ASSESSING THE SAFETY OF PERMISSIBLE L. MONOCYTOGENES LEVELS IN FOOD PRODUCTS IN TERMS OF PUBLIC HEALTH RISK[1]

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Abstract. The article presents the results of a comparative analysis of Listeria monocytogenes risks associated with consumption of the food products containing L. monocytogenes at the levels permitted in the Customs Union, by the Codex Alimentarius Commission, and in the EU for food products. We have determined that with the exposure at the level of the Customs Union hygienic standards (absence of L. monocytogenes in 25 grams of food products), the health risk will not exceed the permissible level. The adoption of the Codex Alimentarius Commission and the EU standards (100 KOE L. monocytogenes/g for marketable products) may result in impermissible levels for the RF population in general and for the most sensitive groups in particular.
Key words: safety assessment, permissible levels, assessment of microbiological risks, L. monocytogenes.

The risks created by microbiological hazards have direct and serious implications for public health. In such a case, the purpose of a microbiological risk assessment is ensuring public health. Risk assessment is the key element in establishing food safety standards to ensure consumer protection and facilitate international trade [1]. The microbiological risk assessment procedure must include the use of quantitative information when assessing the risks. The microbiological risk assessment must be conducted on the basis of a structured approach that includes hazard identification, definitions of hazard characteristics, assessment of the exposure, and identification of the hazard characteristics [2–5].

The microbiological criterion must be used only when it is necessary and the application is practically possible. Such necessity is determined, for example, by the epidemiological data that shows that the food product under review may present risks for public health and that such criterion is critical for consumer protection, or the necessity for such criteria comes from the risk assessment results [4, 6].

There is no doubt that it is necessary to establish the hygienic standards for L. monocytogenes levels in food products since Listeria monocytogenes is a dangerous disease (case mortality equals 21 %) [7]. The U.S. and European researchers have enough evidence that indicate a connection between this disease and the consumption of Listeria contaminated food products, particularly cheese and other dairy products and salads, and to a smaller extent – meat, chicken and fish products [8–12]. Russian researchers also have evidence about the intake of L. monocytogenes with food [13–14].

The hygienic standards adopted in the Customs Union determine the absence of colony-forming units (CFU) in 25 grams of products [15–17]. According to the principle Codex Alimentarius document CAC/GL61-2007 [18], the permissible L. monocytogenes level in food products is established on the basis of likelihood of growth and generation of bacteria in the group of products under review. For the food products that do not support the growth and

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generation of L. Monocytogenes due to its physical and chemical properties, a permissible level of bacteria count in 100 CFU L. monocytogenes/g has been established, and for the food products that promote the growth and generation – absence in 25 g of the product.

In accordance with the EU Regulation 1441/2007 [19], the EU countries have established the levels for the count of L. monocytogenes in the baby food and medicinal products (absence in 25 grams of the product) , for other food products that support the growth and generation of L. monocytogenes as well as for other products that do not support the growth and generation of L. monocytogenes (100 CFU L. monocytogenes/g – during market circulation, and absence in 25 g of the product – before the market launch).

The purpose of the research was to assess the requirements of the technical regulations and Standard sanitary, epidemiological, and hygienic requirements to the products that are subject to sanitary and epidemiological inspection (control) by the Customs Union (hereinafter – CU Standard sanitary requirements), and the standards identified in the international documents (WTO, European Union) for the permissible level of L. monocytogenes in food products for the purposes of public health including the health of sensitive population groups.

At the hazard identification stage, it was determined that L. monocytogenes is a gram-positive facultative anaerobe, nonspore-forming rod. This bacteria refers to the psychrotrophs group which means it is capable of active generation at low temperatures (4–10 °C) and can grow at the temperature of 0 to 45 °C (temperature optimum - 37 °C), with a pH level equal to 4,4 -9,4, and water activity equal to 0,9 in NaCl solution (Miller, 199). The microorganisms are resistant to high water salinity and acidity that unlike most other nonspore-forming bacteria – food infections agents – helps them be preserved under exposure to unfavorable environmental factors [20-21].

L. monocytogenes is found at different stages of food production [21, 22] and is capable of living for an extended period of time in food products, processed plants, household and the environment, particularly when in the fridge of freezer. L. monocytogenes is deposited mostly during the processing of such foods as milk, cheeses (especially soft cheese), ice-cream, raw vegetables, fermented raw meat and boiled sausage links, raw and boiled poultry, raw meat products, and raw and smoked seafood at low temperatures and in high humidity [21–25].

One of the challenges related to the determination of Listeria hazard (which has to do with food products) is absence of clear definition of the cases of disease. In most cases, people refer to a medical facility only in severe cases that require medical intervention. However it is practical to view the ingress of infection as colonization of microorganisms in host organism (adhesion to the mucosa and their growth) resulting in asymptotic forms, gastroenteritis and fever, acute forms, or death. Invasive may lead to perinatal Listeria monocytogenes, meningitis or septicemia. The likelihood of damage to the enteral lining depends on a number of factors including the amount of bacteria in the body, vulnerability of the host, and virulence of the bacteria [26].

In order to exclude L. monocytogenes contamination, such programs as HACCP (Hazard Analysis and Critical Control Points) are launched as well as measures aimed at improving the sanitary conditions in the food industry; as a result, the incidence of Listeria monocytogenes

went down in the USA [27], Great Britain [28], Australia [29] and France [30]. However since that moment the incidence has remained relatively stable [31].

The results of hazard analysis show that the food contaminated with Listeria monocytogenes is considered the main channel of infection and cause of 99% of Listeria monocytogenes [32, 33].

The biggest contributor here is ready-to-use food that supports the growth of L. monocytogenes, has been refrigerated for an extended period of time and can be used without anti- Listeria monocytogenes treatment [25, 34–36], that is the products that were initially treated but later contaminated or cross-contaminated at points of sale or when stored at home.

In registered outbreaks and sporadic Listeria monocytogenes morbidity related to food consumption, the count of *L. monocytogenes* fluctuated from 10^2 to 10^6 in one gram of the product [37]. In the registered outbreaks of Listeria monocytogenes from 1985 to 2005 with the known count of L. monocytogenes in 1 g of the product, particularly stands out an outbreak of 1998-1999 in Finland related to consumption of butter, when 100 CFU L. monocytogenes were present in 1 g of the product [52]. At that time, 18 people got ill including 4 fatal cases. Consequently, we can assume that 100 CFU L. monocytogenes/g of product is a minimal infecting dose.

The presence of L. monocytogenes in human intestines is often transitory. The share of population with L. monocytogenes in the stool sample constitutes 0,5-29% [24]. An average of 2-10% of the population is bacteria carriers with no obvious health problems [24, 38-42]. According to Farber and Peterkin [24], a large amount of healthy carriers signifies that the presence of L. monocytogenes in the stool is not an essential factor that determines the presence of infection.

Pregnancy increases the risk of Listeria monocytogenes but is not a contributory cause of being a carrier [43]. Healthy pregnant women can be carriers of L. monocytogenes but have healthy babies.

More than 20% of the population is considered to fall in the high risk group for Listeria monocytogenes [44, 45]. Healthy children and immune-competent adults have a low risk of severe Listeria monocytogenes.

To evaluate the 'exposure-effect' dependency, we used the 'dose-response' models. In this context, the amount of L. monocytogenes microorganisms that entered the digestive tract is considered the dose. The possibility of the disease development is considered a negative health effect. Such models, as a rule, are built with the use of popular statistical possibility distribution functions, the dependency ratio is determined based on the results of epidemiological researches. One of the simples and frequently used models is an exponential model with one variable [46, 47]:

$$P_i = 1 - \exp[-r_i \cdot N_i], \qquad (1)$$

where P_i – possibility of the disease after taking an I product, N_i – dose of the microorganisms, KOE L. monocytogenes/day, r_i – variable that corresponds with the possibility of the disease after exposure to one single microorganism. Equation (1) is widely used to evaluate the possibility of the disease under exposure to *Listeria monocytogenes* [48]. When

calculating the risk of disease contraction by people with normal immunity, we used the known coefficients by three types of products:

1) smoked fish: $r_1 = 5,6 \cdot 10^{-10}$ [45];

2) chocolate milk: $r_2 = 5,8 \cdot 10^{-12}$ [48];

3) tuna and corn salad (vegetables)»: $r_3 = 1,8 \cdot 10^{-8}$ [48].

When evaluating the risk of contracting a disease after consuming several kinds of the products, we used an additive hypothesis $P = \sum_i P_i$ permissible when operating with small values P_i.

To calculate the possibility of contracting a disease by people in sensitive groups (pregnant and breastfeeding), we used a coefficient $r = 3,15 \cdot 10^{-7}$ [48].

The exposure assessment was conducted on the basis of average daily consumption of the food product groups considered the most likely sources of L. monocytogenes and the permissible level of L. monocytogenes in the food products [48].

When assessing the exposure for the RF population, we used several versions of the daily allowance of food products (vegetables, fruit, fatty products, dairy products, meat products, fish (seafood)): recommended daily allowance of the food products by the RF population [49], actual daily consumption of the food products for pregnant and breastfeeding women which fully provides for their physiological needs in food elements and energy [51].

The level of L. monocytogenes in the food products under the hygienic standards established by the technical regulations of the Customs Union, Codex Alimentarius Commission, and the EU guidelines at the final point of food production was set at 0,04 CFU L. monocytogenes/g [48].

The maximal permissible level of L. monocytogenes in the food product that is ready for consumption and was launched into the market in accordance with the Codex Alimentarius Commission standards and the EU guidelines constituted 100 CFU/g.

The calculation of the amount of L. monocytogenes that entered the body with the food products was conducted on the basis of the following scenarios:

– recommended daily allowance of the food products for the adult population with the level of L. monocytogenes at 0,04 и 100 CFU/g (scenarios 1 and 2);

– actual daily consumption of the food products by the adult population with the level of L. monocytogenes at 0,04 and 100 CFU/g (scenarios 3 and 4);

– consumption based on the optimal daily average food products pack for pregnant women (vegetables and fish) and a recommended daily allowance of other food products with the level of L. monocytogenes at 0,04 and 100 CFU/g (scenarios 5 and 6);

– consumption based on the optimal daily average pack of food products for pregnant women (vegetables and fish) and actual daily consumption of other food products with the level of L. monocytogenes at 0,04 and 100 CFU/g (scenarios 7 and 8);

– consumption in accordance with the optimal daily average pack of products for breastfeeding women (vegetables and fish) and recommended daily consumption of other food products with the level of L. monocytogenes at 0,04 and 100 CFU/g (scenarios 9 and 10);

– consumption in accordance with the optimal daily average pack of products for breastfeeding women (vegetables and fish) and actual daily consumption of other food products with the level of L. monocytogenes at 0,04 and 100 CFU/g (scenarios 11 and 12).

The maximal daily intake of L. monocytogenes with the food products by the above exposure scenarios constituted from 44 (scenario 3) to 185660 (scenario 2) CFU/day.

At the **risk analysis** stage, based on the results of 'dose-response' dependency modelling, we calculated the possibility of development of Listeria monocytogenes for the above exposure scenarios (Table below).

The risk of Listeria monocytogenes development after intake with the food products under different exposure scenarios

Food product groups	Vegetables	Dairy products	Fish	Total risk
Scenario 1	$2{,}76^{-07}$	$2{,}16^{-10}$	$1{,}35^{-09}$	$2{,}77^{-07}$
Scenario 2	$\mathbf{6{,}89^{-04}}$	$5{,}40^{-07}$	$3{,}37^{-06}$	$\mathbf{6{,}93^{-04}}$
Scenario 3	$1{,}41^{-07}$	$1{,}29^{-10}$	$5{,}38^{-10}$	$1{,}42^{-07}$
Scenario 4	$\mathbf{3{,}53^{-04}}$	$3{,}22^{-07}$	$1{,}34^{-06}$	$\mathbf{3{,}54^{-04}}$
Scenario 5	$2{,}76^{-07}$	$7{,}43^{-06}$	$1{,}35^{-09}$	$7{,}71^{-06}$
Scenario 6	$\mathbf{6{,}89^{-04}}$	$\mathbf{1{,}86^{-03}}$	$3{,}37^{-06}$	$\mathbf{2{,}55^{-03}}$
Scenario 7	$1{,}41^{-07}$	$7{,}43^{-06}$	$5{,}38^{-10}$	$7{,}57^{-06}$
Scenario 8	$\mathbf{3{,}53^{-04}}$	$\mathbf{1{,}86^{-03}}$	$1{,}34^{-06}$	$\mathbf{2{,}21^{-03}}$
Scenario 9	$2{,}76^{-07}$	$8{,}69^{-06}$	$1{,}35^{-09}$	$8{,}97^{-06}$
Scenario 10	$\mathbf{6{,}89^{-04}}$	$\mathbf{2{,}17^{-03}}$	$3{,}37^{-06}$	$\mathbf{2{,}86^{-03}}$
Scenario 11	$1{,}41^{-07}$	$8{,}69^{-06}$	$5{,}38^{-10}$	$8{,}83^{-06}$
Scenario 12	$\mathbf{3{,}53^{-04}}$	$\mathbf{2{,}17^{-03}}$	$1{,}34^{-06}$	$\mathbf{2{,}52^{-03}}$

The calculated total risks are either small to negligible and do not differ from the regular daily risk (De minimis level) ($\leq 1\times10^{-6}$), or at a maximal permissible level (1×10^{-6}–1×10^{-4}) for the scenarios with L. monocytogenes intake at the level of the Customs Union's hygienic standards, or impermissible for the population and thus requiring risk-reducing measures (1×10^{-4}–1×10^{-3}), or impermissible for the population (De manifestis level) and requiring emergency risk-reducing measures ($\geq 1\times10^{-3}$) at the level of Codex Alimentarius Commission and the EU standards. High risk levels are developed due to the intake of L. monocytogenes with vegetables by the adult population and additionally with the dairy products for pregnant and breastfeeding women.

When assessing **the uncertainty of the results**, it is necessary to keep in mind that overestimation of the Listeria monocytogenes risk might be caused by the assumption that the amount of L. monocytogenes in all the food products is in the upper limit of the permissible level, that vegetables and dairy products are consumed without prior heat treating that would reduce the level of L. monocytogenes. Overestimation as well as underestimation of the risk may be caused by the 'exposure-response' model developed for certain products, and extrapolation of the dependencies to a group of food products. Underestimation of the risk may result from inaccurate registration of the Listeria monocytogenes cases, especially those that do not have symptoms or are mild cases, as well as from insufficient information about the possibility of contracting Listeria monocytogenes by the most sensitive population groups including those with

dysimmunity. It is necessary to take into account the availability of the information about the development of the disease under exposure of 100 CFU L. monocytogenes/g.

Consequently, assessing the risk of Listeria monocytogenes development under exposure at the level of the hygienic standards of the Customs Union (absence of L. monocytogenes in 25 g of food products) allows us to determine that the health risk will not exceed the permissible level that ensures health safety for the population of the Russian Federation. Adoption of the Codex Alimentarius Commission and the EU standards 100 CFU L. monocytogenes/g (for the marketable products) may result to impermissible risk levels for the population of the RF in general as well as for the most sensitive population groups.

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