Acta vet. scand. 1994, *35,* 11-15.

Occurrence of *Listeria* Species in an Abattoir for Cattle and Pigs in Bosnia and Hercegovina

By *S. Loncarevic, A. Milanovic, F. Caklovica, W. Tham* and *M.-L. Danielsson-Tham*

Department of Food Hygiene, Veterinary Faculty, Sarajevo, Bosnia and Hercegovina and Department of Food Hygiene, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Loncarevic, S., A. Milanovic, F. Caklovica, W. Tham and M.-L. Danielsson-Tham: Occurrence of *Listeria* species in an abattoir for cattle and pigs in Bosnia and Hercegovina. Acta vet. scand. 1994, 35, 11-15. – Altogether 496 samples of meat, lymph nodes, process water and swabs from different places in the abattoir were examined for the presence of *Listeria spp L monocytogenes* was isolated from 31 (6%) and other *Listeria spp.* from 65 (13%) samples *L. monocytogenes* was isolated from 2 of 10 beef meat samples, 4 of 50 pig meat samples and 1 of 21 lymph nodes of pigs. No *Listeria* bacteria were isolated from lymph nodes of cattle. The highest percentage of *Listeria* was recovered from the unclean sections (cattle 22% and pigs 27%) and the highest frequency was observed during the winter months.

meat; lymph nodes; swabs; Listeria monocytogenes.

Introduction

Listeria monocytogenes is recognized as a cause of human disease associated with foodborne outbreaks. Outbreaks of listeriosis have been linked to consumption of coleslaw *(Schlech et al.* 1983), milk *(Fleming et al.* 1985), and cheese *(James et al.* 1985; *Bille & Glauser* 1988). Although there is no evidence of outbreaks due to consumption of red-meat products, some sporadic cases have been reported. The foods incriminated have been home-made pork sausage *(Parodi et al.* 1990) and Cajun pork sausage *(Anon.* 1990). On the other hand, there are many reports of *L. monocytogenes* occurrence in fresh meats, raw meat products and ready-to-eat meat products (see review by *Johnson et al.* 1990). Survival and growth characteristics of *L. monocytogenes;* growth at refrigeration temperature *(Walker et al.* 1990), growth in 10% sodium chloride *(Seeliger & Jones* 1984) and relative resistance to heat *(Donnelly* 1990), as well as its widespread presence in pasture, soil, sewage, poor quality silage, and effluents of abattoirs and meat processing plants, suggest that meat and meat products could be potential vehicles for transmission to man.

The purpose of the present investigation was to study the extent of contamination with *L. monocytogenes* and other *Listeria spp.* in a cattle and pig abattoir in Bosnia and Hercegovina.

Materials and Methods

Altogether 496 samples of meat, lymph nodes, drip-water and swabs from different places in the processing plant were examined (Tables 1, 2 & 3). Samples were collected during the 4 seasons from both the unclean and clean section. The unclean section comprised the stations for bleeding, dehiding or scalding, and evisceration of abdomen and thorax. The clean section included the stations for decapi-

Table 1. Occurrence of *Listeria species* in the cattle section in an abattoir in Bosnia and Hercegovina.

Type of swabs / samples	Number of swabs / samples	Number (%) of positive swabs	
		L monocytogenes	Other *Listeria spp*
Unclean section.			
Equipment	41	2 (5)	4 (10)
Work surfaces	37	3 (8)	7 (19)
Workers' hands	32	3 (9)	5 (16)
Surfaces of carcasses	30	2 (7)	5 (17)
Drip water samples	12	0 (0)	3 (25)
Total	152	10 (7)	24 (16)
Clean section.			
Equipment	38	0 (0)	2 (5)
Workers' hands	7	1 (14)	0 (0)
Surfaces of carcasses	40	1 (2)	2 (5)
Total	85	2 (2)	4 (5)
Grand total	237	12 (5)	28 (12)

tation and washing of heads, splitting and washing carcasses.

Meat *(Musculus gluteobiceps* and *M. gracilis),* each of 500 g, and lymph nodes *(Lnn. mandibularis, Lnn. hepatici, Lnn. intestinales,* and *Lnn. mammarii* or *Lnn. inguinalis)* surrounded by fat tissue, were sampled from carcasses of 20 cattle and 50 pigs in the clean section 30 min after slaughtering (Table 3). The samples were aseptically removed and put into sterile stomacher bags that were then sealed. Cotton swabs moistened with primary enrichment broth (LEB_1, *McClain & Lee* 1987, 1988) were used for swabbing surfaces (4×5 cm^2) of meat, work surfaces (tables, shelves and floor). Each swab was then put into a tube containing 10 ml LEB_1. Drip water from the washed carcasses was collected and 25 ml was transferred to a 100 ml screw cap bottle containing 25 ml of LEB_1 (Tables 1 & 2). All samples were transferred to the laboratory under refrigeration (+4°C) and examined the same day. Isolation and identification of *Listeria spp.* were done according to *McClain & Lee* (1987, 1988). From the meat and lymph node samples respectively, 25 g were cut out and macerated in a stomacher together with 225 ml LEB_1. The macerates, the swab samples and the water samples were incubated at 30°C for 24h. Then 0.1 ml LEB_1 was transferred to 10 ml secondary enrichment broth (LEB_2, *McClain & Lee* 1987, 1988) and incubated at 30°C for 24h. From these LEB_2, 0.1 ml was directly streaked onto LPM (Lithium chlorid-phenylethanol moxalactam) agar plates, and additionally 0.1 ml was streaked after treatment with 0.25% KOH solution. The LPM agar plates were incubated at 30°C for 24h.

Five presumptive *Listeria* colonies were picked from each "positive" plate and streaked onto sheep blood agar for control of purity and hemolytic activity. The strains were tested for Gram reaction, catalase production and motility. For identification of species the following tests were used: CAMP (Christie, Atkins, Munch-Petersen), MR (Methyl Red), VP (Voges-Proskauer), hydrolysis of urea and esculin, nitrate reduction and acid from rhamnose, mannitol, xylose, maltose and glucose.

Table 2. Occurrence of *Listeria species* in the pig section in an abattoir in Bosnia and Hercegovina.

Type of swabs / samples	Number of swabs / samples	Number (%) of positive swabs	
		L. monocytogenes	Other *Listeria spp*
Unclean section.			
Equipment	35	1 (3)	5 (14)
Work surfaces	8	1 (12)	1 (12)
Workers' hands	20	3 (15)	4 (20)
Surface of carcasses	28	3 (11)	8 (29)
Drip water samples	12	1 (8)	1 (8)
Total	103	9 (9)	19 (18)
Clean section:			
Equipment	25	1 (4)	2 (8)
Workers' hands	10	1 (10)	2 (20)
Surface of carcasses	22	1 (4)	4 (18)
Total	57	3 (5)	8 (14)
Grand total	160	12 (7)	27 (17)

Results and discussion

In the present study, *L. monocytogenes* was isolated from 31 (6%) of the samples (meat, lymph nodes, process water and swabs) and other *Listeria spp.* from 65 (13%) (Tables 1, 2, 3). There were no cases where both *L. monocytogenes* and "other *Listeria spp.*" were isolated from the same sample. Thus "other *Listeria spp.*" did not indicate the presence of *L. monocytogenes.*

L. monocytogenes was isolated from 10% of the beef and 8% of the pig meat samples, while other *Listeria spp.* were isolated from 10% in both cases (Table 3). Reports of the incidence of *Listeria spp.* in meat sampled in meat plants are rare. However, in a paper from New Zealand, *L. monocytogenes* was reported to be present in 20% of 25 beef boneless cuts in a meat plant and in 68% of 25 pork cuts in retail display meats *(Lowry & Tiong* 1988). In a Swiss investigation, *L. monocytogenes* was isolated from 17% of 18 raw beef samples, and from 13 % of 31 raw pork meat samples *(Breer & Breer* 1988). However, it is not clear whether these samples were collected at the meat plant or at retail outlets.

In the present study, *L. monocytogenes* was isolated from 5% and other *Listeria spp.* from 14% of the investigated porcine lymph nodes, but not from the bovine lymph nodes. In an investigation from Togo, Africa, hemolytic *Listeria* bacteria were isolated from 2% of 104 and 1% of 118 intestinal lymph nodes from apparently healthy slaughter pigs and cattle, respectively *(Höhne et al.* 1975). As lymph nodes are included in the processing of meat they may be a source of Listeria *(Johnson et al.* 1990). *L. monocytogenes* was isolated from 0-8% and other *Listeria spp.* from 0-19% of environmental samples (work surfaces and equipment) from the cattle section (Table 1). In the New Zealand investigation, *L. monocytogenes* was present in 6 (30%) and other *Listeria spp.* in 3 (15%) environmental samples (work surfaces and knives) at a beef plant *(Lowry & Tiong* 1988).

The highest percentage of *Listeria* positive samples was found during the winter (Table 4). According to *Seeliger* (1969) and *Arm-*

Table 3 *Listeria* incidence in samples of meat and lymph nodes in an abattoir in Bosnia and Hercegovina.

Type of samples	No.of samples / No.of carcasses	Number (%) of positive samples	
		L monocytogenes	Other *Listeria spp*
Beef:			
Meat	20 /20	2 (10)	2 (10)
Lymph nodes	8 / 8	0 (0)	0 (0)
Total	28 / 20	2 (7)	2 (7)
Pig:			
Meat	50 / 50	4 (8)	5 (10)
Lymph nodes	21 / 21	1 (5)	3 (14)
Total	71 / 50	5 (7)	8 (11)
Grand total	99 / 70	7 (7)	10 (10)

strong (1991), listeriosis in animals is most common in winter.

The abattoir investigated has an outdated slaughtering technique, e.g. dehiding of the bovine in the horizontal position by hand, and this may contribute to the spread of *L. monocytogenes.* Technical mistakes, e.g. perforation of udders or abdominal organs, are other sources of contamination. Therefore, it is indispensable that oversights are reduced, particularly at the dehiding, scalding and evisceration stations. Good hygienic practice is very important to reduce contamination risks. To avoid cross-contamination, knives and other instruments should be disinfected regularly. Strict separation of personnel working in the unclean section from those in the clean section, and the avoidance of contacts between fixed installations and carcasses, are other points of great importance.

References

Anonymous: CDC links Cajun pork sausage to Listeriosis case; product recalled. Food Chemic. News. 1990, Jan. 1., p. 35.

Armstrong D. Listeria monocytogenes Infections. In: Evans AS, Brachman PS (eds.): Bacterial Infections of humans – Epidemiology and Control, 2nd ed. Plenum Publ. Co , New York, 1991, pp. 395-402.

Bille J, Glauser M-P: Listeriose – Situation in der Schweiz (Listeriosis in Switzerland). Bulletin des Bundesamtes fur Gesundheitswesen/Bulletin de l'Office fédéral de la santé publique 1988, no 3, 28-29.

Breer C, Breer G: The isolation of *Listeria spp.* in meat and meat products. Proc. 34th Int. Congress Meat Sci. Technol., Part B. 1988, pp. 520-521.

Donnelly CW: Resistance of *Listeria monocytogenes* to heat. In: Miller AJ, Smith JL, Somkuti GA (eds.) Foodborne Listeriosis. Elsevier, The Netherlands, 1990. pp. 189-193.

Fleming DW, Cochi SL, Mac Donald KL, Brondum J, Hayes PS, Plikaytis BD, Holmes MB, Andurier A, Broome CV,Reingold AL· Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. New Eng. J. Med. 1985, *312,* 404-407.

Hohne K, Loose B, Seeliger HPR Recent findings of *Listeria monocytogenes* in slaughter animals of Togo (West Africa). In. Woodbine M (ed.) Problems of listeriosis. Leicester University Press, Leicester, England, 1975, 127-130.

James SM, Fannin SL, Agge BA, Hall B, Parker E, Vogt J, Rung G, Williams J, Lieb L,Salminen C, Prendergast T, Werner SB, Chin J. Listeriosis outbreak associated with Mexican-style cheese. Morbid. Mortal. Weekly Report 1985, *34,* 357-359.

Johnson JL, Doyle MP, Cassens RC· Listeria monocytogenes and other *Listeria spp.* in meat and meat products. J. Food Protect. 1990, *53,* 81-91.

Table 4 *Listeria* incidence on the slaughtering and primary processing lines for cattle and pigs in different seasons in an abattoir in Bosnia and Hercegovina.

Season	Cattle section			Pig section		
	No of samples and swabs	No (%) of positive samples *L monocytogenes*	other *Listeria spp*	No of samples and swabs	No.(%) of positive samples *L monocytogenes*	other *Listeria spp*
Autumn	53	4 (8)	7 (13)	58	5 (9)	9 (16)
Winter	78	7 (9)	11 (14)	64	8 (13)	11 (17)
Spring	74	2 (3)	7 (10)	67	3 (5)	10 (15)
Summer	60	1 (2)	5 (8)	42	1 (2)	5 (12)
Total	265	14 (5)	30 (11)	231	17 (7)	35 (15)

Lowry PD, Tiong I: The incidence of *Listeria monocytogenes* in meat and meat products: Factors affecting distribution. Proc. 34th Int. Congress Meat Sci Technol., part B. 1988, 528-530.

McClain D, Lee WH: Development of USDA – FSIS method for isolation of *Listeria monocytogenes* from raw meat and poultry. J. Ass. Off. Anal. Chem. 1988, *71,* 660-664.

McClain D, Lee WH: Isolation and identification of *Listeria monocytogenes* from meat. USDA / FSIS / Microbiology Division, Laboratory Communication No. 57-9/9, 1987.

Parodi M, Marino P, Balzaretti C, Mauri A, Cainarca M, Cantoni C: Listeriosi sporadica e trasmissione da carne di mariale. (A case report of sporadic listeriosis related to pork-meat in healthy man). G Mal Infett. Parassit. 1990, *42,* 115-116.

Schlech WF, Lavigne PM, Bortolussi RA, Allen AC, Haldane EV, Wort AJ, Martin RS, Hightower AW, Johnson SE, King SH, Nicholls ES, Broome CV: Epidemic listeriosis: Evidence for transmission by food. New Eng. J. Med. 1983, *308,* 203.

Seeliger HPR, Emmerling P, Emmerling H. Listeriosis in Germany Germ. Med. Mth 1969, *14,* 157-163.

Seeliger HPR, Jones D: Genus Listeria. In: Sneath PHA, Maier NS, Sharpe ME, Holt JG (eds.): Bergey's Manual of Systematic Bacteriology, Vol 2. Williams and Wilkins, Baltimore, 1984, pp. 1235-1245.

Walker SJ, Archer P, Banks JG. Growth of *Listeria monocytogenes* at refrigeration temperatures. J. Appl. Bact. 1990, *68,* 157-162

Sammanfattning

Forekomsten av Listeria species i ett slakteri for notkreatur och svin i Bosnien och Hercegovina

Totalt 496 prover, bestående av kott, lymfknutor, processvatten och svabbprover från olika platser på slakteriet, analyserades på forekomst av *Listeria spp L monocytogenes* isolerades från 31 (6%) och andra *Listeria spp* från 65 (13%) av proverna. *L. monocytogenes* isolerades från 2 av 10 prover från notkott, 4 av 50 prover från svinkott och en av 21 svinlymfknutor. Inga *Listeria*-bakterier påvisades i notlymfknutor. Hogsta andelen *Listeria*-bakterier isolerades från de orena avdelningarna. Hogst frekvens noterades under vintermånaderna.

(Received September 14, 1993; accepted September 21, 1993)

Reprints may be requested from: S. Loncarevic, Department of Food Hygiene, Faculty of Veterinary Medicine,
Swedish University of Agricultural Sciences, Box 7009, S-750 07 Uppsala, Sweden.