資料３

食安基発０５２４第１号
平成２４年５月２４日

内　閣　府
　食品安全委員会事務局評価課長　殿

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　食品安全部基準審査課長

食品健康影響評価に係る補足資料の提出依頼について（回答）

平成２４年３月３０日付け府食第３１７号で依頼された、食品中のリステリア・モノサイトゲネスに係る標記について、下記のとおり回答します。

記

① 輸入食品におけるリステリア・モノサイトゲネスの検査をしている食品の輸入実績、検査実績及び違反実績（過去10年分）
また、上記のうち陽性と判定された食品におけるリステリア・モノサイトゲネスの菌数のデータ

輸入食品におけるリステリア・モノサイトゲネスの検査対象は非加熱食肉製品及びナチュラルチーズ（ソフト及びセミソフトタイプ）であり、その実績は別紙のとおり。なお、現状において定量的な判定は行っていないため、菌数のデータはない。

② 国内流通食品について、Codex 基準におけるリステリア・モノサイトゲネスの増殖が起こる RTE 食品及び増殖が起こらない RTE 食品それぞれの汚染実態（汚染量、汚染率）のデータ（過去10年分）

第 528 号
受 24. 5. 24 付
食品安全委員会事務局

国内流通食品の汚染実態のデータについては、コーデックス基準に基づき分類した食品の汚染実態については確認できなかった。

なお、コーデックス基準に基づき分類した食品の汚染実態ではないが、既に提出している「食品におけるリステリアに関する規格基準に係る調査研究（平成 21 年度食品等試験検査）」の表 2、表 4 及び表 5 に国内流通食品の汚染実態について取りまとめられているので、参考としていただきたい。また、このほかに以下の論文を入手したので提出する。

○ Miya S, Takahashi H, Ishikawa T, Fujii T, Kimura B. Risk of Listeria monocytogenes Contamination of Raw Ready-To-Eat Seafood Products Available at Retail Outlets in Japan. Appl Environ Microbiol. 2010 May; 76(10): 3383-3386

（別紙）

リステリア検査対象品目の輸入・検査・違反状況

非加熱食肉製品

年　度	届　出		検　査		違　反	
	件数	重量(t)	件数	重量(t)	件数	重量(t)
平成14年度	2,413	1,845.8	0	0.0	0	0.0
平成15年度	3,199	2,182.4	0	0.0	0	0.0
平成16年度	3,438	2,044.7	46	6.1	1	0.3
平成17年度	3,880	2,143.7	53	15.9	1	0.1
平成18年度	4,781	2,637.0	130	23.2	2	0.1
平成19年度	5,199	2,724.0	272	105.5	5	0.4
平成20年度	4,744	2,421.0	845	297.6	20	1.6
平成21年度	4,529	2,360.2	1,525	748.9	24	5.8
平成22年度	4,857	2,573.2	1,492	826.1	28	10.5
平成23年度	5,238	2,731.6	1,813	758.4	32	4.8
合計	42,278	23,663.6	6,176	2,781.7	113	23.6

ナチュラルチーズ

年　度	届　出		検　査		違　反	
	件数	重量(t)	件数	重量(t)	件数	重量(t)
平成14年度	62,044	56,361.7	139	201.4	0	0.0
平成15年度	61,432	59,862.2	1,349	834.4	2	0.1
平成16年度	60,669	68,978.8	810	724.1	0	0.0
平成17年度	61,838	66,603.0	856	528.5	0	0.0
平成18年度	59,989	67,582.0	747	238.2	4	0.9
平成19年度	59,097	76,301.7	434	212.1	0	0.0
平成20年度	52,920	56,291.4	510	255.6	1	0.0
平成21年度	51,053	59,645.8	491	135.6	0	0.0
平成22年度	48,980	66,742.2	399	451.5	2	0.0
平成23年度	50,621	77,839.1	525	888.2	1	1.2
合計	568,643	656,207.8	6,260	4,469.5	10	2.2

※　数値は輸入食品監視支援システム（FAINS）による検索結果。
　　検査及び違反はリステリア菌によるもの。
　　ナチュラルチーズには検査を実施した水牛由来のチーズ等を含む。

（参考）

輸入食品のリステリア菌検査

以下の検査のいずれかを行った届出を検査分として計上している。

・命令検査：リステリア菌に汚染されている可能性の高い食品（過去に検出事例があった食品等）に対し、国、製造者、品目等を指定し、毎輸入時に行う検査。なお、現在

は、イタリア、デンマーク、フランス及び米国のナチュラルチーズ並びにイタリア、カナダ、スペイン及び米国の食肉製品に対し、製造者の指定等をして行われている。

・モニタリング検査：輸入食品の衛生上の状況を把握するため、過去の輸入実績等を勘案した年間計画に基づき行う検査。なお、本年度は加熱せずに食用に供する非加熱食肉製品及びナチュラルチーズが計画されている。

・その他の検査：初回輸入時や定期的な輸入時に、輸入者としての食品衛生安全確保義務責任の観点から行う検査。

（別紙）

リステリア検査対象品目の輸入・検査・違反状況

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Risk of *Listeria monocytogenes* Contamination of Raw Ready-To-Eat Seafood Products Available at Retail Outlets in Japan[▽]

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Examination of *Listeria monocytogenes* prevalence among ready-to-eat foods in Japan revealed frequent (5.7 to 12.1%) contamination of minced tuna and fish roe products, and the isolates had the same virulence levels as clinical isolates in terms of invasion efficiency and infectivity in cell cultures and a murine infection model, respectively. Premature stop codons in *inlA* were infrequent (1 out of 39 isolates). Cell numbers of *L. monocytogenes* in minced tuna and salmon roe increased rapidly under inappropriate storage temperatures (from a most probable number [MPN] of 10^0 to 10^1/g to an MPN of 10^3 to 10^4/g over the course of 2 days at 10°C). Thus, regulatory guidelines are needed for acceptable levels of *L. monocytogenes* in these foods.

Listeria monocytogenes causes listeriosis in humans mainly through consumption of ready-to-eat (RTE) foods. In Japan, the first reported food-borne listeriosis outbreak occurred in 2001, caused by contaminated cheese (16). Interestingly, this outbreak was detected from routine monitoring in the cheese manufacturing plant. Since this cheese was contaminated with *L. monocytogenes* at a most probable number (MPN) of 10^7/g (16), individuals who had consumed cheese made in the plant were retrospectively examined and were found to have been infected. This was the first and only reported food-borne outbreak in Japan; however, we are unsure if previous or subsequent listeriosis outbreaks have occurred, as there are no official statistics on the incidence of listeriosis, due to the lack of a mandatory notification system (20). On the other hand, a questionnaire-based nationwide surveillance of hospitals estimated that an average of 83 listeriosis cases occur every year, which is equivalent to 0.65 per million inhabitants in Japan (20). Moreover, the pathogen has been detected in surveys of RTE foods at rates similar to those of other industrialized countries (21).

Japan has a unique diet, comprising large quantities of raw RTE seafood, such as sashimi and sushi. Our previous study on *L. monocytogenes* contamination in such foods (11) revealed that minced tuna and fish roe products had high contamination rates (14.3% for minced tuna and 10.0 to 11.4% for fish roe products). In this study, we investigated *L. monocytogenes* prevalence in such RTE foods further, using a larger number of raw RTE seafood and other RTE food products. We also investigated the virulence potential of isolates in invasion efficiency and in a mouse model and determined whether each product type could support the growth of the pathogen. These results can provide baseline data for regulatory guidelines necessary for the safety of such products.

Seafood products and other RTE foods were purchased from 229 different grocery stores and delicatessens located around Tokyo, Japan, between October 2004 and July 2008. By following a two-step enrichment procedure (11), five colonies on Palcam agars (Merck, Darmstadt, Germany) from each enrichment were randomly picked. Serotype was determined by the agglutination method using commercial *Listeria* antiserum (Denka Seiken, Tokyo, Japan). Each isolate was considered to be a different strain if it had a different serotype or multilocus sequence type (MLST), based on six virulence genes

TABLE 1. Prevalence of *L. monocytogenes* in RTE foods available at retail outlets in Japan[a]

Sample type	No. of samples tested	No. of positive samples by mini-VIDAS LMO (%)
Seafood		
Tuna		
Minced tuna	116	14 (12.1)
Tuna block	38	1 (2.6)
Fish roe		
Salmon roe	123	7 (5.7)
Cod roe	164	15 (9.1)
Sushi	36	0 (0.0)
Smoked salmon[b]	33	1 (3.0)
Dried seafood	16	0 (0.0)
Other RTE food		
Natural cheese[b]	65	0 (0.0)
Salad	61	0 (0.0)
Deli sandwich	32	0 (0.0)
Ham[b]	17	0 (0.0)
Total	701	38 (5.4)

[a] Food samples were purchased from 229 different grocery stores and delicatessens located in and around Tokyo, Japan, from October 2004 to July 2008. Screening of *L. monocytogenes* in foods was performed by mini-VIDAS LMO.
[b] Previously, 2.4% (33/1,387 samples) of imported natural cheese, 0% (0/15 samples) of ham, and 5.4% (5/92 samples) of smoked salmon retailed in Japan were reported to be contaminated (21).

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TABLE 2. Serotype distribution of *L. monocytogenes* isolates from minced tuna and fish roe samples[a]

Sample type	No. of isolates					
	Total	By serotype				
		1/2a	3a	1/2b	3b	4b
Minced tuna	15	12		2		1
Salmon roe	6	4		2		
Cod roe	18	5	7	3	1	2
Total	39	21	7	7	1	3

[a] From 36 *L. monocytogenes*-positive samples (14 minced tuna, 7 salmon roe, and 15 cod roe samples) determined by mini-VIDAS LMO (Table 1), 39 isolates were obtained, with 3 food samples producing no isolates. Two isolates with different subtypes (serotypes and/or MLSTs) were obtained from six food samples.

(*prfA*, *inlB*, *inlC*, *dal*, *clpP*, and *lisR*) as described previously (30) (data not shown). Similar to what was found in our previous study, minced tuna and fish roe products were highly contaminated with *L. monocytogenes*, as determined by a mini-VIDAS LMO screening test (bioMérieux Vitek, Marcy l'Etoile, France) (Table 1).

Food processing plants have been found to be the most frequent source of *L. monocytogenes* contamination in many types of foods, including RTE seafood (1, 3, 18, 19, 26, 28). As minced tuna and fish roe products require more processing than other raw seafood products, there is a greater possibility of cross-contamination in such food processing plants. In fact, the contamination rates of minced tuna and fish roe products in Japan were relatively high compared to rates determined in the United States and Europe for other products, such as dairy products (14), vegetables (4), smoked seafood, and meat products (10).

Virulence potential, which differs among *L. monocytogenes* isolates (2, 23, 24), is another important factor in listeriosis risk. Out of 13 known serotypes, three (1/2a, 1/2b, and 4b) are known to be responsible for >90% of human listeriosis cases (17). In this study, approximately 79% (31/39) of the isolates from 36 RTE seafood products comprised these three serotypes (Table 2). The serotypes of the remaining isolates included 3a (7 isolates that were obtained from 7 different cod roe samples purchased from 6 different stores) and 3b (1 isolate obtained from cod roe), which are rarely isolated from human clinical cases (6, 15). Furthermore, almost all (38/39) of the raw RTE seafood isolates were found to encode full-length InlA, a protein required in invasion of host cells (12) (DNA Data Bank of Japan accession numbers AB276379 to AB276437 and AB522784 to AB522794); one serotype 1/2a isolate had a truncated InlA. Even though the number of isolates sequenced was relatively small ($n = 39$), the scarcity of

TABLE 3. *L. monocytogenes* isolates used in the mouse assay

Strain	Serotype	Sampling date	Origin	Reference or source
Food isolates				
2-9	1/2a	19 November 2002	Salmon roe	13
25-8-1	1/2a	9 December 2004	Minced tuna	13
36-25-1	1/2a	2 June 2005	Cod roe	13
40-6-1	1/2a	26 July 2005	Minced tuna	13
22-19-2	3a	16 November 2004	Cod roe	13
34-9-1	3a	28 April 2005	Cod roe	13
29-10-1	1/2b	17 February 2005	Minced tuna	13
40-5-1	1/2b	26 July 2005	Salmon roe	13
50-18-1	1/2b	13 April 2006	Cod roe	This study
9-17	3b	2 February 2003	Salmon roe	13
39-8-1	3b	21 July 2005	Salmon roe	13
50-18-4	3b	13 April 2006	Cod roe	This study
20-5-1	4b	28 October 2004	Cod roe	13
34-18-2	4b	28 April 2005	Cod roe	13
57-2-1	4b	20 July 2006	Minced tuna	This study
Clinical isolates				
C1-117	1/2a		Human	Pathogen Tracker[a]
F2-563	1/2b		Human	Pathogen Tracker
J1-177	1/2b		Human	Pathogen Tracker
J1-169	3b		Human	Pathogen Tracker
J1-094	1/2c		Human	Pathogen Tracker
J1-049	3c		Human	Pathogen Tracker
ATCC 19114	4a		Animal	
ATCC 19115	4b		Human	
CIP103575	4b		Milk	
F2-525	4b		Human	Pathogen Tracker
ATCC 19116	4c		Animal	
ATCC 19118	4e		Animal	
Controls				
ATCC 51782	3a		Cheese	
ATCC 33090 (*L. innocua*)	6a		Animal	

[a] Available at http://www.pathogentracker.net/.

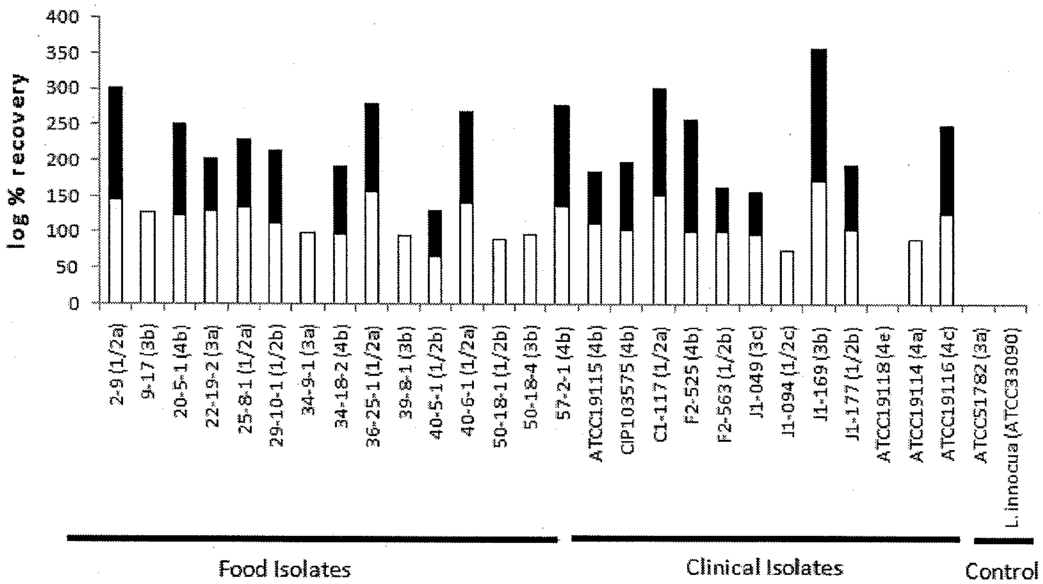

FIG. 1. Virulence of *L. monocytogenes* isolates from RTE seafood in the mouse model. Seven-week-old female BALB/cCrSlc mice were infected via intravenous inoculation at 10^3 to 10^4 CFU. Bacteria were enumerated from the liver (black columns) and spleen (white columns) 3 days after infection. The rate of recovery was determined using the following formula: log (number of cells recovered)/log (number of cells inoculated) × 100. *L. monocytogenes* ATCC 51782, which has attenuated virulence due to the K220T substitution in PrfA (25), and *L. innocua* ATCC 33090 were used as negative controls.

inlA with premature stop codons was in marked contrast to results from other studies (13, 22, 23, 27). This indicates that raw RTE seafood isolates have been through environments where full-length InlA may be required, unlike the other food isolates.

The virulence potential of the seafood isolates was assessed based on *in vivo* bioassays using a mouse model (Table 3 and Fig. 1). Three days after intravenous inoculation with 10^3 to 10^4 CFU, recovery of *L. monocytogenes* from livers and spleens was detected for all the isolates tested, except for one serotype 4e strain (Fig. 1). No recovery was detected in some isolates when homogenized livers were directly plated, but colonies were recovered after enrichment from these liver samples. Statistical analysis (Student's *t* test) revealed no significant differences in infectivity in liver ($P = 0.691$), spleen ($P = 0.274$), or both ($P = 0.882$) between raw RTE seafood and clinical isolates. These data suggest similar levels of virulence between raw RTE seafood isolates and clinical isolates in this animal model. The one raw RTE seafood isolate with truncated InlA was highly infective in liver and spleen, indicating that full-length InlA is not essential in infecting these organs.

We also investigated the possibility that large amounts of *L. monocytogenes* could be ingested through the consumption of contaminated RTE seafood products. To investigate whether raw RTE seafood supports pathogenic growth, we inoculated foods with *L. monocytogenes* (2 strains of serotype 1/2a and 4b, both isolated from fish roe products) and examined them under temperature conditions that could exist during distribution and prior to consumption. A portion (25 g) of each minced tuna and salmon roe sample was inoculated with *L. monocytogenes* at an MPN of 10^0 to 10^1/g and then incubated at 22°C for 6 h and at 5°C or 10°C for 7 days. Although most minced tuna and fish roe products in Japan have a shelf life of less than 3 days, it is possible that they may be consumed after the expiration date. Moreover, certain fish roe products have a 7-day shelf life. Results at room temperature (22°C) were examined to reflect situations such as those in sushi restaurants or small home parties, in which food might remain unrefrigerated for extended periods. Minced tuna and fish roe, which are popular ingredients of sushi, allowed minimal growth of the pathogen, with an increase in cell number to an MPN of 10^2/g, even at room temperature for 6 h (data not shown), while refrigeration (5°C) resulted in an MPN of 10^2/g following 3 and 2 days of incubation in minced tuna and salmon roe, respectively. After a 7-day incubation at 5°C, *L. monocytogenes* cell numbers reached an MPN of 10^3 to 10^4/g. However, increasing the temperature to 10°C resulted in increases of *L. monocytogenes* to an MPN of 10^3 to 10^4/g following only 2 days of incubation and an MPN of 10^7/g after 7 days of incubation. The appearance and odor of all samples were assessed by a panel of five judges to determine the extent of spoilage. At day 2, all products were judged to be unspoiled and safe to eat.

These data raise the concern that raw RTE seafood products available at retail outlets in Japan are at risk for food-borne listeriosis and raise the possibility that these products have already been the cause of illness in the past. Considering the contamination level of RTE foods that have caused listeriosis outbreaks in the past (mostly $\geq 10^4$ CFU/g) (5), the level determined in this study was quite low (Table 4), indicating that the samples were relatively safe in terms of contamination level at the time of purchase. However, this is the case only if they are consumed immediately after purchase. In foods that support growth, cell number is expected to increase at the time of consumption, especially when the food is not properly maintained under refrigeration. The United States also retains a zero-tolerance policy for RTE foods that support growth (9).

TABLE 4. Numbers of *L. monocytogenes* cells in minced tuna and fish roe products purchased from retail stores in Japan, as determined by the MPN method[a]

Sample type	No. of samples tested	MPN/g			
		<0.3	0.3–0.94	1.1–9.3	12–15
Minced tuna	14	10	3	1	
Salmon roe	7	4		2	1
Cod roe	15	6	6	3	
Total	36	20	9	6	1

[a] See reference 7.

In the European Union (EU), regulatory guidelines set different tolerance levels of *L. monocytogenes* contamination depending on whether the food supports growth, with zero tolerance for foods that support growth "before the food has left the immediate control of the food business operator, who has produced it" and a tolerance level of 100 CFU/g for "products placed on the market during their shelf-life" and for foods that do not support growth throughout the shelf life (8). Although most minced tuna and salmon roe products have a short shelf life, they should be categorized as foods that support pathogenic growth. In fact, the U.S. Food and Drug Administration categorizes raw seafood as RTE foods that support *L. monocytogenes* growth (9).

In conclusion, our data suggest that raw RTE seafood may pose risks for food-borne listeriosis. In the United States, preventative regulatory guidelines have been effective in reducing the listeriosis incidence (29), while in Japan, regulation of *L. monocytogenes* is currently limited to dairy products and RTE meat products. Our data will be useful for establishing regulations for microbial food safety that include RTE seafood.

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