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***Listeria monocytogenes* contamination in ready to eat foods**

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Abstract. *Listeria monocytogenes* is a food-borne pathogen responsible for listeriosis, a sickness with a high mortality rate. Listeriosis is largely associated with ready-to-eat (RTE) foods. It is well established that foods that pose the greatest risk of foodborne listeriosis are those RTE foods that have intrinsic characteristics such as pH and water activity that support the growth of *L. monocytogenes*. RTE foods can also become re-contaminated during further processing and handling. Increased handling leads to a higher probability of contamination. Sources of contamination can be food contact surfaces, processing machinery and workers. In our research, *L. monocytogenes* was detected in a RTE salad. Food safety criteria for *Listeria monocytogenes* in RTE foods have been applied from 2006 (Commission Regulation (EC) 2073/2005). Still, human invasive listeriosis was reported to increase during 2009-2013 in the European Union and European Economic Area. Time series analysis for the 2008-2015 period in this area showed an increasing trend of the monthly notified incidence rate of confirmed human invasive listeriosis of the over 75 age groups and female age group between 25 and 44 years old (probably related to pregnancies).

1. Introduction

The genus *Listeria* currently includes 17 recognized species (*Listeria monocytogenes*, *Listeria seeligeri*, *Listeria ivanovii*, *Listeria welshimeri*, *Listeria marthii*, *Listeria innocua*, *Listeria grayi*, *Listeria fleischmannii*, *Listeria floridensis*, *Listeria aquatica*, *Listeria newyorkensis*, *Listeria cornellensis*, *Listeria rocourtiae*, *Listeria weihenstephanensis*, *Listeria grandensis*, *Listeria riparia*, and *Listeria booriae*) of small rod-shaped gram-positive bacteria. Only two of these species, *L. monocytogenes* and *L. ivanovii*, are considered pathogens. *L. monocytogenes* is an important human foodborne pathogen. Importantly, detection of *Listeria* species is often used by the food industry as a marker to detect conditions that allow for presence, growth, and persistence of *L. monocytogenes* [1].

The growth and survival of *L. monocytogenes* is influenced by a variety of factors. In food these include temperature, pH, water activity, salt and the presence of preservatives. The temperature range for growth of *L. monocytogenes* is between -1.5 and 45°C, with the optimal temperature being 30-37°C. Temperatures above 50°C are lethal to *L. monocytogenes*. As *L. monocytogenes* can grow at temperatures as low as 0°C, it has the potential to grow, albeit slowly, in food during refrigerated storage. *L. monocytogenes* will grow in a broad pH range of 4.0-9.6. Although growth at pH<4.0 has



not been documented, *L. monocytogenes* appears to be relatively tolerant to acidic conditions. *L. monocytogenes* becomes more sensitive to acidic conditions at higher temperatures [2]. *L. monocytogenes* is reasonably tolerant to salt and has been reported to grow in 13-14% sodium chloride [3]. Survival in the presence of salt is influenced by the storage temperature. Studies have indicated that in concentrated salt solutions, the survival rate of *L. monocytogenes* is higher when the temperature is lower [2].

Food safety criteria for *Listeria monocytogenes* in ready-to-eat (RTE) foods have been applied from 2006 (Commission Regulation (EC) 2073/2005). This Regulation came into force in January 2006 and requires the following:

- In RTE products intended for infants and for special medical purposes, *L. monocytogenes* must not be present in 25 g of sample (10 sample units);
- *L. monocytogenes* must not be present in levels exceeding 100 colony forming units per gram (CFU/g) during the shelf life of other RTE products (five sample units), and;
- In RTE foods that are able to support the growth of the bacterium, *L. monocytogenes* must not be present in 25 g of sample at the time of leaving the production plant (five sample units); however, if the producer can demonstrate, to the satisfaction of the Competent Authority (CA), that the product will not exceed the limit of 100 CFU/g throughout its shelf life, this criterion does not apply [4].

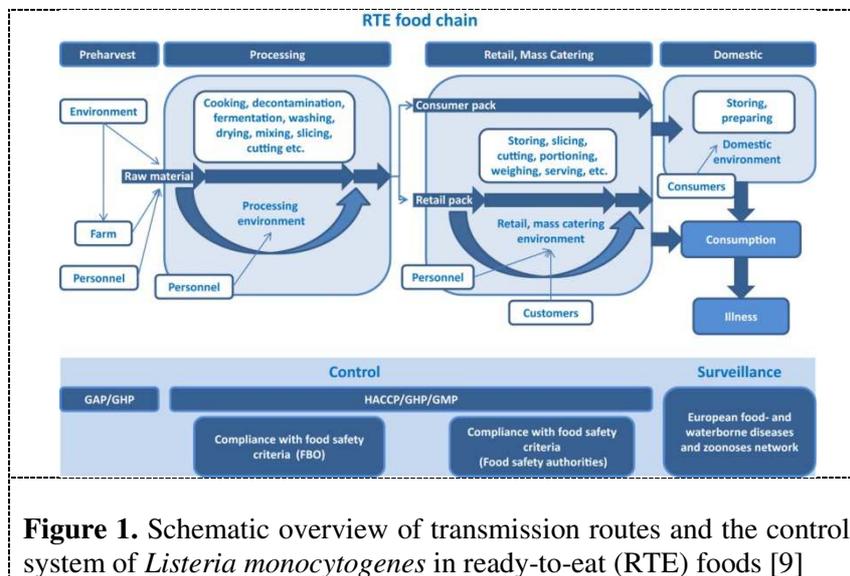
Still, human invasive listeriosis was reported to increase over the period 2009-2013 in the European Union and European Economic Area [5]. A conceptual model was used to identify factors in the food chain as potential drivers for *L. monocytogenes* contamination of RTE foods and listeriosis. Factors were related to the host (i. population size of elderly and/or susceptible people; ii. underlying condition rate), the food (iii. *L. monocytogenes* prevalence in RTE food at retail; iv. *L. monocytogenes* concentration in RTE food at retail; v. storage conditions after retail; vi. consumption), the national surveillance systems (vii. improved surveillance), and/or the bacterium (viii. virulence).

RTE food processing can involve, among other processes, comminution, addition of flavourings, binders, extenders and emulsifiers, etc., addition of preservatives (e.g. lactate, sodium nitrite), decontamination (water, acid), heating (e.g. pasteurising, cooking, baking, boiling, steaming), curing, smoking (hot or cold), fermentation and drying. Most of these steps have the potential to reduce pathogen loads on the RTE food at the time of consumption through microbial inactivation or inhibition of growth. The effectiveness of the control measures depends on the type of food and design of the process. In the case of a mild process (i.e. washing), the pathogen might survive while more intense or severe processes (i.e. sufficient heating) can lead to the elimination of the pathogen. RTE foods may also become re-contaminated during further processing and handling. In the latter case, increased handling leads to a higher probability of contamination [6]. Sources of contamination include food contact surfaces, processing machinery and workers. Contamination with *L. monocytogenes* after heat processing during further handling is one of the most important reasons for contamination. This is due to the capability of *L. monocytogenes* to form biofilms that result in enhanced resistance to disinfectants and antimicrobial agents.

RTE foods can be packed aerobically, under vacuum or in modified atmospheres. Packaging atmosphere can affect the growth of the pathogen during storage and, hence, the final risk. In addition, the amount of growth of *L. monocytogenes* can be affected by the assigned use-by date, since determines the storage time of the product.

Contamination of RTE food in packages that are opened and handled in retail stores (chubs, bricks, etc.) can also occur. Food retail and mass catering establishments are very different from food processing plants. They are open to the public, with customers, sales people, employees, and deliveries coming into the establishment. This can trigger the introduction of *L. monocytogenes* at various points and times of the day. *L. monocytogenes* is regularly found and often widely distributed in retail facilities [7-9]. Retail practices can result in cross-contamination from one RTE product to another, or in contamination from the retail environment, or in both [8]. The persistence of *L. monocytogenes* in a particular environmental site (i.e. slicing machine) at retail can be a niche that facilitates continued

cross-contamination of products from environmental sources. Surveys report that RTE delicatessen meats handled at retail stores have, in general, higher contamination than prepackaged products, indicating the possibility of cross-contamination at retail level [7,8,10] (Figure 1).



Some antimicrobial substances are added to food during production. For example, sorbic acid is sometimes added to prevent the growth of *L. monocytogenes* in foods such as cheeses, and a combination of sorbic acid and benzoic acid is commonly added to prevent the growth of *L. monocytogenes* in foods such as delicatessen-type salads.

Data reported in the zoonoses database show that from 2008 to 2015, as many as 525 human cases of listeriosis, 182 hospitalisations and 37 deaths were reported. Thus, most invasive listeriosis cases appear as sporadic infections and the detected outbreaks are usually small. The 'dairy' food category was responsible for four of these outbreaks causing 44 cases, while 'fish and seafood' and 'meat and meat products' food categories were responsible for 7 and 11 of these outbreaks causing 40 and 126 cases, respectively. In total, these three categories caused 22 (or 59%) strong-evidence food-borne outbreaks, 210 (or 40%) human cases, 125 (69%) hospitalisations and 26 (or 70%) deaths. Food of non-animal origin caused two outbreaks and 34 cases. Some of the outbreaks where the 'other' food category was implicated as the food vehicle could include RTE foods from the three food categories focused on in this Scientific Opinion [9], e.g. sandwiches, buffet meal, mixed foods.

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. The aim of this study was to establish the occurrence of *Listeria* spp., especially *L. monocytogenes* in ready to eat RTE food in Serbia.

2. Materials and Methods

Isolation of *L. monocytogenes* was performed according to the standard method [11]

3. Results and discussion

This study was conducted for one year – from January 2018 to December 2018. Four hundred and eighty-nine samples were collected. *L. monocytogenes* was found in one sample of vegetable salad with mayonnaise.

As *L. monocytogenes* can be found in the environment of food processing plants, RTE food processors should have an effective GMP programme with HACCP system to minimize all potential

sources of food contamination. These should address *L. monocytogenes* in the processing environment. In this regard, the importance of sanitation should not be overlooked. Sanitation management can lead to intervention innovations (e.g., effective remediation) and sanitary design improvements (e.g., equipment and facility). RTE food processors should also strongly consider introducing within their food safety systems one or more validated controls for the elimination of *L. monocytogenes* from their products (e.g., use of a post-lethality treatment). Furthermore, environmental and end-product sampling schemes and the use of microbiological testing as a verification tool to demonstrate the efficacy of the control measures put in place to address *L. monocytogenes* are recommended. Food processing plants should carry out regular environmental sampling to verify the effectiveness of their sanitation program for controlling *Listeria* in the plant environment, and should increase sanitation efforts and control measures in areas where *Listeria* are found.

4. Conclusion

The public health risk from *L. monocytogenes* in RTE food depends on the effectiveness of the control and monitoring procedures which include good agricultural practice at the farm stage and the hazard analysis and critical control points (HACCP) programme and good hygiene practices (GHP) at the processing and retail stages as well as sampling procedures to evaluate compliance with the FSC for *L. monocytogenes*. In this study, the low prevalence of *L. monocytogenes* in RTE foods indicates this pathogen is a low risk for public health.

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