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Seasonal Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in Raw Chicken Meat Using PCR Assay

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Abstract: *Campylobacter jejuni* and *Campylobacter coli* are the most common cause of food-borne bacterial gastroenteritis in both developed and developing countries worldwide. The aim of this study was to detect and determine the seasonal prevalence of *Campylobacter jejuni* and *Campylobacter coli* in raw chicken meat using PCR assay. From July 2009 to July 2010, a total of 350 raw chicken meat samples were purchased from randomly selected retail outlets in Shahrekord and Yasouj, Iran. Overall, 197 meat samples (56.3%) were contaminated with *Campylobacter*. The most prevalent *Campylobacter* species was *Campylobacter jejuni* (92.9%). No significant differences in the prevalence rates were observed between meat samples isolated in Shahrekord (55.2%) and Yasouj (59%).The highest prevalence of *Campylobacter* spp. in chicken meat samples occurred in July (87.5%), Jun (81.8%), September (76.7%) and August (68.8%) respectively, and the lowest prevalence occurred in February (20.7%). Overall, the prevalence of *Campylobacter* spp. in chicken meat samples in summer was significantly ($P < 0.05$) higher than fall and winter. This study shows that seasons of the year influence *Campylobacter* spp. detect ability and the carrier state in market chicken at retail level.

Key words: *Campylobacter* · Chicken meat · Seasonal prevalence

INTRODUCTION

In recent years, the frequency of human enteritis caused by the *Campylobacter* spp. has been on the increase in many developed and developing countries [1-5]. *Campylobacter* spp. mainly *Campylobacter jejuni* and *Campylobacter coli*, have been recognized as a major cause of human gastroenteritis throughout the world [3].

Campylobacter gastroenteritis is a disease mainly taken up with food. In contrast to other bacterial enteritis, an increase of *Campylobacter* populations in foodstuffs is unlikely [5]. Several epidemiological case-control studies have established that ingesting undercooked poultry products significantly increases the risk for acquisition of food-borne campylobacteriosis [6-8]. Campylobacteriosis in humans has shown peak isolation rates during the summer [9]. Conversely, a study conducted by Mattila et al. (1992) in Finland found that *Campylobacter* strains were the leading cause of travelers' diarrhea in the winter (28%), and caused only 7% of these cases in the fall [10].

Poultry carcasses are commonly contaminated with *Campylobacter* in poultry processing plants [4,11]. Contamination during processing occurs directly via intestinal contents or indirectly from bird to bird, via equipment and water [11]. Studies have demonstrated high levels of *Campylobacter* on broiler chickens from farm ranging 0 to100% [12] and retail chickens [13] ranging from 40% to 100% [14]. Generally more flocks remain free of this infection during the cooler months of the year [15].

This study was undertaken to determine the seasonal Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in raw chicken meat using PCR assay in Shahrekord and Yasouj, Iran.

MATERIALS AND METHODS

Samples: From July 2009 to July 2010, 350 chicken meat samples were randomly purchased from 19 retail outlets in Shahrekord and Yasouj, Iran. All samples were taken by using sterilized utensils, placed in separate sterile plastic bags to prevent spilling and cross contamination, and

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were immediately transported to the laboratory in a cooler with ice packs.

DNA Extraction and PCR Conditions and Identification Campylobacter: The samples were processed immediately upon arrival using aseptic techniques. Of each meat sample, 25 g was homogenized and transferred to 225 mL of Preston enrichment broth base (HiMedia Laboratories, Mumbai, India, M899) containing *Campylobacter* selective supplement IV (HiMedia Laboratories, Mumbai, India, FD042) and 5% (v/v) defibrinated sheep blood and incubated for 48 h at 42°C in a microaerophilic condition (85% N_2, 10% CO_2, 5% O_2) [16-17]. DNA from 350 samples was extracted from Preston broth after the enrichment step using a Genomic DNA purification kit (Fermentas, GmbH, Germany, K0512) according to the manufacturer's protocol. The PCR procedures used in this study have been described previously [18]. Three genes selected for the identification of the *Campylobacter* spp., *Campylobacter jejuni*, and *Campylobacter coli* were the *16S rRNA* gene [19], the *mapA* gene, and the *ceuE* gene [20], respectively. The sequences of the three sets of primers used for gene amplification are presented in Table 1. Amplification reactions were performed in a 30 μL mixture containing 0.6 U Taq polymerase (Fermentas, GmbH, Germany), 100 μmol l^{-1} of each dNTP, 0.11 μmol l^{-1} of MD16S1 and MD16S2 primers, and 0.42 μmol l^{-1} of MDmapAl, MDmapA2, COL3 and MDCOL2 primers in the Fermentas buffer (Fermentas, GmbH, Germany). Amplification reactions were carried out using a DNA thermal cycler (Master Cycle Gradiant, Eppendrof, Germany) with the following program: one cycle of 10 min at 95°C, 35 cycles each consisting of 30 s at 95°C, 1 min and 30 s at 59°C, 1 min at 72°C and a final extension step of 10 min at 72°C. The amplification generated 857 bp, 589 bp, and 462 bp DNA fragments corresponding to the *Campylobacter* genus, *Campylobacter jejuni* and *Campylobacter coli*, respectively. *Campylobacter coli* (ATCC 33559) and *Campylobacter jejuni* (ATCC 33560) were used as the positive controls and DNase free water was used as the negative control. The PCR products were stained with 1% solution of ethidium bromide and visualized under UV light after gel electrophoresis on 1.5% agarose.

Statistical Analysis: Data were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), a chi-square test analysis was performed and differences were considered significant at values of $P < 0.05$.

RESULTS AND DISCUSSION

Using PCR techniques, 197 of 350 chicken meat samples (56.3%) were found to be contaminated with *Campylobacter* (Table 2) which is comparable with those reported by others [13, 17, 21-26]. No significant differences in the prevalence rates were observed between meat samples isolated in Shahrekord (55.2%) and Yasoge (59%).

The most prevalent *Campylobacter* species isolated from meat samples was *Campylobacter jejuni* (92.9%); the remaining isolates were *Campylobacter coli* (7.1%). *Campylobacter jejuni* has been reported to be the most frequent species recovered from food of animal origin specially poultry meat [27]. Our results on the prevalence of *Campylobacter jejuni* in raw meat are in agreement with data from other countries [17, 26-28].

Table 2 shows the monthly prevalence of *Campylobacter* spp. in chicken meat samples in Shahrekord and Yasouj, Iran. The table shows, the highest prevalence of *Campylobacter* spp. in chicken meat samples occurred in July (87.5%), Jun (81.8%), September (76.7%) and August (68.8%) respectively, and the lowest prevalence occurred in February (20.7%). Overall, the prevalence of *Campylobacter* spp. in chicken meat samples in summer was significantly ($P<0.05$) higher than fall and winter; however, the difference in the prevalence rates of *Campylobacter* spp. between fall and winter was not statistically significant. This finding is in agreement with other studies that reported peak prevalence rate of *Campylobacter* in poultry meats in the warmer months [13, 17, 29]. In a study conducted by Jacobs-Reitsma *et al.* (1994) reported that *Campylobacter* presence showed seasonal variation, with the highest contamination rate (100%) during the period of June to September, and the lowest (50%) in March [30]. They also indicated that the meteorological data on temperature

Table 1: Primers for polymerase chain reaction (PCR) amplification of campylobacterial DNA for identification DNA

Organism	Primer	PCR product (bp)	Sequence
Campylobacter spp.	*16SrRNA*	857	5' ATC TAA TGG CTT AAC CAT TAA AC 3'
			5' GGA CGG TAA CTA GTT TAG TAT T 3'
Campylobacter jejuni	*mapA*	589	5' CTA TTT TAT TTT TGA GTG CTT GTG 3'
			5' GCT TTA TTT GCC ATT TGT TTT ATT A 3'
Campylobacter coli	*ceuE*	462	5' AAT TGA AAA TTG CTC CAA CTA TG 3'
			5' TGA TTT TAT TAT TTG TAG CAG CG 3'

Table 2: Seasonal prevalence of Campylobacter Campylobacter jejuni and Campylobacter coli in raw chicken meat in Shahrekord and Yasouj, Iran

Month	No. of samples	*Campylobacter* spp. positive	*C. jejuni*	*C. coli*
July	24	21 (87.5%)	19 (90.5%)	2 (9.5%)
August	32	22 (68.8%)	22 (100%)	0 (0.0%)
September	30	23 (76.7%)	23 (81.3%)	0 (0.0%)
October	36	16 (50.0%)	14 (100%)	2 (12.5%)
November	37	13 (35.1%)	12 (92.3%)	1 (7.7%)
December	33	15 (45.5%)	14 (93.3%)	1 (6.7%)
January	24	10 (41.7%)	9 (90.0%)	1 (10.0%)
February	29	6 (20.7%)	5 (83.3%)	1 (16.7%)
March	30	18 (60.0%)	16 (88.9%)	2 (11.1%)
April	25	16 (64.0%)	16 (100%)	0 (0.0%)
May	28	19 (67.9%)	17 (89.5%)	2 (10.5%)
June	22	18 (81.8%)	16 (94.1%)	2 (11.1%)
Total	350	197 (56.3%)	183 (92.9%)	14 (7.1%)

showed some relation to the presence of *Campylobacter* in broiler flocks; elevated temperatures coincided with high isolation rates. The fact that research findings show a 0 to 100% range of broilers testing positive for *Campylobacter jejuni* gives further creditability to a seasonal trend, as is suggested by these studies [15]. In another study, in agreement with our results, the highest isolation rates of *Campylobacter jejuni* in retail market broiler in North Carolina were during the warmer months of the year, from May to October (87-97%), and the lowest were in December (7%) and January (33%) [17]. However, in a study conducted by Stern and Line (1992) the season of year was not revealed in the prevalence of *Campylobacter* among retail-level chicken carcasses sampled.

In conclusion, the results of this study showed the importance of chicken meats as potential sources of *Campylobacter* spp. infection in people. Furthermore, to ensure food safety, poultry meats must be cooked properly before consuming. Also, this study shows that seasons of the year influence *Campylobacter* spp. detect ability and the carrier state in market chicken at retail level.

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