

THE OCCURENCE OF THERMOPHILIC *CAMPYLOBACTER* (*C. jejuni*) IN RAW MILK

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Summary: Consumption of raw milk or contaminated milk with *Campylobacter jejuni* (*C. jejuni*) has been described as a cause of human enteritis *Campylobacter* enteritis associated with consumption of raw milk is still being occurred in developed and in under developed countries. In this study, the presence of thermophilic campylobacters were examined in 120 raw milk samples from individual milking cows in Kars and Ardahan districts. Thermophilic campylobacters were isolated from 6 samples (5.0 %) out of 120 and these were identified as *C. jejuni* based on morphological and biochemical tests.

Keywords: Thermophilic *Campylobacter*, raw milk, microbiological quality.

Çiğ Sütlerde Termofilik *Campylobacter* (*C. jejuni*) Varlığının Araştırılması

Özet: Çiğ süt veya *Campylobacter jejuni* (*C. jejuni*) ile kontamine olmuş süt insanların enteritis olgularının bir etkeni olarak tanımlanmaktadır ve çiğ süt bağlantılı *Campylobacter* enteritis olguları gelişmiş ve gelişmekte olan ülkelerde halen görülmektedir. Bu çalışmada Kars ve Ardahan bölgesinde sağlıklı 120 inekten alınan çiğ sütlerde termofilik *Campylobacter* türlerinin varlığı araştırıldı. 120 örnekten 6'sında (% 5.0) termofilik *Campylobacter* izole edildi ve bunlar morfolojik ve biyokimyasal testlere dayanarak *C. jejuni* olarak tanımlandı.

Anahtar sözcükler: Termofilik *Campylobacter*, çiğ süt, mikrobiyolojik kalite.

INTRODUCTION

Thermophilic *Campylobacter* spp. particularly *C. jejuni* and *Campylobacter coli* (*C. coli*) are major human intestinal pathogens worldwide. *C. jejuni* and *C. coli* that cause human enteritis are commensals of livestock particularly poultry but also cattle, sheep, pigs, dogs and cats. Even campylobacteriosis a self-limiting infection there is an increase in the cases of *Campylobacter* infections¹. The high incidence of human infections may be due to the low infection dose for *C. jejuni* which ranges from 100 to 500 organisms². Identified vehicles of infections are drinking and recreational waters, raw cows' milk and under cooked poultry meat. *Campylobacter* spp. can be isolated with high frequency from poultry and poultry products derived from them, from cattle and a variety of wild animals and are also present in the natural environment. Campylobacteriosis may occur as epidemics or as sporadic infections and nearly 70 % of the sporadic infections are due to consumption of contaminated poultry but sporadic cases also derive from drinking contaminated water, raw milk or milk contaminated after pasteurization and exposure during the foreign travel but the aetiological fractions varies remarkably by district and season^{3,4}. Although the majority of cases are sporadic, outbreaks involving consumption of contaminated raw milk and untreated

water occur^{5,7}. *Campylobacter* species are found commonly in dairy cow faeces⁸⁻¹⁰ and therefore can enter the supply of raw milk from bovine faeces. The recovery of identical *C. jejuni* biotypes from ground water and dairy cattle suggests transmission between water and livestock¹¹. Dairy cattles are farmed on small-scale family run farms and they have also very close contact with the members of family and on-farm consumption of raw milk is common in the area of Kars and Ardahan. Therefore, there may be a potential risk of *Campylobacter* infections in humans and animals. In the current study, the prevalence of thermophilic *Campylobacter* in raw milk was analysed.

MATERIAL and METHODS

A total of 120 raw milk samples collected from individual milking cows from house-hold farms in Kars and Ardahan were examined in this study to determine the presence of thermophilic campylobacters. Samples were collected aseptically and brought to the laboratory maintaining cold chain and analysed immediately. Isolation and identification of thermophilic campylobacters were performed based on the method of Food and Drug Administration (FDA)¹². One ml milk samples were inoculated in to 9 ml *Campylobacter* Enrichment Broth Base (Bolton

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formulation, AM7526, Acumedia) and were incubated at 37°C for 2-4 hours and then at 42°C for 20-44 hours in a microaerobic environment (Campygen CN025A, Oxoid). After incubation, they were inoculated on to mCCDA (Modified Campylobacter Blood-Free Selective Agar Base, CM 739, Oxoid) medium and incubated at 42°C for 24-48 hours in a microaerobic atmosphere as above. Suspected small and weak 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, all colonies showing morphology similar to those of *Campylobacter* spp. were examined by phase-contrast microscopy (x 1000) and 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, including cephalothin and nalidixic acid antibiotic disc susceptibility tests, growth on the media containing 1.0 % glycine and 3.5 % NaCl, H₂S production in TSIA (Triple Sugar Iron Agar), nitrate reduction, growth at 25°C, 35-37°C and 42°C. The tests used for the identification of *Campylobacter* spp. are summarised in Table 1.

Table 1. The tests used for the characterisation and identification of *Campylobacter* spp.¹².

Tablo 1. *Campylobacter* türlerinin karakterizasyonunda ve identifikasyonunda kullanılan testler¹².

Tests	<i>C. jejuni</i>	<i>C. coli</i>	<i>C. lari</i>
Hippurate hydrolyse	+	-	-
Nitrate reduction	+	+	+
H ₂ 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	-	-	-

S: Sensitive, R: Resistance, Di: Different

RESULTS and DISCUSSION

Contaminated milk has long been recognized as a source of human *Campylobacter* infection and *Campylobacter* has been isolated from samples of raw milk on sale to the public¹³. The isolation rate of campylobacters from raw milk can be low and there could be a significant dose-response relationship between the risk and degree of illness and the amount of milk consumed. However, contaminated milk can cause campylobacteriosis with high incidence rates. Wood et al.¹³ reported *Campylobacter* outbreaks associated with consumption of raw milk during youth activities from 1981 through 1990. Twenty outbreaks were identified in 11 different states. 458 outbreak associated cases occurred among 1013 people who drank raw milk, with an overall rate of 45 %. Kalman et al.¹⁴ reported an milkborne *Campylobacter* infection in Hungary where 500 to 600 visitors consumed unpasteurized milk on an animal farm sale. 52 people from a wide age range fell ill, primarily with inflammatory enteritis. These cases were identified with 34 *Campylobacter* positivity; 30 with *C. jejuni* and 4 with *C. coli*. Jones et al.¹⁵ monitored an outbreak occurred among school children mainly in the 2-4 and 5-7 year old age groups and it was established from epidemiological and microbiological data that 2500 children were infected. The source of the epidemic was almost certainly contaminated milk. Recently, an outbreak of *C. jejuni* enteritis occurred among people who had attended a meal where raw milk was served. Thirteen of 20 people who had attended the meal became ill. *C. jejuni* was cultured from five of six stools that were submitted to hospital. Raw milk consumption was strongly associated with the illness ($p=0.0072$, Fisher exact test)¹⁶.

The high prevalence of *C. jejuni* in cattle faeces in winter, compared with summer parallels the greater frequency of milk-borne outbreaks during the first months of the year¹⁷. Furthermore, all the milk-borne outbreaks of *Campylobacter* enteritis have been caused by *C. jejuni/coli*, the type exclusively isolated from faeces. As campylobacters have been found in healthy cows at counts of about 10⁵ per g of faeces, only a few grams of faeces are needed to contaminate a bulk tank to produce a potentially infective dose in a glass of milk theoretically¹⁸. Several workers found that the viable counts of campylobacters introduced into raw cow's milk rapidly decreased after inoculation¹⁹⁻²². This and the low isolation rates of campylobacters in milk may be caused by the

antibacterial action of the lactoperoxidase system in milk²³.

Rohrbach et al.²⁴ examined 292 raw milk samples and isolated campylobacters from 36 (12.3 %). Larkin et al.²⁵ isolated *C. jejuni* in two (5.0 %) raw milk samples out of 41. Manus and Lanier²⁶ analyzed 237 raw milk samples but could isolate *C. jejuni* from only one (0.4 %) sample. On the contrary, Moustafa²⁷ isolated *C. jejuni* from 82 raw milk samples out of 92 in Egypt whereas Beumer et al.²³ reported *C. jejuni* in 41(4.5 %) raw milk samples out of 904. Loewenherz et al.²⁸ could isolate *C. jejuni* in milk and milk products at the level 5.0 %. However, Hutchinson et al.²⁹ analysed milk samples from 40 milking cows and only two milk samples from two milking cows contained *C. jejuni*. Ozkoc³⁰ examined raw milk and milk products sold in the markets in Istanbul and no *Campylobacter* was found in each 50 samples of raw milk, cheese and ice-cream. Richard et al.³¹ also could not isolate any *Campylobacter* spp. from raw milk or raw milk products. In this study, a total of 120 raw milk samples collected locally analysed microbiologically for the prevalence of *Campylobacter* spp. Thermophilic *Campylobacter* spp. were isolated from 6 (5.0 %) out of 120 samples examined. All of these isolates were identified as *C. jejuni*. Our results are in agreement with the previous studies and indicate the potential danger inherent in the consumption of raw milk. Cases have also been reported^{32,33} due to failed milk pasteurization and inadequately pasteurized milk. The possibility of such outbreaks will remain until all milk is heat treated. 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 farm level and in the dairy to avoid infection with this bacteria. Pasteurization of milk must be carried out thoroughly and no milk must be allowed for distribution to the general public without pasteurization. As the number of samples used in this study is limited the true incidence of *C. jejuni* in milk in Kars and Ardahan districts is to be determined. In addition, epidemiological studies are required to determine the incidence of campylobacteriosis in humans and animals in this location. It is concluded that milk is still among the risk group of food in respect of *Campylobacter* infections in Kars and Ardahan districts.

REFERENCES

- 1 **Humphry T:** The significance of *Campylobacter* species as foodborne pathogens, SOFHT Focus, 26 Spring. Available from: <http://www.soft.co.uk/sf26camp.htm>, 1999.
- 2 **Robinson DA:** Infective dose of *Campylobacter jejuni* in milk. *Br Med J*, 282: 1584, 1981.
- 3 **Anonymous:** Seattle-King County Department of Public Health, 1984, Surveillance of the flow of *Salmonella* and *Campylobacter* in a community. Communicable Disease Control Section. Seattle-King County Department of Public Health, Seattle, Wash, USA.
- 4 **Pearson AD, Healing TD:** The surveillance and control of *Campylobacter* infection. Communicable Disease Review (CDR), Volume 2, Review 12. 1992.
- 5 **Bean NH, Goulding JS, Lao C, Angulo FJ:** Surveillance for foodborne disease outbreaks-United States, 1988-1991. CDC Surveillance Summaries, Morbid. Mortal. *Weekly Rep*, 45 (5): 1-66, 1996.
- 6 **Headrick ML, Korangy S, Bean NH, Angulo FL, Altekruze SF, Potter ME, Klontz KC:** The epidemiology of rawmilk associated foodborne disease outbreaks reported in the United States, 1973 through 1992. *Am J Public Health*, 88: 1219-1224, 1994.
- 7 **Morgan D, Gunneberg C, Gunnell D, Healing T D, Lamerton S, Soltanpoor D, Lewis A, White DC:** An outbreak of *Campylobacter* infection associated with the consumption of unpasteurised milk at a large festival in England. *Eur J Epidemiol*, 10: 581-585, 1994.
- 8 **Atabay H, Corry JEL:** The isolation and prevalence of campylobacters from dairy cattle using a variety of methods. *J Appl Microbiol*, 84: 733-740, 1998.
- 9 **Giacoboni GI, Itoh K, Hirayama K, Takahashi E, Mitsnoka T:** Comparison of fecal *Campylobacter* in calves and cattle of different ages in Japan. *Jpn J Vet Med*, 55: 555-559, 1993.
- 10 **Humphry TJ, Beckett P:** *Campylobacter jejuni* in dairy cows and raw milk. *Epidemiol Infect*, 98: 263-269, 1987.
- 11 **Stanley KN, Wallace JS, Currie JE, Diggle PJ, Jones K:** The seasonal variation of thermophilic campylobacters in beef cattle, dairy cattle and calves. *J Appl Microbiol*, 85: 472-480, 1998.
- 12 **Anonymous:** *Campylobacter jejuni*. Food and Drug Administration (FDA), Available from: <http://www.vf.cfsan.fda.gov>, Date of arriving: 12.02.2000.
- 13 **Wood RC, MacDonald KL, Osterholm MT:** *Campylobacter* enteritis outbreaks associated with drinking raw milk during youth activities. A 10-year review of outbreaks in the United States. 268 (22): 3228-30, 1992.
- 14 **Kalman M, Szollosi E, Czermann B, Zimayni M, Szekeres S, Kalman M:** Milkborne *Campylobacter* infection in Hungary. *J Food Prot*, 63 (10): 1426-1429, 2000.
- 15 **Jones PH, Willis AT, Robinson DA, Skirrow MB, Josephs DS:** *Campylobacter* enteritis associated with the consumption of free school milk. *J Hyg*, 87 (2): 155-62, 1991.
- 16 **Robinson DA, Jones DM:** Milk-borne *Campylobacter* infection. *J British Medical*, 282: 1374-1376, 1981.
- 17 **Peterson MC:** *Campylobacter jejuni* enteritis associated with consumption of raw milk. *J Environ Health*, 65 (9): 20-21, 24, 26, 2003.
- 18 **Waterman CS, Park RWA:** A research for the source of *Campylobacter jejuni* in milk. *J Hyg Camb*, 92: 333-337, 1984.

- 19 **Barrell RAE**: The survival of *Campylobacter coli/jejuni* in unpasteurized milk. *J Infection*, 3: 348-352, 1981.
- 20 **Oosterom J, Engels GB, Peters R, Pot R**: *Campylobacter jejuni* in cattle and raw milk in The Netherlands. *J Food Prot*, 45: 1212-1213, 1982.
- 21 **De Boer E, Hartog BJ, Borst GHA**: Milk as a source of *Campylobacter jejuni*. *Netherlands Milk and Dairy Journal*, 38: 183-194, 1984.
- 22 **Doyle MP, Roman DJ**: Prevalence and survival of *Campylobacter jejuni* in unpasteurised milk. *Appl and Environ Microbiol*, 44: 1154-1158, 1982.
- 23 **Beumer RR, Noomen A, Marijs JA, Kampelmacher EH**: Antibacterial action of the lactoperoxidase system on *Campylobacter jejuni* in cow's milk. *Netherlands Milk and Dairy Journal*, 39: 107-114, 1985.
- 24 **Rohrbach BW, Draughon FA, Davidson PM, Oliver SP**: Prevalence of *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Salmonella* in bulk tank milk: risk factors and risks of human exposure. *J Food Prot*, 55 (2): 93-97, 1992.
- 25 **Larkin LL, Vasavada PC, Marth EH**: Incidence of *Campylobacter jejuni* in raw milk as related to its quality. *Milchwissenschaft*, 46 (7): 428-430, 1991.
- 26 **Manus C, Lanier JM**: *Salmonella*, *Campylobacter jejuni* and *Yersinia enterocolitica* in raw milk. *J Food Prot*, 50 (1):51-55, 1987.
- 27 **Moustafa MK**: Occurrence of *Campylobacter jejuni* in raw milk in Assuit city. *Assuit Vet Med J*, 22: 101-104, 1990.
- 28 **Loewenherz LK, Melthmann M, Mildrandt C**: Occurrence of *Campylobacter jejuni* in various foods of animal origin. *Fleischwirtschaft*, 76 (9): 958-961, 1996.
- 29 **Hutchinson DN, Bolton FJ, Hinchliffe PM, Dawkins HC, Horsly SD, Jessop EG, Robertshaw PA, Counter DE**: Evidence of udder excretion of *Campylobacter jejuni* as the cause of milk-borne *Campylobacter* outbreak. *J Hyg*, 94(2): 205-15, 1985.
- 30 **Özkoç EE**: İstanbul Anadolu yakasında satışı sunulan çiğ süt ve bazı süt ürünlerinde *Campylobacter jejuni*'nin mevcudiyeti, Yüksek Lisans Tezi, İstanbul Üniversitesi, Sağlık Bilimleri Enstitüsü, İstanbul. 1998.
- 31 **Richard LV, Little AA, Patton CM, Timothy JB, Lillian AO**: Serotyping and serology studies of *Campylobacteriosis* associated with consumption of raw milk. *J Clinical Microbiol*, Nov, 998-1000, 1984.
- 32 **Birkhead G, Vogt RL, Heun E, Evetli P**: A multiple-strain outbreak of *Campylobacter enteritis* due to consumption of inadequately pasteurized milk. *J Infect Dis*, 157(5): 1095-1097, 1988.
- 33 **Fahey T, Morgan D, Gunneburg C, Adak GK, Majid F, Kaczmarek E**: An outbreak of *Campylobacter jejuni enteritis* associated with failed milk pasteurization. *J Infect*, 31 (2): 137-143, 1995.

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