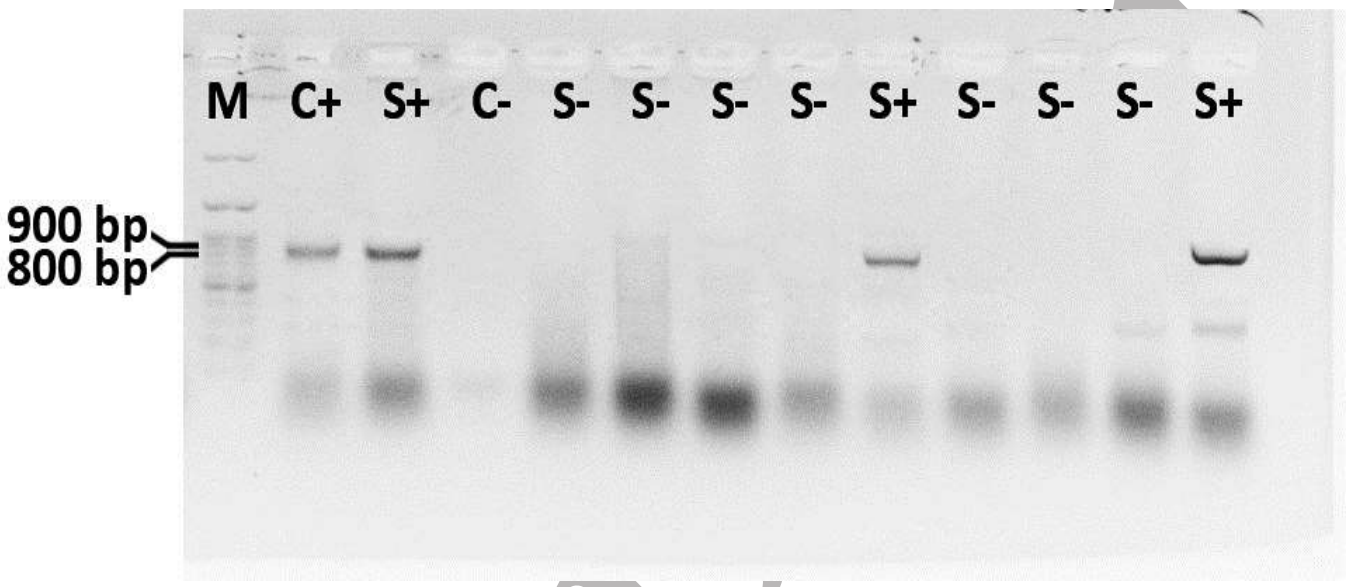

Figure1: PCR assay for detecting *Campylobacter* sex-specific genes; M: 100 bp marker, C+: Positive control (857 bp), C-: Negative control, S+: Positive sample, and S-: Negative sample.

3.2. Identification of *Campylobacter jejuni* Species

Among the 16 samples that were positive for the *Campylobacter* genus, 8 samples (50%) were reported positive for *C. jejuni* species DNA in the PCR assay (Figure 2).

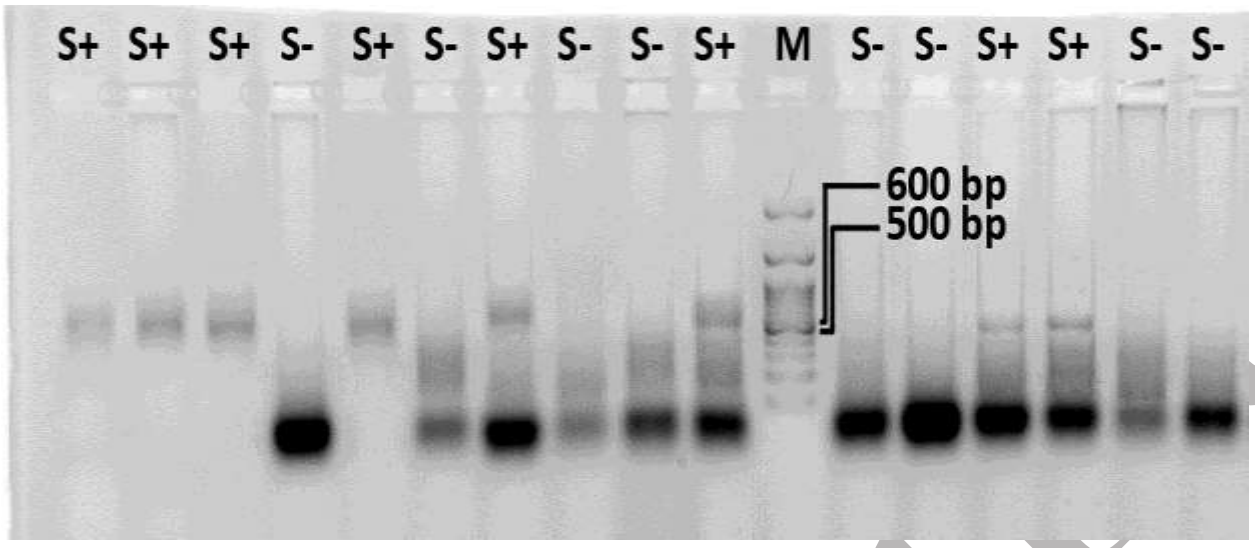

Figure2: PCR assay for detecting *Campylobacter jejuni* species-specific genes; M: 100 bp marker, C+: Positive control (589 bp), C-: Negative control, S+: Positive sample, and S-: Negative sample.

3.3. Identification of *Campylobacter coli* Species

Within the 16 samples positive for the *Campylobacter* genus, 10 samples (62.5%) were determined to be positive for *C. coli* species DNA using the PCR method (Figure 3, Table 2).

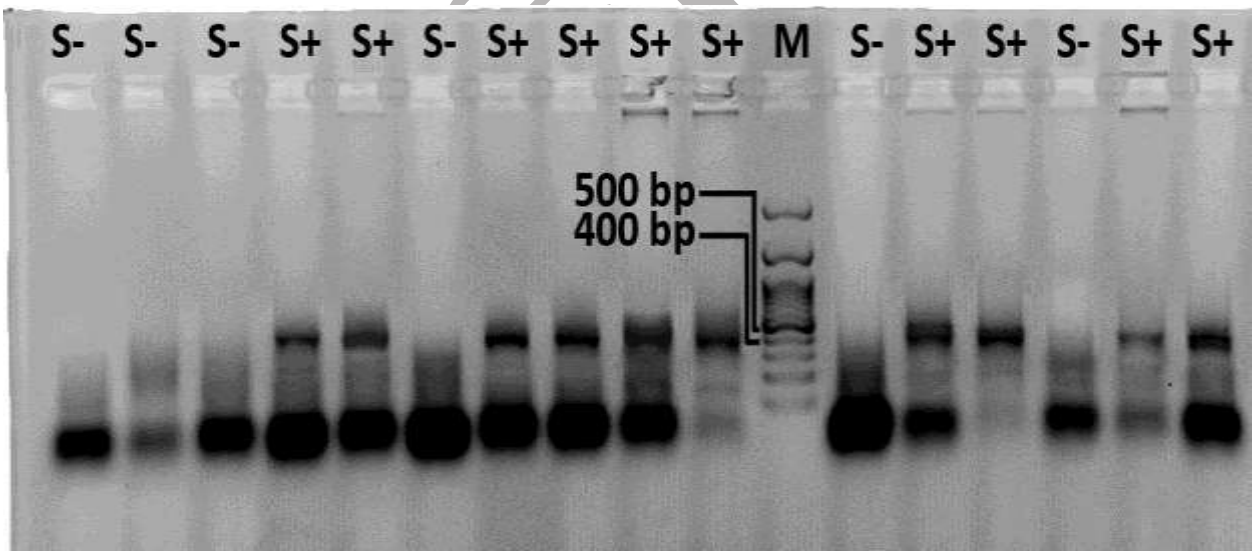

Figure3: PCR assay for detecting *Campylobacter coli* species-specific genes; M: 100 bp marker, C+: Positive control (462 bp), C-: Negative control, S+: Positive sample, S-: Negative sample.

Table 2. Number and percentage of samples positive for *Campylobacter* spp. (*C. jejuni* and *C. coli*).

Category	Among *Campylobacter* positive samples (n=16)		Among total samples (n=200)	
	No.	%	No.	%
Campylobacter genus				
Positive	16	100%	16	8%
Negative	0	0%	184	92%
C. jejuni				
Positive	8	50%	8	4%
Negative	8	50%	192	96%
C. coli				
Positive	10	62.5%	10	5%
Negative	6	37.5%	190	95%

4. Discussion

Several studies have demonstrated that Campylobacter can translocate from the intestinal tract to internal organs such as the liver by penetrating the mucosal barrier, subsequently colonizing these sites. These findings challenge the traditional perception of Campylobacter as a harmless commensal in chickens and raise concerns for both poultry and public health, especially considering the link between contaminated chicken liver and human infections (6).

The prevalence observed in this study is similar to that reported in Denmark (9–15%) and China (12%) (13). However, some studies have reported higher rates: Noormohamed et al. (2012) found 67% contamination in liver and gizzard samples from retail chickens, Berang et al. (2019) reported 83% in broiler carcasses, and Gharajalar et al. (2020) found 43% contamination in chicken livers (13–15).

The prevalence of Campylobacter is known to vary by region, season, and production system. Higher rates are often observed in summer and autumn (16). In contrast, the present study was conducted in winter, which may account for the lower contamination rate compared to studies with longer or seasonally broader sampling periods.

Khalili et al. (2009) reported a 3.3% prevalence of C. jejuni in cecal samples from Kerman, which aligns with our findings (18). Other studies have shown varied distributions of C. jejuni and C. coli. For example, Noormohamed et al. (2012) found similar frequencies for both species, while Franciska et al. (2017) reported C. coli as predominant in laying hens and C. jejuni in broilers (13,19). In contrast, Cox et al. (2021) reported 72% C. jejuni and 28% C. coli in chicken livers (20). Similarly, Iranian studies often report C. jejuni as the dominant species (21,22).

Differences in species prevalence may be influenced by host species, production systems, or antibiotic pressure, as C. coli tends to be more resistant (23,24). Moreover, sampling methods affect detection rates;

studies that sample multiple tissues (e.g., liver, gizzard, feces) tend to report higher prevalence than those using fewer or more limited sample types.

In conclusion, the present study reports a relatively low prevalence of Campylobacter in broiler flocks in Kerman, potentially due to better farm management, seasonal factors, or sampling methodology. Broader studies using standardized sampling and diagnostic techniques are needed for a clearer understanding of Campylobacter epidemiology in poultry.

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Authors' Contribution

1- Study concept and design: Shafiei.H

2- Acquisition of data: Jajarmi.M, Kalidari.M

3- Analysis and interpretation of data: Shafiei.H, Kalidari.M, Jajarmi.M, Zamani-Ahmadmahmudi.M

4- Drafting of the manuscript: Jahedi.M, Hajipour.P

5- Critical revision of the manuscript for important intellectual content: Shafiei.H

6- Statistical analysis: Jajarmi.M, Zamani-Ahmadmahmudi.M

7- Administrative, technical, and material support: Jajarmi.M, Zamani Ahmadmahmudi.M, Hajipour.P

8- Study supervision: Shafiei.H

Conflict of Interest

The authors are responsible for the content of this article and declare that they have no competing interests.

Ethics

Ethical approval was deemed unnecessary, as all sampling and testing procedures were performed on carcasses obtained from slaughterhouses, thereby eliminating the need for the use of experimental animals.

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Data Availability

The data that support the findings of this study are available on request from the corresponding author.

In this study, DeepSeek AI and Grammarly were used solely for improving the text's phrasing and enhancing its writing quality. No AI tools were employed for generating or shaping the content itself.

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