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Rapid identification and quantification of *Campylobacter jejuni* by culture and real-time PCR from slaughtered poultry carcasses in Tehran, Iran

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Abstract

Campylobacter species are the main cause of infectious gastroenteritis in human beings. Consumption of poultry meat has been proposed as one of the important sources of campylobacteriosis in human beings. The aim of this study was to investigate the prevalence of *Campylobacter jejuni* (*C. jejuni*) in freshly slaughtered poultry in Tehran using culture and molecular methods. It is a descriptive-analytical and cross-sectional study. Fifty samples of whole fresh poultry were randomly collected from supply centres in Tehran from January to February 2024. The standard method of enrichment and cultivation in modified Charcoal Cefoperazone Deoxycholate agar (mCCD) was used to identify *Campylobacter* spp. DNA was extracted from the confirmed colonies using a commercial kit. Then the real-time PCR method by amplification of the specific region of *C. jejuni* (ORF-*C*) was used to determine the *C. jejuni*. Out of 50 examined samples, 19 samples (38%) showed positive *Campylobacter* spp. cultures. The results of the Molecular tests showed that *C. jejuni* was isolated from 13 samples (26%). The results indicated *Campylobacter* contamination in poultry carcasses in Tehran. Quality control must be improved to reduce contamination in slaughterhouses.

Introduction

Food-borne diseases are one of the most serious public health problems worldwide (1). Poultry meat is one of the most consumed animal protein sources in many countries, including Iran (2). Various bacterial pathogens are transmitted to consumers through meat and poultry products, including *Salmonella*, *Escherichia coli*, *Listeria* and *Campylobacter* (3, 4). *Campylobacter* is the most common cause of gastroenteritis in human beings in many developing and developed countries (5, 6).

The members of the genus *Campylobacter*, which belong to the family *Campylobacteraceae*, are Gram-negative, rod-shaped, curved, narrow, spiral-shaped, motile and mostly microaerophilic bacteria. Two species of *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) are the

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most important disease-causing species in human beings isolated from poultry carcasses (5). Studies have shown that 95 % of human cases of campylobacteriosis are caused by *C. jejuni*, 4 % by *C. coli* and the remaining 1 % by other species (7). This disease is a zoonosis transmitted from animals such as cattle, sheep, pigs and poultry to human beings via the faecal-oral route. This infection can occur as mild and self-limiting diarrhoea up to bloody diarrhoea (8).

Although in most cases campylobacteriosis occurs as self-limiting enteritis without the need for antibiotic treatment, severe campylobacteriosis, especially in patients with a weak immune system, can lead to long-term enteritis and septicaemia (9). Although *Campylobacter* is not considered a pathogenic agent for poultry and *Campylobacter* infections in poultry do not usually cause clinical symptoms, the consumption of undercooked poultry meat and poultry products is the most important source of infection for human beings (10). Contamination of chicken carcasses during slaughter, handling and transportation also increases the incidence of campylobacteriosis. One of the main reasons for the contamination of chicken carcasses with *Campylobacter* is the high presence of this bacterium in the intestines of poultry that have reached slaughter age, leading to contamination of the carcass during the slaughter process (11, 12). Some reports estimate that 20 to 40 percent of individual *Campylobacter* infections are caused by eating poultry meat. In the United States, it is reported that 80% of *Campylobacter* infections are foodborne (13).

According to the studies mentioned, the possibility of contamination of broilers with this bacterium is high, and there is also a risk of contamination by human beings. To control contamination, it is therefore necessary to check for the presence of these pathogens, to know the risk factors that cause contamination and to control them. In addition to the points mentioned above, hygiene in slaughterhouses should also be considered for control purposes. Therefore, we investigated the prevalence of *C. jejuni* in freshly slaughtered chickens in Tehran using culture and molecular methods (real-time PCR).

Materials and Methods

Sample collection

This study is descriptive-analytical and cross-sectional. Using the sample size calculation formula and assuming a precision of 14% and a confidence level of 95%, the minimum required sample size was calculated as 50 samples. Tehran, as the capital of Iran with a large population and diverse food sources, presents a high-risk environment for studying campylobacteriosis, particularly because of its numerous poultry markets and slaughterhouses that facilitate comprehensive sampling for assessing *Campylobacter* contamination. Fifty samples of whole fresh poultry were randomly collected from supply centres in Tehran from January to February 2024. Samples were collected from five different slaughterhouses (10 samples each) using a simple random sampling method over four weeks. The poultry samples were taken on the day of slaughter, fresh and not frozen, and their production and packaging dates were checked. All samples were transported to the Microbiology Laboratory of Islamic Azad University, Shabestar Branch, Iran, as quickly as possible under aseptic conditions by using insulated containers and ice packs at a temperature of 0 to 4ºC and tested immediately.

Preparation of the samples

According to Instruction No. 17219 of the National Standard of Iran, to obtain samples from fresh poultry, the skin and subcutaneous tissue of the breast area are cut with a sterile scalpel blade and a certain amount is taken.

Isolation and identification of Campylobacter species

Microbiological isolation and biochemical identification of Campylobacter spp

Campylobacter spp. were identified by a culture method according to Instruction No. NRLASD/FQ/P/037 of the Standard Operating

Procedure of Iran Veterinary Organization. For this purpose, 10 grams of the sample were added to 90 ml of 0.1% sterile peptone buffer water (Merck, Germany). The mixture was homogenized well and incubated at 41.5 degrees Celsius for 24 hours. Then 0.1 and 0.01 dilutions were prepared and inoculated into two plates of modified Charcoal Cefoperazone Deoxycholate agar medium (mCCD) (Merck, Germany) and incubated again under microaerophilic conditions at 41.5 ^{0}C for 44 ± 4 hours. To confirm and separate *Campylobacter* species, individual grown colonies are analysed using several biochemical tests: Gram staining (identifying Gram-negative, curved or spiral-shaped rods), catalase test (positive in most species except *C. sputorum* and *C. concisus*), oxidase test (positive in most species), hippurate hydrolysis test (positive in *C. jejuni*), and resistance to cephalothin and nalidixic acid (typical for *Campylobacter* species, particularly *C. jejuni*) (14).

Molecular detection of Campylobacter jejuni

Extraction of bacterial DNA from the confirmed colonies was performed using the High Pure PCR Template Kit (Roche, Switzerland) and according to the manufacturer's instructions. The primer (15) and probe sequence from Table 2 synthesized by Sinaclon, Iran, was used to amplify the *C. jejuni*-specific region of the ORF-C sequence.

Real-time PCR was performed using the RealQ Plus 2x Master Mix for Probe Kit (Ampliqon). Table 1 presents the detailed composition of the real-time PCR reaction mix, including the volumes and final concentrations of each component (15).

Table 1. Real-time PCR reaction mix components and volumes

Component	Volume (µL)	Final concentration
PCR Master Mix	12.5	1x
Forward primer (10 µM)	1	0.4 µM
Reverse primer (10 µM)	1	0.4 µM
Probe (10 µM)	0.25	0.1 µM
DNA template	5	-
Sterile deionized water	5.25	-
Total	25	-

Amplification and detection were performed using the Rotor-Gene Q (Qiagen). Real-time PCR amplification was performed using the program according to the study by Sails *et al.* (15). The thermal cycling conditions included an initial step at 50°C for 2 minutes, followed by 95°C for 10 minutes, and 45 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. For the negative controls, the DNA template was replaced by sterile water. *Campylobacter jejuni* ATCC33560 was used as a positive control. A positive result was defined as a clear exponential amplification curve above the threshold, as per the standard protocol. Positive and negative controls performed as expected. The primer and probe sequences, as detailed in Table 2, are critical for the specificity of the assay.

Results

Prevalence of contamination in culture and molecular assays

In general, the results of the culture of the samples showed that out of 50 fresh poultry samples, 19 samples (38%; 95% CI: 25–51%) had a positive *Campylobacter* culture and according to the results of the complementary molecular tests in 19 positive samples, *C. jejuni* infection was detected in 13 samples (26%; 95% CI: 14–38%) (Figure 1). Figure 2 shows the amplification curve of the real-time PCR.

Table 2. Primer and probe sequences

Primers and probe name	Oligonucleotide sequences (5′ to3′)	Ref.	Product size (bp)
82F	TTGGTATGGCTATAGGAACTCTTATAGCT	15	116 bp
197R	CACACCTGAAGTATGAAGTGGTCTAAGT		
CJTP2	FAM-TGGCATATCCTAATTTAAATTATTTACCAGGAC-MGB		

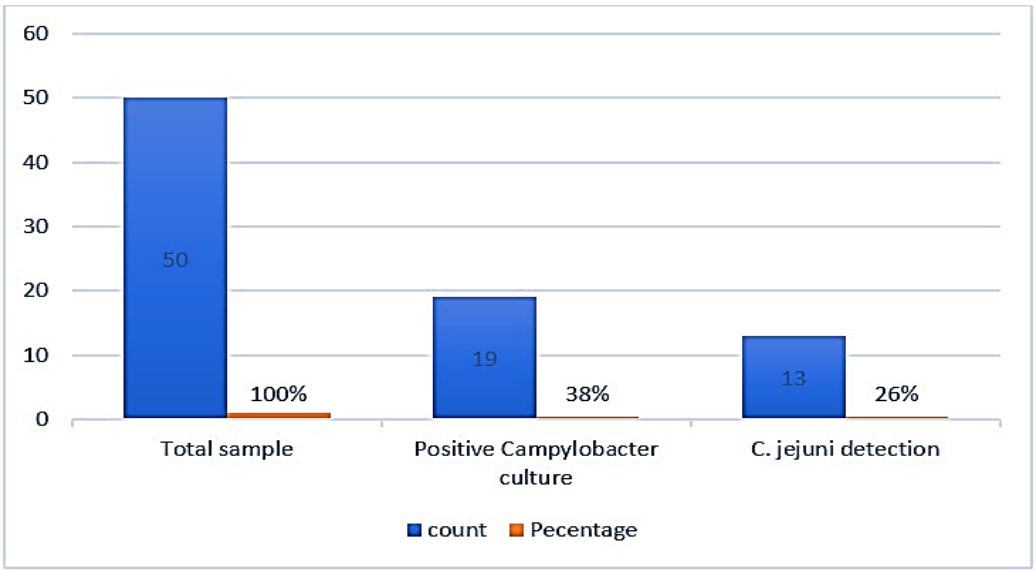

Fig. 1. Number and percentage of *Campylobacter*-infected samples

Discussion

Foodborne diseases are one of the most important health problems in the world. Since *Campylobacter* as the most common bacterium causing gastroenteritis is associated with the consumption of poultry meat (16, 17), we decided to investigate the prevalence of this bacterium in slaughtered poultry in Tehran using culture and molecular (real-time PCR) methods. The results of the present study show that out of a total of 50 poultry meat samples from Tehran slaughterhouses, 19 samples (38%) were infected with *Campylobacter* species using the culture method, which is consistent with the reports from other studies around the world and in Iran. In the Iranian studies, the prevalence of poultry infections with *Campylobacter* in different cities such as Isfahan, Shahr-e-kord, and Tehran were reported to be 56.1, 47 and 49.5, respectively (18-20). In the study by Fani *et al*. (21), 31 samples (31%) of 100 carcass samples from an industrial poultry slaughterhouse in the city of Gonabad were infected with *Campylobacter*.

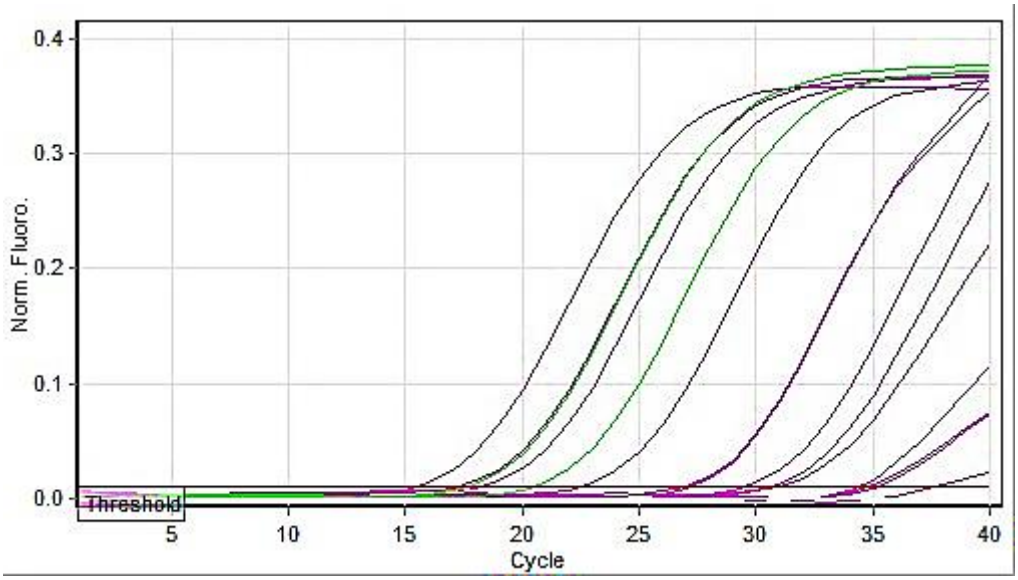

Fig. 2. Amplification curve of ORF-C by real-time PCR

The results of the study by Nasiri and Motalebi (22), showed that 208 samples (37.7%) out of 522 samples of poultry meat and its edible contaminants were infected with *Campylobacter* species. In the study by Sadeghi *et al*. (23), out of 100 chicken meat samples collected from 22 districts of Tehran, 35 samples were infected with *Campylobacter* species. The results of Pourahmadi et al. (24) showed that 217 samples (66.2%) of 328 chicken carcass samples from slaughterhouses in the city of Jahrom were infected with *Campylobacter*.

In other countries, contamination levels of 30 to 90 percent were reported. In a study of 300 poultry carcasses in Senegal, 168 samples (56%) were found to be infected with *Campylobacter* (25). In the study by Nobile *et al*. (26) to investigate the prevalence of *Campylobacter* species in raw poultry meat samples in southern Italy, 43 samples (20.67%) out of a total of 208 samples were contaminated with *Campylobacter*. The infection rate was also 49.90% in Ireland and 48% in Pakistan (27, 28). According to the results of various studies, the different levels of contamination can be attributed to the degree of infection of the poultry in the different areas, the time intervals between the studies, the different slaughter methods and the compliance with hygiene principles in the different stages of slaughter.

Since the PCR method is capable of detecting *Campylobacter* at the species level compared to the culture method (15), we used the real-time PCR method to identify the species *C. jejuni* in the present study. The results of the molecular assays in our study showed that of the 19 positive samples in the culture method, 13 samples (26%) were associated with *C. jejuni* and the rest with other *Campylobacter* species. The results of other studies also showed that the isolation of *C. jejuni* was far more common than other species, so that in the study by Fani *et al*. (21) out of 31 positive *Campylobacter* samples, 19 samples (61.29%) were *C. jejuni*. The results of the study by Nasiri and Motalebi (22) show that 78.4% of the *Campylobacter* species isolated from the total samples were *C. jejuni*. In the study by Raeisi *et al*. (29), 40.8% of the poultry meat samples were infected with *C. jejuni* and in the study by Hoseinpour *et al*. (30), 31% of the poultry meat samples were infected with *C. jejuni*. Hussain *et al*. (28) in Pakistan reported a prevalence of *C. jejuni*

in chicken meat samples of 72%. In another similar study by Whyte *et al*. (27) in Ireland, the prevalence of *C. jejuni* in chicken meat was reported to be 84.6%.

Other studies have reported higher *Campylobacter* contamination in summer (4); the winter sampling window in the present study may partly explain the comparatively lower rate observed here.

In general, it can be said that, according to the results of the present study, *Campylobacter* infections, especially *C. jejuni*, still occur with different percentages in poultry meat in slaughterhouses in Iran, and it is therefore necessary to carry out continuous testing and to inform consumers to cook poultry fully before consumption to reduce campylobacteriosis. Further, in order to reduce *Campylobacter* contamination, it is necessary to observe personal and environmental hygiene in slaughterhouses, avoid contact between carcasses and especially with the contents of the digestive tract, use suitable water for correct and complete washing in the slaughter process and apply the principles of hygiene at all stages of slaughter.

Conclusion

The results of the present study showed that out of 50 fresh chicken samples, 19 samples (38%) were infected with all types of *Campylobacter* species by the culture method and 13 samples (26%) were associated with *C. jejuni* species by additional testing with the molecular method. This result demonstrates both the efficiency of the molecular method in rapidly identifying the contaminating species and the presence of the risk of *Campylobacter* contamination in the poultry sold in Tehran, which may call into question the consumption of this product. In order to reduce the contamination of poultry meat and poultry products with *Campylobacter* species and other similar microorganisms, the principles of environmental and personal hygiene in slaughterhouses must be observed, and regular testing under the highest possible supervision should take place.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Artificial Intelligence Statement

During the preparation of this manuscript, the authors used ChatGPT-4 solely for language editing and grammatical correction.

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