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Original Article

Outbreak of *Salmonella* Braenderup Infection Originating in Boxed Lunches in Japan in 2008

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There have been only 2 reports of a large-scale foodborne outbreak arising from *Salmonella* enterica serotype Braenderup infection worldwide. On August 9, 2008, an outbreak originating in boxed lunches occurred in Okayama, Japan. We conducted a cohort study of 786 people who received boxed lunches from a particular catering company and collected 644 questionnaires (response rate: 82%). Cases were defined as those presenting with diarrhea (≥4 times in 24h) or fever (≥38℃) between 12 am on August 8 and 12 am on August 14. We identified 176 cases (women/men: 39/137); younger children (aged <10 years) appeared to more frequently suffer severe symptoms. Three food items were significantly associated with higher risk of illness; *tamagotoji* (soft egg with mixed vegetables and meat) (relative risk (RR): 11.74, 95% confidence interval (CI): 2.98-46.24), pork cooked in soy sauce (RR: 3.17, 95% CI: 1.24-8.10), and vinegared food (RR: 4.13, 95% CI: 1.60-10.63). Among them, only the RR of *tamagotoji* was higher when we employed a stricter case definition. *Salmonella* Braenderup was isolated from 5 of 9 sampled cases and 6 food handlers. It is likely that unpasteurized liquid eggs contaminated by *Salmonella* Braenderup and used in *tamagotoji* caused this outbreak.

Key words: boxed lunch, cohort study, foodborne diseases, *Salmonella* Braenderup, unpasteurized liquid eggs

Salmonella species are common etiological agents of bacterial gastroenteritis outbreaks worldwide [1-8]. *Salmonellae* are estimated to cause 1.4 million illnesses and 400 deaths annually in the United States [9] and 39,085 illnesses annually in Japan [10]. According to passive surveillance in Japan, various serotypes of *Salmonella* were detected in 2008, as follows: *Salmonella* Enteritidis: 341 cases (31.5%); *Salmonella* Infantis: 105 cases (9.7%); *Salmonella* Typhimurium: 82 cases (7.6%); *Salmonella* Saintpaul: 70 cases (6.5%); and *Salmonella* Braenderup: 65 cases (6.0%) <http://idsc.nih.go.jp/iasr/virus/graph/salm2008.pdf; accessed September 2, 2010>.

There have been 2 reports of large-scale outbreaks arising from *Salmonella* Braenderup infection worldwide. In 1993, the ingestion of food highly contaminated with *Salmonella* Braenderup resulted in an acute gastroenteritis outbreak in Switzerland [11], which was caused by mishandling of food during manufacture. In this outbreak, 215 cases of gastroenteritis were identified between October 14 and November 1. The cases developed severe and typical gastroenteri-

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tis. Children were more severely affected than adults, and 6 of 29 required hospitalization. In another example of a foodborne outbreak caused by *Salmonella* Braenderup, a large multistate outbreak occurred after exposure to Roma tomatoes in restaurants in the United States [12]. There were 125 confirmed cases in 16 states and 12 probable cases in this outbreak. The Roma tomatoes were traced back to a single tomato-packing plant.

On August 11 and 12, 2008, Okayama City Public Health Center (OCPHC) received reports of a suspected foodborne outbreak from 4 business establishments in Okayama city. The people affected had a variety of symptoms including abdominal pain, fever, nausea and diarrhea. To identify the source, food sanitation inspectors from OCPHC conducted face-to-face or telephone interviews about the affected peoples' food consumption and symptoms. All had eaten a boxed lunch served by "catering company A" on August 9, 2008. Further enquiries revealed that there were many individuals with similar gastrointestinal symptoms who had consumed the same food. We therefore conducted epidemiologic, microbiologic, and environmental investigations to identify the extent of the outbreak and the probable vehicle of infection, and to advise on the appropriate control measures.

Materials and Methods

Epidemiologic investigation. To identify the source of infection, we conducted a cohort study of individuals who had eaten boxed lunches served by "catering company A" on August 9. Self-administered questionnaires were distributed to those who ordered a boxed lunch on August 9. Questionnaires were not distributed to those whose cases had been reported to OCPHC on August 11 and 12, as OCPHC food sanitation inspectors had already asked the same questions either in an in-person interview or by telephone, and their data were included in the study. We collected demographic and clinical data, as well as data on the types of boxed lunch (*i.e.*, lunch of the day, children's lunch, and noodle lunch) and individual food items. With regard to younger children under 10 years of age, parents or nurses answered the questionnaires. Cases were defined as those who presented with diarrhea (looser stools than normal ≥ 4 times in 24 h) or fever (≥ 38℃) between 12 am on August 8 and 12 am on August 14.

We calculated attack rates (ARs) according to age group (10-year categories) and sex. We further calculated the ARs, relative risk (RR) and 95% confidence intervals (CI) of each type of boxed lunch and food item. For food items found to have a statistically significant association with illness in the univariate analysis, we calculated Mantel-Haenszel RRs for each food item, stratified by a potential confounder. In a supplementary analysis, we restricted cases to those who presented with diarrhea (looser stools than normal ≥ 4 times in 24 h) and fever (≥ 38℃) between 12 am on August 8 and 12 am on August 14. A p value less than 0.05 (two-sided) was considered statistically significant. The analysis was performed using Epi Info Version 3.5.1 (CDC, Atlanta, GA, USA).

Microbiologic and environmental investigation. We retrieved stool specimens from 9 patients who were still ill at the time of the interview. These stool samples were sent for enteric bacterial analyses at OCPHC. Some stool samples were collected by medical providers and sent to private laboratories. We cultured stool samples according to standard methods for enteric bacterial pathogens, including *Salmonella* species, *Yersinia*, *Campylobacter* species, pathogenic *Escherichia coli*, *Clostridium perfringens*, *Aeromonas* species, *Plesiomonas*, *Vibrio* species, *Bacillus cereus*, and *Staphylococcus aureus* [13]. To identify the *Salmonella* species, suspected colonies were confirmed using appropriate biochemical tests. Isolates were serotyped according to the Kauffman and White scheme using somatic (O) and flagellar (H) antigens [14].

Beginning August 12, food sanitation inspectors of OCPHC visited "catering company A" to inspect whether it complied with the Food Sanitation Act and the Municipal Ordinance for the Food Sanitation Act. The inspections focused on food processing, hygiene conditions, efficacy of in-house control, and the related health status of the kitchen staff. We collected stool samples from all food handlers at company A. In addition, we collected swabs for culture from the kitchen utensils, equipment, and work surfaces. The stool samples from food handlers and the swabs were tested for the same bacterial enteric pathogens as the cases.

Results

Epidemiologic investigation. A total of 786 boxed lunches were distributed on August 9, and we collected 644 questionnaires, 601 of which were self-administered (overall response rate: 82%). We identified 176 persons (women/men: 39/137) who met the case definition. The median age of the cases was 38 years (range, 1–71 years). The AR was slightly higher among women (34.5%) compared with men (27.0%) (Table 1). The AR in those who were aged < 10 years (76.7%) was higher than in those in older age groups (≥ 10 years, 25.9% [153/590]) (Table 1). Table 2 shows the distributions of each clinical symptom by age group. The risk of developing severe clinical symptoms, including fever and vomiting, was higher among young children (aged < 10 years). More children sought medical attention. We observed no lethal cases.

An epidemic curve plotted using information about the time of onset of the first symptom in 156 cases indicated a common-source pattern (Fig. 1). In addition, of the 146 cases who gave precise times for both the onset of their illness and having eaten the boxed lunch, the median incubation period was 24.0 h (range, 3.1–98.0 h).

Table 3 shows the number of cases and ARs of each type of boxed lunch served on August 9. The AR was 1.2% among those who had eaten a noodle lunch. Compared with those who had eaten the noodle lunch, those who consumed the lunch of the day or the children's lunch had a substantially higher risk of developing illness (RR: 22.09, 95% CI: 13.13–156.01; RR: 62.25, 95% CI: 8.78–441.23, respectively).

Table 1 Number of cases and attack rates by age group and gender, Japan, August 2008

Age groups	Number of cases			Number of population at risk			Attack rates (%)		
	Women	Men	Total	Women	Men	Total	Women	Men	Total
0–9	12	11	23	15	15	30	80.0	73.3	76.7
10–19	0	2	2	0	4	4	N/A	50.0	50.0
20–29	7	21	28	23	78	101	30.4	26.9	27.7
30–39	4	38	42	14	104	118	28.6	36.5	35.6
40–49	5	24	29	15	103	118	33.3	23.3	24.6
50–59	4	26	30	24	135	159	16.7	19.3	18.9
60–69	6	12	18	20	57	77	30.0	21.1	23.4
70–79	1	3	4	2	11	13	50.0	27.3	30.8
Total	39	137	176	113	507	620	34.5	27.0	28.4

N/A, not available.

Table 2 Frequencies of clinical symptoms among cases (n = 176), Japan, August 2008

Clinical symptoms	Adults and children (≥ 10 years old) (n = 153)		Children (< 10 years old) (n = 23)		Total (n = 176)	
	Number	Percent	Number	Percent	Number	Percent
Diarrhea	152	99.3	21	91.3	173	98.3
Abdominal pain	128	83.7	20	87.0	148	84.1
Fever (≥ 38℃)	101	66.0	21	91.3	122	69.3
Headache	76	49.7	3	13.0	79	44.9
Chills	61	39.9	4	17.4	65	36.9
Nausea	49	32.0	7	30.4	56	31.8
Vomiting	21	13.7	8	34.8	29	16.5
Sought medical attention	91	59.5	19	82.6	110	62.5
Hospitalization	2	1.3	1	4.3	3	1.7

Table 3 Boxed lunch-specific attack rates and relative risks, Japan, August 2008

Types of box lunch	Ate food			RR	95% CI
	Cases	Total	AR (%)		
Lunch of the day	111	417	26.6	22.09	13.13–156.01
Children's lunch	24	32	75.0	62.25	8.78–441.23
Noodle lunch	1	83	1.2	1	

AR, attack rate; RR, relative risk; CI, confidence interval.

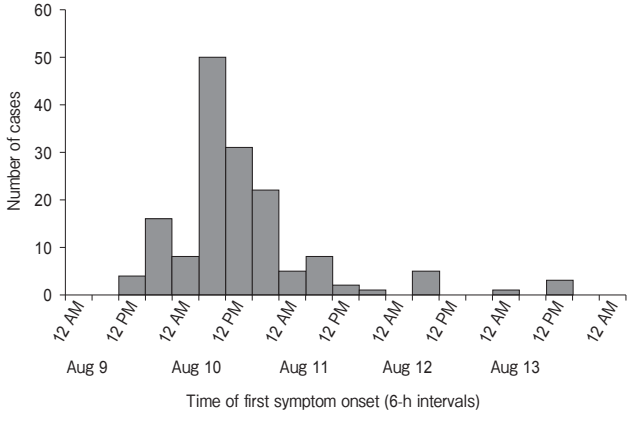

Fig. 1 Epidemic curve of cases of *Salmonella* Braenderup infection, Japan, August 2008 (date of onset of illness was available for 156 cases).

Of the individual food items, we found three that were significantly associated with a higher risk of illness; *tamagotoji* (RR: 11.74, 95% CI: 2.98–46.24), pork cooked in soy sauce (RR: 3.17, 95% CI: 1.24–8.10), and vinegared food (RR: 4.13, 95% CI: 1.60–10.63) (Table 4). *Tamagotoji* is runny scrambled eggs with vegetables and ground meat. Since the RR of *tamagotoji* was considerably higher than that of the other 2 food items, we considered that the RRs of pork cooked in soy sauce and vinegared food were confounded by *tamagotoji*. When we stratified by *tamagotoji*, pork cooked in soy sauce was not significantly associated with illness (Mantel-Haenszel RR: 2.31, 95% CI: 0.94–5.70), whereas vinegared food was still significantly associated with illness (Mantel-Haenszel RR: 3.15, 95% CI: 1.16–8.53). Note that, although both pork cooked in soy sauce and vinegared food were associated with more than doubled risk of illness, their Mantel-Haenszel RRs were lower than the corresponding crude RRs shown in Table 4. It should also be noted that the noodle lunch included pork cooked in soy sauce and vinegared food but not *tamagotoji* (Table 4).

To minimize disease misclassification bias, we conducted a supplementary analysis by employing a stricter case definition, *i.e.*, those who presented with diarrhea and fever between 12 am on August 8 and 12 am on August 14. The RRs of *tamagotoji*, pork cooked in soy sauce, and vinegared food were ∞ (95% CI: undefined), 2.85 (95% CI: 0.73–11.20), and 3.77 (95% CI: 0.95–14.91), respectively. From these findings, we considered that *tamagotoji* was most likely associated with a higher risk of illness.

Microbiologic and environmental investigation. A total of 9 stool specimens from cases were submitted to the laboratory of OCPHC, and 5 were positive for *Salmonella* Braenderup. The other 4 stool specimens did not yield *Salmonella* Braenderup or other enteric pathogens. Of the 20 patients who consulted doctors and whose samples were sent to a private laboratory, three were positive for *Salmonella* O7 (including *Salmonella* Braenderup), and 5 were positive for *Salmonella* (detailed information about serotype was unknown).

Inspection of "catering company A" identified numerous problems with sanitation and hygiene. The inspection revealed very poor hygienic conditions in the areas used for food preparation and storage. Although food preparation surfaces, utensils, and equipment were not properly sanitized, cultures of environmental swabs did not yield *Salmonella* Braenderup or other enteric pathogens.

Interviews with a manager and a review of the food handlers' health records revealed that no gastrointestinal illness was reported among the staff members around this outbreak. Not all food handlers used gloves properly, and some did not change gloves at appropriate times. Food handlers lacked accurate knowledge about food safety and did not receive

Table 4 Food-specific attack rates and relative risks among subjects who ate boxed lunches, Japan, August 2008

Food items	Types of boxed lunch[a]			Ate food			Did not eat			RR	95% CI
	D	C	N	Cases	Total	AR (%)	Cases	Total	AR (%)		
Calamari cutlet	+	+	+	152	496	30.6	6	28	21.4	1.43	0.70–2.94
French fries	+	+	+	144	482	29.9	6	26	23.1	1.29	0.63–2.65
Shredded cabbage	+	+	+	131	444	29.5	19	67	28.4	1.04	0.69–1.56
Tamagotoji[b]	+	+	−	157	448	35.0	2	67	3.0	11.74	2.98–46.24
Pork cooked in soy sauce	+	+	+	149	458	32.5	4	39	10.3	3.17	1.24–8.10
Vinegared food	+	+	+	140	390	35.9	4	46	8.7	4.13	1.60–10.63
Food cooked in soy sauce	+	+	+	134	436	30.7	9	47	19.1	1.61	0.88–2.94
Japanese pickles	+	+	+	116	392	29.6	25	101	24.8	1.20	0.82–1.74
Chinese noodles	−	−	+	0	32	0.0	136	500	27.2	0.00	Undefined
Wheat noodles	−	−	+	1	35	2.9	135	496	27.2	0.11	0.02–0.73
Buckwheat noodles	−	−	+	0	14	0.0	136	516	26.4	0.00	Undefined
Rice	+	−	−	105	330	31.8	29	123	23.6	1.35	0.95–1.93
Rice ball	−	+	+	25	92	27.2	111	417	26.6	1.02	0.70–1.48

AR, attack rate; CI, confidence interval; D, lunch of the day; C, children's lunch; N, noodle lunch; RR, relative risk; +, Included; −, Not included.

[a] Lunch of the day, children's lunch, and noodle lunch were served to 417, 32, and 83 subjects, respectively.

[b] *Tamagotoj*i is runny scrambled eggs with vegetables and ground meat.

appropriate education about safe food preparation. Food handlers ate boxed lunches cooked by "company A" regularly. Of the 14 food handlers in "company A," 6 were found to harbor *Salmonella* Braenderup. They were advised by food sanitation inspectors to consult their doctors.

Because the epidemiologic investigation showed that *tamagotoji* was associated with illness, OCPHC food sanitation inspectors determined the recipe for the *tamagotoji* from the kitchen chef. The *tamagotoji* contained burdock roots, carrots, green onions, unpasteurized liquid eggs and ground meat. After the items had been purchased, the burdock roots, carrots, and green onions were stocked in a walk-in cooler, while the unpasteurized liquid eggs and ground meat were placed in a freezer. At 10 am on August 8, employee B started washing and cutting carrots and green onions and stored them in the refrigerator. At 3 am on August 9, employee B stir-fried the ground meat, cooked burdock roots, carrots, and green onions with seasoning and water in a huge pan. Employee B heated these ingredients fully but did not measure the temperature with a food thermometer. Although the unpasteurized liquid eggs had been placed in the refrigerator to thaw 2 days before, they were not thawed completely. Unpasteurized liquid eggs were added to the stir-fried ingredients in a huge pan. It is likely that the *tamagotoji* was undercooked because the unpasteurized liquid eggs were only partly thawed and were heated quickly. Cooked *tamagotoji* was placed in the same container as was used for the unpasteurized liquid eggs, chilled to around 35℃ with a vacuum cooler and then stored in the refrigerator for 1 h until it was placed in the lunchboxes between 6 and 7 am on August 9 by the food handlers. The boxed lunches were distributed to each business establishment by delivery vehicles that had no cold storage, and were then left at room temperature in the business establishments.

Discussion

The present findings indicate that this foodborne outbreak was caused by the consumption of *tamagotoji* in boxed lunches served by "catering company A" on August 9, 2008. We identified *Salmonella* Braenderup in 5 of 9 sampled cases, which implied that the most likely causative pathogen was *Salmonella* Braenderup. We found that younger children (aged < 10 years) suffered severe symptoms, such as fever and vomiting more frequently, which is consistent with previous reports [11, 15]. It has also been reported that children with foodborne diseases caused by other serotypes of *Salmonella* present with more severe

symptoms [16].

The ingredients of *tamagotoji* were burdock roots, carrots, green onions, unpasteurized liquid eggs and ground meat. *Salmonella* has been frequently detected in hens' eggs [17–19]. It was reported that 8.3% of unpasteurized liquid eggs are contaminated by *Salmonella* in Japan [20]. By contrast, in the United States, 19% of unpasteurized liquid eggs were reported to be *Salmonella* Enteritidis-positive [21]. In addition, foodborne outbreaks of *Salmonella* have been found to be caused by unpasteurized liquid eggs and whole eggs [1, 6, 22–24]. In general, *tamagotoji* is not heated sufficiently; thus, it is most likely that among the ingredients of *tamagotoji*, unpasteurized liquid eggs were the causative ingredient in this foodborne outbreak. We consider 2 possible routes of infection. First, there is the possibility that the unpasteurized liquid eggs were not heated sufficiently. Employee B reported that the unpasteurized liquid eggs had been only partially defrosted, implying that, when cooked, the unpasteurized liquid eggs were not heated sufficiently to eliminate *Salmonella* Braenderup. Second, the investigation suggested that cross-contamination of heated *tamagotoji* could have occurred via the container that had previously come into contact with uncooked unpasteurized liquid eggs that may have been contaminated by *Salmonella* Braenderup. According to information from the egg preparation plant, however, there had been no other foodborne outbreak resulting from the unpasteurized liquid eggs manufactured by this plant.

We propose 3 recommendations to prevent foodborne disease caused by unpasteurized liquid eggs. First, when unpasteurized liquid eggs are used, they should be heated until the center reaches 75℃ for 1 min, as measured by a food thermometer, to eliminate *Salmonella*. Second, when food is prepared for high-risk persons such as children and the elderly, it is highly recommended that pasteurized liquid eggs be used instead of unpasteurized liquid eggs. This could prevent large, serious foodborne outbreaks and is consistent with the recommendation made by Morse *et al.* [25]. Third, kitchen utensils, equipment, and work surfaces should be washed thoroughly and disinfected adequately when they come into contact with unpasteurized liquid eggs. One report found that *Salmonella* Enteritidis can survive for 2 weeks or more on a stainless steel bowl [26].

Three limitations should be noted. First, we collected most of the food consumption and disease information using self-administered questionnaires. It is likely that the cases recalled their food consumption as accurately as possible, whereas the non-cases did not. Some non-cases might have responded that they ate all of the food items even though they consumed only a portion of the boxed lunch. This possible differential information bias could have led to the underestimation of the current findings. Second, the frequencies of subjective symptoms (*e.g.*, headache and chills) in children younger than 10 years old were likely to be underestimated because these symptoms were assessed by their parents or nurses. Therefore, although these frequencies were higher among adults, we should cautiously interpret these results. Note, however, that we defined the cases on the basis of more objective symptoms such as the number of diarrhea episodes and body temperature. Finally, most subjects had eaten all of the food items in the boxed lunch, which made it difficult to identify specific food item(s) as a cause of the foodborne disease.

In conclusion, this foodborne outbreak was caused by the consumption of *tamagotoji* in boxed lunches served by "catering company A" on August 9, 2008. It is most likely that unpasteurized liquid eggs contaminated by *Salmonella* Braenderup caused the outbreak. With regard to the infection route, there is a possibility that the unpasteurized liquid eggs were not heated sufficiently, and that cross-contamination of heated *tamagotoji* could have occurred by reuse of the container that had previously come into contact with uncooked unpasteurized liquid eggs contaminated by *Salmonella* Braenderup. It is highly recommended that unpasteurized liquid eggs should be heated sufficiently; that pasteurized, rather than unpasteurized, liquid eggs should be given to high-risk subjects, such as children and the elderly; and that kitchen equipment exposed to unpasteurized liquid eggs should be washed thoroughly to prevent cross-contamination.

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