Bulletin UASVM, Veterinary Medicine 68(2)/2011
pISSN 1843-5270; eISSN 1843-5378

Incidence of *Listeria species* from Food Products

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Abstract. By its implication in the public health, the food contamination with *L. monocytogenes* raises an important economic problem concerning the food industry. The presence of *Listeria* spp. proved to be a useful indicator during all the stages of the food processing chain.

A total number of 30 strains of *Listeria* spp. were investigated for identification/ confirmation. These strains were isolated from animal meat (raw minced meat, muscular tissues, sausages and other pork and beef preparations), poultry and diary products.

The bacterial identification included morphological (Gram staining, microscopic examination, motility, oblique illumination of colonies on blood-free agar) and biochemical (catalase, beta-hemolysis on 5% sheep blood agar, CAMP test, trehalose, mannitol, manose, rhamnose, xilose reactions) methods. *L. monocytogenes* strains were serotyped for serovar identification.

The investigations finally revealed: 8 strains (26.66%) *L. monocytogenes* (out of which 7 strains *L. monocytogenes* serotype 1a, and 1 strain *L. monocytogenes* serotype 4b), 19 strains (63.33%) *L. innocua*, 1 strain (3.33%) *L. grayi*, and 2 strains (6.66%) *L. welshimeri.*

Keywords: epidemiology, *Listeria monocytogenes*, zoonosis, listeriosis, raw food products

INTRODUCTION

Epidemiological studies have demonstrated that *Listeria monocytogenes*, an opportunistic pathogen for humans and animals, is transmitted by food.

Although diary products have been described as major source outbreaks of listeriosis, other raw or recontaminated products of animal or vegetable origin may serve as vehicles of transmission of this pathogen. *Listeria* spp. has been isolated from poultry, red meat and meat products, although these foods have not been associated with outbreaks of human listeriosis. *Listeria* spp. are capable of growing on both raw and coked meat at refrigeration temperatures. During the transformation processes from raw meat into meat products *Listeria monocytogenes* can be introduced, and the amount depends on the extent of contamination, personal and general hygienic measures, as well as the process parameters.

Different commercial food products (raw milk and dairy, vegetables, raw meat, poultry and fish), as well as fast food preparations are frequently contaminated by *Listeria* germs and prove to be the source of *Listeria* infection manifested by different clinical aspects

of listeriosis (septicemia, meningitis, encephalitis, abortive disease), as result of the digestive transmission of these germs to humans.

MATERIALS AND METHODS

A total number of 30 strains of *Listeria* spp. were investigated for identification/ confirmation. These strains were isolated from animal meat: 16 strains (53.33%) in raw meat - raw minced meat, muscular tissues, poultry; 10 strains (33.33%) in meat products - sausages and other pork and beef preparations, and 4 strains (13.33%) in diary products (Figure 1).

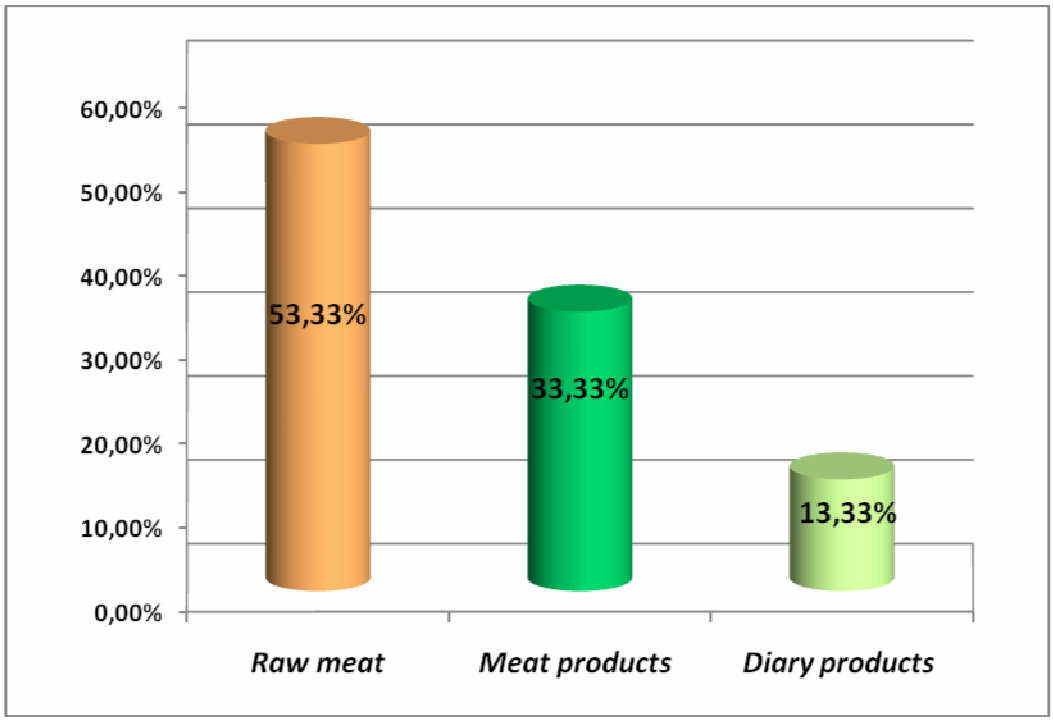

Fig. 1. The percentage of investigated strains.

The international recommended tests [5,6,7,8] were performed.

The strains were cultivated on 5% blood agar; after incubation 18 hrs at 35-37°C, the morphological aspects of the isolated colonies were examined, including beta haemolytic activity.

For the bacterial identification there were included morphological methods (Gram staining, microscopic examination, motility) and biochemical methods (catalase, CAMP test, and carbohydrates use reactions).

The Gram stained smears were microscopically examined by 90 HI.

Typical colonies were tested for oxidase and catalase.

The mobility test was performed by inoculating a loop of culture in a tube with 3‰ agar, after 18 hrs incubation at 35-37°C, the mobility of the culture being examined.

The carbohydrates use was tested by inoculatind media containing simple broth with 1% glucose, trehalose, D-mannitol, D-manose, L-rhamnose, D-xilose, and brohm-thymol blue as pH indicator; after incubation 18 hrs at 35-37°C, the fermentation reactions were read [11, 12].

The CAMP test was performed by streaking a beta-haemolytic *Staphylococcus aureus*

strain ATCC 25923 and *Rhodococcus equi* strain ATCC 6939, in single straight lines in parallel, on a sheep blood agar plate. The tested *Listeria* strains were streaked perpendiculary, in between the two indicator organisms, without touching them (separated by 1-2 mm). After incubation 18 hrs at 35-37°C, a positive reaction was considered of an enhanced zone of beta-haemolysis at the intersection of the test and indicator strains.

L. monocytogenes strains were serotyped for serovar identification with hiperimmune rabbit adsorbed sera *Listeria monocytogenes* serotype 1a and *Listeria monocytogenes* serotype 4b [2, 3].

RESULTS AND DISCUSSIONS

All of the 30 examined strains were positive for *Listeria* spp. (Tabel 1). The investigations finally revealed:

- 8 strains (26.66%) *L. monocytogenes* (out of which 7 strains *L. monocytogenes* serotype 1a, and 1 strain *L. monocytogenes* serotype 4b);
- 19 strains (63.33%) *L. innocua*;
- 1 strains (3.33%) *L. grayi*;
- 2 strains (6.66%) *L. welshimeri*.

Tab. 1.

Identification of *Listeria* spp. strains (No. %)

Tests \ Species		*L .monocytogenes* serotype 1a		*L .monocytogenes* serotype 4b		*L. innocua*		*L. grayi*		*L.welshimeri*	
		No.	%	No.	%	No.	%	No.	%	No.	%
Beta-haemolysis		7	23.33	-	-	-	-	-	-	-	-
CAMP Test	*Staphylococcus aureus*	7	23.33	1	3.33	-	-	-	-	-	-
	Rhodococcus equi	-	-	-	-	-	-	-	-	-	-
Mobility		7	23.33	1	3.33	-	-	-	-	-	-
Oxidase Test		-	-	-	-	-	-	-	-	-	-
Catalase Test		7	23.33	1	3.33	19	63.33	1	3.33	2	6.66
Acid from:	Glucose	7	23.33	1	3.33	19	63.33	1	3.33	2	6.66
	Trehalose	2	6.66	1	3.33	2	6.66	-	-	-	-
	D-Mannitol	-	-	-	-	-	-	1	3.33	-	-
	D-Manose	7	23.33	1	3.33	19	63.33	-	-	2	6.66
	L-Rhamnose	7	23.33	1	3.33	1	3.33	-	-	1	3.33
	D-Xilose	-	-	-	-	2	6.66	-	-	-	-
Serological identification	*L.monocytogenes* 1a	7	23.33	-	-	-	-	-	-	-	-
	L.monocytogenes 4b	-	-	1	3.33	-	-	-	-	-	-

Raw minced meat (Tabel 2) had the highest incidence of *Listeria* spp., with 9 (30%) positive strains. The high incidence of *Listeria* spp. in raw meats can be attributed to fecal contamination during evisceration, or to food handling.

In muscular tissue there were revealed 6 strains (20%) of *Listeria* spp: 4 strains *L. innocua*, 1 strain *L. monocytogenes* and 1 strain *L. grayi*.

In chicken meat was found 1 strain (3.33%) *L. monocytogenes*.

The incidence of *Listeria* spp. in meat products was found: 6 strains (20%) in fresh sausages (3 strains *L. monocytogenes* and 3 strains *L. innocua*).

L. monocytogenes strains were serotyped and they revealed:

- 7 strains *L. monocytogenes* 1a (serotype circulating in Romania);

- 1 strain *L. monocytogenes* 4b (serotype isolated from poultry carcass).

L. monocytogenes 1a, the serotype circulating in Romania, was identified both in raw meat and meat products. *L. monocytogenes* 4b, the serotype wide spread in West-Europe, was detected in poultry carcass, imported from Italy.

L. innocua was identified in 19 strains (63.33%) among *Listeria* species. There were detected predominantly: 8 (26.66%) strains in raw minced meat, 3 (10%) strains in fresh sausages, and 3 (10%) in pork muscular tissue. *L. innocua* was the most common specie of the genus *Listeria* detected in raw meat, while other *Listeria* species were less frequently [4, 9, 10].

L. welshimeri was revealed in 2 strains (6.66%) from powder milk.

L. grayi, 1 strain (3.33%), was detected in beef muscular tissue.

Tab. 2.

Incidence of *Listeria* species from food products (No. %)

Species / Products		*L. monocytogenes* serotype 1a		*L. monocytogenes* serotype 4b		*L. innocua*		*L. grayi*		*L.welshimeri*	
		No.	%	No.	%	No.	%	No.	%	No.	%
Raw minced meat (pork,beef)		1	3.33	-	-	8	26.66	-	-	-	-
Muscular tissue of	horse	-	-	-	-	1	3.33	-	-	-	-
	pork	-	-	-	-	3	10.0	-	-	-	-
	beef	1	3.33	-	-	-	-	1	3.33	-	-
Poultry carcass		-	-	1	3.33	-	-	-	-	-	-
Paste of Romanian sausages		1	3.33	-	-	-	-	-	-	-	-
Fresh sausages		3	10.0	-	-	3	10.0	-	-	-	-
Bacon in processing		-	-	-	-	1	3.33	-	-	-	-
Smocked chop		1	3.33	-	-	-	-	-	-	-	-
Pork shoulder blade		-	-	-	-	1	3.33	-	-	-	-
Romanian pressed cheese		-	-	-	-	1	3.33	-	-	-	-
Powder milk		-	-	-	-	-	-	-	-	2	6.66
Raw milk		-	-	-	-	1	3.33	-	-	-	-
TOTAL STRAINS		7	23.33	1	3.33	19	63.33	1	3.33	2	6.66

CONCLUSIONS

- By implication in the public health, the contamination of food with *L. monocytogenes* raises an important economic problem concerning the food industry. The presence of *Listeria* spp. proved to be an useful indicator during all the stages of the food processing chain.
- The high incidence of *Listeria* spp. in raw meats can be attributed to fecal contamination during evisceration, or to food handlers.
- *L. monocytogenes* 1a, the serotype circulating in Romania, was identified in both raw meat and meat products.
- *L. monocytogenes* 4b, the serotype wide spread in West-Europe, was detected in poultry carcass, imported from Italy.
- *L. innocua* was the most common specie of the genus *Listeria* detected in raw meat, while other *Listeria* species were less frequently.

ACKNOWLEDGMENTS

This work was supported by the strategic grant POSDRU/ ID 76888, Project „Doctoral programme for training scientific researchers" cofinanced by the European Social Found within the Sectorial Operational Program Human Resources Development 2007–2013.

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