

Ready-to-eat meat products as a source of *Listeria monocytogenes*

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Abstract

In 2015 in the European Union member states listeriosis caused 270 deaths. Food is the route of transmission in 99% of all human infection cases. Several studies from different countries have shown that the presence of *Listeria monocytogenes* in food can be as high as 58.3%. One of the most important ways to protect food from these microorganisms is to prevent the spread of the bacteria at processing plants at different stages of food production chain. The ability of *L. monocytogenes* to survive in extreme conditions and to form biofilms on various surfaces is a significant challenge for food safety. Removal of these bacteria from niches in processing plants is difficult and requires the use of sanitisers and precise equipment cleaning. The presence of *L. monocytogenes* in processing environment at slaughterhouses, deli meat factories or in retail may be a reason of cross-contamination. Proper hygienic systems applied by workers in food preparing places and knowledge about different routes of spreading of these bacteria may effectively decrease the risk of food contamination. Standardised legal regulations and control of meat product manufacture should be a fundamental way to protect food from *L. monocytogenes* contamination.

Keywords: ready-to-eat meat products, *Listeria monocytogenes*, contamination, food chain.

Introduction

Listeriosis is one of the most dangerous foodborne zoonoses with a high mortality rate reaching 20%–30% (32, 33, 39). Although it is a relatively rare illness, with the notification rate of 0.46 cases per 100,000 persons in 2015 in the European Union (EU), most of the infections required hospitalisation (97.4%) (9). At the same year 2,206 confirmed human listeriosis cases and 270 deaths were noted. In Poland in 2015, 70 people suffered from *Listeria monocytogenes* infection, with the notification rate of 0.18 (9). A significant increasing trend during 2011–2015 was observed in the EU member states, *i.e.* from 1,516 cases in 2011 to 2,242 in 2014 (9). In high-risk persons, *e.g.* the elderly, new-borns, or people with weakened immune system, listeriosis may manifest as meningitis, meningoencephalitis, septicaemia, and abortion (29, 31, 32, 36, 39). The disease is caused by a ubiquitous microorganism – *L. monocytogenes*, a Gram-positive and non-spore-forming bacterium which can grow under anaerobic and aerobic conditions and survive even in extreme environmental conditions, a such

as high concentration of salts, wide temperature range (–0.4°C–45°C), pH between 4 and 9.6 (40), and water activities ≥ 0.92 (25). All these features make *L. monocytogenes* a foodborne pathogen extremely dangerous for humans, which can occur both in food and food production environment. These bacteria are often present in freezers, warehouses, and on surfaces in processing plants which have contact with food or raw products (12, 28). *L. monocytogenes* has been typed into four serotypes of which only three (1/2a, 1/2b, 4b) are involved in 95% of all human listeriosis cases (42).

As mentioned above, *L. monocytogenes* has a remarkable ability to survive under extreme environmental conditions, which has an impact on the spread of these bacteria in a broad range of foodstuff. Contaminated food is the most important route of transmission of these microorganisms to humans and it is responsible for about 99% listeriosis cases (25). Several studies have shown that different kinds of ready-to-eat (RTE) foods of animal origin were a vehicle for transmission of *L. monocytogenes* to consumers (21, 24). According to the latest EFSA report, in 2015 in the EU

member states, the levels of contamination of RTE products were 3.5% of 2,847 of tested fish units and 4% of 2,366 of tested meat samples (9).

RTE meat production chain

The major steps in RTE meat production are farms, slaughterhouses, manufactures, shops, restaurants, and transport of products between these places. In each step of this food chain there are different possible sources of *L. monocytogenes* contamination. Reduction or elimination of the microorganisms from these places will be helpful in limiting the bacteria in the final food products. Most studies based on the “farm to fork” concept have shown that contamination of the final products usually occurs in processing plants or at the retail level and less often directly from food-producing animals (19, 26). Furthermore, human factor plays an important role, especially employees who do not comply with proper hygiene rules and may be vectors of *L. monocytogenes* along the food chain (Fig. 1).

It is important to distinguish potential routes of spreading of *L. monocytogenes* to know how to protect food and production environment from the bacterial contamination, as well as how different processes and environmental conditions influence the growth of the bacteria at different stages of the food chain. As it was mentioned, these microorganisms are able to survive under extremely different environmental conditions (25). Furthermore, they form biofilms on the surfaces of the processing equipment which may be a reservoir of these microorganisms for a long period, since *L. monocytogenes* is resistant to disinfectants, UV light, and desiccation (8, 33). It is difficult to remove bacterial biofilms, which makes them one of the most important hazards in food production process. There are few types of sanitisers which may be used in food environment and most of them are based on acid-anionic, halogen, hydrogen peroxide, quaternary ammonium, and chlorine (21, 34). Moreover, disinfection itself is not enough to eliminate *L. monocytogenes* from the production areas. It is also necessary to remove all food waste which may limit the contact of sanitiser agents with the bacterial biofilm by effective washing and rinsing of the surfaces before decontamination (33). Biofilm formation is often supported by accumulation of food residues in specific niches, like meat choppers or minced meat machines (8). It has been shown that *L. monocytogenes* is capable to form biofilm on stainless steel and glass or even polyvinyl chloride and polyethylene surfaces (4). The structure of the biofilm shows a great variety, from monolayer of cells to knitted chain network and honeycomb-like structure. Biofilm forming is influenced by *L. monocytogenes* factors, such as biofilm-associated protein (Bap), protein SecA2, and flagella, as well as environmental conditions, like temperature and adhesion properties of the surfaces (18). No correlation between *L. monocytogenes* molecular lineages or serovars and biofilm formation capacity has been observed (4, 7, 8). Additionally, it has been shown

that these bacteria are able to form biofilms with other microorganisms which may be present in the production environment, e.g. *Pseudomonas* or *Staphylococcus*. This may increase the strength of the biofilm structure and its resistance to the majority of cleaning and disinfection agents (18).

Livestock as a source of *L. monocytogenes* in food

Farms are the first stage of the RTE food production. At this level, the most important aspect is ensuring the proper state of the livestock health, which depends, among others, on proper veterinary management and quality of feed (11, 19). However, Hellström *et al.* (19) demonstrated that the presence of *L. monocytogenes* in pigs does not necessarily mean that pork meat products will be contaminated with these bacteria. From a total of 1,962 samples collected at farms (rectal swab and feed) and slaughterhouses (tonsils, pluck, carcass swabs), 119 (6%) were positive for *L. monocytogenes*. The highest prevalence of these bacteria was detected in tonsils (24%) and pluck sets (5%) while the carcasses were contaminated only in 1%. Genotyping investigation has shown that in the case of direct contact of carcass or pluck sets with tonsils there is a possibility to transfer *L. monocytogenes* to meat. It was also documented that proper methods during the slaughter can limit the prevalence of these bacteria in the final product. Some isolates of *L. monocytogenes* were detected in minced meat from the negative carcass, suggesting that contamination of meat occurred after processing as an effect of cross-contamination. In addition, these results suggest that *L. monocytogenes* was transferred to the products after slaughter of the animals as the meat from contaminated and not-contaminated carcasses was mixed (19, 26). In another study, prevalence of *L. monocytogenes* was much lower in carcasses (2.4%) and in the intestines (9.3%) than in the ground meat obtained from these animals (50.2%) (26). This may also suggest that transmission of the bacteria is more likely post-slaughter, e.g. from the environment of slaughterhouse.

Storage of meat and RTE food

Storage time and conditions are important aspects in safety of RTE foods. During production of this kind of food it is important to use a raw meat free of *L. monocytogenes*. According to the EFSA guidelines, storage time should not exceed 15 days for red and 3 days for poultry meat at 7°C and 4°C, respectively (10). However, contamination of meat products may also occur during and after processing (heat treatment, smoking, fermentation process) (23). Studies on kinetics of growth of *L. monocytogenes* in sliced packed salami showed that storage conditions had an impact on the survival of these bacteria. In the case of a low-dose inoculated samples stored at 5°C the time needed to reach the detection limit

of *L. monocytogenes* was more than 16 days in air packing and more than 23 days in vacuum packing. For the same samples kept at 25°C *L. monocytogenes* has not been identified in air-packed or vacuum-packed conditions after 6 and 18 days, respectively. These results showed that higher temperature of storage and air packing decreased the level of *L. monocytogenes* faster than lower temperature and vacuum packing (14).

L. monocytogenes in processing environment

At RTE producing plants there are several dedicated zones, *i.e.* raw meat warehouse, preparing and packing areas, as well as final product stockrooms. In each of these locations there are possible sources of contaminations or cross-contamination, routes of spreading of *L. monocytogenes*, and potential problems with hygiene control.

Cold stores are the places where raw meat transported from slaughterhouses is kept. This is a critical area from which *L. monocytogenes* can be spread to other parts of the production facility. As it was

mentioned, the bacteria are able to form biofilms on many surfaces, including floor drains (Fig. 1). The presence of *L. monocytogenes* in drains may result in the spread of the microorganism by water aerosol or during floor cleaning. The bacteria can also be transported with clothes and boots of workers, resulting in contamination of other zones of the plant (8, 34).

Contamination of RTE food may also be connected with slicing machines. Several surveys have shown that in food producing plants it is difficult to overcome contamination of the processing environment. One of the important preventing procedures is to properly separate places like cutting rooms and cooling zones or raw meat delivery corridors from other spaces, such as delivery areas of heat-treated products, packing rooms, or places with smoking and boiling equipment.

A proper hygienic system can prevent the cross-contamination with *L. monocytogenes*, which is a fundamental risk factor in RTE food facilities (33). A proper hygienic system can prevent the cross-contamination with *L. monocytogenes*, which is a fundamental risk factor in RTE food facilities (33).

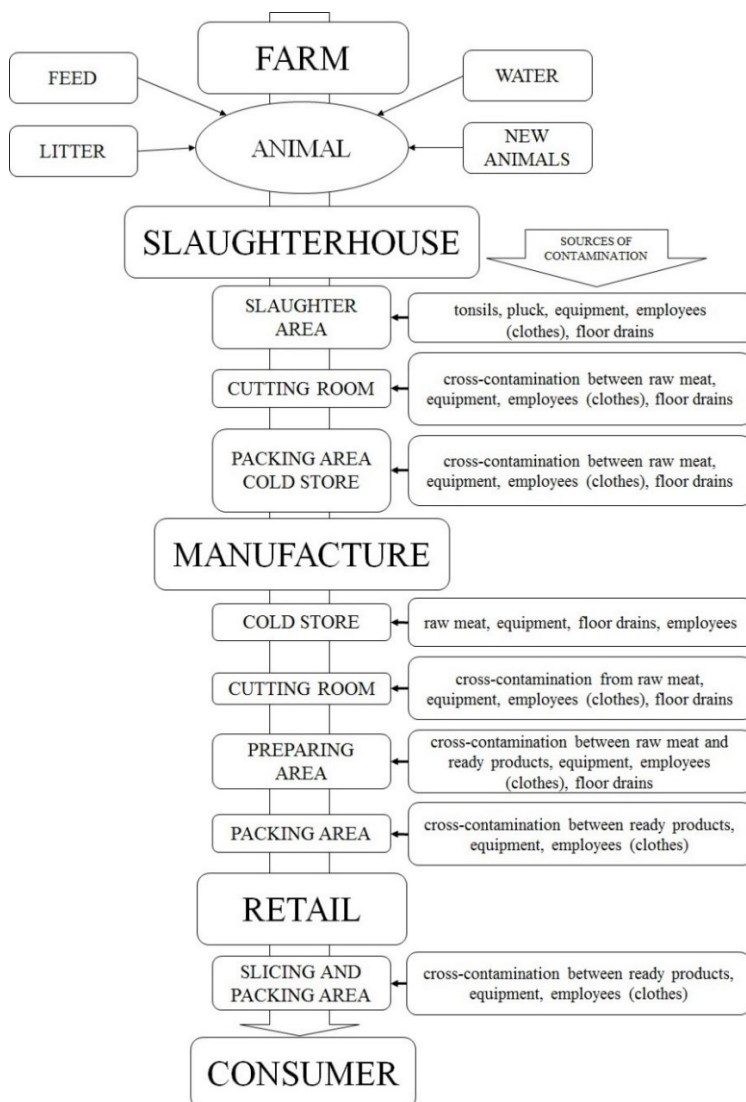


Fig. 1. Sources of *L. monocytogenes* at different stages of RTE meat production chain

Therefore, to maintain a high hygiene standard and sterility in production areas, elaboration of effective methods of cleaning and disinfection of unavailable niches, where routine disinfections are ineffective, is needed (16, 20). It is necessary to select effective sanitisers (alkaline-, acid-, alcohol- and QAC-based products) and to determine their minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) to choose the best solution for a given production facility (34).

***L. monocytogenes* in retail**

Many studies demonstrated that deli products sliced in retail shops often have a higher level of bacterial contamination than products prepared in deli meat factories (3). As tested in the USA, approximately 83% of listeriosis cases caused by deli meats were associated with products sliced at retail (35). Preparation of such RTE food in shops increases the risk of cross-contamination of products from different manufactures (21). Chaitiemwong *et al.* (3) showed that risk of higher concentration of bacteria increased when deli products were sliced separately for individual customers. Furthermore, it was documented that the last slice often had a higher number of *L. monocytogenes* than the previous ones (3). In smaller shops and local deli meat factories inadequate quality or number of equipment used for preparation of RTE foods may also cause a higher contamination risk of the final sliced products. Moreover, lack of the appropriate sanitisers and inaccurate washing of polyethylene and wooden cutting boards may result in the persistence of *L. monocytogenes* and cross-contamination of other food products. Guat Goh *et al.* (15) revealed that the number of *L. monocytogenes* on chicken meat in comparison with the number of bacteria found on the cutting board depended, among others, on the material from which the cutting board was made. In the case of the transfer of bacteria from wooden board to chicken meat (30 min after cutting the contaminated raw meat on the same cutting board), the probability of cross-contamination was 73.5%, whereas in the case of a plastic cutting board it amounted to 90.2% (15).

***L. monocytogenes* in RTE food – legal regulations**

To establish legal regulations concerning the prevalence of *L. monocytogenes* in food, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) established international standards called Codex Alimentarius. One of the Codex documents (CAC/GL 61-2007) refers to the standards in food hygiene to control *L. monocytogenes* in meat products (17). Another important international legislative document is ISO 22000:2005 which is applied in Food Safety Management Systems (FSMS) based on

Good Hygiene Practices and Production (GHMP), as well as in Hazard Analysis and Critical Control Points (HACCP) (19). However, despite well-regulated global standardised rules of hygiene in food production chain, there are still many countries where problems with food contamination with *L. monocytogenes* are difficult to solve. This situation is often due to the lack of sufficient knowledge about the risk of infection, both on the part of consumers and food manufacturers.

In the European Union the criteria on the presence of *L. monocytogenes* in RTE food are the same as in the Codex and they are specified in Commission Regulation (EC) No. 2073/2005 as later amended. It has been established that food business operators are responsible for application of the relevant criteria for different kinds of food. The number of *L. monocytogenes* must not exceed the limit of 100 cfu/g during the shelf life of food which is unable to support the growth of these bacteria, or *L. monocytogenes* is absent in 25 g in RTE food which is able to support the growth of these microorganisms (5). The infective dose in most of listeriosis cases is $>10^4$ cfu/g and for immunocompromised patients it could be even 100 cfu/g (39). According to the recent EFSA report concerning the food contamination with *L. monocytogenes*, for RTE products on the market less than 0.3% of single samples and 0%–1.4% of batched samples were found to comply with the criterion of ≤ 100 cfu/g of Regulation (EC) No 2073/2005. However, higher levels of non-compliance (primarily presence in 25 g) have been reported in similar samples of the comparable processing stage, from 0% to 3.5% in single samples and from 0% to 6.1% in batch samples, respectively. For the RTE products of meat origin, contamination with *L. monocytogenes* was 1.62% for batch samples and 2.07% for single samples, respectively (9).

There are many publications describing the presence of *L. monocytogenes* in RTE food products (Table 1). It can be concluded that food contamination with these microorganisms is still a problem, both in developed and developing countries.

Conclusions

Listeriosis is a very dangerous zoonosis, which, despite its low incidence, remains a major public health concern due to high mortality rate. Several studies have shown that RTE food is one of the most important vehicles responsible for human infections. To ensure the safety of such meat products one has to pay attention to many factors at different stages of the production chain. It is important to standardise food law and procedures for hygiene and sanitation in processing plants in different countries. Strains of *L. monocytogenes* display differences in virulence and epidemic potential, and a limited number of serotypes have been responsible for human foodborne infections. Molecular typing of these bacteria plays an important role in epidemiology and foodborne outbreak investigation.

Table 1. Contamination of RTE products in different countries

Country	Food product	Number of samples tested	Percentage of positive samples	Reference
Europe				
Serbia	Cooked meat products	311	0.96%	2
	Cured meat products	111	4.5%	
Spain	Cooked meat products	35	5.71%	13
	Raw-cured products	57	5.26%	
	Dry-cured products	37	2.7%	
Sweden	Heat treated meat products	507	1.2%	28
Italy	Salami	112	20.5%	6
Portugal	Dry-cured ham	44	2.3%	31
United Kingdom	Cooked meat	1,321	2.2%	30
	Pâté	411	0.24%	
	Fermented meats	316	1.9%	
Austria	Cooked sausage-Pâté	112	4.5%	41
	Fermented sausages	65	1.5%	
Poland	Cooked sausage	84	2.38%	27
Slovakia	Ľudová salami	11	54%	24
	Ham sausage	8	37.5%	
	Ipeľská sausage	12	58.3%	
Turkey	Cooked red meat	31	6.4%	43
Asia				
Japan	RTE raw meats	96	3.1%	38
	Heat treated meat products	1,091	0.8%	
Malaysia	Sausages	30	13.3%	29
China	Cooked meat	62	6.5%	37
	Cooked meat	55	7.2%	
South America				
Chile	Cooked sausage	41	13%	32
	Pâté	62	55%	
Colombia	Sausage	169	7.69%	11
	Ham	10	6.13%	
	“Choriso”	67	4.03%	
Trinidad and Tobago	Deli meats	70	1.4%	22
Brazil	Pork sausage	138	39.8%	36
North America				
Canada	Fermented sausage	100	4%	1

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