

## OCCURRENCE OF *YERSINIA ENTEROCOLITA* IN TURKISH WHITE CHEESE CONSUMED IN KARABÜK REGION-TURKEY

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### Abstract

*In this study, occurrence of a pathogen Yersinia enterocolitica in white cheese was studied. 100 white cheese samples were collected from Karabük of which 50 samples from open markets, 30 samples from A, B, C firms and 20 samples from supermarkets. Occurrence of Yersinia enterocolitica in the samples were analysed by using the ISO method which is a conventional methodology for the investigation of foodborne pathogens. It has been isolated and identified in 8 samples (8%) out of 100 white cheese samples which may be considered as potential health hazard for public.*

**Key words:** *Yersinia enterocolitica, white cheese*

### Karabük Yöresinde Tüketime Sunulan Beyaz Peynirlerde *Yersinia enterocolitica* Varlığının Araştırılması

*Bu çalışmada gıda kaynaklı bir patojen olan Yersinia enterocolitica'nın beyaz peynirlerde varlığı araştırılmıştır. Araştırmada, Karabük piyasasından sağlanan 3 farklı firmaya ait (A, B, C) 30 adet beyaz peynir örneği, 20 adet paketlenmemiş beyaz peynir örneği ve pazarlardan sağlanan 50 adet beyaz peynir örneği olmak üzere toplam 100 adet beyaz peynir örneğinde çalışılmıştır. Örneklerde Yersinia enterocolitica'nın varlığının analizi için, gıda kaynaklı patojen mikroorganizmaların araştırılmasında konvansiyonel bir metot olan ISO kullanılmıştır. Yersinia enterocolitica, 100 beyaz peynir örneğinin 8'inde (%8) izole edilmiş ve tanımlanmıştır. Bu örneklerin halk sağlığı açısından potansiyel zarar oluşturabileceği düşünülmektedir.*

**Anahtar Kelimeler:** *Yersinia enterocolitica, beyaz peynir*

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## INTRODUCTION

*Yersinia enterocolitica* is a bacterium indigenous to the gastrointestinal tract of warm-blooded animals and is associated with human and animal diseases (1, 2). Diarrhea, gastroenteritis and mesenteric lymphadenitis are the most frequent clinical manifestations of human yersiniosis (3).

Several studies indicated that yersiniosis in humans was caused by consumption of contaminated food (4). *Y. enterocolitica* has been isolated from a variety of foods, including meat, raw oysters poultry, raw milk, pasteurized milk and dairy products, salads and vegetables, fruits and from water (1, 5, 6, 7), however strains 0:3, 0:8, 0:5,27 and 0:9 are the most commonly implicated in infection (8, 9).

Milk (raw or pasteurized) and milk products, particularly cheese and ice cream, have frequently been found to be contaminated with *Y. enterocolitica* (10). *Y. enterocolitica* can grow to large numbers at refrigeration temperatures, so milk contaminated with that organism could become a significant health risk for consumers. The presence of *Yersinia spp.* in traditional fresh cheese could be attributed to different factors such as the use of raw milk and the eventual contamination from human handlers, environment and water (2, 9, 10, 11, 12).

Turkish white cheese is probably the most popular, and economically the most important variety of cheese in Turkey. In the traditional or artisanal manufacture of Turkish white cheese, milk may or may not be pasteurized and the curd is handled extensively by the cheese maker. Cheesemilk was applied to pasteurization at 72-74 °C for 15-20 sec and ripened at 12-15 °C for 30-60 days and than stored at 5 °C (13, 14).

Our aim was to identify and detect *Yersinia enterocolitica* in Turkish white cheese samples consumed in Karabük Region.

## EXPERIMENTAL

### *Samples Collection*

In this study, 100 white cheese samples of which 50 from open markets, 30 packaged from A, B, C firms and 20 unpackaged samples from supermarkets were analysed. All white cheese samples were collected from Karabük region. All the samples were placed in sterile glass jars and were carried to the laboratory in a cold container and were used for analysis, immediately (15,16).

### *Analyses of Samples*

Samples were analysed for *Y. enterocolitica* using one of the methods prescribed by the ISO (16). *Y. enterocolitica* 0:8 strain WA, *Y. enterocolitica* 0:3 strain E 61264, *Y. enterocolitica* 0:9 BL 87/46 were used as reference strains in this study. *Y. enterocolitica* strains were provided by Hacettepe University Department of Food Engineering.

In isolation process, Peptone, Sorbitol and Bile Salts (PSB) Broth were used as enrichment and *Yersinia* Selective Agar Base (Oxoid CM 653) was used as a selective agar.

For detection of *Y. enterocolitica*, a sample of 25 g was weighed into sterile glass jars homogenized with 225 ml Peptone, Sorbitol and Bile Salts (PSB) Broth and incubated at 22-25 °C for 5 days. Presence of the microorganism was determined using Cefsulodin-Irgasan-Novobiocine (CIN; Oxoid CM 653, Oxoid selective supplement SR 109 E) Agar, incubated at

30 °C for 24 h. Five colonies that are thought to be *Yersinia spp.* (on *Yersinia* selective agar, dark red colonies surrounded by a transparent border) were picked from the plating media.

After incubation at 30 °C for 24 h in Nutrient Agar (Difco 0126-0140), organisms were submitted to Gram staining and the following biochemical tests: Catalase and oxidase tests, glucose fermentation with or without gas production, urease production, H<sub>2</sub>S production, presence of tryptophan deamination, lysine decarboxylase, ornitin decarboxylase, sucrose fermentation, rhamnose fermentation, citrate, esculin hydrolysis and indole production, Voges-proskauer test, methyl red test, motility at 22-26 °C and 37 °C.

## RESULTS AND DISCUSSION

*Y. enterocolitica* was isolated from 8 (16%) of the 50 samples which were collected from open markets. *Y. enterocolitica* was isolated from neither 20 unpacked samples which were collected from supermarkets nor 30 samples which were collected from A, B, C firms. Data presented in Table 1 indicate an overall *Y. enterocolitica* prevalence of 8% in cheese samples.

Table 1. Prevalence of *Y. enterocolitica* in the examined white cheese

White cheese samples	The Number of Samples	The Number of Samples Isolated <i>Y. enterocolitica</i>	Prevalence of <i>Y. enterocolitica</i> %
White Cheese Samples			
From Open Market	50	8 samples	8 (16%)
Samples of Known A, B, C Firms	30	No colonies observed	0 %
Unpacked Samples From Supermarkets and Groceries	20	No colonies observed	0 %
Total	100	8	8 (8%)

In the present study, the number ranging from 1 to 100 were given to all cheese samples. The first 30 cheese samples belong to firms (A, B, C). The rest of cheese samples, belong to unpacked cheese, sold in supermarkets and open markets. Existence of *Yersinia enterocolitica* was observed in 36, 39, 43, 49, 59, 61, 67 and 70 numbered cheese samples which are collected from open markets and its biochemical tests were performed. Samples number 67 and 70 were urease negative and were regarded according to the information of the method employed. In the method used (ISO,1994) with the nonpathogenic strains the possibility of negative urease activity was emphasized. The results of the biochemical tests are shown in Table 2.

According to the results, proportion of *Y. enterocolitica* in 50 samples, which were collected from open markets, was very high (16%). These results may depend on presence of unpasteurised milk, contamination that may occur in production, during storage or because of nonhygienic conditions (17,18).

*Y. enterocolitica* was not detected in the white cheese of known firms and in the unpacked white cheese of supermarkets and groceries. This shows that all processes are operated in hygiene rules from production to consumption.

The results show that people are taking risk with respect to their health by consuming the white cheese made by villagers and sold in open markets.

Several authors have reported the detection of *Y. spp.* and particularly *Y. enterocolitica* in the cheese in Turkey. Among these, there is some data reporting lower isolation rates than those reported in the present study. For instance, Sağun (19), has investigated and has determined the

presence of 2.4% *Y. enterocolitica* in a total of 41 white cheese examined. Some researchers reported that even higher isolation ratios were detected compared to the results of this study. For instance, Aytaç and Özbaş (20) have studied the presence of *Y. enterocolitica* in white cheese and they have determined 19 out of 66 (28.8%) samples positive of *Y. enterocolitica*. Moreover, Yücel et. al (21) have also studied cheese samples and they have examined the samples for the presence of *Yersinia* spp. In 100 samples, 14 were determined to be contaminated with *Yersinia* spp. *Y. enterocolitica* is the most commonly isolated species with the percentage of 35.7.

**Table 2.** Results of biochemical test

Biochemical Tests	Reference strains and positive samples										
	0:3	0:8	0:9	36	39	43	49	59	61	67	70
Catalase	+	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-	-	-
Gas production	-	-	-	-	-	-	-	-	-	-	-
H <sub>2</sub> S	-	-	-	-	-	-	-	-	-	-	-
Urease	+	+	+	+(z)	-	+	+	+	+	-	-
Tryptophane deaminase	-	-	-	-	-	-	-	-	-	-	-
Lysine decarboxylase	-	-	-	-	-	-	-	-	-	-	-
Ornithine decarboxylase	+	+	+	-	+	-	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	-	-	-	-	-	-	-	-	-	-	-
Citrate	-	-	-	-	-	-	-	-	-	-	-
Esculine	-	-	-	-	+	-	+	+	+	-	+
Indole	-	+	+	-	+	-	+	+	+	-	+
Voges-proskauer 37 °C	-	-	-	-	-	-	-	-	-	-	-
Voges-proskauer 25 °C	+	+	+	+	+	+	+	+	+	+(z)	+
Methly Red	+	+	+	+	+	+(z)	+	+	+	+	+
Motility test 37 °C	-	-	-	-	-	-	-	-	-	-	-
Motility test 22-26 °C	+	+	+	+	+	+	+	+	+	+	+

(z): weak reaction

The occurrence of *Y. enterocolitica* in cheese samples has been investigated in several countries. For instance, Brindani and Freschi (22) have reported *Y. enterocolitica* in one of the 24 cheese samples (4.2%) in Italy. Boer and Kuik (23) have investigated 50 cheese samples and they have isolated *Y. enterocolitica* in one sample (2%) in Netherlands. Brodsky (24) has mentioned that he has isolated *Y. enterocolitica* in one sample of the 112 cheese samples (0.9%) in Canada. These findings have lower rates in comparison with our study.

There are also reports stating the lack of *Yersinia* spp. among the samples that were studied by Tornadijo et al (25) in Spain and Karplyuk et al (26) in Russia.

As a result, foodborne outbreaks have been reported in recent years. This has attracted our attention in terms of both the economic and public health consequences of microbial

contamination of processed dairy products. The need for the sanitary procedures during production and avoiding the contamination after pasteurization is of great importance.

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