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PRESENCE OF *LISTERIA* SPECIES IN FRESH MEATS FROM RETAIL MARKETS IN SERBIA

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Listeria spp. are Gram positive, short, non-sporing rods, microaerophilic. Of the six species currently recognized, Listeria monocytogenes is the most important as it causes a range of infections in humans and animals. The organism can be found in a wide variety of habitats including the soil, food processing environments and raw foods. The ability of the organism to grow at refrigeration temperatures is of major importance in food production. This study examines the presence of Listeria species in fresh meat. 29 samples (chicken, pork and beef) meat. This bacteria was found in 82.7% of analyzed samples; 7 L. innocua, 8 L. monocytogenes and 9 L. welshimeri (of all isolates). L. innocua prevailed in pork meat (40%), L. monocytogenes in chicken and pork meat (30%), and L. welshimeri in beef meat (44.4%).

KEYWORDS: fresh meat, *Listeria* spp., *L. monocytogenes*

INTRODUCTION

Food producers are bound to deliver healthy, wholesome and safe products to the market. Meat and meat products are classified as epidemiologically hazardous food which may be contaminated with bacteria from the *Listeria* genus. These microaerophilic Gram-positive rod-shaped bacteria can contaminate fresh meat during processing from many sources: air, contaminated water and/or during the distribution. Studies have confirmed that feces of healthy humans contain *Listeria* organisms (1, 2, 3). Lymph glands have been also shown to be sources of contamination (4). Some of the species such as *L. monocytogenes* and *L. ivanovii* have been addressed as causative agents of severe food infections. Listeriosis has been listed as a rare disease in relation to the other food-borne infections but with exceptionally high fatality rate (20-30%) (5). The manifestations of listeriosis include septicemia, meningitis (or meningoencephalitis), encephalitis, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion or stillbirth. At present the infective dose of *L. monocytogenes* is unknown,

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although it is believed to vary with the strain and susceptibility of the patient (6). Sometimes in susceptible persons, fewer than 10^3 cfu/g or ml may cause disease (7). Listeriosis is a serious disease, primarily transmitted through various foods: milk, milk products, meat and meat products, fish, eggs and egg products, fruits and vegetables. It is particularly difficult to control, since it is ubiquitous and widespread in the environment, and since it possesses physiological characteristics that allow growth under conditions that are usually adverse for most other pathogenic bacteria. The consumption of contaminated sausages lead to an outbreak in the USA during 1998, involving 110 cases with 4 deaths (8). In another case in France, between 1999 and 2000, as a result of consumption of pork, 7 deaths had been seen out of 26 listeriosis cases (8). Especially hazardous are undercooked meat products. *L. monocytogenes* is resistant to high salt concentrations and low temperatures, which enables its survival in sausages and dried meat products.

Many studies are focused on the detection of *Listeria* spp. in various food commodities in order to prevent epidemic of human listeriosis. Much attention has been paid on the incidence of pathogenic *Listeria* spp. strains. Taking into consideration that the existing data on the incidence of *Listeria* food contamination in Serbia are rather scarce, the aim of this study was to investigate the occurrence of *Listeria* spp. in various types of fresh meat from local retail markets.

EXPERIMENTAL

Material

For the experiment, 29 samples of fresh chicken, pork and beef meat were purchased from retail markets in Novi Sad (Serbia). Growth media used in the experimental work were obtained from HiMedia (Mumbai, India).

Isolation of *Listeria* species

For the isolation of *Listeria* species, a two-stage enrichment procedure in Fraser broth was used (9). After sterilization, selective supplements (ferric ammonium citrate, acriflavin hydrochlorid, and nalidixic acid) were added to the broth. Meat samples were incubated in Erlenmeyer flasks with Fraser broth at 30°C for 24 h. After that, 0.1 mL of broth were transferred into 10 mL tubes of Fraser broth for secondary enrichment and incubated at 37°C for the next 48 h. The content was transferred with inoculation loop to PALCAM and Oxford agar plates. The plates were incubated at 37°C for 48 h. The suspect *Listeria* colonies were further confirmated on the basis of Gram stain, catalase, oxidase, and motility tests.

Identification of the isolated species

The VITEK® 2 system (bioMerieux, France) and VITEK® 2 Gram-Positive (GP) identification card were used for identification of *Listeria* spp. The GP identification card is based on established biochemical methods and newly developed substrates. There are

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43 biochemical tests measuring carbon source utilization, enzymatic activities and resistance. Final identification results are available in approximately eight hours or less.

RESULTS AND DISCUSSION

Listeria spp. were identified in 82.7% of the investigated meat samples. Their presence was significant in all types of meat investigated. The obtained data showed that pork meat was the most contaminated (90%) (Table 1).

Table 1. Prevalence of the *Listeria* spp. in different types of meats

Meat type	Number of contaminated samples	*Listeria* spp. Prevalence (%)
Chicken	8	80
Pork	9	90
Beef	7	77.8

From the samples of chicken, pork and beef the following species were isolated: *L. innocua* (7 isolates), *L. monocytogenes* (8 isolates) and *L. welshimeri* (9 isolates). *L. innocua* was the most frequent in pork meat (40%). *L. monocytogenes* was more prevalent in chicken and pork (30%) than in beef meat (22.2%), whereas *L. welshimeri* dominated in beef meat (44.4%) (Fig. 1).

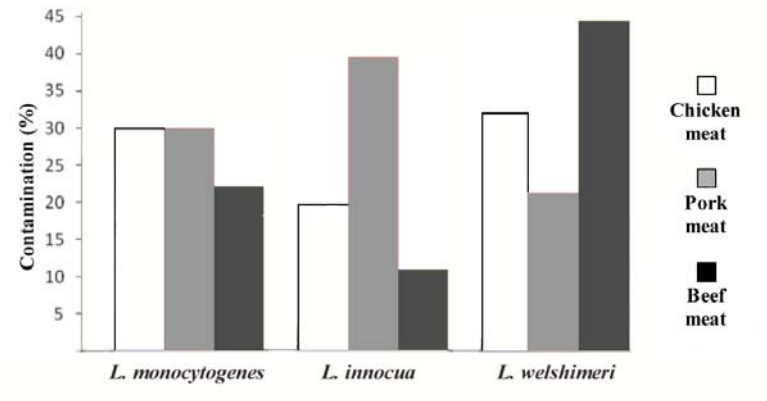

Figure 1. Distribution of *L. innocua, L. monocytogenes* and *L. welshimeri* in the analyzed meat types

In general, *L. welshimeri* was the species with the highest incidence (isolated in 31% of samples) followed by *L. monocytogenes* and *L. innocua* with slightly lower but still significant incidence (27.6% and 24.1%) (Fig. 2).

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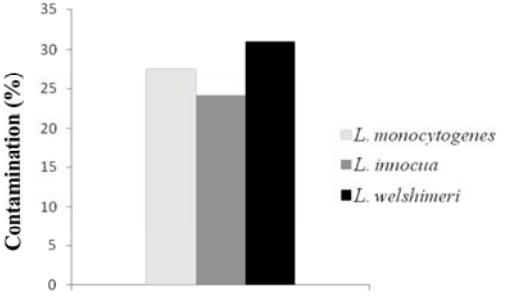

Figure 2. Isolated *Listeria* species in the fresh meat

It is evident from the present study that the prevalence of *Listeria* was significant in the fresh meat samples. It should be noted that pathogenic *L. monocytogenes* was present in all meat types. In contrast to other pathogenic microorganisms, this bacterium has ability to grow and reproduce in a range of temperature, from - 1.5 to 45°C (10), which is a serious problem for food manufacturers. According to the general opinion, meat contamination with bacteria occurs during the skin removal (11).

Listeria species were detected in swabs taken from skins of beef ribs and rounds as well as in those taken after the skin removal (2). Rayser et al. (12) reported *Listeria* contamination in 89% of minced beef meat, over 70% of minced poultry and turkey meat and 96% of pork sausages. Besides *L. monocytogenes, L. innocua* and *L. welshimeri* were also identified. Skovgaard et al. (13) showed that the occurence of *L. innocua* was more frequent than *L. monocytogenes* in beef and poultry meat. Also, *L. innocua* was the dominating species in poultry meat and poultry products in the study reported by Kosek-Paszkowska et al. (14).

L. monocytogenes was isolated in 16% of fresh sausages, 15.8% mechanically cut poultry meat, 10.7% skinless sausage, 6.8% minced meet, and 4.3% fermented durable sausages (15). Over the period 2000-2003, under the scrutiny of the USDA and FDA, 713 samples of various food commodities were recalled from retail markets (16). The recalled products included fresh pork, poultry and beef meat, dairy products, seafood, ice creams, fresh, and processed fruits and vegetables. Meat accounted for about one half of the recalled products. Microbial contaminants were the reason for 43.9% recall events. *L. monocytogenes*, *E. coli,* and *Salmonella enteritidis* were the main pathogens in the suspected foods. *L. monocytogenes* accounted for 186 product recalls which accounted for 26% of the total recall events. *L. monocytogenes* was present in 19.3% of tested chicken meat and 7.1% of beef meat (17). Especially concerning fact is that it is capable of not only surviving in vacuum packed meat products (18) but of reproducing under these conditions (19).

CONCLUSION

This study has confirmed a high prevalence of *Listeria* in meat, which implies that chances of contracting listeriosis are greatly increased when consuming inadequately

cooked meat and meat products. Control for the *Listeria* presence in every stage of food processing and distribution, including retail market, raising the hygienic and sanitary standards are key prerogatives in the prevention of listeriosis outbreaks.

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ПРИСУСТВО *LISTERIA* ВРСТА У СВЕЖЕМ МЕСУ ИЗ МАЛОПРОДАЈНИХ ОБЈЕКАТА У СРБИЈИ

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Listeria врсте су Грам позитивне, аспорогене, микроаерофилне бактерије које су способне да расту у опсегу температура од 4-37°C. Налазе се у земљишту, сировој храни и производном окружењу. Најзначајнија врста као узрочник инфекција људи и животиња (листериоза) је *Listeria monocytogenes*. За безбедност намирнице је важна чињеница да је ова бактерија способна да се размножава на температури хлађења. Овај рад показује присуство *Listeria* врста у свежем месу. Укупно је испитано 29 узорака пилећег, свињског и јунећег меса. Ове бактерије су установљене у 82,7% узорака; 7 *L. innocua*, 8 *L. monocytogenes* и 9 *L. welshimeri* (од свих изолата). *L. innocua* је била доминантна у свињском месу (40%), *L. monocytogenes* у пилећем и свињском месу (30%) и *L. welshimeri* у јунећем месу (44,4%).

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