

Full Length Research Paper

Detection and identification of *Campylobacter* spp. from retail raw chicken, turkey, sheep and goat meat in Ahvaz, Iran

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Campylobacter species are common bacterial pathogens causing gastroenteritis in humans worldwide and the consumption of poultry meats is suspected to be the leading cause of this illness. This study was therefore conducted to determine the prevalence of *Campylobacter* spp. from retail raw meats in Ahvaz, Iran. From July 2009 to February 2010, a total of 215 raw meat samples from chicken (n = 60), turkey (n = 50), sheep (n = 50) and goat (n = 45) were purchased from randomly selected retail outlets in Ahvaz, Iran and were evaluated for the presence of *Campylobacter*. *Campylobacter* spp. isolated from 60 of 215 meat samples (27.9%) examined. The highest prevalence of *Campylobacter* spp. was found in chicken meat (61.7%), followed by turkey meat (36.0%), sheep meat (6.0%) and goat meat (4.4%). The most prevalence *Campylobacter* species isolated from meat samples was *Campylobacter jejuni* (88.3%), the remaining isolates were *Campylobacter coli* (11.7%). All 60 *Campylobacter* strains identified as *C. jejuni* and *C. coli* were also positive by using polymerase chain reaction (PCR). Significantly higher prevalence rates of *Campylobacter* spp. ($P < 0.05$) were found in the meat samples taken in summer (44.1%). Furthermore, to ensure food safety, poultry meats must be properly cooked before consuming.

Key words: *Campylobacter*, raw meat, chicken, turkey, sheep, goat.

INTRODUCTION

Campylobacter species are gram-negative, non-spore-forming microaerophilic organisms and are the most common food borne bacteria cause of human enteric diseases in several industrialized and developing countries (Skirrow, 1994; Mead et al., 1999; Oberhelman and Taylor, 2000; Han et al., 2007). Within the genus *Campylobacter*, *Campylobacter jejuni* and *Campylobacter coli* are the predominant species isolated

from fresh meat and poultry and are the most common species associated with human campylobacteriosis (Corry and Atabay, 2001). Most cases of campylobacteriosis associated with handling raw poultry, eating raw or undercooked contaminated meats (especially poultry and/ or by-products), cross-contamination of raw or cooked foods and lack of hygiene (Corry and Atabay, 2001; Suzuki and Yamamoto, 2009).

Disease caused by *Campylobacter* usually manifests at diarrhea, fever and severe abdominal pain. Although, most human cases are sporadic and outbreaks are relatively rare (Ketly, 1997), more serious consequences of campylobacteriosis include the autoimmune-mediated

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

Organism	Primer	PCR product (bp)	Sequence
<i>Campylobacter jejuni</i>	<i>mapA</i>	589	5 CTA TTT TAT TTT TGA GTG CTT GTG 3 5 GCT TTA TTT GCC ATT TGT TTT ATT A 3
<i>Campylobacter coli</i>	<i>ceuE</i>	462	5 AAT TGA AAA TTG CTC CAA CTA TG 3 5 TGA TTT TAT TAT TTG TAG CAG CG 3

demyelinating neuropathies Guillain-Barre and Miller Fisher syndromes (Nachamkin et al., 1998).

There have been many studies regarding isolation of *Campylobacter* spp. from food samples, especially poultry meat. Wide variation (0 - 100%) in the prevalence of *Campylobacter* in fresh poultry meat have been reported in different countries (Willis and Murray, 1997; Yun-Sook et al., 2006, Suzuki and Yamamoto, 2009, Sallam, 2007, Whyte et al., 2004). Although, much attention has been focused on poultry meat, red meat also remains the most common cause of food borne general outbreaks of infectious intestinal disease (Little et al., 2008). Currently, there is limited information on the prevalence of *Campylobacter* in raw meat in Iran. The aim of the present study was to determine the prevalence of *Campylobacter* species in raw retail chicken, turkey, sheep and goat meat in Ahvaz, Iran.

MATERIALS AND METHODS

Sample collection

From July 2009 to February 2010, a total of 215 raw meat samples from chicken (n = 60), turkey (n = 50), sheep (n = 50), and goat (n = 45) were purchased from randomly selected retail outlets in Ahvaz, Iran. All samples were placed in separate sterile plastic bags to prevent spilling and cross contamination and were immediately transported to the laboratory in a cooler with ice packs.

Microbiological analysis

The samples were processed immediately upon arrival at the lab by using aseptic techniques. Each meat sample (10 g) was homogenized and transferred to 90 mL of Preston enrichment broth base containing *Campylobacter* selective supplement IV (HiMedia Laboratories, Mumbai, India) and 5% (v/v) defibrinated sheep blood. After incubation at 42°C for 24 h in a microaerophilic condition (85% N₂, 10% CO₂, 5% O₂), 0.1 mL of the enrichment was then streaked onto *Campylobacter* selective agar base containing an anti-biotic supplement for the selective isolation of *Campylobacter* species (HiMedia Laboratories, Mumbai, India) and 5% (v/v) defibrinated sheep blood and incubated at 42°C for 48 h under the same condition. One presumptive *Campylobacter* colony from each selective agar plate was subcultured and tested by standard micro-biological and biochemical procedures (Bolton et al., 1992).

DNA extraction and polymerase chain reaction conditions

Campylobacter jejuni and *C. coli* isolates identified by

bacteriological methods were tested by polymerase chain reaction (PCR).

The PCR procedures used in this study have been previously described (Denis et al., 1999). Briefly speaking, 1 mL of pure culture of *Campylobacter jejuni* and *C. coli* were centrifuged at 13000 g for 5 min at room temperature. The DNA was then extracted using a genomic DNA purification kit (Fermentas, GmbH, Germany, K0512) according to the manufacturer's protocol. Two genes selected for the identification of the *C. jejuni*, and *C. coli* were the *mapA* gene (Stucki et al., 1995) and the *ceuE* gene (Gonzalez et al., 1997), respectively. The two 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 MDmapAI, MDmapA2, COL3 and MDCOL2 primers in the Fermentas buffer (Fermentas, GmbH, Germany). Amplification reactions were carried out using a DNA thermal cycler (Master Cycle Gradient, Eppendorf, 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, 589, and 462 bp DNA fragments corresponding to the *Campylobacter* genus, *C. jejuni* and *C. coli*, respectively. *C. coli* (ATCC 33559) and *C. 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 ultra-violet (UV) light after gel electrophoresis on 1.5% agarose.

Statistical analysis

Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), chi-square test and fisher's exact two-tailed test analysis were performed and differences were considered significant at values of P < 0.05.

RESULTS AND DISCUSSION

In this study, 215 meat samples of chicken, turkey, sheep and goat were analyzed microbiologically for the prevalence of *Campylobacter* species. Out of a total 215 raw meat samples, 60 (27.9%) isolates could be classified as *Campylobacter*. The most prevalence *Campylobacter* species isolated from meat samples was *Campylobacter jejuni* (88.3%); the remaining isolates were *C. coli* (11.7%). All 60 *Campylobacter* strains identified as *C. jejuni* and *C. coli* were also positive using PCR. The highest prevalence rates of *Campylobacter* species were found in chicken (61.7%), followed by turkey meat (36.0%), sheep meat (6.0%) and goat meat (4.4%).

Table 2. Prevalence of *Campylobacter* spp. isolated from chicken, turkey, sheep and goat meat in Ahvaz, Iran.

<i>C. coli</i>	<i>C. jejuni</i>	<i>Campylobacter</i> spp. positive	No. of samples	Meat sample
2 (5.4%) ^a	35 (94.6%) ^a	37 (61.7%) ^a	60	Chicken
3 (16.7%) ^a	15 (83.3%) ^a	18 (36.0%) ^b	50	Turkey
2 (67.7%) ^b	1 (33.3%) ^b	3 (6.0%) ^c	50	Sheep
0 (0.0%) ^a	2 (100%) ^a	17 (4.4%) ^c	45	Goat
7 (11.7%)	53 (88.3%)	60 (27.9%)	215	Total

Results expressed as the number of *Campylobacter*-positive samples / number of samples analyzed (%).

^{a, b, c}Values in the same column with different superscripts are significantly different ($P < 0.05$).

There were significant differences ($p < 0.05$) in the level of contamination with *Campylobacter* among different raw meat samples; however, no significant differences were found between sheep meat and goat meat samples.

The prevalence of *Campylobacter* spp. in the chicken meat samples was relatively high, which is comparable to those by other (Zhao et al., 2001; Rahimi and Tajbakhsh, 2008; Suzuki and Yamamoto, 2009; Sallam, 2007; Hossain, et al., 2007; Taremi et al., 2006; Whyte et al., 2004; van Nierop et al., 2005); however, higher contamination rates have also been reported (Han et al., 2007; Yilidrim et al., 2005; Pamuk and Akgun, 2009).

The contamination rate of turkey meat samples observed in this study was in agreement with those reported by Whyte et al. (2004) in Ireland (37.5%), Alter et al. (2005) in Germany (25.6%), Rahimi and Tajbakhsh, (2008) in Isfahan, Iran (27.4%) and Zhao et al. (2001) in Maryland (14.5%). However, Ghafir et al. (2007) reported higher prevalence of *Campylobacter* in turkey carcasses in Belgium (72.5 - 80.4%). Also, in a study conducted in Estonia, *Campylobacter* species were isolated from 73.3% of turkey meat samples (Praakle-Amin et al., 2007).

These variations in (Table 2) *Campylobacter* populations may be due to the differences in hygienic conditions during breeding, cross contaminations that may occur during differences in hygienic conditions during breeding, cross contaminations that may occur during defeathering, eviscerating and cutting of carcasses in portions and some other environmental factors such as, the temperature of water in the scalding tank, the low aerotolerance of *Campylobacter* spp., the methods and the medium used in the isolation of campylobacters as it is well known that some medium are more selective than others. Epidemiological studies show that contamination of water by the wild birds in the poultry processing plants, cross contaminations during defeathering and eviscerating of internal organs play important role in the occurrence of campylobacteriosis (Kazwala, et al., 1990; Elmali and Yaman, 2004; Franchin et al., 2007).

In the present study, 6.0 and 4.4% of retail sheep and goat meat samples were found to be *Campylobacter* positive, respectively, these levels are comparable with the recorded by Whyte et al. (2004) from Ireland, Little et al. (2008) from the UK and Zweifel et al. (2004) from

Switzerland. But higher than previously reported by Raji et al. (2000), Phillips et al. (2006), Hussain et al. (2007). Higher prevalence in the present study may be due to cross contamination during manual skinning, evisceration and processing in the slaughterhouse or in the butcher shops.

In the present study *C. jejuni* was far more common than *C. coli*. *Campylobacter jejuni* has been reported to be the most frequent species recovered from food of animal origin specially poultry meat (Zanetti et al., 1996; Hussain et al., 2007; Ghafir et al., 2007; Son et al., 2007). The results on the prevalence of *C. jejuni* in raw meat are in agreement with data from other countries (Whyte et al., 2004; Hussain et al., 2007; Ghafir et al., 2007; Suzuki and Yamamoto, 2009).

The prevalence of *Campylobacter* in all raw chicken, turkey, sheep and goat meat samples were significantly higher in the summer season (44.1%), which is in agreement with previous studies that reported peak prevalence in the warmer months (Willis and Murray, 1997; Rahimi and Tajbakhsh, 2008; Yun-Sook et al., 2006). For example Willis and Murray, (1997) reported that highest recovery rates of *Campylobacter* in retail broiler carcasses were obtained during the warmer months of the year (87 - 97%) than during the winter (7 - 33%).

In conclusion, the results of this study showed the importance of poultry meats as potential sources of *Campylobacter* spp. infection in people who consume chicken, and turkey meat. Good manufacturing practices and food safety assurance programmers aim to reduce meat contamination with *Campylobacter*.

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