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Salmonella Enteritidis outbreak associated with a school-lunch dessert: cross-contamination and a long incubation period, Japan, 2001

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SUMMARY

A *Salmonella* Enteritidis (SE) outbreak in Japan was investigated with an observational study, analytical epidemiology and bacteriological examination (including phage typing). The outbreak occurred among 96 schoolchildren, and was caused by SE phage type 1. The outbreak source was dessert buns served at a school lunch (RR 42·55, 95% CI 5·93–305·11, $P<0{\cdot}001$). The buns were probably cross-contaminated from eggs from a factory with a history of SE-contaminated products. The incubation period was longer than usual (3–16 days, median 8 days). A low contaminating dose may account for the long incubation period and low attack rate. Outbreak detection was hampered by the absence of routine *Salmonella* surveillance in Japan. The investigation was complicated by concurrent illnesses from other SE phage types. It was successful, in part, because adequate food samples were available for microbiological testing.

INTRODUCTION

Since 1989, *Salmonella* Enteritidis (SE) has been the most commonly isolated serotype of human salmonellosis in Japan [1]. Worldwide, when infections are traced to a source, SE infections are frequently associated with undercooked eggs or egg products [1–5]. Secondary infection following environmental contamination with SE can also occur [1].

On 10 October 2001, the Toyohashi City Health Centre (TCHC) was notified of a possible SE outbreak. By 12 October, 66 SE cases had been detected throughout Toyohashi city (population 370 000), Aichi prefecture. Most of the reported cases were in elementary and pre-schoolchildren. Routine surveillance for SE does not exist in Japan, so departure from expected numbers of infections could not be determined. However, the accumulation of SE cases in young children within such a short period was perceived to be abnormal and an investigation was begun. The initial hypothesis generating interviews identified school lunches, served to approximately 34 000 students in 52 elementary and 22 junior high schools in Toyohashi city, as a common food exposure among schoolchildren. No other common food item, grocery store, restaurant or other event was identified in the initial investigation.

In August 2001, a previous SE outbreak affecting at least 30 persons and possibly associated with steamed eggs from a delicatessen had occurred in

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this city. The delicatessen used liquid eggs from Factory A. While food samples from the outbreak were not available, SE was isolated from subsequent frozen and pasteurized liquid egg samples from Factory A, one of three major suppliers of liquid egg products in Toyohashi city.

To help determine the source and other characteristics of the October outbreak, the TCHC and the Field Epidemiology Training Programme (FETP) performed a series of epidemiological and laboratory investigations. These investigations especially focused on school lunches and factories producing liquid egg, including Factory A.

METHODS

Active case finding and descriptive epidemiology

From 10 October, investigators collaborated with the Association of Medical Practitioners of Toyohashi City and the Aichi Prefecture Health Department to identify all culture-positive patients. Cases were defined as persons who resided in the Toyohashi area who became ill after 1 September 2001, and had a stool culture positive for SE. Serotyping was done at TCHC. Each case or adult household member of a child case was interviewed, mainly by telephone, by TCHC food inspectors. Information was collected on symptoms, illness onset, and exposure before onset to egg or egg-related products, groceries, restaurants, food-related events, sports activities, and direct animal exposures.

Analytical epidemiology

On 18 October, a questionnaire about food-related events was sent to schoolmasters of all elementary schools, nursery schools and kindergartens in Toyohashi city by Toyohashi City Board of Education.

A retrospective cohort study of all elementary and junior high school students in Toyohashi was conducted to implicate possible common sources of exposure to SE among cases. Relative risks, Taylor series 95% confidence intervals for relative risks and χ^2 P values were calculated with Epi-Info, version 6 (CDC, Atlanta, GA, USA).

Microbiological investigations

Hospital laboratories sent all *Salmonella* O9 isolates, including SE, to the TCHC for serotyping. Food, environmental and stool samples from food handlers were cultured and serotyped at the TCHC Laboratory. *Salmonella* Enteritidis phage typing was performed at the Department of Bacteriology, National Institute of Infectious Diseases (NIID). Pulsed-field gel electrophoresis (PFGE) was done at Aichi Prefecture Laboratory and the Department of Bacteriology, NIID. Antibiotic susceptibility testing was done at Toyohashi City Medical Association Laboratory Centre.

Observational studies

Between 15 and 19 October 2001, the TCHC and FETP inspected three out of four school-lunch kitchens, four out of 28 elementary schools that reported SE cases, five bread/rice factories, a dessert factory (Factory B), three liquid egg factories (including the implicated Factory A).

Environmental/food samples and stool specimens of food handlers were taken from the observed facilities. These samples were tested according to standard procedures [6, 7].

RESULTS

Active case finding and descriptive epidemiology

From 1 September to 31 October 2001, 163 confirmed cases were identified throughout Toyohashi city; 36 were pre-schoolchildren or still at home, 110 were in elementary school, three were in junior high school and 14 were of high school age or older. The median age of all SE cases was 8 years (range, 8 months to 74 years); 87 (53%) were male. Twelve cases (mean age 5 years, range 1–10 years) were hospitalized; there were no fatal cases.

Of the 150 SE isolates from case patients, 102 (68%) were phage type (PT) 1, 36 were PT47, 10 were PT4, one was PT1b and one was untypable. PT47 was dominant in late September (Fig.) mainly among pre-schoolchildren. However, PT1 was dominant after 4 October (Fig.). All PT1 cases which occurred after 4 October were schoolchildren or their contacts.

The phage types of *Salmonella* isolates in the pre-school and elementary schoolchildren were different, and the two groups did not share common individuals or exposures. Since the pre-school and schoolchildren cases appeared to be separate outbreaks, this paper will focus on PT1 cases in the elementary and junior high schools. There were 93 SE PT1 cases from 28 out of 52 elementary schools

Table. *SE PT1 cases of elementary and junior high school according to lunch centre*

	Elementary school		Junior high school	
Lunch centre	No. of lunches served (no. of schools served)	No. of cases (no. of related schools)	No. of lunches served (no. of schools served)	No. of cases (no. of related schools)
West	5862 (11)	36 (7)	2709 (6)	1 (1)
East	5366 (12)	21 (6)	3502 (6)	2 (1)
North	5269 (14)	20 (7)	2695 (5)	0 (0)
South	5417 (13)	15 (7)	2914 (5)	0 (0)
Own kitchen	770 (2)	1 (1)	—	—
Total	22 684 (52)	93 (28)	11 820 (22)	3 (2)

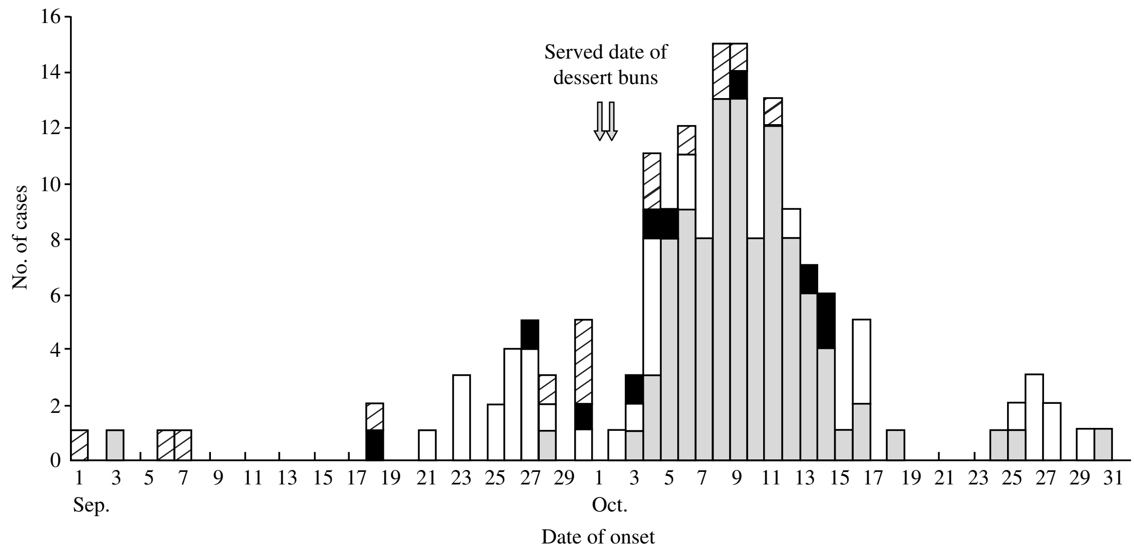

Fig. Epidemic curve of SE cases according to phage typing, Toyohashi, 2001 ($n=163$). ▨, PT1 ($n=102$); □, PT47 ($n=36$); ■, PT4 ($n=10$); ▨, others ($n=15$).

in the school district. There were 0–9 cases from each elementary school (median: one person) and three from two junior high schools (Table). PFGE and antibiotics susceptibility tests were identical in the PT1 cluster in schoolchildren.

All elementary schoolmasters in Toyohashi city were interviewed through a questionnaire survey. Elementary-school cases or their parents were interviewed by telephone with standardized questionnaires. The only common exposure we identified was eating the school lunch. No other common circulated foods or events were implicated.

Observational studies

Schools

Four school-lunch kitchens prepared side dishes for 21 914 students in 50 elementary schools and 11 820 students in 22 junior high schools in Toyohashi city. Two elementary schools prepared food in their own kitchens and served 770 students. The menu was basically the same among these kitchens but the date each meal was served was different to avoid a shortage of fresh foods. A total of 96 SE PT1 cases ate lunch meals prepared at four lunch centres and one at their own kitchen (Table).

Three lunch centres (East, West and South) were inspected. The kitchen inspections revealed no problems with sanitation, hygiene, or safety assurance of food. There were common, well-documented and microbiologically relevant procedures for handling eggs and chicken in all lunch centres. They served only cooked foods. Ninety-eight environmental samples taken mainly from areas associated with eggs and chicken were negative for SE. According to regulations, stool sampling was do done every

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2 weeks for all food handlers from September to October, and SE was not detected. The 352 stored school-lunch samples which had been served during the 2 weeks preceding this outbreak were negative for SE.

We observed hygienic practices and school-lunch serving procedures in four elementary schools. School lunch is basically served by the children themselves. Although table-cleaning procedures used when serving lunch were not adequate in some classrooms, all 98 samples from four elementary schools were negative for SE.

School-lunch food prepared outside of the schools

Bottled milk for the school lunch at all schools was from one factory and the same milk product was sold on the market. Five factories served bread and rice for all schools. In some of the bread/rice factories, sanitation practices were inadequate in the rice cooking line, storage containers for bread or rice, and hand-washing equipment. However, the case distribution was not related to the areas served by the problem factories. Forty-eight stool samples from food handlers, 63 environmental samples and seven food samples were obtained from the factories and were negative for SE.

Dessert buns served at school lunch

Dessert buns (chestnut paste wrapped with jelly made from starch) from Factory B were served at 52 schools (70%) on either 1 or 2 October. In the remainder of the schools the dessert buns were not served because of school events. Only dessert buns were served to all PT1 cases in the same condition and these buns were served only for the school lunches in Toyohashi city.

On 18 and 19 September, Factory B used unpasteurized liquid and shelled eggs from Factory A for a trial production of cream puffs. These were made by only a few staff and we could not obtain information about details of cream puff production and cleaning of the equipment used. As for the commercial production the steel machine components were normally washed and immersed in hot water after each procedure, and plastic components were washed and steamed. However, the investigators found that the bean-jam-filling machine, which was used for cream puff production, had many parts, so it was possible that cleaning may not have been adequate.

On 20 September, Factory B produced dessert buns in the same place using the same bean-jam-filling machine and plastic containers that had been used to make cream puffs. This was the first time Factory B produced a ready-to-eat dessert for a school lunch. Eggs were not used to make the dessert buns. The Factory B employees did not follow the recipe for dessert buns, as ordered by the school-lunch committee. This included heating the dessert buns to 93 °C for 5 min. While the machines heated the dessert buns after they were produced, the machine thermometer was broken at the time of our investigation, therefore the dessert bun temperatures were not measured. The dessert buns were then wrapped individually in cellophane bags and stored in a freezer before shipping. On 17 October 36 environmental samples and three food samples were collected. These and stool samples from all food handlers in Factory B were negative.

Liquid egg factories including Factory A

All three liquid egg factories in Toyohashi city were inspected. We found problems in production and sanitation in one factory (Factory A). Although 2 out of 11 egg-related samples from Factory A collected on 16 October were positive for SE PT47, SE PT1 was not isolated by us. One positive egg sample was pasteurized and frozen and one was not pasteurized. In Factory A, pasteurization of liquid eggs did not comply with regulations. A direct epidemiological linkage between SE PT47-positive samples and cases was not identified, because these positive samples were not from shipped products. Twenty-three environmental samples from Factory A were negative for SE.

Because of the August SE outbreak, frozen and pasteurized liquid eggs from Factory A were collected twice in September 2001 in the earlier investigations. SE PT1 was isolated from these samples and the PFGE pattern was indistinguishable from PT1 cases of elementary schoolchildren who were ill in October. A direct epidemiological linkage was not found between this lot of positive liquid egg and human cases. Twenty-six environmental samples and 10 egg-related samples from the other two liquid egg factories were negative for SE.

Further epidemiological investigations

Because of the investigation of liquid egg Factory A and dessert Factory B, a limited retrospective

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cohort study of schoolchildren in Toyohashi city was conducted. The exposed group comprised all children in schools where dessert buns were served. Consumption was assumed if the child was present at school on the day the dessert was served. We confirmed that all schoolchildren with SE PT1 attended elementary or junior high school on the day the dessert buns were served. All cases developed illness after the date buns were served. Considering the usual range for *Salmonella* incubation periods, we first analysed those who became ill within 5 days of exposure. This showed a significant association between illness and dessert bun consumption (RR 11·52, 95% CI 1·56–84·91, $P=0{\cdot}002$; relative risk was calculated adding one for each element because of 'zero' cells). We found cases only in schools that served the dessert buns. Other foods served at school lunches were not associated with illness.

One child became ill 23 days after the dessert buns were served. This case was from an elementary school with two other cases, therefore secondary transmission was likely. For the remaining 95 PT1 cases in the school outbreak, the incubation period was 3–16 days (median 8 days). The relative risk calculated from SE PT1 schoolchildren cases with a 3–16 days' incubation period was 42·55 (95% CI 5·93–305·11, $P<0{\cdot}001$), thus strengthening the association between dessert consumption and illness.

Attack rates calculated from all the SE PT1 cases with a 3–16 days' incubation period who were served buns were 0·5% (92/18 571) in elementary schoolchildren, 0·07% (3/4265) in junior high schoolchildren and 0% in teachers.

Additional microbiological investigation on the dessert buns

Because the analytical and descriptive epidemiology implicated the dessert buns, three more samples of dessert buns were tested in mid-December 2001, even though 10 buns in the first screening were negative for SE. Additional tests on the dessert buns were performed using the standard procedures. One of the three additional tests was positive for SE PT1. The PFGE pattern of the bun isolate was indistinguishable from those of the cases. This pattern rarely occurs in Japan (H. Izumiya, personal communication). The contamination dose was <30 organisms/100 g with the MPN (most probable number) method.

DISCUSSION

School-lunch system and foodborne outbreaks

In Japan, school-lunch systems have been provided for almost all elementary schools and some junior high schools. After the catastrophic *Escherichia coli* O157 outbreak (5591 cases) associated with school lunch in 1996 [8], food safety systems in school kitchens were rapidly developed. The numbers of outbreaks and related cases decreased. However, this outbreak shows that additional precautions should be emphasized for foods prepared in places other than the school kitchen.

Implicated food for PT1 cases

Dessert buns produced by Factory B were implicated as the source of PT1 cases among schoolchildren. This conclusion was supported by several findings. First, the analytical epidemiology revealed that dessert buns were associated with the SE infection among schoolchildren. Second, SE was isolated from the same single production of dessert buns, and the phage types of the isolates from cases and the dessert were the same. Third, the PFGE patterns from cases and the dessert were indistinguishable. This pattern is uncommon in Japan. Fourth, between 4 October and 18 October, PT 1 cases occurred only in exposed schoolchildren and family members. Fifth, the epidemic curve suggested a point-source outbreak; person-to-person transmission could not explain the outbreak. Finally, other common sources of infection could not be found.

Since the epidemiological and microbiological evidence argues that the buns were the outbreak source, we assume that cross-contamination may have occurred in the bean-jam-filling machine or containers. Unfortunately, it was not possible to verify this hypothesis through environmental sampling, interviews, or review of records. There was, however, evidence to suggest heating of the dessert buns was inadequate after they were produced.

Factory B had not previously handled eggs or produced dessert buns. Although the implicated liquid egg sample could not be tested, the liquid egg Factory A, which had provided Factory B with egg products, had several problems with safety assurance. This is the second reported outbreak in Japan associated with school-lunch desserts prepared outside of school kitchens. The previous one occurred in 1997 and involved 1371 cases from four prefectures. It was caused by SE-contaminated sponge cakes produced by a confectioner [1].

After the outbreak in 2001, the bean-jam-filling machine, containers, and other equipment were disinfected. Staff were instructed to keep the cooking manual available, and conduct ongoing hygiene education.

Incubation period and attack rate

The previously reported incubation period for salmonellosis is 6–72 h, usually approximately 12–36 h [9]. Our investigation found a longer SE incubation period than previously reported in the literature. In this outbreak, the incubation period was a median of 8 days (range 3–16 days) compared with the usual 12–36 h. We believe the incubation period in this outbreak is accurate for several reasons. First, person-to-person infection was not a major factor in this outbreak. Secondary transmission was documented in only three instances to younger members within households. The outbreak-related cases were not clustered in any one school, but were scattered in 30 schools, averaging only 3·2 cases per school. Sixteen schools had only one case. These isolated cases had the same incubation period (5–11 days, median 7 days) as the other cases in the outbreak. Second, consumption of dessert buns after the serving day was unlikely, because this was prohibited by schoolteachers. Third, environmental contamination from the dessert buns was unlikely because the buns were wrapped. Finally, there was no evidence of environmental SE contamination in schools. Although we could not completely rule out secondary transmission from asymptomatic or unreported cases for all cases, the most plausible explanation for onset of illness long after the implicated exposures is a long incubation period.

In other *Salmonella* outbreak investigations, the incubation periods have been long if the ingested dose was low [10–12]. We could not determine the contamination level of the dessert buns from the one positive sample, but the contamination level was probably low and variable, causing a long and wide incubation period. Although only 1 out of 13 samples was positive, the contamination was below the level of detection with the MPN method.

Attack rates calculated from reported cases were very low; 0·5% in elementary school and 0·07% in junior high school. We could not include the suspected cases in this calculation, because we could not investigate cases that did not attend clinics or did not submit stool specimens. From the data on buns tested, the prevalence of contaminated buns was estimated to be 7·7% (1/13). Using this to estimate the number of exposed people, the attack rates are still low (6·3% in elementary school and 0·9% in junior high school). Low-level contamination could be the reason for this. Host susceptibility may play a part in the different attack rates in elementary and junior high schools.

Usefulness of analytical epidemiology

In Japan, health authorities have not been familiar with the usefulness of analytical epidemiology. Our experience demonstrated the usefulness of cohort studies to identify a source of infection in the initial absence of supporting laboratory data. After realizing that dessert buns could be implicated through the findings of descriptive and analytical epidemiology, we decided to examine more dessert buns. Finally, from the additional bacteriological examination, we could isolate SE from the implicated food. To enable faster action, the importance of analytical epidemiology should be recognized. In this outbreak, and in many other institutional foodborne outbreaks in Japan, a large number of foods and environmental samples were tested. In future outbreaks, epidemiology should be used at an earlier stage to implicate suspected foods, and to allow more targeted testing of food and environmental samples.

The uniformity of food consumption in institutions frequently makes conventional epidemiological studies, such as case-control and cohort studies, difficult. However, in this outbreak, it was possible to identify large groups of exposed and unexposed schools. Dessert consumption was assumed if the child was present on the day the dessert was served. Because direct interviewing of ill and well children is difficult, it was not possible to directly verify this assumption. However, we believe this assumption is reasonable in Japan, because it is customary for all elementary schoolchildren to eat all foods served to them at school.

Usefulness of the food-retention system in Japan

Since 1997, the Ministry of Health, Labour and Welfare (MHLW, formerly MHW) of Japan advises restaurants and caterers to store portions of raw food materials and cooked dishes for more than 2 weeks at temperatures below −20 °C. Many large-scale cooking facilities and those that have social responsibilities, such as in schools, day-care centres and hospitals follow this advice. In this outbreak, many food items were kept in freezers under microbiologically ideal conditions. This system and the intensive bacterial examination performed in TCHC laboratory made it possible to identify suspicious foods and, accordingly, in conjunction with epidemiological investigation, identify contamination routes.

Importance of routine *Salmonella* surveillance

A significant limitation of *Salmonella* surveillance in Japan is the lack of reporting and investigation of individual cases of salmonellosis or sporadically occurring *Salmonella* isolates. While foodborne disease outbreaks, including those caused by *Salmonella*, are reportable in Japan, the lack of individual case reporting may hinder prompt outbreak detection.

The national and international distribution of foods, including processed foods, is expanding. Foods can be stored longer and transportation is more efficient than in the past. Consequently, outbreaks of food poisoning are becoming more complex [13]. To conduct better investigation of foodborne outbreaks, comparison of PFGE patterns and phage typing of related isolates with stored foods is very useful [14].

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