

## THERMOPHILIC *CAMPYLOBACTER* SPP. ON FROZEN POULTRY CARCASSES

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**Summary:** A total of 50 frozen poultry samples were collected from the markets in Kars district and analyzed for the presence of thermophilic *Campylobacter* spp., *Campylobacter jejuni* (*C. jejuni*), *Campylobacter coli* (*C. coli*) and *Campylobacter lari* (*C. lari*). *Campylobacter* spp. were isolated and identified according to the method of Food and Drug Administration (FDA). Thermophilic *Campylobacter* spp. were isolated at the level of (29 samples) 58.0 % from 50 frozen chicken carcasses. 21 (42.0 %) of these isolates were identified as *C. jejuni*, 7 (14.0 %) isolates were *C. coli* and 1 (2.0 %) isolate was identified as *C. lari*.

It is concluded that poultry carcasses consumed in their area pose a risk for human health.

**Keywords:** Thermophilic *Campylobacter* spp., poultry, frozen carcass.

### Donmuş Piliç Karkaslarında Termofilik *Campylobacter* Türleri

**Özet:** Kars bölgesinde *Campylobacter* türlerinin halk sağlığı açısından oluşturduğu riski belirlemek amacıyla marketlerden toplanan toplam 50 donmuş tavuk örneği termofilik *Campylobacter* türleri, *Campylobacter jejuni* (*C. jejuni*), *Campylobacter coli* (*C. coli*) ve *Campylobacter lari* (*C. lari*) yönünden analiz edildi. *Campylobacter*'lerin izolasyon ve identifikasyonu Gıda ve İlaç Dairesinin (FDA) önerdiği metoda göre yapılmıştır. 50 donmuş tavuk karkasının % 58.0'inden (29 örnek) termofilik *Campylobacter* spp. izole edildi. Bu izolatların 21'i (% 42.0) *C. jejuni*, 7'si (% 14.0) *C. coli* ve 1'i *C. lari* olarak tanımlanmıştır.

Bu yörede satışa sunulan kanatlı karkaslarının insan sağlığı açısından bir risk taşıdığı sonucuna varılmıştır.

**Anahtar sözcükler:** Termofilik *Campylobacter* spp., piliç, donmuş karkas.

### INTRODUCTION

*Campylobacter*s affect human health in a negative manner<sup>1-4</sup> and cause big economic losses<sup>5,6</sup>. They are found in the microflora of faeces, intestine, ceacal, internal organs and carcass<sup>7-10</sup>. Thermophilic *Campylobacter* are microaerophilic and capnophilic microorganisms<sup>11</sup> requiring about 5.0 % O<sub>2</sub>, 10.0 % CO<sub>2</sub> and 85.0 % N<sub>2</sub> for their growth<sup>12</sup> and cause many diseases such as food poisoning<sup>5</sup>, Guillain-Barré Sendrom (GBS), Miller Fisher Sendrom (MFS)<sup>13,14</sup>, hemolytic uremic sendrom<sup>15</sup>, obstructive hepatitis<sup>16</sup>, reactive arthritis, pancreatitis, peritonitis, meningitis<sup>5</sup>. Although *Campylobacter*s are microaerophilic, capnophilic and have low aerotolerance activity<sup>12</sup> *C. jejuni* is considered to be one of the microorganism in the risk group for poultry<sup>17-20</sup> and red meat<sup>7,9,21-24</sup>. In recent years, studies regarding the prevalence of *Campylobacter* spp. have been focused on epidemiology and application of rapid methods in practise<sup>19,25</sup> and studies to reduce the numbers of *Campylobacter*s in poultry carcasses before consumption is also being carried out<sup>26</sup>.

In this study, the isolation and identification of thermophilic *Campylobacter* spp. in chicken carcasses sold in the markets of Kars district were aimed to

determine the potential risk of *Campylobacter*s in respect of human health.

### MATERIAL and METHODS

A total of 50 frozen chicken carcasses in their packages were examined in this study. The samples were collected aseptically and brought up to the laboratory maintaining cold chain and analysed immediately. Isolation and identification of thermophilic *Campylobacter*s were performed based on the method of Food and Drug Administration (FDA)<sup>27</sup>. Samples taken from the surface and deeper tissues of frozen carcasses were used in the microbiological analysis. 25 g of samples from chicken carcasses were transferred into sterile polyethylene bags and 100 ml *Campylobacter* enrichment broth base (AM7526, Acumedia) was added for each bag. They were initially incubated at 37°C for 2-4 hrs in a microaerobic environment (*Campygen* CN025A, Oxoid) and subsequently incubated at 42°C for microaerobically 20-44 hrs. Then, 0.1 ml broth samples were inoculated onto mCCDA (Modified *Campylobacter* Blood-Free Selective Agar Base, CM 739, Oxoid) medium and incubated at 42°C for microaerobically 24-48 hrs in a microaerobic atmosphere. Suspected small and weak

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grown colonies were inoculated into *Campylobacter* enrichment broth base (AM7526, Acumedia) and incubated at 37°C for 2-4 hrs in a microaerobic environment (Campygen CN025A, Oxoid), they were inoculated onto mCCDA (Modified *Campylobacter* Blood-Free Selective Agar Base, CM 739, Oxoid) medium and incubated at 42°C for microaerobically 24-48 hrs in a microaerobic atmosphere. At the end of incubation, grey colonies were Gram stained. Hippurate hydrolyse test was performed on Gram (-), suspicious colonies. Further biochemical tests were performed on hippurate positive and negative colonies and Gram (-) colonies with spiral shaped bacteria, using cephalothin and nalidixic acid antibiotic disc susceptibility test, growth on the media containing 1.0 % glycine and 3.5 % NaCl, H<sub>2</sub>S production in TSIA (Triple Sugar Iron Agar), nitrate reduction, growth microaerobically at 25°C, 35- 37°C and 42°C<sup>27</sup>. The tests used for the identification of *Campylobacter* spp. are summarised in Table 1. The results were istatically analysed using Chi-square test<sup>28</sup>.

**Table 1.** The tests used for the characterisation and identification of *Campylobacter* spp.

**Tablo 1.** *Campylobacter* türlerinin karakterizasyon ve identifikasyonunda kullanılan testler.

Tests	<i>C. jejuni</i>	<i>C. coli</i>	<i>C. lari</i>
Hippurate hydrolyse	+	-	-
Nitrate reduction	+	+	+
H <sub>2</sub> S/TSIA	-	Di	-
Growth:			
25°C	-	-	-
35-37°C	+	+	+
42°C	+	+	+
Resistance:			
Cephalothin	R	R	R
Nalidixic acid	S	S	R
Growth:			
% 1.0 Glycine	+	+	+
% 3.5 NaCl	-	-	-

Se: Sensitive, Re: Resistance, Di: Different

## RESULTS and DISCUSSION

In this study, 50 packed frozen chicken carcasses were analysed microbiologically for the prevalence of *Campylobacter* spp. Thermophilic *Campylobacter* spp. were isolated from 29 (58.0 %) frozen chicken carcasses out of 50 samples examined. 21 (42.0 %) of these isolates were identified as *C. jejuni*, 7 (14.0 %) of them were *C. coli* and 1 (2.0 %) isolate was identified as *C. lari*. The rates and the levels of thermophilic

*campylobacters* on frozen chicken carcass samples are summarized in Table 2.

**Table 2.** The tests used for the characterisation and identification of *Campylobacter* spp.

**Tablo 2.** *Campylobacter* türlerinin karakterizasyon ve identifikasyonunda kullanılan testler.

Carcass No	Thermophilic campylobacters		
	<i>C. jejuni</i> (%)	<i>C. coli</i> (%)	<i>C. lari</i> (%)
50	21 (42.0%)	7 (14.0 %)	1 (2.0 %)

There have been many studies regarding isolation of thermophilic *campylobacters* from frozen chicken carcasses. The results obtained in our study showed similarity with the results obtained in some studies and also differences with the results obtained in other studies. Boer and Hahne<sup>18</sup> reported the isolation of *C. jejuni* at the level of 61.0 % in 170 chicken carcasses out of 279 samples. Kazwala et al.<sup>29</sup> isolated thermophilic *campylobacters* from 80 chicken carcasses out of 100 samples examined and 82.2 % of these isolates were identified as *C. jejuni* biotype 1 and 7.6 % of the isolates were identified as *C. jejuni* biotype 2 whereas *C. lari* and *C. coli* were found at the levels of 7.6 % and 2.6 % respectively. Kwiatek et al.<sup>21</sup> analysed 203 fresh chicken carcasses and isolated thermophilic *campylobacters* from 163 carcasses at the level of 80.3 % 88 (54.0 %) of these isolates were identified as *C. jejuni*, 65 (39.9 %) of isolates were *C. coli* and 10 (6.1 %) of isolates were *C. lari*. Nielsen and Nielsen<sup>30</sup> reported the isolation of thermophilic *Campylobacter* spp. on all of 156 chicken samples analysed. 85.0 % and 15.0 % of their isolates were identified as *C. jejuni* and *C. coli* respectively. Ozer and Ergun<sup>31</sup> examined 50 frozen chicken carcasses and isolated *C. jejuni* from 48 samples at the level of 96.0 % whereas Steinhauserova and Fojtivila<sup>25</sup> isolated thermophilic *campylobacters* from frozen chicken carcasses at the level of 89.0 % and identified them as *C. jejuni* using PCR. Futhermore, Zhao et al.<sup>23</sup> examined frozen chicken carcasses sold in the markets in Washington. They isolated *Campylobacter* spp. at the levels of 70.7 % in 184 samples. The results obtained in our study are lower than those reported by Kwiatek et al.<sup>21</sup> Nielsen and Nielsen<sup>30</sup> Steinhauserova and Fojtivila<sup>25</sup>, Zhao et al.<sup>23</sup> in respect of total thermophilic *Campylobacter* numbers. Comparing the numbers of samples for *C. jejuni* and *C. lari*, our results are also lower than those found by Boer and Hahne<sup>18</sup>, Kazwala et al.<sup>29</sup>, Kwiatek et al.<sup>21</sup>, Nielsen and Nielsen<sup>29</sup>, Ozer and Ergun<sup>31</sup>. Regarding of *C. coli*, our

results are higher than those reported by Kazwala et al.<sup>29</sup> and show similarity with the results of Nielsen and Nielsen<sup>30</sup>. Our results indicated that *C. jejuni* is the predominant species and this is in agreement with the results of other researchers. Stern et al.<sup>32</sup> could not isolate any *C. jejuni* in 40 frozen chicken pieces but, isolated *C. jejuni* from 5 fresh chicken samples out of 40 at the level of 12.5 %. Some authors monitored 360 chicken samples during June, September, December and March in 1985 and isolated *C. jejuni* at the levels of 25.6 %, 34.4 %, 32.2 % and 26.7 % respectively. Atanassova and Ring<sup>33</sup> examined a flock of broiler (509) before and after slaughter and found thermophilic *Campylobacter* spp. at the level of 41.1 % before slaughter and at the level of 45.9 % after slaughter. These results show that our results are higher than those mentioned above. These variations in *Campylobacter* populations may be due to the 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 media used in the isolation of campylobacters as it is known that some media are more selective than others<sup>21</sup>. Epidemiological studies<sup>29,34-37</sup> 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. Particularly, internal organs and carcasses can be contaminated due to the rupture of intestines<sup>36,38</sup>. Also, consumption of under cooked poultry meat and other foods such as salads and bread that have become cross contaminated from raw meats in the kitchen are the important risk resources of *Campylobacter* contamination<sup>5,18,39</sup>. Although some methods are being developed<sup>26</sup> and the cooling process applied during the processing of carcasses causes a reduction in the microbial numbers of carcasses<sup>17</sup>, poultry meat is still among the risk group of food in respect of *Campylobacter* infections<sup>23,40</sup> because the organism survives on chicken held at refrigeration temperatures<sup>41</sup> and has also been isolated from chicken carcasses held at -15°C for 30 weeks<sup>42</sup> and at -18°C for 2<sup>43</sup> and 12 months<sup>44</sup> and chicken livers held at -20°C for 84 d<sup>38</sup>. Furthermore, *C. jejuni* remained viable at -20 and -70°C and quickly replicated after the sample thawed<sup>45</sup>. Some researchers<sup>17,46</sup> suggested that cross contamination in the processing plant is virtually impossible to

eliminate. Thus, the production of poultry products that are free of campylobacters depends upon whether or not live birds contain the organisms prior to processing.

The results of this study confirmed that *C. jejuni* is the predominant species ( $P < 0.05$ )<sup>28</sup> found on frozen chicken carcasses. Since *C. jejuni* survives after freezing it may be concluded that the increasing use of frozen meat may have contributed to an increase in its incidence. Thus, the presence of thermophilic *Campylobacter* spp. poses a risk for public health and therefore risk factors should be taken into consideration to minimize *Campylobacter* infections in humans. In order to achieve this, prevention measures should be implemented at the flock level and in the kitchen to avoid infection with this bacteria. In addition cross contamination during slaughter and processing should be reduced. As the number of samples analysed in this study is small, the true incidence of *C. jejuni* in poultry meat consumed in this area requires more comprehensive studies.

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