

# *Listeria* species in raw and ready-to-eat foods on the market of Bosnia and Herzegovina



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## Abstract

*Listeria* is a food-borne pathogen that poses a great threat to human health. Severe symptoms followed by a high mortality rate are mainly caused by *L. monocytogenes*. Despite the zero-tolerance policy, the genus *Listeria* is still present in raw and ready-to-eat food. There are no recent studies regarding contamination of food with *Listeria* species in Bosnia and Herzegovina. This research is focused on obtaining basic information on the level of food contamination with this

alarming pathogen. In this study, a total of 238 samples were analysed, where 105 samples were various types of raw food and 133 samples were ready-to-eat foods, and 18 samples tested positive for *Listeria* species. After isolation and detection, the confirmation of positive isolates of *Listeria* spp. was conducted using the biochemical kit API® *Listeria*.

**Key words:** *Listeria*; *Listeria monocytogenes*; raw food; RTE food

## Introduction

Despite significant advances in technology and food production over the past two decades, the risk of foodborne illnesses has increased (Shamloo et al., 2019). When it comes to food-borne pathogens, one of the most fatal causative agents is *Listeria monocytogenes*, which is especially dangerous for pregnant women, the elderly, immune-compromised individuals, and newborns

(Bintsis, 2017; Shamloo, 2019). During the 1990s and 2000s, several outbreaks of foodborne listeriosis were observed worldwide (Voetsch et al., 2007). These events provoked strict applications of the HACCP system with a zero-tolerance policy regarding the presence of *L. monocytogenes* in food (Mengoni and Apraiz, 2003). Despite the zero-tolerance policy, the genus *Listeria* today

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is abundantly present in food and still poses a threat to human health.

*Listeria* species are Gram-positive aerobic or facultative anaerobic bacteria (Jemmi and Stephan, 2006). The invasive form of listeriosis and gastroenteritis are mainly caused by *L. monocytogenes* where symptoms are: fever, diarrhoea, headache, arthralgia, chills, and vomiting (Bintsis, 2017; Thakur et al., 2018). Human infections by *L. monocytogenes* are responsible for hospitalisation rates as high as 91%. *Listeria* poses a great threat to human health because it can multiply at the refrigerator temperature and therefore persists in the food-processing environment (Jemmi and Stephan, 2006).

Foodstuffs most commonly associated with *Listeria* infections are products such as raw milk, soft cheese (produced from raw milk), raw meat products, and salads (Jemmi and Stephan, 2006). Hygiene weak points in milk processing and animal slaughtering are critical points for occurring contamination. Further, cross-contamination from food-processing equipment is a possible source of *Listeria* species in processed food (Jemmi and Stephan, 2006). To our knowledge, there are no recent studies regarding the food contamination with *Listeria* species on the Bosnian and Herzegovinian market. Thus, the aim of this study is to assess the market situation considering the presence of *Listeria* species in raw and ready-to-eat foods.

## Material and methods

Detection and isolation of *Listeria* spp. were performed on the following types of foodstuffs of animal origin (Table 1): meat, poultry meat, seafood, meat products (hot dogs, ham, cold cuts etc.), fresh milk, dairy products (hard and soft cheeses, cheese spreads, etc.) and other foods of animal origin.

A total of 238 samples (Table 1) were analysed, where 105 samples were types of raw food (Category I) and 133 samples were various types of Ready-to-eat (RTE) food (Category II). Samples of fresh and RTE products were collected in individual quantities of 300 g. Pasteurised, durable and semi-durable products were sampled in the original packaging. Samples were collected randomly in all cantons in the Federation of Bosnia and Herzegovina over a one-year period. Samples were collected from places including shopping malls, butcher shops, restaurants, pastry shops, retail shops, and marketplaces. Whilst maintaining the cold chain, samples were delivered on the same day of sampling to the laboratory, where microbiological analyses were started within the next 24 hours. Analyses were conducted in the Laboratory for the Microbiological Examination of Foodstuffs at the Faculty of Veterinary Medicine, University of Sarajevo. After obtaining the results of microbiological analyses, biochemical identification of *Listeria* species was performed.

Methods for detecting and enumerating *Listeria monocytogenes* and *Listeria* spp. were conducted according to the official method EN ISO 11290-1:2017. Preparation of samples for analysis and decimal dilutions were carried out according to the EN ISO 6887:(1-5), depending on the sample type. Isolation and detection of *Listeria* spp. were carried out according to the international standard method EN ISO 11290-1:2017 - Horizontal method for detection and enumeration of *Listeria* spp. - Part 1: Detection method.

Following the principles of asepsis, approximately 50 g of sample was homogenised in a laboratory blender (Lab-Blender 3500, Seward) for 30-60 seconds. After homogenisation, 25 g was taken for primary enrichment. Primary enrichment was carried out using enriched broth with reduced selectivity

(Half-Fraser Broth, Fluka) followed by incubation at 30°C for 25±1 hours. After primary enrichment, the bacterial culture was transferred to enrichment broth with full selectivity (Fraser Broth, Fluka) and incubated at 37°C for 24±2 hours. Cultures obtained from the second enrichment were plated on Agar *Listeria* acc. to Ottaviani and Agosti (ALOA) (Sigma Aldrich, Fluka). After incubation for 24-48±2 hours at 37°C, the characteristic green-blue colonies of *Listeria monocytogenes* surrounded by an opaque halo were confirmed by specific morphological tests. Moreover, grown green-blue colonies without an opaque halo were also tested with the API test to identify *Listeria monocytogenes* and other *Listeria* species.

Confirmation of *Listeria* spp. positive isolates was conducted using the biochemical kit API® *Listeria* (BioMérieux, France). Inoculation of suspected colonies was performed by taking one or more identical colonies and inserting them into ampoules containing 0.85% NaCl solution, thus preparing a suspension with a density equivalent to 1.0 McFarland, measured with a densitometer (DENSIMAT® BioMérieux, France).

The results were interpreted in two steps: visually using a reading table, after which the results were entered into the attached result forms, and using the original software (APILAB PLUS® BioMérieux, France).

For all statistical data processing, Fisher's exact test was applied. Tests were performed using MINITAB® Release 14.11.1 software (Minitab® Statistical Software Inc., State College, PA, USA).

## Results

Of the samples analysed (238) after isolation on selective media and identification by API biochemical kit, 18 positive samples were isolated, where 13 isolates were from raw food (Category

I) and 5 isolates were from RTE food (Category II), as presented in the Table 1.

A statistically significant difference was observed in the distribution of genus *Listeria* between raw and RTE foods, while there was no observed significant difference in the distribution of any of *Listeria* species ( $P>0.05$ ).

## Discussion

In the present study, 18 *Listeria* spp. isolates were obtained from 238 tested samples. Morphological and biochemical identification discovered 6 *Listeria* species (*L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. grayii*, *L. invanovii* and *L. seeligeri*). Despite the absence of PCR identification, the determination of species within genus *Listeria* by biochemical properties utilising an API *Listeria* kit is a highly accurate method (Setiani et al., 2015).

We observed a significant difference in the prevalence of *Listeria* spp. between two categories of food: Raw foods (Category I) and RTE foods (Category II), which is similar to other studies (Ibrahim and Mac Rae, 1991; Pesavento et al., 2010), while significant differences in the distribution of *Listeria* species between two categories of foods were not observed.

*L. monocytogenes* was the most common species since it was present in 4.76% of tested samples of raw foods and 1.50% of tested samples of RTE foods. These findings are similar to those obtained in other studies where the observed prevalence of *L. monocytogenes* was highest in raw meat samples compared to RTE samples (Pesavento et al., 2010). The presence of *Listeria monocytogenes* in raw products is of lesser importance, due to the thermal processing of food, compared to its presence in RTE products. Despite the low proportion of *L. monocytogenes* isolates in RTE foods, its presence is significant since it can cause very severe symptoms in elderly, immunocompromised

**Table 1.** Prevalence of *Listeria* species in the isolated strains

Product type	No. of samples	No. of isolates	<i>L. monocytogenes</i> No.	<i>L. innocua</i> No.	<i>L. seeligeri</i> No.	<i>L. welshimeri</i> No.	<i>L. grayii</i> No.	<i>L. ivanovii</i> No.
<b>Category I: Raw foods</b>								
Chicken burger	9	2	0	2	0	0	0	0
Fresh veal meat	5	1	0	1	0	0	0	0
Hot dogs	5	0	0	0	0	0	0	0
Beef	10	2	1	0	1	0	0	0
Chicken fillet	20	2	1	0	0	1	0	0
Bosnian sausages	10	2	2	0	0	0	0	0
Bosnian ćevapčići	10	0	0	0	0	0	0	0
Patties	10	1	0	1	0	0	0	0
Burgers	5	1	0	0	0	0	1	0
Sea food	6	0	0	0	0	0	0	0
Raw milk	15	2	1	0	0	0	0	1
<b>Total:</b>	<b>105</b>	<b>13</b> (12.38%)	<b>5</b> (4.76%)	<b>4</b> (3.81%)	<b>1</b> (0.95%)	<b>1</b> (0.95%)	<b>1</b> (0.95%)	<b>1</b> (0.95%)
<b>Category II: Ready-To-Eat foods</b>								
Chicken ham	10	0	0	0	0	0	0	0
Fresh cheese	10	2	1	0	0	1	0	0
Chocolate milk	5	0	0	0	0	0	0	0
Pasteurised milk	10	0	0	0	0	0	0	0
Sterilised milk	5	0	0	0	0	0	0	0
Semi-hard cheese	14	1	0	1	0	0	0	0
Powdered milk	5	0	0	0	0	0	0	0
Butter	16	0	0	0	0	0	0	0
Butter with rapeseed oil	5	0	0	0	0	0	0	0
Sour cream	10	1	0	0	0	0	1	0
Yogurt	10	0	0	0	0	0	0	0
Bey's soup	12	0	0	0	0	0	0	0
Ice cream	10	1	1	0	0	0	0	0
Fruit yogurt	11	0	0	0	0	0	0	0
<b>Total:</b>	<b>133</b>	<b>5</b> (3.75%)	<b>2</b> (1.50%)	<b>1</b> (0.75%)	<b>0 (-)</b>	<b>1</b> (0.75%)	<b>1</b> (0.75%)	<b>0 (-)</b>
<b>Fisher exact test, p</b>		<b>0.0267</b>	<b>0.2482</b>	<b>0.1766</b>	<b>0.4435</b>	<b>0.9999</b>	<b>0.9999</b>	<b>0.4435</b>

patients, children, and pregnant women (Pesavento, 2010). Furthermore, among food-borne zoonoses, listeriosis is considered to have the highest mortality rate (Pouillot et al., 2016).

Pesavento et al. (2010) isolated *Listeria* spp. from raw meat and retail products. Six different *Listeria* species were found, similar to our research: *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii*, and *L. grayii*. Analogous to our results, their study showed a higher presence of *L. monocytogenes* in raw foods than in RTE foods. Furthermore, we observed a higher occurrence of *Listeria* spp. (13.33%) in raw milk compared to ready-to-eat dairy products (4.5%). These findings are expected since dairy products undergo obligatory pasteurisation or sterilisation before entering the market. According to the meta-analysis conducted by Liu et al. (2020), the overall *L. monocytogenes* prevalence in meat and poultry products, including raw and RTE products, was highest (8.9%) among various foods. In alignment with this, we can note an insignificant but observably higher occurrence of *L. monocytogenes* in meat and poultry products compared to milk and dairy products in the present study.

Food safety is a topic of great concern both for developed countries, and developing countries such as Bosnia and Herzegovina. Due to its wide distribution and endurance to various adverse conditions during growth and development, *L. monocytogenes* is considered one of the most important foodborne pathogens (Kiš et al., 2019). According to the ECDC (2020), 2502 confirmed cases of human listeriosis were reported in the EU/EEA countries, with an overall age-standardised notification rate was 0.42 cases per 100,000 population. A meta-analysis by De Noordhout (2014) conducted showed that *L. monocytogenes* caused 23,150 infections worldwide, with a fatality rate of 23.59%.

As stated above, a high prevalence of *L. monocytogenes* in raw foods would not pose a significant threat to consumers themselves. However, cross-contamination is a more significant risk because raw products (especially meat products) are often stored or handled in close proximity to other foods, including RTE products (Liu et al., 2020). This situation can lead to cross-contamination of *L. monocytogenes* onto other foods, thus increasing the risk to consumer health. Even if we assume that all entities in the food business operate in accordance with HACCP principles, this does not exclude the possibility that cross-contamination will occur in consumer homes. In order to find the source and route of *Listeria* species contamination, the next step should be PCR identification and DNA fingerprinting performing pulsed-field gel electrophoresis (PFGE) on isolated strains.

## Conclusions

Listeriosis is a zoonosis that causes very severe symptoms and the presence of *Listeria* strains in food poses a considerable safety concern. Even though its occurrence in RTE food was lower than in raw food, there is a higher potential consumer risk associated with RTE food. These results confirm the necessity of conducting good manufacturing and hygiene practices in order to secure the microbiological safety of RTE foods. The presence of *L. innocua*, *L. welshimeri*, *L. grayii* and *L. seeligeri* in food is probably the result of contamination during processing, since these microorganisms are commonly found in soil, water, waste, and food processing facilities, and are rarely capable of infecting animals. The origin of *L. monocytogenes*, and other *Listeria* species in food could be better explained by DNA fingerprinting utilising PFGE, but that method is outside the scope of this paper.

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## Listeria vrste u sirovoj hrani i hrani spremnoj za konzumiranje na tržištu Bosne i Hercegovine

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*Listeria* je patogen prenosiv hranom i predstavlja veliku opasnost za ljudsko zdravlje. Teški su simptomi praćeni visokom stopom mortaliteta većinom prouzročeni *L. monocytogenes*. Bez obzira na politiku nulte tolerancije, rod *Listeria* je još uvijek prisutan u sirovim proizvodima i proizvodima spremnim za konzumiranje, predstavljajući veliku opasnost za zdravlje ljudi. Za sada još u bliskoj budućnosti ne postoje studije o kontaminaciji hrane vrstama *Listeria* u Bosni i Hercegovini. Ovim istraživanjem htjeli smo dobiti osnovne

podatke o količini kontaminiranosti hrane ovim patogenom. U studiji je analizirano ukupno 238 uzoraka hrane, pri čemu su u 105 uzorka bile različite vrste sirove hrane te 133 uzorka hrane spremne za konzumaciju (gotove hrane). Od ukupno 238 uzoraka, 18 uzoraka je bilo pozitivno na *Listeria* vrste. Nakon izolacije i detekcije, potvrđivanja pozitivnih izolata *Listeria* spp. je provedena pomoću biokemijskog kita API® *Listeria*.

**Gljučne riječi:** *Listeria*, *Listeria monocytogenes*, sirova hrana, gotova hrana