

Prevalence of Campylobacter Jejuni and Coli in Sheep Carcasses by Using Cultural and PCR Methods

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Article information	Abstract
Article history: Received: 15 Nov 2012. Accepted: 30 Jan 2013 Available online: 25 Feb 2013 ZJRMS 2013; 15 (12): 28-31	Background: Campylobacter species are common bacterial pathogens causing gastroenteritis in humans worldwide. Materials and Methods: A total of 148 randomly sheep carcasses were sampled by surface section of neck meat taken immediately after slaughter analyzed using microbiological examinations. Results: Campylobacter spp. was isolated from 10.13% meat cultures samples examined. Among these 80% sample were <i>C. jejuni</i> and 20% sample were <i>C. coli</i> . Using PCR assays, the number of positive campylobacters increased to 11.48%. Of these positive samples, 82.35% were <i>C. jejuni</i> and 17.65% were <i>C. coli</i> . Significantly higher prevalence rates of Campylobacter spp. ($p < 0.05$) were found in the meat samples taken in summer (47.05%). Conclusion: The PCR is a reliable and sensitive method which can be used as a diagnostic technique for the detection of campylobacter in lamb samples.
Keywords: Campylobacter jejuni Campylobacter coli PCR Sheep	Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.

Introduction

Campylobacter is a curved, rod-shaped, nonspore forming, motile, and Gram-negative organism belonging to the Campylobacteriaceae. There are several species and subspecies in this family, among them *C. jejuni* and *C. coli* are the ones identified as strains responsible for most campylobacter infection cases in man. Campylobacter, especially *C. jejuni*, is a major cause of gastroenteritis worldwide in humans and animals [1]. Humans can be infected if they have a direct contact with animals and contaminated food. Infective dose of less than 500 cells can cause infection. Disease caused by campylobacter usually manifests at diarrhea, fever and severe abdominal pain. Although, most human cases are sporadic and outbreaks are relatively rare, more serious consequences of campylobacteriosis include the autoimmune-mediated demyelination neuropathies Guillain-Barre and Miller Fisher syndromes. Intestinal tract of sheep and goats mainly colonize *C. jejuni*, *C. coli*, *C. hyointestinalis* and *C. fetus*. These types are labeled as the cause of abortion in sheep and goats [2]. The intestinal contents may leak on to the carcass during the slaughtering process, meat is a major source of human campylobacteriosis the relative direct and indirect contributions of cattle and sheep to sporadic human infections are currently unknown. There is little evidence to suggest that red meat is an important risk factor for human infection, either from case-controlled studies or prevalence studies on meat. This possibly reflects the more hygienic slaughter procedure for ruminants than poultry.

Variables, such as herd size and type, season, age of animal, sample site, sample frequency and isolation method, geography, diet and husbandry practices, have been suggested to account for differences [3, 4].

There have been many studies regarding isolation of Campylobacter spp. from food samples. Wide variation (0-100%) in the prevalence of campylobacter in fresh lamb have been reported in different countries [3-6]. 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 [6]. The aim of the present study was to determine the prevalence of *C. jejuni* and *C. coli* in lamb in Rafsanjan, Iran.

Materials and Methods

Sample collection: This descriptive cross-sectional study was performed From April 2011 to February 2012, a total of 148 randomly sheep carcasses were sampled in slaughter house of Rafsanjan, Iran. Sampled were by surface section of neck meat taken immediately after slaughter analyzed using microbiological examinations. 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: ISO 10272-1:2006(E) method is used for identification of the campylobacter species.

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 antibiotic 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 [5].

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 [5]. 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 and the ceuE, respectively. The two sets of primers used for gene amplification are presented in Table 1. Amplification reactions were performed in a 30 ml mixture containing 0.6 U Taq polymerase (Fermentas, GmbH, Germany), 100 mmol l⁻¹ of each dNTP, 0.11 mmol l⁻¹ of MD16S1 and MD16S2 primers and 0.42 mmol l⁻¹ of MDmapA1, 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 [5].

Statistical analysis: Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS-17.1. 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

Out of 148 meat samples of sheep were analyzed microbiologically for the prevalence of *Campylobacter* species, 15 samples proved positive upon culture accounting for 10.13% of the total samples. According to biochemical tests 12 samples (80%) of the strains belonged to the *C. jejuni* and 3 samples (20%) to *C. coli*. Using PCR technique, 17 positive samples were detected which comprises 11.48% of all specimens. Based on PCR assay 14 samples (82.35%) of the strains isolated were belonged to the *C. jejuni*, while the remaining 3 samples (17.65%) belonged to *C. coli*. All the culture positive samples were also PCR positive. In the remaining cases the results from both culture and PCR were simultaneously negative. Table 2 shows prevalence of *Campylobacter* spp. isolated from sheep meat in different seasons in Rafsanjan, Iran. Figure 1 is a representative of the gel image for polymerase chain reaction detection of the *Campylobacter* spp. *C. jejuni* (589 bp), and *C. coli* (462 bp).

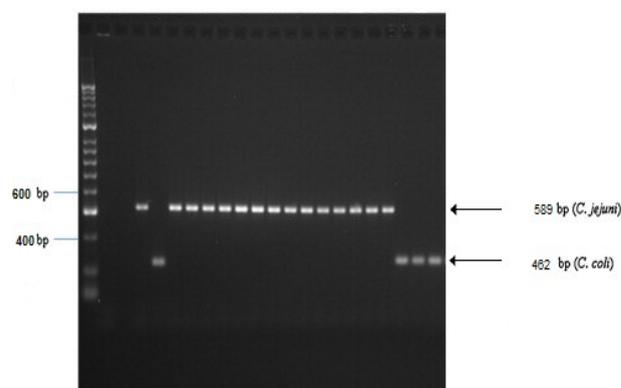


Figure 1. Multiplex PCR products for identification of *Campylobacter* isolates. Lane 1: hyper ladder Bioline II; lane 2: *Salmonella Typhimurium*, negative control; lane 3: Empty, negative control; lane 4: *C. jejuni*, positive control, 589-bp fragment; lane 5: *C. coli* RM2228, positive control, 462-bp fragment; lanes 6 to 19 *C. jejuni*, 589-bp fragment; lanes 20 to 22 *C. coli*, 462-bp fragment

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

Organism	Primer	PCR product (bp)	Sequence
<i>Campylobacter jejuni</i>	mapA	589	5' CTA TTT TAT TTT TGA GTG CTT GTG3'
			5' GCT TTA TTT GCC ATT TGT TTT ATT A3'
<i>Campylobacter coli</i>	ceuE	462	5' AAT TGA AAA TTG CTC CAA CTA TG3'
			5' TGA TTT TAT TAT TTG TAG CAG CG3'

Table 2. Prevalence of *Campylobacter* spp. isolated from lamb in different seasons in Rafsanjan, Iran

Season	NO. of collected sample	<i>Campylobacter</i> spp. positive	<i>C. jejuni</i>	<i>C. coli</i>
Spring	37(25%)	5(29.42%)	5(35.71%)	0(0%)
Summer	37(25%)	8(47.05%)	6(42.86%)	2(66.66%)
Fall	37(25%)	3(17.65%)	2(14.29%)	1(44.44%)
Winter	37(25%)	1(5.88%)	1(7.14%)	0(0%)
Total	148(100%)	17(11.48%)	14(82.35%)	3(17.65%)

Discussion

In this study we found a high frequency of *Campylobacter* spp. in sheep (11.48%). The prevalence of species distribution of campylobacters in this study was *C. jejuni* 14 (82.35%), *C. coli* 3 (17.65%). The previously reported prevalence of campylobacters among sheep in Iran were *C. coli* (33.3%) and *C. jejuni* (67.7%) [5]. Few studies have been conducted on the prevalence and incidence of *Campylobacter* bacteria in sheep in Iran. The most research studies have been conducted *Campylobacter* in poultry. In the sheep meat samples was relatively high, which is comparable to those by other [7, 8]. However, higher contamination rates have also been reported [9-12]. According to our biochemical tests used for strain differentiation, 80% of positive samples were belonged to *C. jejuni* and the remaining 20% categorized as *C. coli* and there were no uniform distribution patterns of these two strains. In the present study, sensitivity and specificity of the PCR method as compared to the culture were 100% and 88.23%, respectively. Since PCR method was capable of detecting more positive cases compared to the culture method, it seems that PCR method is a good substitute for the culture method in detecting *Campylobacter* in samples from sheep. Based on our results, PCR found to be sensitive enough, fast and reliable that could act as appropriate substitute for culture or at least as a supplementary method, when culture yielded negative results. The contamination rate of sheep meat samples observed in this study was in agreement with those reported by Raji et al. in Negeria (3.54%), Sahin et al. in USA (9.3% *C. jejuni*) [8,13]. Reported higher prevalence of *Campylobacter* in sheep carcasses by Zweifel et al. in Switzerland (17.5%) [7], Schilling et al. (2012) in Germany (25%) [14], Scott et al. in Ontario (18.54%) [15], Strachan et al. in UK (78%) [9], Dempster

et al. in New Zealand (48% *C. Fetus*) [11], Findik et al. in turkey (84%) [12]. Variables, such as herd size and type, season, age of animal, sample site, sample frequency and isolation method, geography, diet and husbandry practices, have been suggested to account for differences [3-4]. The prevalence of campylobacter in sheep samples were significantly higher in the summer season (47.05%), which is in agreement with previous studies that reported peak prevalence in the warmer months [3-5]. In conclusion, the results of this study showed the importance of sheep meats as potential sources of *Campylobacter* spp. infection in people who consume sheep meat. Good manufacturing practices and food safety assurance programmers aim to reduce meat contamination with campylobacter.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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