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Assessing the prevalence of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* infections in milk and dairy products in different sales outlets

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Abstract

Yersinia infection is ubiquitous disease, causing considerable damage to human health and has the possible fatal risk if child is infected. The main pathogenic groups are *Yersinia pseudotuberculosis*, *Yersinia enterocolitica*, and *Yersinia pestis*. The aim of the study was to study the prevalence of infection with *Y. pseudotuberculosis* and *Y. enterocolitica* in milk and dairy products in different sales outlets. The results of the study revealed high prevalence of infection in domestic dairy production (from 7.1% to 33.3%).

Keywords: Food safety; milk; dairy products; *Yersinia pseudotuberculosis*; *Yersinia enterocolitica*.

Introduction

Yersinios is a group of acute infectious diseases caused by Gram-negative enterobacteria of the genus *Yersinia*, characterized by general intoxication, rash, liver, spleen, gastrointestinal tract, joints, and other organs and systems lesion, severity and susceptibility to the development of exacerbations and relapses [1].

Pathogens to human are the following three types of bacteria of the genus Yersinia: *Yersinia pseudotuberculosis*, *Yersinia enterocolitica*, causing gastrointestinal diseases, and *Yersinia pestis*, etiological agent of bubonic and pneumonic plague. In healthy adults, infection with *Y. enterocolitica*, as a rule, leads to a limited gastroenteritis and/or mesenteric lymphadenitis, forming microabscesses or granulema lesion with central necrosis [2,3]. However, it should be remembered that patients with immunodeficiency gastroenteritis can lead to severe bacteremia in which the mortality rate approaching to 50% [2]. The most susceptible to disease of yersiniosis group are children under the age of 5 yrs, with one third of all cases according to different authors accounted for the age under 1 yr. Thus, in this population infection has often an unfavorable prognosis and often fatal [4-6].

In the pathogenesis of yersiniosis caused by *Y. pseudotuberculosis* and *Y. enterocolitica*, in the human body the general mechanism of the process is dominated. The main route of transmission is nutritional. Thus, initially seeding of ileum and colonization of Peyer's patches within the first hours after the bacteria enter the human body occurs. Next, bacterial cells are transported in mesenteric lymph nodes, and then spread systemically through the blood stream to eventually localize in the liver and spleen [7].

As mediated by transmission through animal products, yersiniosis infection most often occurs in violation of the rules of handling and storage of food, it is very important to carry out sufficient monitoring of the process at different stages. Among the most susceptible animal products are milk and dairy products. Data for identifying *Y. enterocolitica* in dairy products are described in various studies worldwide [8,9]. Special attention to the issue of safety of dairy products on Yersinia infection

is associated with ability of *Y. enterocolitica* infection to grow in the raw milk and keep it viable at low temperatures for a long time. As a result, the consumption of contaminated dairy products is a high risk of yersiniosis infection. Thus, the consumption of raw milk and a variety of dairy products is widespread. Dairy products are sold both in the official sales outlets, and on the natural markets in different countries [10-13]. Extensive use of milk in the diet of different groups of the population, especially for cooking for children under 5 yrs dictates the need to consider the prevalence of infection of dairy products and milk.

The aim of our study was to access the prevalence of infection with bacteria yersinia (*Y. pseudotuberculosis* and *Y. enterocolitica*) in milk and dairy products in some stage of implementation in different sales outlets.

Methods

Design of the study

To conduct the study, we developed a protocol that matches the design of cross-sectional survey. Realization of the program of research on the stage of collecting the material was carried out from September 2013 to August 2014. To the stage set, all the sales outlets have been mapped for the randomization of the sample. After this, the route has been applied, corresponding to the direction from east to west along the diagonal line. All located directly on the line or in the nearest location points were included in the study. There included, such as spontaneous markets on the periphery, implement products of the farm, as well as shops, mini supermarkets, mainly in the central parts of the city. Total number of sales outlets was 42. Sampling of dairy products including products is shown in Table 1.

After sampling the products, they are immediately placed in the vacuum aseptic containers, then in refrigerated containers and transported immediately to the laboratory for further study.

Bacteriological separation of Y. pseudotuberculosis *and* Y. enterocolitica

Key importance in determining the infection of the product is a traditional bacteriological confirmation. However, the bacteriological method allows to determine causative agent of yersiniosis not more than 10% of cases. Duration of the study was 21 to 28 days [14].

For analysis product samples were taken in an amount of 25 g, then grinded (in case of solid products – butter, cheese, ice cream) and placed in an accumulation storage in a ratio of 1:10. For sowing supernatant was used. The scheme of bacteriological examination included "enrichment" of the test material at low temperature, the use of differential diagnostic medium for the isolation and identification of cultures, the definition of their biotype, serotype, virulence. During the "cold enrichment" test material in Pepto-potassium medium was placed in a refrigerator and held it up to the first positive culture (no more than 10 days). Seeding on solid nutrient media was conducted from Pepto-potassium medium at 2-3, 5-7, and 10 days. Fence subsequent material was with a special loop of the upper third of the layer of the medium (except surface of the solution). View of colonies and keeping their morphology was carried out in

Table 1: Characteristics of dairy products sample (n = 630).

Name of the product	Number of the samples (n)	Quantity of the samples (%)
Pasteurized cow milk	58	9.2
Fresh cow milk	63	10.0
Cheese	63	10.0
Home-made cheese	51	8.1
Sour cream	51	8.1
Home-made sour cream	42	6.7
Kefir	42	6.7
Home-made kefir	56	8.9
Yogurt	48	7.6
Butter	15	2.4
Home-made butter	15	2.4
Ice cream	126	20.0
Total	**630**	**100**

Table 2: Characteristics of colonies *Y. pseudotuberculosis* and *Y. enterocolitica* on solid nutrient media Endo and Hottinger [15].

Species	Medium Endo		Medium Hottinger	
	24 h	48 h	24 h	48 h
Y. pseudotuberculosis	Small colonies (dewdrop) round, convex, shiny, colorless	Colonies diameter of 0.1-0.2 mm, large, convex, shiny with smooth edges, colorless	Colonies diameter of 0.1-0.2 mm, bluish-white translucent with smooth edges, with mucous slightly elevated center (under the microscope look like pyramids or with converging to the center of the face)	Colony diameter of 0.2-0.5 mm, transparent with a smooth edge. In polymorphic population – some colonies with irregular nodular scalloped edges
Y. enterocolitica	The same	The same, but with tints of pink	Colony diameter of 0.1-0.2 mm and 0.5 mm, round, convex with even margins, translucent with bluish soft consistency	Colony diameter of 0.3-0.5 mm, more convex with a smooth edge, transparent

48 hours, according to the characteristics given in Table 2 [15].

Conducting DNA extraction of Y. pseudotuberculosis *and* Y. enterocolitica *by polymerase chain reaction*

Polymerase chain reaction (PCR) – a highly sensitive method for detecting DNA of Yersinia in various biological samples is widely used in laboratory practice of European countries and in some well-equipped laboratories, medical institutions in Russia [14]. For carrying out this type of study, foods were homogenized in buffered saline in the ratio of 1:10 was thoroughly stirred for 15 minutes and then centrifuged at 12,000 rpm for 10 min. Thereafter, the precipitate was suspended in 300-500 mcl of distilled water a number of technical procedures was performed. PCR was performed according to the manufacturer's instructions kits for PCR "AmpliSens (R) *Y. pseudotuberculosis*" and "AmpliSens (R) *Y. enterocolitica*". To account the results of PCR electrophoresis was made on a 1.5% agarose gel. After staining the material with ethidium bromide, the gel was viewed in a transilluminator with UV wavelength equal to 310 nm. After the reaction, the amplified DNA fragments (bands in the gel, luminescent under UV light) were identified in size by comparing with the molecular weight marker, or positive control [14,15].

Statistical Analysis

The results were analyzed using standard methods of descriptive statistics. The basic idea of variables – absolute numbers and their percentage (*n*, %). The statistical significance of differences in the two groups was determined by the Chi-square test (χ^2). The procedure for the statistical analysis was performed in the program SPSS, version 20 for Windows (IBM Ireland Product Distribution Limited, Ireland). The critical level of significance of statistical differences was set when the $p < 0.05$.

Results

During the study, it was found that in dairy products, such as the results of bacteriological research, and according to the PCR method often had positive results in separation *Y. enterocolitica* (Table 3).

Most susceptible product was butter. From these dairy products more often in relation to the rest of the dairy products bacteria of the genus *Yersinia* was distinguished (*Y. pseudotuberculosis* – 13.3% and *Y. enterocolitica* – 33.3%). Thus, even at the level of product sales of factory production in the legal sales point, there was one case (6.7%) of infection products with *Y. enterocolitica* (Table 3). Apparently, this is related to the duration and temperature of storage of the product.

To a lesser degree of contamination from bacteria of the genus *Yersinia* of all kinds of home-made cheese was exposed. That may be associated with the peculiarities of the production of cheese at home. Among a large part of the factory of dairy products – pasteurized

milk, cheese, sour cream, kefir, yogurt, butter, and ice cream colony growth or genetic material of *Y. flora* were not detected (Table 3).

When making comparisons within groups of dairy products, based on the characteristics of their production, factory-made products/home products all positive and negative results of a study by PCR were selected. Categories were divided according to the common names – "milk", "cheese", "sour", "butter". Products categories, "ice cream" and "yogurt", were not included due to lack of similar comparison groups in mass and home production. So, we identified significant differences in the prevalence of Yersinia infection pathogens in the category of "cheese" ($p < 1^{-7}$) and "butter" ($p < 0.03$) (Table 4). Other names of dairy products are not subject to statistical analysis of the differences in mean zero content comparison groups of variables.

Discussion

In the Republic of Kazakhstan, due to geographical and socio-cultural characteristics in different regions, there is a territorial uneven incidence of yersiniosis. In some areas it is higher compared to the other, so, for example, in the general structure of the identified cases of yersiniosis in the South Kazakhstan region accounts – 16.4%, Zhambyl – 13.3%, East Kazakhstan 10.5%, Almaty 3.4%, 2.6%, Pavlodar, Akmola 1.5%. In other areas, including Kostanay annually only sporadic cases are recorded [15]. It should be noted that the data on the incidence of yersiniosis may differ from the *status quo*. So the official statistics in Kazakhstan accounted for only intestinal form of intestinal yersiniosis (enteritis caused by *Y. enterocolitica*). And yersiniosis caused by *Y. pseudotuberculosis* is not separately recorded. At the same time, if systematic

Table 3: Yersinia identification characteristics in dairy products by bacteriological and PCR methods, *n* (%).

Name of the product	Quantity of the samples	Bacteriological method		PCR method	
		Y. pss.[1]	*Y. ent.*[2]	*Y. pss.*[1]	*Y. ent.*[2]
Pasteurized cow milk	58	–	–	–	–
Fresh cow milk	63	10 (15.9%)	13 (20.6%)	10 (15.9%)	13 (20.6%)
Cheese	63	–	1 (1.6%)	–	2 (3.2%)
Home-made cheese	51	8 (15.7%)	12 (23.5%)	8 (15.7%)	12 (23.5%)
Sour cream	51	–	–	–	–
Home-made sour cream	42	3 (7.1%)	5 (11.9%)	3 (7.1%)	5 (11.9%)
Kefir	42	–	–	–	–
Home-made kefir	56	9 (16.1%)	14 (25%)	9 (16.1%)	14 (25%)
Yogurt	48	–	–	–	–
Butter	15	–	1 (67%)	–	1 (67%)
Home-made butter	15	2 (13.3%)	5 (33.3%)	1 (6.7%)	5 (33.3%)
Ice-cream	126	–	–	–	–
Total	**630**	**32 (5.1%)**	**49 (7.8%)**	**31 (4.9%)**	**49 (7.8%)**

[1]*Y. pseudotuberculosis*.
[2]*Y. enterocolitica*.

Table 4: The evaluation of Yersinia infection of dairy products by PCR method according to the nature of their production (factory-made products/home products), *n* (%).

Variable	Factory-made products	Home-made products	P-evaluation
Milk	–	23 (36.5%)	–
Cheese	2 (3.2%)	20 (39.2%)	1^{-7}
Sour cream	–	8 (19%)	–
Kefir	–	23 (41.1%)	–
Butter	1 (6.7%)	6 (40%)	0.03

microbiological diagnosis of infectious disease cases pseudotuberculosis, along with yersiniosis, are yet detected [16].

It should be noted that the incidence of yersiniosis is everywhere, so in 2012 two domestic outbreaks were recorded caused by bacteria of the genus Yersinia in Poland, in the province of Mazowieckie. In both cases four children aged 0-14 yrs suffered in the general, one of them was hospitalized [17].

In previous studies on the safety of milk and dairy products, it was found that the detection of *Y. enterocolitica* (73.8% of positive results) happens in practice much more often than in the milk does not enter into the process of treatment (64.7%), which, of course, contradicts many results obtained in similar studies worldwide [13,18-21].

According to the results of the Iranian province of Isfahan research bacteriological confirmation of contamination of dairy products was observed only in 9.42% and 5.07% of the total number of samples of milk and milk products for the presence of *Y. pseudotuberculosis* and *Y. enterocolitica*, respectively. These indicators are only slightly higher than we received. This fact can be explained by the inclusion in the study of Iranian scientists more traditional names (derived from domestic production) dairy products [18].

Great attention to the production and storage of food products, especially milk and dairy should be paid to the cold chain. Food storage at temperatures below −12°C is most secure to prevent microbial contamination of foods. However, with regard to storage in the frozen remains problematic stability and the ability to reproduce psychrophilic (cryophilic) microorganisms, which concerns Yersinia and Listeria, Campylobacter, and some other microorganisms. To guarantee the safety of perishable products it is need to track and prevent possible violations (breaks) in the cold chain on the way of the delivery of products to the end user [22].

Now milk and dairy products are widely distributed in the diet of children in particular and the general adult population. The content of milk balanced composition of proteins, minerals, fats, and vitamins is essential for healthy and nutritious food person. Moreover, the production of various milk products such as cheese, sour cream, butter, yogurt, sour cream, became an integral component of its use in the practice of the production of food products, both at home for personal use production and limited implementation and the level of mass production and sale in grocery hypermarkets. Every day millions of people use milk and dairy products in their diet. Thus, the hygienic quality of milk and dairy products is very important, especially for mass production. It is unacceptable the breach in the production and storage of food products, including dairy, due to the fact that for a variety of bacterial infections it can serve as a breeding ground or condition for breeding. As a consequence of non-compliance with the necessary rules, we can observe outbreaks of infectious diseases that are detrimental to the health of human and material, as they require overhead organism and financial means to purchase medicines [18].

Conclusions

According to the results of the study, we have revealed a high prevalence of contamination of milk and dairy products with bacteria of the genus Yersinia (*Y. pseudotuberculosis* and *Y. enterocolitica*) that are at the stage of implementation in different outlets (from 1.6% to 33.3%). A large proportion of products were home-made products, mainly to be implemented on natural markets and legalized outlets (from 7.1% to 33.3%). Most often the positive results of detection of Yersinia bacteria were fixed in home-made butter – from 6.7% to 33.3% by PCR. The main reason for such a high incidence of contamination of milk and dairy products with *Y. pseudotuberculosis* and *Y. enterocolitica* according to the literature should be considered a violation of sanitary-epidemic rules for the production and storage of these types of food products. However, to create a complete picture on the issue it is necessary to conduct larger studies with the stages of the study of all parts of the epidemic process.

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