NOTE *Public Health*

Prevalence and contamination levels of *listeria monocytogenes* in ready-to-eat foods in Tokyo, Japan

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ABSTRACT. We surveyed prevalence and contamination levels of *Listeria monocytogenes* in ready-to-eat foods between 2000 and 2012 in Tokyo. *L. monocytogenes* was isolated from 52 (1.7%) out of 2,980 samples. Comparing the prevalence in the study period, 2.2% were positive in the former period (2000–2005) and 1.2% in the latter (2006–2012). Using the most probable number (MPN) technique, 32 samples were contaminated with fewer than 0.3 *L. monocytogenes*/g, 10 samples with 0.3–1.0/g and 4 samples with more than 1.0/g (the maximum was 2.3/g). The most common serovar was 1/2a, followed by 1/2b, 4b and 1/2c. We revealed that ready-to-eat foods in Tokyo were contaminated with *L. monocytogenes,* although the contamination levels were low.

KEY WORDS: *Listeria*, *Listeria monocytogenes*, MPN, ready-to-eat food, serotype

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Listeria monocytogenes is a food-borne pathogen. Although it causes mild, non-specific and influenza-like symptoms in healthy adults, it can cause invasive listeriosis culminating in sepsis and meningitis in the immunocompromised, and the elderly. When pregnant women are infected with this organism, it is particularly devastating for unborn or newly delivered babies [4].

Only one food-borne outbreak caused by *L. monocytogenes* has been reported in Japan, which was due to natural cheese in 2001 [10]. In the U.S.A., European countries and Australia, outbreaks were often reported due to dairy foods, meat products, deli meat, fish products and vegetables [4].

In 2007, the Codex Alimentarius Commission has presented the guidelines to control *L. monocytogenes* in foods [2], and microbiological criteria for *L. monocytogenes* were adopted in ready-to-eat (RTE) foods prepared for consumption without further heating required as an annex in the document in 2009 [3]. The Codex determined that samples should contain *L.monocytogenes* no more than 100 cfu/g for foods in which growth will not occur and that samples should show absence in 25 g for foods in which growth can occur. The Codex gave the following examples of factors that can control growth of *L. monocytogenes* in foods: pH below 4.4, water activity (Aw) <0.92, a combination of factors (pH, Aw) e.g., a combination of pH <5.0, Aw <0.92 and freezing.

In Japan, soft or semi-soft cheese and unheated meat products contaminated with *L. monocytogenes* have been prohibited for import or commercial use since 1993 [12]. These foods should have shown absence of this organism in 25 g. To follow the Codex microbiological criteria, the standard for soft/semi-soft and semi-hard cheese and unheated meat products was fixed at ≤100 cfu/g in 2014 [13, 14]. In the present study, we surveyed the prevalence and the extent of contamination of this organism and other *Listeria* spp. in many types of RTE foods in Tokyo, Japan. As preservatives have been developed to control *Listeria* spp. in RTE foods recently, the period of collection was divided into the former and the latter to compare the prevalence between the two periods. And, pH and Aw were determined for the RTE foods found to be contaminated with *L. monocytogenes*, to determine whether growth of this organism could occur.

A total of 2,980 samples were obtained from 2000 to 2012 from retail shops and food-processing plants in metropolitan Tokyo and surrounding area. These included 626 dairy products, 1,491 meat and meat products, 718 seafood samples and 145 pickled vegetable products (Table 1). The period of collection was divided into "former" (from 2000–2005) and "latter" (from 2006–2012). The total number of samples collected in the former and the latter period was 1,710 and 1,270, respectively.

For each sample, one-step enrichment method and/or two-step enrichment method was adopted. One-step enrichment method was performed as follows. Aseptically composited 25-g portions of samples were homogenized with 225 m*l* of enrichment medium and then incubated at 30°C for 48 hr. For enrichment, EB broth (Merck, Darmstadt, Germany) was used for dairy products, and UVM broth (Merck) was used for all other samples. Two-step enrichment method was performed as follows, 25-g portions of test samples were added to 225 m*l* of primary enrichment medium; half-Fraser broth (Merck) and incubated at 30°C for 24 hr. Primary en-

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Table 1. Prevalence of *Listeria spp*. in ready-to-eat foods in Tokyo (2000–2012)

Samples	Period	No. of samples examined	No. of positive samples (%)					
			L. monocytogenes	*L. innocua*	*L. seeligeri*	*L. welshimeri*	*L. grayi*	*Listeria* spp.
Dairy products								
Natural cheese (Soft/semi-soft)	2000–2005	127	1 (0.8)	4 (3.1)				5 (3.9)
	2006–2012	161		1 (0.6)				1 (0.6)
	Subtotal	288	1 (0.3)	5 (1.7)				6 (2.1)
Natural cheese (Hard/semi-hard)	2000–2005	217	1 (0.5)					1 (0.5)
	2006–2012	73						0 (0)
	Subtotal	290	1 (0.3)					1 (0.3)
Dehydrated powdered infant formula	2000–2012	39						0 (0)
Butter	2000–2012	8						0 (0)
Cream	2000–2012	1						0 (0)
Total		626	2 (0.3)	5 (0.8)				7 (1.1)
Ready-to-eat raw meats and meat products								
Ready-to-eat raw meats	2000–2005	83	3 (3.6)	8 (9.6)		5 (6)		12 (14.5)
	2006–2012	13						0 (0)
	Subtotal	96	3 (3.1)	8 (8.3)		5 (5.2)		12 (12.5)
Unheated meat products	2000–2005	85	13 (15.3)	6 (7.1)		2 (2.4)		19 (22.4)
	2006–2012	43	1 (2.3)	2 (4.7)		2 (4.7)	1 (2.3)	3 (7)
	Subtotal	128	14 (10.9)	8 (6.3)		4 (3.1)	1 (0.8)	22 (17.2)
Specifically heated meat products	2000–2005	27				3 (11.1)		3 (11.1)
	2006–2012	10						0 (0)
	Subtotal	37				3 (8.1)		3 (8.1)
Heat treated meat products (packaged after being heat treated)	2000–2005	474	2 (0.4)	4 (0.8)		1 (0.2)		5 (1.1)
	2006–2012	617	7 (1.1)	2 (0.3)		2 (0.3)		10 (1.6)
	Subtotal	1091	9 (0.8)	6 (0.5)		3 (0.3)		15 (1.4)
Heated meat products (heat treated after being packaged)	2000–2012	57						0 (0)
Dried meat products	2000–2012	40						0 (0)
Cooked meats	2000–2005	37				1 (2.7)		1 (2.7)
	2006–2012	5						0 (0)
	Subtotal	42				1 (2.4)		1 (2.4)
Total		1491	26 (1.7)	22 (1.5)		16 (1.1)	1 (0.1)	53 (3.6)
Ready-to-eat seafood samples								
Fresh fish	2000–2005	38	1 (2.6)	3 (7.9)				4 (10.5)
	2006–2012	48	4 (8.3)	7 (14.6)	4 (8.3)		1 (2.1)	11 (22.9)
	Subtotal	86	5 (5.8)	10 (11.6)	4 (4.7)		1 (1.2)	15 (17.4)
Shellfish	2000–2005	111	1 (0.9)	1 (0.9)	1			3 (2.7)
	2006–2012	49						0 (0)
	Subtotal	160	1 (0.6)	1 (0.6)	1 (0.6)			3 (1.9)
Boiled seafood	2000–2012	19						0 (0)
Roe	2000–2005	79	5 (6.3)	13 (16.5)	1 (1.3)			17 (21.5)
	2006–2012	45	1 (2.2)	4 (8.9)			1 (2.2)	6 (13.3)
	Subtotal	124	6 (4.8)	17 (13.7)	1 (0.8)		1 (0.8)	23 (18.5)
Delicatessen of seafood	2000–2005	108	2 (1.9)	10 (9.3)	1 (0.9)			12 (11.1)
	2006–2012	90	2 (2.2)	1 (1.1)			1 (1.1)	4 (4.4)
	Subtotal	198	4 (2)	11 (5.6)	1 (0.5)		1 (0.5)	16 (8.1)
Dried seafood	2000–2005	131	5 (3.8)	9 (6.9)				12 (9.2)
Total		718	21 (2.9)	48 (6.7)	7 (1)		3 (0.4)	69 (9.6)
Pickled vegetables								
Pickled vegetables	2000–2005	145	3 (2.1)					3 (2.1)

richment cultures (0.1 m*l*) were inoculated into 10 m*l* of secondary enrichment medium; Fraser broth (Merck) and incubated at 37°C for 48 hr. Each enrichment culture was plated out on PALCAM agar and incubated at 30°C for 48 hr. The

Table 2. MPN value of *L. monocytogenes* in ready-to-eat foods (2000–2012)

Food samples	No. of samples examined	MPN value (/g)		
		<0.3	0.3–1	>1
Natural cheese	2	1		1[a)]
Ready-to-eat raw meats	3	3		
Unheated meat products	13	10	3	
Heat treated meat products (packaged after being heat treated)	7	3	4	
Fresh fish	5	2	1	2[b)]
Roe	5	5		
Delicatessen of seafood	4	2	1	1[c)]
Dried seafood	4	4		
Pickled vegetables	3	2	1	
Total	46	32	10	4

a) Semi-soft cheese made in France, b) Minced tuna, c) "Matsumaezuke", a kind of pickle of squid and seaweeds.

cultures of some samples were plated out on ALOA (Merck) or CHROMagar Listeria (CHROMagar, Paris, France) plates and incubated at 37°C for 24–48 hr. From each plate, colonies presumptively identified as *Listeria* spp. were streaked onto trypticase soy agar (Eiken Chemical, Tokyo, Japan) and incubated at 30°C for 24 hr to obtain pure culture. Confirmation that isolates were *Listeria* spp. was carried out by performing the Henry illumination test, catalase reaction, motility test, CAMP test, and assessing carbohydrate utilization of rhamnose, xylose and mannitols based on the use of ISO11290-1 method [8].

Quantitative determination was conducted using the most probable number (MPN) method with 3 tubes. Briefly, 25-g portions of test samples were added to 225 m*l* of culture medium and homogenized. Then, 10 m*l* of the homogenate was added to each of the 3 empty tubes, and 1 m*l* and 0.1 m*l* of the homogenate to each of 3 tubes of 10 m*l* culture medium, which were incubated at 30°C for 48 hr. For the culture medium, EB broth was used for dairy products, and UVM broth was used for all other samples. After incubation, each culture was plated out on PALCAM agar and incubated at 30°C for 48 hr. From each plate, colonies presumed to be *Listeria* spp. were checked for confirmation. MPN value was estimated on the basis of the number of positive tubes obtained from each set of dilution series.

Cultures identified as *L. monocytogenes* were serotyped using commercially available *Listeria* anti-sera (Denka Siken Co., Tokyo, Japan).

The pH of the samples was estimated using a pH meter by measuring the filtrate of samples with distilled water. The Aw of the samples was measured using a Decagon Model CX-2 (Decagon Devices, Inc., Pullman, WA, U.S.A.).

The prevalence of *Listeria* spp. in RTE foods is shown in Table 1. *L. monocytogenes* was isolated from 52 (1.7%) out of 2,980 samples. Comparing the prevalence of *L. monocytogenes* in the study period, 37 (2.2%) out of 1,710 samples were found positive in the former period, and 15 (1.2%) out of 1,270 samples in the latter.

A risk assessment report of *L. monocytogenes* in foods by the Food Safety Commission of Japan states that the percentage of retail foods contaminated with *L. monocytogenes* was 2.2% for imported natural cheeses, 0% for domestic natural cheese, 0% for other dairy goods, 3.89% for unheated meat products, 2.26% for fresh seafood, 7.19% for seafood products and 18.8% for pickled vegetables [6]. A previous risk assessment conducted by the FDA/USDA/CDC showed that the percentages for contaminated foods in the U.S.A. were 2.5% for cheese, 0.3% for dairy goods other than cheese, 3.0% for processed meat products, 7.0% for fresh seafood, 9.5% for seafood products and 2.1% for pickles [5]. Comparing prevalence of contamination among reports, the percentage of food contaminated with *L. monocytogenes* of natural cheese (0.3%) and pickled vegetables (2.1%) was low, and that of unheated meat products (10.9%) was high in our study.

The period of the survey (2000–2012) was divided into two parts, the former and the latter. The contamination of food products, such as unheated meat products and roe, tended to be reduced in the latter half of the study period. Recently, good hygienic practices [2] and use of preservatives, such as sodium benzoate, sodium propionate, potassium sorbate and nisin [19], have been introduced to limit contamination with *Listeria* spp. in many manufacturing facilities in Japan and overseas. As such, contamination with this pathogen may tend to be reduced.

In pickled vegetables, *L. monocytogenes* was detected in 3 (cucumber pickled in rice-bran paste, 2.1%) of 145 samples. It has been reported in Hokkaido where factory contamination with *L. monocytogenes* was suspected during the production of "asazuke", Japanese light pickles [11]. In 2012, an outbreak of enterohemorrhagic *Escherichia coli* O157 due to consumption of pickled Chinese cabbage occurred in a number of districts [7]. Therefore, pickled vegetables should be applied general principles of food hygiene, and their sanitary facilities must be improved in the factory setting to prevent secondary contamination from the production line in the same way as dairy foods or meat products.

From the positive samples, 46 were assessed for numbers of *L. monocytogenes* by MPN. Thirty-two of these were contaminated with <0.3 *L. monocytogenes*/g, 10 with 0.3–1.0/g

Table 3. pH and Aw of ready-to-eat foods contaminated with *L. monocytogenes*

Type of foods	Samples	MPN value (/g)	pH	Aw
Unheated meat products	Salami sausage	<0.3	7.0	0.93
	Salami sausage	<0.3	6.4	0.83
	Raw ham	0.4	6.1	0.91
	Raw ham	<0.3	6.0	0.89
Heat treated meat products (packaged after being heat treated)	Sausage	0.36	6.4	0.98
Fresh fish	Minced tuna	0.92	5.8	0.99
	Minced tuna	<0.3	6.1	0.98
	Minced tuna	2.3	6.2	0.99
	Minced tuna	<0.3	6.9	0.98
Roe	"Tarako"	<0.3	6.0	0.95
Delicatessen of seafood	Jellyfish with sea urchin eggs	<0.3	5.8	0.86

Table 4. Serotypes of *L. monocytogenes* isolated from ready-to-eat foods (2000–2012)

Food samples	No. of isolates of each serotypes (%)							
	1/2a	1/2b	1/2c	4b	3a	3b	3c	Total
Natural cheese	1 (50)	1 (50)						2
Ready-to-eat raw meats and meat products	17 (51.5)	6 (18.2)	2 (6.1)	6 (18.2)		1 (3)	1 (3)	33
Ready-to-eat seafood samples	10 (40)	5 (20)	5 (20)	3 (12)	1 (4)	1 (4)		25
Pickled vegetables	2 (66.7)	1 (33.3)						3
Total	30 (47.6)	13 (20.6)	7 (11.1)	9 (14.3)	1 (1.6)	2 (3.2)	1 (1.6)	63

and 4 with >1.0/g (Table 2). The samples contaminated with >1.0/g were a semi-soft cheese type made in France (value, 1.6/g), 2 minced tuna samples (values, 2.1/g and 2.3/g) and "matsumaezuke," which is a kind of pickle of squid and seaweeds (value, 1.5/g).

The pH and Aw of 11 samples out of *L. monocytogenes*-positive samples are shown in Table 3. Samples that were pH ≥4.4, Aw ≥0.92 and pH ≥5.0 + Aw ≥0.94 were 1 of 4 unheated meat products, 1 heat treated meat product (packaged after being heat treated), 4 fresh fish samples and 1 of 2 fish products (Table 3).

In this survey, it was almost less than 1/g, and the highest value observed was 2.3/g. However, in this study, *L. monocytogenes* was detected in foods where pH and Aw supported growth [3]. Although the MPN value of *L. monocytogenes* being low, it is possible for the organism to grow in those foods. It has been reported that generation times of *L. monocytogenes* are 36 hr at 4.4°C and 10 hr at 10°C [18]. Furthermore, despite the fact that raw RTE seafood in Japan has a short shelf life, cell numbers of *L. monocytogenes* in minced tuna and salmon roe increased rapidly under inappropriate storage temperatures of 10°C (from a MPN of 10° to 10^1/g to a MPN of 10^3 to 10^4/g over a course of 2 days at 10°C) [15]. The Codex guideline prescribes keeping the temperature at less than 6°C for production and transportation [2]. As the Codex, raw RTE seafood should be kept at less than 6°C in Japan.

Serovars of 63 isolates identified as *L. monocytogenes* isolated from RTE foods included 1/2a, 30 strains (47.6%); 1/2b, 13 strains (20.6%); 4b, 9 strains (14.3%); 1/2c, 7 strains (11.1%); 3b, 2 strains (3.2%); 3a and 3c, 1 strain (1.6%), respectively (Table 4). In the report of Food Safety Commission of Japan, that was 1/2a (52.2%), followed by 1/2c (17.2%), 1/2b (15.3%) and 4b (10.2%) [6]. These findings were similar. On the other hand, the most common serotype from case of listeriosis in Japan during 1958–2001 was 4b (59.9%), followed by 1/2b (26.4%) and 1/2a (5.8%) [6]. The most common serotype was different between isolates obtained from humans and food, probably due to differences in pathogenicity. It has been reported that *L. monocytogenes* can be attenuated, depending on the mutation of virulence-associated genes, such as *prfA*, *inlA*, *inlB* and *plcA* in lineage II [17]. The exhibit of mutations result in a premature stop codon in *inlA* is different for each serotype, 19.1% in 1/2a, 14.3% in 1/2b, 100% in 1/2c and 0% in 4b [9]. Otherwise, after 2000, serotype 4b was still commonly reported in cases in the U.S.A. [1], but serotype 1/2a showed an increasing trend in Europe [16]. The common serotype in the outbreak was 4b before the year 2000. However, serotype 1/2a increased after 2000 [6]. It is possible that the prevalent serotype of *L. monocytogenes* is changing recently.

The RTE foods marketed in Tokyo were contaminated with this organism, although the contamination levels were low. There are few incidents or outbreaks of *L. monocytogenes* in Japan, but Japan Nosocomial Infections Surveillance estimated that 200 listeriosis cases occur in a year [6]. Considering the high mortality rate this disease presents with, many of these are likely to have been fatal. In addition, listeriosis is largely attributable to foodborne transmission of the microorganism. It is important to continue surveys of

prevalence and contamination levels of *L. monocytogenes* in RTE foods in Tokyo, Japan.

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