

Contamination of Poultry Products with *Listeria monocytogenes* at Poultry Processing Plants

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ABSTRACT. This study aimed to confirm that poultry products packed at poultry processing plants have already been contaminated with *Listeria monocytogenes* and that poultry products contaminated with *L. monocytogenes* are derived from broiler flocks infected with *L. monocytogenes*. *L. monocytogenes* was isolated from 16.8% (58/345) of chicken breast products and 2.3% (8/345) of chicken liver products. In contrast, *L. monocytogenes* was isolated from the pooled cecal content sample from only 1 (4%) of 25 flocks and was never isolated from any pooled dropping samples collected from 25 farms. The results of our study indicate that cecal content does not seem to be an important source of *L. monocytogenes* in poultry products.

KEY WORDS: *Listeria monocytogenes*, poultry processing plant, poultry product.

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Listeria monocytogenes is a ubiquitous pathogen in the environment and is capable of causing infections in humans. The bacterium is primarily transmitted to humans through contaminated foods [12]. In the United States of America, 24 confirmed foodborne listeriosis outbreaks were reported during 1998–2008, resulting in 359 cases of illness, 215 hospitalizations and 38 deaths [1]. In Japan, a foodborne listeriosis outbreak caused by contaminated cheese was first documented in 2001 [7]. Since this outbreak, no foodborne listeriosis has been reported. On the other hand, Okutani *et al.* [9] conducted a nationwide surveillance for listeriosis and estimated that there was an average of 83 cases of listeriosis per year and an incidence of 0.65 cases per million of the population in Japan during 1996–2002. Ochiai *et al.* [8] has reported that 28.7% (37/129) of domestic poultry products retailed in the Tokyo metropolitan area of Japan were contaminated with *L. monocytogenes*; 3 serovars, viz., 1/2a, 1/2b and 4b, were frequently isolated from these products. These 3 serovars are common in human listeriosis [13].

In Japan, consumption of raw meat and liver from poultry is fairly common; such consumption is a potential source for human listeriosis. There are several papers reporting that the environment in poultry processing plants is a source of *Listeria* contamination of raw poultry products [2, 3, 5]. We have

inferred that poultry products packed in poultry processing plants have already been contaminated with *L. monocytogenes* and that the bacteria isolated from *L. monocytogenes*-positive products are derived from broiler flocks that are infected with *L. monocytogenes*.

To test this hypothesis, we conducted 2 surveys in cooperation with 3 poultry processing plants, A, B and C, in eastern Japan. These 3 processing plants belong to different broiler production companies, and each processing plant handles only those broilers raised in the farms which contract with the same company as the plant. The processing capacity of these plants was 32,000, 12,000 and 10,000 birds per day, respectively. At plants A and C, processing lines and equipment were cleaned using high-pressure water, disinfected with alkali surfactants and then subjected to high-pressure water cleaning after slaughter of the final flock of the day. At plant B, processing lines and equipment were cleaned by high-pressure water, disinfected with quaternary ammonium compounds and amphoteric surfactants and then subjected to high-pressure water cleaning after slaughter of the final flock of the day.

Survey 1 was conducted at plants A and B from September 2011 through February 2012. This survey was aimed at confirming that poultry products packed in poultry processing plants have already been contaminated with *L. monocytogenes*. Sampling was performed at each plant 11 times (i.e., every 2 weeks). On each sampling day, 5 packs (2 kg/pack) of chicken breasts or livers from each of the first 2 flocks were collected. If the environment in poultry processing plants is a significant source of *L. monocytogenes* contamination of poultry products, poultry products from the first flock on each slaughter day would be contaminated by *L. monocytogenes* in the environment of the plants. In addition, the following flock would be also directly affected by

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Table 1. Detection of *L. monocytogenes* in breast and liver products collected at plant A

	Date of sampling											Total	
	September		October		November		December		January		February		
	26	11	24	7	21	5	19	16	30	13	27		
First flock	A ^{a)} 1 ^{b)}		B: 1	C: 1	E: 1	F: 1	H: 1	J: 1	K: 1	L: 1	N: 1	B: 3	
Breast products	2 ^{c)}	4	3	1	0	4	3	0	0	1	0	18	
Liver products	1	2	1	0	0	0	1	1	0	0	0	6	
Second flock	A: 2		B: 2	D: 1	E: 2	G: 1	I: 1	J: 2	K: 2	M: 1	O: 1	P: 1	
Breast products	0	0	2	1	0	2	2	0	0	0	0	7	
Liver products	0	1	0	0	0	0	0	0	0	0	0	1	

a) Farm code, b) Flock code, c) The number of positive products among 5 products taken.

Table 2. Detection of *L. monocytogenes* in breast products collected at the plants

	N ^{a)}	Number of positive products among 5 breast products from each flock					
		0	1	2	3	4	5
Plant A	5	1	1	1	0	0	2
Plant C	20	7	8	3	2	0	0

a) The number of flocks investigated.

the *L. monocytogenes* contamination of the flock slaughtered immediately before.

Survey 2 was conducted at plants A and C from August 2012 through December 2012. Twenty-five flocks were investigated to confirm that *L. monocytogenes* isolates from *L. monocytogenes*-positive products are derived from broiler flocks that are infected with *L. monocytogenes*. These flocks were scheduled to be the first flock slaughtered on each slaughter day. Of the 25 flocks, a total of 20 flocks of 3 broiler farms were scheduled to be slaughtered at plant C, and the remaining 5 flocks of 5 broiler farms were scheduled to be slaughtered at plant A. One week prior to slaughter of each flock, 5 pooled dropping samples (approximately 25 g each) were collected from the flock house. At the plants, 4 pooled cecal content samples (approximately 25 g each) were collected from 20 birds from each selected flock. One pooled cecal content sample was obtained from the cecal contents of 5 birds. In addition, 5 packs (2 kg/pack) of breasts or livers were collected from the flock.

Samples collected at the 3 plants among the 2 surveys were transported to the Institute for Food and Environment Sciences in Tokyo Kenbikyo-in Foundation (IFES) via express delivery, under refrigeration. At the laboratory, samples were refrigerated at $4 \pm 2^\circ\text{C}$ and examined within 24 hr after arrival.

L. monocytogenes was isolated as previously described [11]. One isolate per *L. monocytogenes*-positive sample was serotyped by using commercial specific antisera according to the manufacturer's instructions (Denka Seiken Co., Ltd., Tokyo, Japan). Wilcoxon signed-rank test was used for comparing *L. monocytogenes* contamination rates of poultry products between first and second flocks, and other statistical analysis was performed using Fisher's exact test.

In survey 1, 110 breast and 110 liver products from 22 flocks of 16 broiler farms (A–P) were collected at plant A,

and *L. monocytogenes* was isolated from 25 (22.7%, 25/110) breast products and 7 (6.4%, 7/110) liver products (Table 1). The contamination rate was significantly higher ($P < 0.001$) in breast products than in liver products. The contamination rate (32%, 24/70) in breast products collected during September–December 2011 was significantly higher ($P < 0.001$) than in those collected during January–February 2012 (3%, 1/40). The contamination rate was statistically higher ($P < 0.02$) in breast products from the first flocks than in those from the second flocks slaughtered each day. The contamination rate was also statistically higher ($P < 0.05$) in liver products from the first flocks than in those from the second flocks.

On the other hand, at plant B, 110 breast and 110 liver products from 22 flocks of 16 broiler farms were investigated, and no *L. monocytogenes* was isolated from any products.

In survey 2, *L. monocytogenes* was isolated from 17 (68%) of the 25 flocks investigated. In 16 of the 17 *L. monocytogenes*-positive flocks, *L. monocytogenes* was isolated only from breast products. The remaining *L. monocytogenes*-positive flock was slaughtered at plant A, and *L. monocytogenes* was isolated from 1 pooled cecal content sample, 5 breast products and 1 liver product. No *L. monocytogenes* was isolated from any pooled dropping samples. *L. monocytogenes* contamination rate was markedly higher ($P < 0.001$) in breast products (26.4%, 33/125) than in liver products (0.8%, 1/125). Two or more of 5 breast products were *L. monocytogenes*-positive in 3 (60%) of the 5 flocks slaughtered at plant A, but in 5 (25%) of the 20 flocks slaughtered at plant C (Table 2). At plant A, *L. monocytogenes* was isolated from 13 (52%) of 25 breast products, and the 13 *L. monocytogenes*-positive breast products were derived from 4 (80%) of 5 flocks investigated. On the other hand, in plant C, *L. monocytogenes* was isolated from 20 (20%) of 100 breast products, and the 20 *L. monocytogenes*-

Table 3. Serovars of *L. monocytogenes* isolates obtained from 2 plants A and C

		Samples	N	Serovars		
				1/2a	1/2b	4b
Survey 1	Plant A	Breast products	25	3	22	0
		Liver products	7	4	3	0
Survey 2	Plant A	Breast products	13	0	12	1
		Liver products	1	0	1	0
		Pooled cecal contents	1	1	0	0
	Plant C	Breast products	20	20	0	0
Total			67	28	38	1

positive breast products were derived from 13 (65%) of the 20 flocks investigated. The contamination rate was significantly ($P=0.002$) higher in breast products from plant A than in those from plant C.

A total of 67 *L. monocytogenes*-positive samples were obtained, and these 67 isolates were comprised of 3 serovars (Table 3), viz., 1/2a (42%, 28/67), 1/2b (57%, 38/67) and 4b (1%, 1/67). Serovar 1/2b was the most common serovar (89%, 34/38) in breast products collected at plant A, but accounted for 50% (4/8) of isolates from liver products. There was a statistically significant difference ($P=0.02$) in the distribution of serovar 1/2b between breast and liver products. At plant C, all isolates belonged to serovar 1/2a. In survey 2, the isolate from the pooled cecal content sample collected at plant A belonged to serovar 1/2a, although isolates from poultry products collected at the plant belonged to 2 different serovars, viz., 1/2b and 4b.

Cox *et al.* [4] reported that no *L. monocytogenes* was isolated from 115 cecal samples, while the bacterium was isolated from 27 (26%) of 105 poultry carcasses. In survey 2, although *L. monocytogenes* was isolated from 26.4% of chicken breast products tested, the bacterium was never isolated from any pooled dropping samples collected at the farms tested and was isolated from only 1 pooled cecal content sample collected at plant A. This confirms the result of Cox *et al.* [4]. The serovar 1/2a of the isolate from the one pooled cecal content sample in survey 2 was not obtained from any poultry products collected at plant A. Therefore, it was not possible to determine where *L. monocytogenes* obtained from poultry products came from.

It has been reported that the presence of *L. monocytogenes* in poultry products is primarily caused by contamination during processing and that some *L. monocytogenes* strains can persist for several months in poultry processing plants [6, 10].

The results of our study indicate that cecal content does not seem to be an important source of *L. monocytogenes* in poultry products. *L. monocytogenes* on the surface of processing line that comes to contact with poultry products is more likely to be a source of *L. monocytogenes* contamination, as, once *L. monocytogenes* contaminated processing plants, it is reported that the bacterium persists for several months [6, 10]. Although *L. monocytogenes* contamination rates were statistically higher in poultry products from the first flocks than in those from the second flocks, its cause is

yet to be elucidated by collecting environmental samples at processing plants before the beginning of slaughtering broiler flocks. The higher *L. monocytogenes* contamination rate in breast products collected at plant A during September–December suggests that *L. monocytogenes* can propagate better in processing environments with higher temperatures. López *et al.* [6] have reported that poultry carcasses were less contaminated with *L. monocytogenes* in March compared with April and May and suggest that the possibility of certain seasonal trends associated with the isolation of *L. monocytogenes* from broiler carcasses. Their results and suggestions are consistent with ours.

Considering surveys 1 and 2 together, *L. monocytogenes* was isolated from 16.8% (58/345) of breast products and 2.3% (8/345) of liver products, and the serovars of the isolates were 1/2a, 1/2b and 4b. It could be confirmed that poultry products shipped from poultry processing plants were already contaminated with *L. monocytogenes*. However, the contamination rates and the distribution of the serovars in this study are not representative of poultry products throughout Japan for the following 3 reasons. The first reason is that the contamination rates of *L. monocytogenes* in poultry products collected at the 3 plants tested were considerably different from each other. Japan has more than 150 poultry processing plants, annually slaughtering over 300,000 broilers. If plants producing poultry products with a high *L. monocytogenes* contamination rate, like plant A, predominate, the contamination rate of poultry products would be higher than that observed in this study. The second is that only the first 2 flocks in one slaughter day were investigated in the present study. Generally, more than 3 flocks are slaughtered at one poultry processing plant on a slaughter day in Japan. Poultry products derived from the third-to-last flocks would be less contaminated with *L. monocytogenes* than the first 2 flocks. The last reason is that there may be a seasonal variation in the contamination of poultry products by *L. monocytogenes*, as noted above. To estimate *L. monocytogenes* contamination rate of poultry products shipped from poultry processing plants, a survey should be planned taking into account the above factors.

The cleaning and disinfection programs of processing lines and equipment after slaughtering the last flock in a slaughter day hold the key to reducing *L. monocytogenes* contamination rate of poultry products shipped from poultry processing plants. The cleaning and disinfection programs

of processing lines and equipment implemented at plant B may prevent *L. monocytogenes* colonization on the product contact surfaces, because no *L. monocytogenes* was isolated from any poultry products collected at the plant. The disinfectant used at plant B was different from that used at plants A and C. However, it is too early to conclude that quaternary ammonium compounds are more effective in killing *L. monocytogenes* than alkali surfactants. This is because the effectiveness of disinfectants can be influenced by numerous factors, such as temperature and the presence of organic debris, before application of disinfectants. Since no environmental samples were collected at any of the plants investigated in this study, we could not compare the effect of the cleaning and disinfection programs implemented at plant B to those implemented at the others.

In conclusion, the findings of the present study indicate that poultry products are contaminated with *L. monocytogenes* adhering to product contact surfaces in processing environments. Comparison of *L. monocytogenes* contamination rates of environmental samples among the 3 plants will help to identify control measures for preventing colonization of *L. monocytogenes* in processing environments of poultry processing plants. In order to achieve this goal, we recognize the necessity and importance of collecting environmental samples before slaughtering the first flock and after slaughtering the last flock on the same day in several processing plants and identifying where *L. monocytogenes* colonizes. We will initiate the new study as soon as situation allows us to do so.

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