SHORT COMMUNICATION

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Prevalence of *Listeria monocytogenes* in sprouts and vegetable mixtures and salads sold in retail shops in Lublin, eastern Poland – preliminary study

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Abstract

Introduction. In 2022–2023, examinations were carried out for the presence of a pathogenic bacterium *Listeria monocytogenes* in ready-to-eat (RTE) vegetable products (sprouts and vegetable mixtures and salads) sold for immediate consumption in retail shops located in Lublin, eastern Poland. The identification of *Listeria* strains were performed according to the Polish Standard and accomplished with the Microgen Listeria-ID System.

Results. A high prevalence of *L. monocytogenes* infections was found in the unprocessed sprouts of plants belonging to the cabbage (Brassicaceae) family – kale (30.8%), broccoli (18.8%) and radish (7.1%). The mean prevalence of infections in this family (18.6%) was significantly higher compared to the sprouts of plants belonging to other families, none of which were infected ($P<0.05$). In the minimally processed RTE mixtures and salads, *L. monocytogenes* was detected in 6.1% of samples. The presence of following non-pathogenic species of the *Listeria* genus was also found In the examined RTE products: *L. innocua*, *L. grayi*, and *L. seeligeri,* which occurred both in sprouts and vegetable mixtures and salads in the prevalence of 13.8% and 10.2%, respectively.

Conclusions. The obtained results demonstrate a high prevalence of *L. monocytogenes* in sprouts, especially those of the cabbage family, which was markedly greater compared to vegetable mixtures. The results suggest a need for continuation of research on the occurrence of *L. monocytogenes* in the sprouts of plants belonging to the cabbage family. If the results of this study are subsequently confirmed, the application of suitable prevention measures are to be highly recommended.

Key words

Listeria monocytogenes, sprouts, cabbage family, minimally processed RTE food, retail shops, Poland

INTRODUCTION

Listeria monocytogenes is a facultatively anaerobic Gram-positive, non-sporing rod-shaped bacterium that causes listeriosis in man and animals. It is the only pathogenic species within *Listeria* genus comprising, according to Townsend et al., 17 species [1, 2]. In man, the disease occurs most frequently as meningoencephalitis, sepsis and uterus infection causing miscarriage. The populations at risk are pregnant women, infants, the elderly and immunocompromised individuals [1, 3]. The mortality rate is high, up to 20–30% [4].

The infection of humans occurs almost exclusively through the alimentary route which accounts for 99% of listeriosis cases in the USA [3], where the number of the listeriosis cases per year is estimated as 1,600, of which 260 people die [5]. In Poland in 2022, there were only 142 cases of this disease [6].

The risk of disease is increased by some traits of the bacteria which enable them to persistence in the natural environment, and ease of propagation in the organisms of humans and animals. They include the ability to propagate and survive at low temperatures (0.5–9.3°C), formation of biofilms, formation of persister cells highly resistant to environmental stressors, ability to intracellular propagation and tolerance to disinfectants [3].

In the external environment, *L. monocytogenes* occurs in soil, water, sewage as well as in plants and plant products [4, 7]. The bacterium is often isolated from raw vegetables, the consumption of which has been often identified as a cause of numerous epidemics [1, 7]. Since several decades, epidemiologists have been interested in plant sprouts as a potential source of *Listeria,* sold as ready-to-eat (RTE) products which comprise an important element of the vegetarian diet. As the cultivation of edible sprouts requires a high humidity and temperature, together with the presence of various nutrients, such an environment creates a risk of development for various pathogens, including *Listeria* [8]. The other known cause of alimentary infections with *Listeria monocytogenes* bacteria is consumption of minimally processed vegetable mixtures and/or salads, classified among RTE foods [1, 9–14]. In Poland, legal Act PN-EN ISO 11290–1:2017–07 recommends the methods for the detection and enumeration of Listeria in foods which were followed in the current study [15].

In USA in 1997, in anticipation of the possible risk of gastrointestinal diseases that could be induced by the consumption of infected sprouts, the National Advisory Committee on Microbiological Criteria for Foods [16]

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specified the appropriate methods of cleaning, storing and disinfection of seeds intended for the production of edible sprouts, to protect consumers against alimentary infections, including listeriosis. Nevertheless, since then, at least two epidemics of listeriosis caused by consumption of sprouts have been described in USA (2008 and 2014, the latter caused by infected mung bean), together with five hospitalizations and two deaths [1]. *Listeria* strains were isolated many times from sprouts [8, 17, 18] which stimulated a search for better prevention measures.

OBJECTIVE

The aim of the present study was to detect *Listeria monocytogenes* and other species of this genus in sprouts of five vegetable species and 10 kinds of vegetable mixtures and salads sold in retail shops in Lublin, eastern Poland.

MATERIALS AND METHODS

Samples. In 2022–2023, 65 unprocessed samples of vegetable sprouts (kale, broccoli, radish, mung bean and leek) and 52 samples of minimally processed vegetable mixtures and salads were obtained from retail shops in the city of Lublin in eastern Poland. Sprouts were acquired immediately from substrate, not cut and packed in plastic boxes. Minimally processed vegetables comprised mixtures of various cut plants (lettuce, arugula, cabbage, beet, kale, spinach) or salads made from leafy, not cut vegetables (spinach, kale, lamb's lettuce, arugula). All sprouts and minimally processed vegetable mixtures and salads were purchased in original packages with the permissible date of consumption displayed. The sampled sprouts and vegetable mixtures were stored in shops at the temperature 2–8°C and analyzed immediately after purchase. The samples of the products were bought randomly in various retail shops and were produced by various manufacturers.

Detection of *Listeria* spp. and *Listeria monocytogenes* by culture and metabolic tests. The identification tests by culture were performed according to the Polish Norm PN-EN ISO 11290–1:2017–07 [15].

To 25 grams of an examined sample (from sprouts and vegetable mixtures or salads), 225 ml of Half Fraser broth for introductory multiplication were added. Obtained suspension was homogenized for 5 min using the Bag mixer 400 SW (Interscience, France) and then incubated at 30°C for 24 hrs. Afterwards, 0.1 ml of culture was transferred to 10 ml of Fraser broth and again incubated at 37°C for 48 hrs. The broth culture was inoculated into a selective solid media (Oxford Agar and ALOA) enabling isolation and identification of *Listeria* spp. and incubated at 37°C for 48 hrs. The grown colonies were identified with Gram staining and Microgen Listeria-ID System, enabling the identification of *Listeria monocytogenes* and five other *Listeria* species.

The above bacteria can be identified from selective or non-selective agar by using Microgen Listeria-ID, a system consisting of a single strip containing 12 wells. Of which, 11 wells are filled with substrates for biochemical reactions comprising esculin hydrolysis and fermentation of 10 sugars (mannitol, xylose, arabitol, ribose, rhamnose, trehalose, tagatose, glucose-I-phosphate, methyl D-glucose, methyl-D-mannose). Well number 12 is used for detection of haemolysis reaction by adding a haemolysin reagent to the bacterial suspension. Identification of isolates is achieved by recording the results visualised by a colour change after 18–24 hours incubation. The results are analysed using the Microgen Identification System Software (MID-60).

Statistical analysis. The differences between groups were analyzed with Mann-Whitney non- parametric test, using STATISTICA v. 6.0 package (Statsoft, Tulsa, Oklahoma, USA). A value of $P \leq 0.05$ was assumed to be significant.

RESULTS

Obtained results of sprouts examination shows that the presence of total *Listeria* strains was detected only in the sprouts of plants belonging to cabbage family (Brassicaceae), namely in kale (*Brassica oleracea* var. *sabellica*) and broccoli (*Brassica oleracea* var. *italica*), which are cultivars of the cabbage (*Brassica oleracea*), and in radish (*Raphanus sativus*) (Tab. 1). The percentage of sprouts infected with all identified species of *Listeria* was 39.5% and proved to be significantly higher ($P<0.01$) compared to parallelly examined sprouts from other families which were not infected with *Listeria* strains: mung bean (*Vigna radiata*) from pea family (*Fabaceae*), leek (*Allium ampeloprasum*) from amaryllis family (*Amaryllidaceae*) and mixed sprouts from various families.

Table 1. Prevalence of *Listeria* in various sprouts

Sprout Bacterial species	Kale (*Brassica oleracea* var. *sabellica*)	Broccoli (*Brassica oleracea* var. *italica*)	Radish (*Raphanus sativus*)	Total *Brassicaceae*	Mung bean (*Vigna radiata*)	Leek (*Allium ampeloprasum*)	Mixed sprouts	Total Non-*Brassicaceae*	*Brassicaceae* versus Non-*Brassicaceae*	Total sprouts
Listeria monocytogenes	4/13 (30.8%)*	3/16 (18.8%)	1/14 (7.1%)	8/43 (18.6%)	0/11 (0)	0/6 (0)	0/5 (0)	0/22 (0)	P = 0.045 Diff. significant	8/65 (12.3%)
Listeria innocua	2/13 (15.4%)	4/16 (25.0%)	3/14 (21.4%)	9/43 (20.9%)	0/11 (0)	0/6 (0)	0/5 (0)	0/22 (0)	P = 0.033 Diff. significant	9/65 (13.8%)
Listeria grayi	0/13 (0)	0/16 (0)	0/14 (0)	0/43 (0)	0/11 (0)	0/6 (0)	0/5 (0)	0/22 (0)	P = 1.000 Diff. not significant	0/65 (0)
Total *Listeria* spp.	6/13 (46.2%)	7/16 (43.8%)	4/14 (28.6%)	17/43 (39.5%)	0/11 (0)	0/6 (0)	0/5 (0)	0/22 (0)	P = 0.0016 Diff. highly significant	17/65 (26.1%)

*positive/examined (percent); Diff. – difference; $P \leq 0.05$ – difference assumed as significant; $P \leq 0.01$ – difference assumed as highly significant.

Table 2. Prevalence of various *Listeria* species in vegetable mixtures

Vegetable Species	Spinach (*Spinacia oleracea*)	Kale (*Brassica oleracea* var. *sabellica*)	Mixture with iceberg lettuce** (*Lactuca sativa* var. *capitata*)	Mixture with romaine lettuce** (*Lactuca sativa* var. *longifolia*)	Mixture with red cabbage** (*Brassica oleracea* var. *capitata*)	Mixture with corn salad** (*Valerianella locusta*)	Mixture with arugula** (*Eruca vesicaria*)	Mixture with beet greens** (*Beta vulgaris*)	Mixture of different vegetables#	Mixture of young leaves of spinach and beet greens**	Total mixtures
L. monocytogenes	2/3* (66.7%)	0/2 (0)	0/3 (0)	0/3 (0)	0/2 (0)	0/8 (0)	0/9 (0)	0/4 (0)	0/11 (0)	1/4 (25.0%)	3/49 (6.1%)
L. innocua	0/3 (0)	0/2 (0)	0/3 (0)	0/3 (0)	0/2 (0)	0/8 (0)	0/9 (0)	0/4 (0)	0/11 (0)	0/4 (0)	0/49 (0)
L. ivanovii	0/3 (0)	0/2 (0)	0/3 (0)	0/3 (0)	0/2 (0)	0/8 (0)	0/9 (0)	0/4 (0)	0/11 (0)	0/4 (0)	0/49 (0)
L. seeligeri	1/3 (33.3%)	1/2 (50.0%)	0/3 (0)	0/3 (0)	0/2 (0)	0/8 (0)	0/9 (0)	0/4 (0)	0/11 (0)	0/4 (0)	2/49 (4.1%)
L. grayi	0/3 (0)	0/2 (0)	2/6 (33,3%)	0/3 (0)	1/2 (50.0%)	0/8 (0)	0/9 (0)	0/4 (0)	0/11 (0)	0/4 (0)	3/49 (6.1%)
Total *Listeria* spp.	3/3 (100%)	1/2 (50.0%)	2/6 (33.3%)	0/3 (0)	1/2 (50.0%)	0/8 (0)	0/9 (0)	0/4 (0)	0/11 (0)	1/4 (25.0%)	8/49 (16.3%)

* positive/examined (percent); ** main component(s) of mixture containing also other vegetable species; # no dominant component occurred; $ percent of *Listeria* strains isolations

From medical point of view, a high prevalence of infection of plants from the cabbage family with the pathogenic species *Listeria monocytogenes* (18.6%) was noteworthy, which was also significantly higher compared to cultivated plants from other families (P<0.05). The prevalence was highest in kale (30.8%), lower in broccoli (18.8%), and the lowest in radish (7.1%) (Tab. 1). The plants from the cabbage family were also infected in a high percentage with the saprophytic *Listeria innocua* species (20.9%), which was also significantly higher (P<0.05) compared to plants from other families. No infection was found with *Listeria grayi*, the other saprophytic species.

Results of examination of the minimally processed RTE vegetable mixtures and salads for the presence of *Listeria* strains are presented in Table 2. In the examined samples, *L. monocytogenes* and saprophytic species *L. seeligeri* and *L. grayi* were found, but no *L. innocua* and *L. ivanovii* were detected. In total, a 6.1% prevalence of the infection with pathogenic *L. monocytogenes* in vegetable mixtures and salad samples was insignificantly smaller (P>0.05), compared to the analogous infection of sprouts (12.3%). Similarly, the prevalence of the infection of vegetable mixtures and salad samples with saprophytic *Listeria* strains amounted to 10.2%, which was also insignificantly smaller (P>0.05) compared to the analogous infection of sprouts (13.8%).

DISCUSSION

The obtained results indicate that the sprouts of cultivable plants belonging to the cabbage family (Brassicaceae) may be infected in large proportion with the pathogenic bacterium *Listeria monocytogenes,* as well as with other, non-pathogenic species belonging to the *Listeria* genus. Although the reason for this common infection is not fully known, it might possibly be due to the high nutritional value of the taproots of cabbage family plants, and/or by the properties of seeds containing a high-calorie mustard oil (used for treating bronchitis and fever [19]. It was proved experimentally that Listeria monocytogenes can easily colonize the sprouts of broccoli, radish and alfalfa [20, 21].

In previous research in the USA, the presence of *Listeria monocytogenes* was detected in the sprouts of following plants: mung bean, soya, alfalfa, and broccoli [8, 17, 18]. In Korea, Kim et al. [22] stated the presence of this pathogen in one of 20 sprouting seeds of alfalfa and its absence in rapeseed. In the range of other studies performed in Canada, USA, India and Latvia, *L. monocytogenes* was not detected in the sprouts of various vegetables [23–26]. Among sprouts (in which the presence of *L. monocytogenes* was stated in the current study), the pathogen was not detected by Thunberg et al. [9] on the territory of USA in the sprouts of broccoli, alfalfa, mung bean, and soya, but was detected by Zhang et al. [18], also in USA, in one broccoli sprout and 2 alfalfa sprouts, which together formed only 0.11% of a large sample of 2,652 sprouts belonging to different species of cultivated plants. To the best of the knowledge of the authors of the current study, *L. monocytogenes* had not been detected until recently in the sprouts of kale and radish; therefore the present work reports for the first time the presence of this pathogen in sprouts of these 2 vegetable species.

The results of the current study conform with earlier studies by Kordowska-Wiater et al. [10], who also found on the territory of Lublin province, the presence of *L. monocytogenes* in ripe, frozen kale and broccoli vegetables, in both cases at the level of 1.3%. The relatively common detection of this pathogen in sprouts of alfalfa (*Medicago sativa*) reported worldwide, could be explained by the common cultivation of these sprouts as a forage crop of high nutritional value. Nevertheless, the prevalence of alfalfa infection never exceeded 5%, and was often many times lower compared to the prevalence of kale and broccoli infection with *L. monocytogenes* stated by the authors of the current study.

The mean prevalence of non-pathogenic species of *Listeria* in the examined sprouts (13.8%) in the current study appeared to be greater compared to analogous values reported from Canada [23], USA [17] and Latvia [26], which were within 0–4.4% limits, but lower compared to the prevalence reported by Saroj et al. [25] from India – 30.6%.

In the presented study, the presence of *L. monocytogenes* was found in 6.1% of the samples from vegetable mixtures and salads sold by retail shops in the city of Lublin. Thus, in this area, the epidemiologic situation seems to be similar compared to the results of earlier authors who found the presence of this pathogen in the samples of vegetable salads

sold in Malaysia [11], Brazil [12], Türkiye [13], England [27, 23] and northwestern Poland [14], within the range 1.2–22.5%. The lower result was obtained by Seo et al. [29], who did not detect the presence of *L. monocytogenes* in vegetable salads sold in Seoul, Korea.

The mean prevalence of non-pathogenic *Listeria* species in the examined samples of vegetable mixtures and salads (10.2%) in the current study was higher compared to analogous values observed in England [27, 28] and Brazil [12], which ranged from 2.4–4.3%, but lower compared to those obtained by Szymczak et al [14] in northwestern Poland and by Ponniah et al. [11] in Malaysia – 17.7% and 33.3%, respectively.

CONCLUSIONS

In the examinations of ready-to-eat (RTE) vegetables sold in retail shops in the city of Lublin, eastern Poland, a high prevalence of infections with pathogenic bacterium *Listeria monocytogenes* was found in the sprouts of plants belonging to cabbage family (Brassicaceae). This was significantly higher compared to the sprouts of other plant families in which no infections were found. The prevalence of *L. monocytogenes* in vegetable mixtures and salads was about twice as low as in sprouts. The obtained results indicate the need for a continuation of studies on the prevalence of *L. monocytogenes* in the sprouts of the cabbage family, also by using of molecular methods, and undertaking adequate prevention measures in the case of confirmation of positive results, as obtained in this study.

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