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

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Yayın Kodu: 2004/27-A

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 insanları enterik olgularının bir etkini olarak tanyanmaktadır ve çiğ süt bağlantılı Campylobacter enteritis olguları gelişmiş ve gelişmekte olan ülkelerde hala görülmektedir. Bu çalışmada Kars ve Ardahan bölgelerinde sağlık 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 testleri dayanıklı C. jejuni olarak tanımlanladı.

Anahtar słów: Termofilik Campylobacter, çiğ süt, microbiyolojik 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. Campylobacterosis 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 foraging travel but the aetiologic fractions varies remarkably by district and season3,4. Although the majority of cases are sporadic, outbreaks involving consumption of contaminated raw milk and untreated water occur5-7. Campylobacter species are found commonly in dairy cow feces8-10 and therefore can enter the supply of raw milk from bovine feces. The recovery of identical C. jejuni biotypes from ground water and dairy cattle suggests transmission between water and livestock11. Dairy cattle 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 incubated 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 hydrolysis 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.

<table>
<thead>
<tr>
<th>Tests</th>
<th>C. jejuni</th>
<th>C. coli</th>
<th>C. lari</th>
</tr>
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<tbody>
<tr>
<td>Hippurate hydrolysis</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>H₂S/TSIA</td>
<td></td>
<td>Di</td>
<td>-</td>
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<tr>
<td>Growth:</td>
<td>25°C</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>35-37°C</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>42°C</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Resistance:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalothin</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Growth:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% 1.0 Glycine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>% 3.5 NaCl</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

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 public⁹. 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.¹⁰ 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.¹¹ 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.¹² 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=.0072, Fisher exact test)⁶.

The high prevalence of C. jejuni in cattle feaces in winter, compared with summer parallels the greater frequency of milk-borne outbreaks during the first months of the year⁷. 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 10⁷ per g of feaces, only a few grams of feaces are needed to contaminate a bulk tank to produce a potentially infective dose in a glass of milk theoretically⁸. Several workers found that the viable counts of campylobacters introduced into raw cow’s milk rapidly decreased after inoculation⁹. This and the low isolation rates of campylobacters in milk may be caused by the
antibacterial action of the lactoperoxidase system in milk\(^2\).

Rohrbach et al.\(^2\) examined 292 raw milk samples and isolated campylobacters from 36 (12.3 %). Larkin et al.\(^3\) isolated \(C.\) jejuni in two (5.0 %) raw milk samples out of 41. Manus and Lanier\(^4\) analyzed 237 raw milk samples but could isolate \(C.\) jejuni from only one (0.4 %) sample. On the contrary, Moustafa\(^5\) isolated \(C.\) jejuni from 82 raw milk samples out of 92 in Egypt whereas Beumer et al.\(^6\) reported \(C.\) jejuni in 41 (4.5 %) raw milk samples out of 904. Loewenherz et al.\(^7\) could isolate \(C.\) jejuni in milk and milk products at the level 5.0 %. However, Hutchinson et al.\(^8\) analysed milk samples from 40 milking cows and only two milk samples from two milking cows contained \(C.\) jejuni. Ozkoc\(^1\) 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.\(^2\) 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\(^2\) 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 prevalence of \(C.\) jejuni in milk in Kars and Ardanah 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 Ardanah districts.

REFERENCES

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