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Yersinia enterocolitica and Shigella spp. in pasteurized milk

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Research Article

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ABSTRACT

In this study, it was planned to investigate the presence and serotypes of Yersinia enterocolitica (Y. enterocolitica) in various pasteurized milk samples, collected from retail markets in Kırıkkale region, using classical isolation and identification. Pasteurized milk samples were taken for isolation in the study; aseptically, 25 g of the sample was added to 225 ml of Peptone Sorbitol Bile Buyyon (PSBB), and incubated at 10 ° C for 10 days.

On day 10, the enrichment media was removed from the incubator and thoroughly mixed. From the enrichment media, 0.1 ml in 0.5% saline was transferred to 0.5% KOH and stirred for 2-3 seconds. One loop to the Mac Conkey plate and CIN plate were inoculated successively. After 1 day of incubation, the CIN plates were examined. Small (1-2 mm in diameter) colonies with a sharp-edged dark red center, the entire edge of which is surrounded by a clear colorless zone, were selected for identification. Colonies showing characteristic features after incubation were identified by a rapid identification system (BBL, Crystal). Y. enterocolitica could not be isolated in any of the 100 pasteurized milk samples that were examined, but yeast was detected in 6 of the milk samples and Shigella spp. were detected in one of the samples.

The reason why Yersinia spp. could not be isolated in our study, might be that the pasteurization process prevented the reproduction of Yersinia spp., but did not prevent the growth of Shigella spp. It is thought that working with a higher number of samples may increase the isolation rate, and Y. enterocolitica and Shigella spp., which are important sources of infection, should be examined in pasteurized milk.

Keywords: Pasteurized milk, Shigella, Yersinia enterocolitica

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Giriş

Milk is a complete food, especially for children and elderly people. It has high protein content, various minerals, oils, and vitamins. It's the primary source of nutrition for young mammals. And milk, cheese, cream, butter, yogurt and various dairy products, such as kefir and ice cream, are also consumed in quantities (Momtaz, 2012). Millions of people consume milk and dairy products per day. Among the types of Yersinia, especially Y. enterocolitica is within the 28 pathogen bacteria identified by the World Health Organization, which is found to be transmitted directly or indirectly by milk and milk products. This pathogen, which can lead outbreaks in healthy people, is attracting attention because Y. enterocolitica is a zoonotic factor and can overcome pasteurization when there is a large amount of Y. enterocolitica in raw milk. Y. enterocolitica can reproduce very quickly and very easily in pasteurized milk if the pasteurization process is wrong and/or insufficient. Because many microorganisms that prevent the growth of Y. enterocolitica are inhibited and eliminated by pasteurization; Y. enterocolitica reproduces and becomes a source of infection for humans (Schiemann, 1987). Products obtained from infected animals play an important role in the transmission of Y. enterocolitica to humans. Water and nutrients contaminated by these animals have a very important role in contamination (Sağun & Ergün, 1996). Contaminated foodstuffs play the biggest role in the transmission of Y. enterocolitica infections to humans. Y. enterocolitica from foods such as milk (especially chocolate milk), cheese, ice cream, meat (beef, sheep, pork, poultry), seafood, hamburger, sauce, mushroom salad and pudding, as well as vegetables such as carrots, tomatoes, green salad, mushrooms are also isolated (Lee, 1979; Morris et al, 1976; Sdliemarin, 1978). The fact that Y. enterocolitica can reproduce even at +4°C, shows that contaminated food kept in the refrigerator is also risky (Butler, 2000).

There are limited studies on the topic of Y. enterocolitica in pasteurized milk in Türkiye. Özbaş and Aytaç (1992) studied for the isolation of Y. enterocolitica, which is considered a pathogen in terms of human health, from milk and dairy products. For this purpose, 66 white cheese and 60 pasteurized milk samples collected in different periods were examined. Soyutemiz et al. (2000)

studied 100 raw milk samples collected from 47 different settlements covering 9 different cities in the Western Anatolia region (Bursa, Balıkesir, Bandırma, Kütahya, Eskişehir, Çanakkale, Burdur, İzmir, Manisa) between March and September 1999. Y. enterocolitica was isolated in 20 of 100 raw milk samples. All classes were determined to be serotype 0:3.

Güven et al. (2010), a total of 750 samples were collected, including 150 ice cream samples, 150 raw milk samples, 150 fresh cheese (white cheese) samples, 150 chicken drumsticks, and 150 minced meat samples, from three cities in northeastern Türkiye (Kars, Ardahan, and Iğdır). Of the 750 food samples analyzed, 57 samples (7.6%) were considered positive for Yersinia species. Eighteen (2.4% in total) isolated from 6 feta cheese, 4 ice cream, 2 chicken drumsticks, 4 minced meat, and 2 raw milk samples were evaluated as pathogenic Y. enterocolitica.

In 2011, an outbreak of Y. enterocolitica was detected in Pennsylvania due to the lack of pasteurization (Longenberger et al., 2014). An outbreak of Y. enterocolitica was detected in the United States of America again in 2015. The outbreak was associated with pasteurized milk from a local dairy; contamination of milk after pasteurization has been cited as a source (Ackers et al., 2000). In 2019, an outbreak of Y. enterocolitica originating from pasteurized milk was reported in Pennsylvania (Gruber et al., 2021).

In a study conducted in Egypt, Y. enterocolitica isolation was stated to be 10% in the samples of raw milk and dairy products. The highest isolation rate was determined from raw milk of 22%, followed by fermented milk of 12%, pasteurized milk of 4%, and salty ripened cheese of 2% (Ahmed, HA, 2018).

Ibrahim and Hallaç (2021) conducted a study to determine the prevalence of Yersinia species, especially Y. enterocolitica, in milk and dairy products in Isfahan, Iran. During one year, a total of 285 commercial and conventional dairy products and 267 pasteurized and raw milk samples were collected; analysis was done by PCR test. Of the 32 samples, Shigella spp. was detected that 50% of them were identified as Shigella dysenteriae, 18.75 % were identified Shigella sonnei and Shigella flexneri, and 12.5% were identified as Shigella boydii. Shigella spp. is a zoonotic bacterial pathogen that is among the foodborne pathogens and causes clinically severe diarrhoea. Studies show that there is a relationship between bacillary dysentery and Shigella spp. in developing countries. 160 million cases in a year have been linked to Shigella spp.; it has been defined as a factor that causes symptoms such as diarrhea, spasm, and shock, which is a great threat to society (Zhang et al., 2018). An outbreak of Shigella sonnei was detected from fresh pasteurized milk cheese in Spain in 1995-1996. (Garcia-Fulguerias et al., 2001). From a total of 231 raw milk samples obtained from different animals, 4 (0.87%) Shigella spp. were detected by multiplex PCR (Demirci et al., 2022).

This study aimed to investigate the presence and serotypes of Y. enterocolitica with classical isolation and identification methods in various pasteurized milk samples collected from retail markets in the Kırıkkale Region.

Materials And Methods

Sampling

Sampling: In this research, 100 packages of pasteurized milk samples belonging to 3 different companies, collected from retail markets in the Kırıkkale Region, were used with classical isolation and identification procedures. In the study, pasteurized milk samples were delivered to the laboratory as soon as possible. In Table 1, the numbers and types of pasteurized milk samples collected from 3 different companies are presented.

Table 1. *The numbers and types of pasteurized milk collected from 3 different companies*

Companies					
A		B		C	
Numbers of Samples	Types of Pasteurized milk	Numbers of Samples	Types of Pasteurized milk	Numbers of Samples	Types of Pasteurized milk
13	Strawberry	10	Strawberry		
8	Banana	12	Banana		
12	Lactose-Free Semi-Fat				
10	Chocolate	15	Chocolate		
4	3% Semi-Fat	10	3% Semi-Fat		
2	Full Fat	3	Full Fat	1	Full Fat

Isolation and Identification

The samples were processed according to the FDA method (FDA, 2017). Aseptically, 25 g of the sample was weighed, transferred in 225 ml of Peptone Sorbitol Bile Buyyon (PSBB), homogenized for 30 seconds and incubated at 10 ° C for 10 days.

If it is suspected that high levels of Yersinia spp. are present in the pasteurized milk sample, it was planned to inoculate 0,1 ml of the milk sample on Mac Conkey Agar and Celfsulodin-Irgasan-Novobiocin (CIN) Agar.

On day 10, the enrichment water was removed from the incubator and thoroughly mixed. From the enrichment water, 0.1 ml in 0.5% saline was transferred to 0.5% KOH and stirred for 2-3 seconds. One loop was inoculated on to the Mac Conkey and CIN plates successively. After 1 day of incubation, the CIN plates

were examined. Small (1-2 mm in diameter) colonies with a sharp-edged dark red center, the entire edge of which is surrounded by a clear colorless zone, were selected for identification. Colonies showing characteristic features after incubation were identified by a rapid identification system (BBL, Crystal).

Results

In this study, Y. enterocolitica could not be isolated in 100 of the pasteurized milk samples that were examined, but yeast was isolated in 6 milk samples and Shigella spp. was detected in one of the samples. The isolation and identification results of 7 pasteurized milk are given in Table 2 indicating the companies, types, and identified microorganisms of pasteurized milk.

Table 2. *The companies, types, and identified microorganisms of 7 pasteurized milk.*

Milk samples	Result of Identification		
	Y. enterocolitica	Yeast	*Shigella spp.*
C-1 (Company A 1 Full-Fat Pasteurized Milk)	-	-	+
Ç-1 (Company A 1 Chocolate Pasteurized Milk)	-	+	-
L-1 (Company A 1 Chocolate Pasteurized Milk)	-	+	-
G-1 (Company A 1 Chocolate Pasteurized Milk)	-	+	-
D-1 (Company A 1 Pasteurized Milk with Banana)	-	+	-
K-1 (Company B 1 3% Semi-Skimmed Pasteurized Milk)	-	+	-
I-1 (Company A 1 Strawberry Milk)	-	+	-

For positive control, a milk sample was contaminated with 1 CFU/ml Y. enterocolitica (Y. enterocolitica ATCC 9610) standard strain and studied with the same method; Y. enterocolitica was isolated and identified.

Discussion

Products obtained from infected animals play an important role in the transmission of Y. enterocolitica to humans (Emrullah and Özer, 1996). Contaminated foodstuffs play the biggest role in the transmission of Y. enterocolitica infections to humans. It has also been reported that pasteurization is not sufficient when Y. enterocolitica is present in large quantities in raw milk. The isolation of Y. enterocolitica from pasteurized milk by many researchers supports this view (Tacket et al., 1984). Y. enterocolitica can reproduce rapidly and easily in pasteurized milk, caused by incorrect and/or insufficient pasteurization, or post-pasteurization recontamination. Because many microorganisms that prevent the growth of Y. enterocolitica are inhibited and eliminated by pasteurization, Y. enterocolitica reproduces rapidly in milk, becoming a source of infection for humans (Lee, 1979; Greenwood & Hooper, 1985; Asakawa et al., 1979).

In Morocco, milk and dairy products total of 227 milk and dairy samples were examined for the investigation of the presence of Y. enterocolitica and reproduction was observed in 11 out of 30 raw milk (36.6%), 1 out of 20 pasteurized milk (5%), 15 out of 63 traditional fermented milk (23.8%), 7 out of 94 cheese, and 1 out of 20 cream samples (5%) (Hamama et al., 1992)

In Northern Ireland, Y. enterocolitica and the incidence of enterocolitica-like organisms were investigated in raw and pasteurized milk samples; it was found that 34 of 150 raw milk, 5 of 20 bottled raw milk, and 4 of 50 pasteurized milk were contaminated (Walker & Gilmour, 1992)

In the Province of Isfahan, Iran, Yersinia species and especially Y. enterocolitica were examined in 285 commercial and conventional dairy products, 267 pasteurized and raw milk samples during one year. The culture result showed that 52 (9.42%) and 28 (5.07%) of the total 552 dairy and dairy samples contained Yersinia species and Y. enterocolitica, respectively. Of the Y. enterocolitica isolates 24 were found to be positive (4.59%) in the PCR. Yersinia species and Y. enterocolitica have been reported to have the highest prevalence in raw cow's milk and traditional cheese. Yersinia species and Y. enterocolitica were reported to be negative in pasteurized cow milk, raw camel milk, commercial ice cream, commercial cheese, yogurt, butter and curd samples. Pasteurization is the best way to reduce the burden, especially of Yersinia species. The ability for Yersinia species to reproduce in yogurt, curd, and butter were stated to be low in this study (Rahimi et al., 2014).

A study was conducted on milk contamination by Y. enterocolitica in Alsace, France. Samples of bulk raw milk, processed raw, or pasteurized milk were collected from the tanks of the dairy plant and producers. 233 raw milk samples were examined as a result of the study, Y. enterocolitica was found in 127 samples (78 out of 101 bulk milk samples, 21 out of 92 samples from individual manufacturers and 28 out of 40 retail samples). Y. enterocolitica was detected in 3 of the 37 pasteurized milk examined (Vidon & Delmas, 1982)

Milk has high protein, mineral, fat, and vitamin values for humans; it is an important food. In addition, dairy products such as cheese, cream, butter and ice cream made from milk are consumed quite a lot. Y.

enterocolitica is among the important pathogens thar are detected to be directly or indirectly contaminated with milk and dairy products determined by WHO. Y. enterocolitica attracts attention due to its zoonotic nature and its ability to overcome pasteurization when present in high amounts in raw milk. Y. enterocolitica can also reproduce quickly and easily in pasteurized milk by infecting milk in case of incorrect or insufficient pasteurization or as a result of post-pasteurization recontamination. Because many microorganisms that prevent the reproduction of Y. enterocolitica are inhibited by the pasteurization process and provide advantages for its reproduction. Due to this situation, it will be useful for public health to check Y. enterocolitica in these products from time to time. It is thought that the study of a higher number of samples may increase the isolation rate, and Y. enterocolitica, which is an important source of infection, should be studied in milk. In addition, detection of Shigella spp. in pasteurized milk is important from the point of view of public health.

Kaynaklar

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