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 as 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 slaughter process, meat is a major source of human contents may leak on to the carcass during the cause of abortion in sheep and goats [2]. The intestinal

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 1-1 of each dNTP, 0.11 mmol 1-1 of MD16S1 and MD16S2 primers and 0.42 mmol 1-1 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 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).

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Primer</th>
<th>PCR product (bp)</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter jejuni</td>
<td>mapA</td>
<td>589</td>
<td>5 CTAT TTT TAT TTT TGA GTG CTT GTG3</td>
</tr>
<tr>
<td>Campylobacter coli</td>
<td>ceuE</td>
<td>462</td>
<td>5 AAT TGA AAA TGG CTC CAA CTA TGG3</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Season</th>
<th>NO. of collected sample</th>
<th>Campylobacter spp. positive</th>
<th>C. jejuni</th>
<th>C. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>37(25%)</td>
<td>5(29.42%)</td>
<td>5(35.71%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Summer</td>
<td>37(25%)</td>
<td>8(47.05 %)</td>
<td>6(42.86%)</td>
<td>2(66.66%)</td>
</tr>
<tr>
<td>Fall</td>
<td>37(25%)</td>
<td>3(17.65%)</td>
<td>2(14.29%)</td>
<td>1(4.44%)</td>
</tr>
<tr>
<td>Winter</td>
<td>37(25%)</td>
<td>1(5.88%)</td>
<td>1(7.14%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Total</td>
<td>148(100%)</td>
<td>17(11.48%)</td>
<td>14(82.35%)</td>
<td>3(17.65%)</td>
</tr>
</tbody>
</table>

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 Campylobacters 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 Nigeria (3.54%), Schilling 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.

Acknowledgements

This paper is the result of the approved research project of the Young Researchers Club, Shahr-e-Kord Islamic Azad University, Shahr-e-Kord; No. 88556.

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.

Funding/Support

Islamic Azad University, Shahr-e-Kord Branch.

Corresponding author at:
Young Researchers Club, Islamic Azad University, Shahr-e-Kord Branch, Shahr-e-Kord, Iran
E-mail: reza.vet64@gmail.com

References


