Acta vet. scand. 1994, *35,* 149-154.

Occurrence of Listeria Species in Broilers Pre- and Post-Chilling in Chlorinated Water at Two Slaughterhouses

By *S. Loncarevic, W. Tham* and *M.-L. Danielsson-Tham*

Department of Food Hygiene, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Loncarevic, S., W. Tham and M.-L. Danielsson-Tham: Occurrence of Listeria species in broilers pre- and post-chilling in chlorinated water at two slaughterhouses. Acta vet. scand. 1994, 35, 149-154. – Altogether 323 pooled samples of neck skins from 1615 broilers from 2 processing plants (A and B) were examined for the presence of *Listeria* species. The broilers were sampled pre-chilling – after leaving the final rinser but before entering the chiller with chlorinated water – and post-chilling – immediately upon leaving the chiller. Free available chlorine in the chilling water varied from 2 to 15 ppm in plant A and was about 10 ppm in plant B. *Listeria monocytogenes* was only isolated from broilers in plant A sampled post-chilling (58% of 62 samples). *L. innocua* was isolated from 19% and 39% of broilers sampled pre-chilling in plants A and B, respectively. Post-chilling, *L innocua* was isolated from 3% and 6% of samples from plants A and B, respectively.

L. monocytogenes; Listeria innocua; broiler neck skins.

Introduction

Outbreaks of human listeriosis have been associated with coleslaw *(Schlech et al.* 1983), milk *(Fleming et al.* 1985), soft cheese *(James et al.* 1985, *Bille* 1988), paté *(McLauchlin et al.* 1991) and pork tongue in aspic *(Goulet et al.* 1993). Investigators have also identified turkey franks *(Barnes et al.* 1989) and chickens *(Kerr et al.* 1988, *Schwartz et al.* 1988, 1990, *Kaczmarski & Jones* 1989) as sources of sporadic cases. The reported prevalence of *Listeria monocytogenes* in raw broiler meat range from 0% to 64% (Table 1).

Swedish poultry processing plants – in contrast to most other countries – use extra chlorinated water when rinsing carcasses along the processing line (from defeathering to chilling) as well as during chilling. This extra chlorination is a requirement of the food regulations and the free available chlorine in the rinsing water and chiller water should be a minimum of 10 ppm. The chilling usually takes place in a so-called screw chiller.

The purpose of the present study was to investigate the occurrence of *Listeria* species in broilers: a) pre-chilling – after leaving the final rinser but before entering the chiller b) post-chilling – immediately upon leaving the chiller.

Materials and methods

Two processing plants were included in this study, plant A where ca 11 million broilers are slaughtered annually and plant B that processes ca 4 million. The broilers were sampled

Table 1. Occurrence of *L monocytogenes* in raw broilers.

Country	Type of sample	Origin	No. of samples assayed	% of samples positive for *L monocytogenes*	References
United Kingdom	frozen	kitchens	64	64[a]	*Kwantes & Isac* 1975
Norway	ready-for-sale	slaughterhouses	90	61	*Rørvik & Yndestad* 1991
England & Wales	skin and carcass	retail outlet	100	57	*Pini & Gilbert* 1988.
Canada	chicken legs	retail outlet	16	56	*Farber et al* 1989
United Kingdom	neck skins	slaughterhouse	30	50	*Hudson & Mead* 1989
United Kingdom	fresh	kitchens	38	50[a]	*Kwantes & Isac* 1975
Taiwan	carcass	local markets	16	50	*Wong et al.* 1990
Denmark	neck skins	slaughterhouse	17[b]	47	*Skovgaard & Morgen* 1988
Japan	carcass	retail outlet, slaughterhouse	300	41	*Nakama et al.* 1992
United Kingdom	ready-to-eat	retail outlet	102	27	*Kerr et al.* 1990
Switzerland	not known	not known	56	25	*Breer & Schopfer* 1988
United States	ready-to-cook	retail outlet	90	23	*Bailey et al.* 1989
Australia	frozen	retail outlet	80	15	*Varabioff* 1990
California, USA	fresh & semi frozen	supermarket	160	13	*Genigeorgis et al.* 1989
Sweden	meat and skin	slaughterhouse	45	0	*Ternstrom & Molin* 1987

a Both hemolytic and non-hemolytic *Listeria spp* included.
b Each sample consisted of 10 neck skins.

pre-chilling – after leaving the final rinser but before entering the chiller with chlorinated water – and post-chilling – immediately after passing through the chiller. Neck skins were aseptically removed and sampled 5 and 5 into sterile plastic bags. Thus, each pooled sample consisted of 5 broiler neck skins and weighed 26 – 35 g. Altogether 323 such samples (124 from plant A and 199 from plant B) from 1615 broilers were collected and examined for the presence of *Listeria* species. In plant A, the samples were collected on 3 different occasions during April and May representing 23 breeders, and in plant B twice during July and August representing 6 breeders. The samples were refrigerated and sent together with ice packs to the laboratory. They reached the laboratory within 24h and were kept at 4°C until examination.

The free available chlorine in the chilling water was determined colorimetrically in comparators with orthotoluidin at the slaughterhouse several times during the day of slaughter.

Enrichment and cultural procedures for detection and isolation of *Listeria* were done according to the method of International Dairy Federation (Anon. 1990) with slight modification. From each pooled sample, 25 g was cut out and macerated with 225 ml LEB (Listeria Enrichment Broth) in a stomacher. The *Listeria* enrichment broth base consisted of 30 g Tryptone Soya broth (Oxoid CM129), 6 g Bacto Yeast Extract (Difco 0127-01-7) and 1000 ml water. The medium was completed by adding 3 selective agents (2.3 mg acriflavine HCl, 9.2 mg nalidixic acid and 11.5 mg cycloheximide) to 225 ml of the *Listeria* enrich-

Table 2. Prevalence of *Listeria spp.* in pooled samples of broilers neck skins collected pre- and post-chilling in chlorinated water.

Plants	Sampling period/ No. of breeders	*Number of samples*[a]		Number (%) of pooled samples positive for the presence of *L. monocytogenes*		*L. innocua*	
		pre-chill	post-chill	pre-chill	post-chill.	pre-chill	post-chill.
A	I / 2	20	20	n.d.	11 (55)[b]	2 (10)	1 (5)[b]
	II / 9	18	18	n.d.	10 (56)	1 (6)	n.d.
	III / 12	24	24	n.d.	15 (63)	9 (38)	1 (4)
Total		62	62	n.d.	36 (58)	12 (19)	2 (3)
B	I / 1	50	50	n.d.	n.d.	n.d.	n.d.
	II / 5	49	50	n.d.	n.d.	39 (80)	6 (12)
Total		99	100	n.d.	n.d.	39 (39)	6 (6)

a = one sample consisted of five neck skins.
b = one sample harboured both *L. monocytogenes* and *L. innocua.*
n.d. = not detected.

ment broth. The macerate was incubated at 30°C for 48h and then 0.1 ml was streaked onto *Listeria* Selective Medium (Oxford Formulation) plates (Oxoid: agar base CM856 and supplement SR140). The plates were incubated at 37°C for 48h. Five typical colonies were isolated from each "positive" plate and tested for cell shape, Gram reaction, hemolytic reaction on horse blood agar, tumbling motility (10h at 20°C), fermentation of rhamnose, xylose, hydrolysis of esculin and production of catalase *(Seeliger & Jones* 1986). All strains of *L. monocytogenes* were serotyped with *Listeria* O Antiserum type 1 and 4 (Difco laboratories, Detroit, Michigan, USA).

Results

Two *Listeria* species – *L. monocytogenes* and *L. innocua* – were found in the 323 investigated samples. *L. monocytogenes* was only isolated from processing plant A in broilers after passing through the chiller (Table 2). Broiler neck skins from both plants yielded *L. innocua,* however, more frequently pre- than post-chilling (Table 2). *L. monocytogenes* and *L. innocua* were isolated from the same sample in 1 case only, post-chilling in plant A. Of the 5 strains isolated from this sample, 1 was *L. monocytogenes* and 4 *L. innocua.*

All isolated *L. monocytogenes* strains belonged to serogroup 1/2. The free available chlorine in the chilling water varied from 2 to 15 ppm in plant A (Table 3) and was about 10 ppm in plant B. All the post-chilling samples from plant A that were positive for the presence of *L. monocytogenes* derived from broilers chilled in water having ≤10 ppm free available chlorine.

Discussion

All the samples positive for the presence of *L. monocytogenes* derived from post-chilled broilers in processing plant A. At each of the 3 sampling periods in plant A, 55, 56 and 63%, respectively, of the samples collected after chilling harboured *L. monocytogenes* (Table 2). *Hudson & Mead* (1989), who also sampled neck skins, reported that whereas no *L.*

Table 3. Occurrence of *L. monocytogenes* in post-chill samples from plant A in relation to chlorine concentration in the chilling water.

Free available chlorine (ppm)	No. of samples positive for *L. monocytogenes*	No of samples negative for *L monocytogenes*	Total no of samples
2	1	–	1
4	2	–	2
6	11	4	15
10	22	18	40
15	–	4	4
Total	36	26	62

monocytogenes was found in chickens immediately after bleeding, this species was found in 50% after passing the chiller without chlorination but before packaging. The results from the present study of plant A and that of *Hudson & Mead* are in agreement with the hypothesis of *Johnson et al.* (1990) that a limited number of *Listeria*-contaminated poultry carcasses may contaminate a large number of carcasses in the chilling water. Similarly, *Lillard* (1990) found that the incidence of *Salmonella* on broiler carcasses was higher after passage through the non-chlorinated chiller. Pre-chilling (after the final washer), the incidence of *Salmonella* was 14% while post-chilling it was 37%.

In the present study, no *L. monocytogenes* was isolated from plant B, possibly due to a sufficient antimicrobial concentration of free available chlorine (10 ppm) in the chiller. *Genigeorgis et al.* (1989) reported the non-attendance of *Listeria spp.* in neck skins from chickens sampled after passage through a chiller tank with water containing 20 ppm total chlorine. In plant A, on the other hand, the chlorine concentration varied considerably during the sampling time and was sometimes as low as 2 ppm. This may be one explanation of the frequent occurrence (58%) of *L. monocytogenes* in the samples from post-chilled broilers in plant A.

Acknowledgement

Financial support provided by Medical Inspector Gustav Kjerrulf's Research Foundation through the recommendations of Swedish Veterinary Association is gratefully acknowledged.

References

Anonymous: Milk and milk products. Detection of *Listeria monocytogenes.* Provisional International IDF standard 143:1990. International Dairy Federation Brussels, Belgium 1990.

Bailey JS, Fletcher DL, Cox NA · Recovery and serotype distribution of *Listeria monocytogenes* from broiler chickens in the Southeastern United States. J. Food Protect. 1989, *52,* 148-150.

Barnes R, Archer P, Strack J, Istre GR Listeriosis associated with consumption of turkey franks. Morbid. Mortal. Week. Rep. 1989, *38,* 267-268.

Bille J. Anatomy of a foodborne listeriosis outbreak. Foodborne Listeriosis. Proc. Symp. Sept. 7, 1988, Wiesbaden, FRG. Behr's Verlag, Hamburg 1988, pp. 30-36.

Breer C, Schopfer K. Listeria and food. Lancet, 1988, *ii,* 1022.

Farber JM, Sanders GW, Johnston MA: A survey of various foods for the presence of *Listeria* species. J. Food Protect. 1989, *52,* 456-458.

Fleming DW, Cochi SL, MacDonald KL, Brondum J, Hayes PS, Plikaytis BD, Holmes MB, Audurier

A, Broome CV, Reingold AL: Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. 1985, *312,* 404-407.

Genigeorgis CA, Dutulescu D, Garayzabal JF: Prevalence of *Listeria spp.* in poultry meat at the supermarket and slaughterhouse level. J. Food Protect. 1989, *52,* 618-624 & 630.

Goulet V, Lepoutre A, Rocourt J, Courtieu A-L, Dehaumont P, Veit P: Épidémie de listériose en France. Bilan final et résultats de l'enquête épidémiologique. (Listeriosis epidemic in France. Results and conclusion of the epidemiological inquiry). Bull. Épidemiol. Hebd. 1993, No. 4, 13-14. In French.

Hudson WR, Mead GC. Listeria contamination at a poultry processing plant. Lett. Appl. Microbiol. 1989, *9,* 211-214.

James SM, Fannin SL, Agee BA, Hall B, Parker E, Vogt J, Run G, Williams J, Lieb L, Salminen C, Prendergast T, Werner SB, Chin J: Listeriosis outbreak associated with Mexican-style cheese. California. Morbid. Mortal. Week. Rep. 1985, *34,* 357-359.

Johnson JL, Doyle MP, Cassens RG: Listeria monocytogenes and other *Listeria spp.* in meat and meat products – A review. J. Food Protect. 1990, *53,* 81-91.

Kaczmarski EB, Jones DM: Listeriosis and ready-cooked chicken. Lancet 1989, *i,* 549.

Kerr KG, Dealler SF, Lacey RW: Materno-fetal listeriosis from cook-chill and refrigerated food. Lancet 1988, *ii,* 1133.

Kerr KG, Rotowa NA, Hawkey PM, Lacey RW. Incidence of *Listeria spp* in pre-cooked, chilled chicken products as determined by culture and Enzyme-linked Immunoassay (ELISA). J. Food Protect. 1990, *53,* 606-607 & 629.

Kwantes W, Isaac M: Listeria infection in West Glamorgon. In: Woodbine M (ed): Proc. 6th Intern. Symp. on Problems of Listeriosis, Nottingham, 1974. Leicester Univ. Press, Leicester, 1975, pp. 112-114.

Lillard HS: The impact of commercial processing procedures on the bacterial contamination and cross-contamination of broiler carcasses. J. Food Protect. 1990, *3,* 202-204 & 207.

McLauchlin J, Hall SM, Velani SK, Gilbert RJ: Human listeriosis and paté: a possible association. Brit. Med. J. 1991, *303,* 773-775.

Nakama A, Maruyama T, Kokubo Y, Iida T, Umeki F: Incidence of *Listeria monocytogenes* in foods in Japan. Abstract of the 11th Intern. Symp. on Problems of Listeriosis, Copenhagen, 1992, pp. 317-318.

Pini PN, Gilbert RJ: The occurrence in the U.K. of *Listeria* species in raw chickens and soft cheeses. Int. J. Food Microbiol. 1988, *6,* 317-326.

Rørvik LM, Yndestad M: Listeria monocytogenes in foods in Norway. Int. J. Food Microbiol. 1991, *13,* 97-104.

Schlech WF, Lavigne PM, Bortolussi RA, Allen AC, Haldane EV, Wort AJ, Hightower AW, Johnson SE, King SH, Nicholls ES, Broome CV: Epidemic listeriosis – evidence for transmission by food. N. Engl. J. Med. 1983, *308,* 203-206.

Schwartz B, Broome CV, Brown GR, Hightower AW, Ciesielski CA, Gaventa S, Gellin BG, Mascola L, and the Listeriosis study group: Association of sporadic listeriosis with consumption of uncooked hot dogs and undercooked chicken. Lancet 1988, *ii,* 779-782.

Seeliger HPR, Jones D. Genus *Listeria.* In: Sneath PAH., Mair NS, Sharpe ME and Holt JG (eds.): Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore, 1986, pp. 1235-1245.

Skovgaard N, Morgen C-A. Detection of *Listeria spp.* in faeces from animals, in feeds, and in raw foods of animal origin. Int. J. Food Microbiol. 1988, *6,* 229-242.

Ternstrom A, Molin G: Incidence of potential pathogens on raw pork, beef and chicken in Sweden, with special reference to Erysipelothrix rhusiopathiae. J. Food Protect. 1987, *50,* 141-146 & 149.

Varabioff Y: Incidence and recovery of *Listeria* from chicken with a pre-enrichment technique. J. Food Protect. 1990, *53,* 555-557 & 624.

Wong H-C, Chao W-L, Lee S-J: Incidence and characterization of *Listeria monocytogenes* in foods available in Taiwan. Appl. Environ. Microbiol. 1990, *56,* 3101-3104.

Sammanfattning

Forekomsten av Listeria *hos slaktkycklıng.*

Halsskinn från 1615 slaktkycklingar slaktade på 2 slakterier (A och B) undersoktes på forekomst av *Listeria*-species. Samlingsprov om 5 halsskinn togs ut omedelbart före resp. efter passage av vattenkylare (spinchiller). *Listeria monocytogenes* kunde endast påvisas i prover från slakteri A, och dar endast i halsskinn uttagna efter spinchiller. Av dessa var 58% positiva på *L. monocytogenes. L. innocua* isolerades

från 19% av proverna från slakteri A och 39% från slakteri B före passage av spinchiller. Efter spinchillern var frekvensen *L. innocua* 3% resp. 6%. Halten fri obunden klor i kylvattnet varierade mellan 2 och 15 ppm i slakteri A, medan slakteri B hela tiden höll 10 ppm.

(Received June 9, 1993, accepted January 28, 1994)

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.