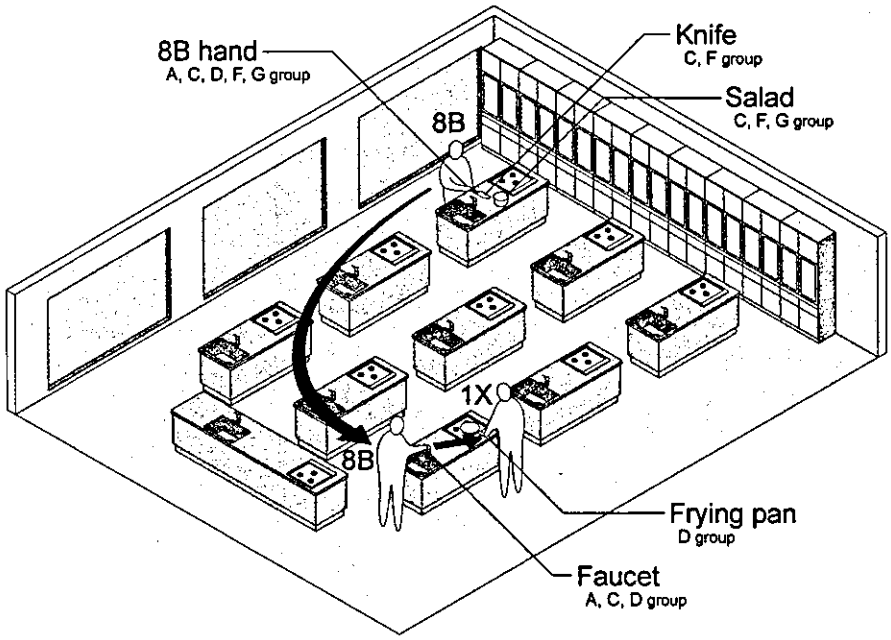

FIGURE 1. *Transmission of* S. aureus *originating from the hands of study participant 8B. The 14 strains of* S. aureus *originating from the hands of study participant 8B were divided into five ribogroups. Strains belonging to the same groups were also isolated from a knife used by study participant 8B, a salad prepared by study participant 8B, a faucet used by study participant 8B, and a frying pan handle touched by an unknown person (1X), who is thought to have also touched the faucet used by study participant 8B. The surmised diffusion of* S. aureus *from hands and utensils is illustrated above.*

of 739 trade school students. In the present study, *S. aureus* was isolated from the hands of 5 (19.2%) of 26 participants, which show the contamination rates are comparable to other studies.

Of the seven total ribogroups, five were related to the hands of participant 8B. Groups A, C, and D were isolated from one of participant 8B's hands before food preparation activities were also isolated from a faucet, so it was surmised that they were transmitted from participant 8B's hand to the faucet (Fig. 1). This faucet is located at a food preparation area different from the one used by participant 8B. Because participant 8B was in the back of the kitchen, adjacent to a window, further study was needed to clarify the link between participant 8B and the faucet. It was determined through video evidence and interviews with participant 8B and other participants that participant 8B rinsed his hands at the faucet before engaging in food preparation activities. Additionally, group D was isolated from a frying pan handle, so it is surmised that this ribogroup was transmitted there by someone (person 1X) who touched the faucet. Group C included ribogroups not only from the subject faucet but also from a knife and salad. All were also isolated from study participant 8B's hands, so it is supposed that they were found on participant 8B's hand. Groups F and G were surmised to have been similarly transmitted to the knife and salad.

Group C was already found on the hand of 8B before food preparation activities, and *S. aureus* was not detected in raw foodstuffs that were used for salad. Hand and knife samples were taken simultaneously, and the knife had been disinfected before use. Therefore, it is reasonable to consider that the salad and knife were contaminated by participant 8B.

On interviewing participant 8B, we found that he had atopic dermatitis with skin inflammation. A total of 4.60 log CFU per hand of *S. aureus* was collected from the hand of participant 8B during food preparation activities, and 2.0 log CFU per knife of *S. aureus* was collected from a knife handled by participant 8B. Although the knife was gripped by the other hand of a participant who was subjected to hand sampling, the transfer rate is roughly calculated as 0.25%. Chen et al. *(6)* reported that the bacterial transfer rate of hand to spigot was 0.01 (−1 SD), 0.16 (mean), and 1.95% (+1 SD), and the hand-to-lettuce bacterial transfer rate was 0.06 (−1 SD), 0.76 (mean), and 8.91% (+1 SD). The estimated transfer rate in our study was in the reported range.

Figure 2 illustrates how the possible transmission of *S. aureus* took place in the environment examined for the current study. It was suspected that groups A and C were transmitted from the hand of participant 8B to a salad and faucet; that group D was transmitted from the faucet to the frying pan via person 1X, whose hands were negative for *S. aureus* before cooking; and that group F was transmitted to a knife by participant 8B while performing food preparations.

Bacterial transmission in the kitchen occurs during food handling and preparation. Humphrey et al. *(14)* reported that *Salmonella* contaminated hands, utensils, and work surfaces during preparation of dishes in the kitchen. *Campylobacter jejuni (23), Campylobacter* spp. *(13),* and small round structured virus *(18)* have been identified in preinoculated foods to cause cross-contamination with other surfaces and sites in the kitchen. Our findings identify the ability of foodborne disease to become disseminated from food handlers.

In an immunological study of an *S. aureus*–based food poisoning incident, Wei and Chiou *(31)* isolated 27 strains of *S. aureus,* and used PFGE to type them. Their findings indicated that specimens collected from food poisoning patients and from a wound on the hand of a food preparation worker were of the same genotype, and they reported that *S. aureus* originating from the food preparation worker was the cause of that food poisoning. People who work in food preparation and who have a skin inflammation should not only wash and disinfect their hands but also wear gloves

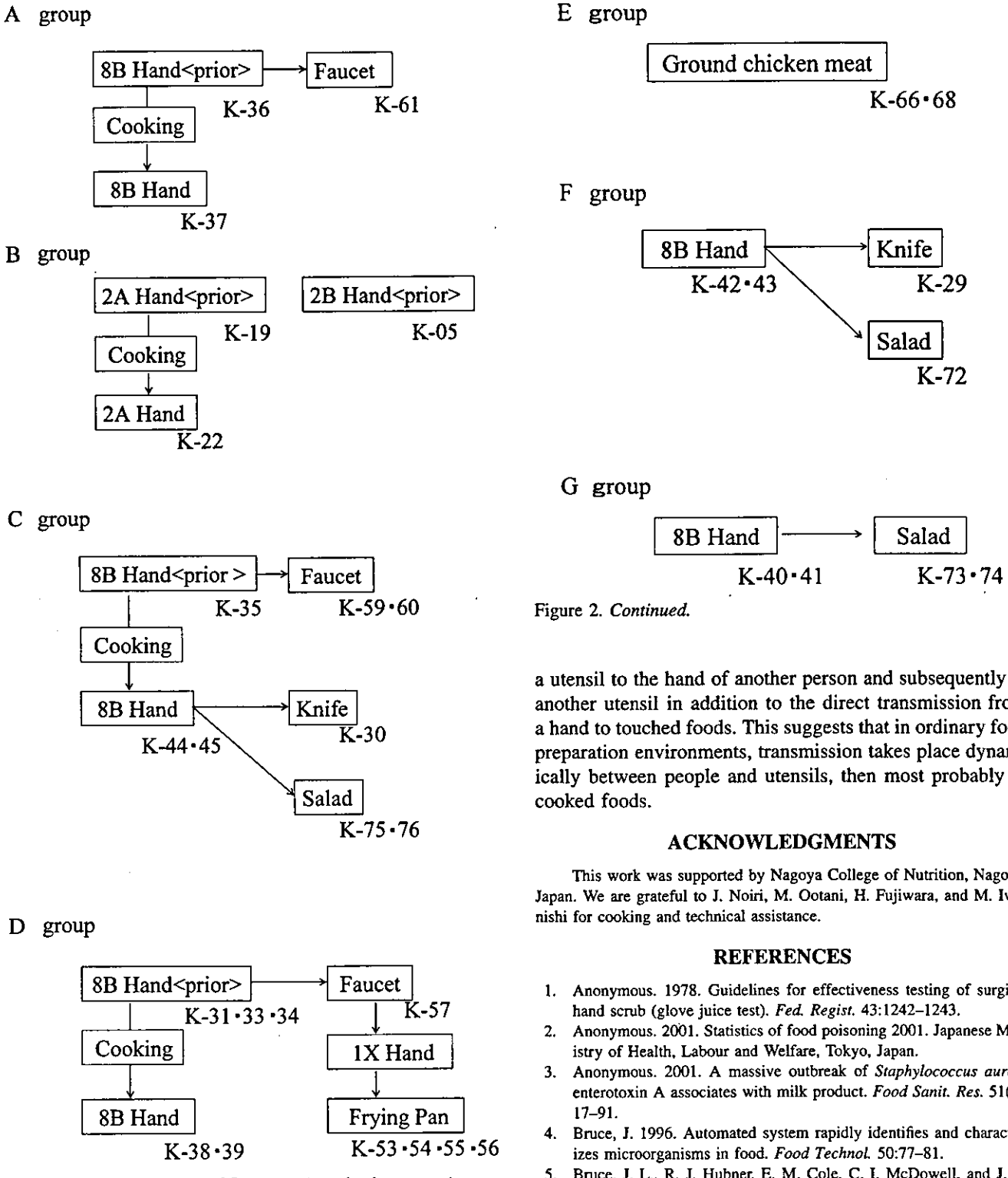

FIGURE 2. *Transmission of* S. aureus *in a food preparation environment. The transmission patterns were drawn by the results from riboprint pattern analysis, yielded 34 strains of* S. aureus, *and were then surmised based on* S. aureus *origins. K- is isolate code of strain, <prior> means prior to food preparation activities.*

Figure 2. *Continued.*

to prevent the contamination of bacteria from their hands to handling foods.

The current study has shown that it is possible for *S. aureus* to be transmitted from the hand of one person via a utensil to the hand of another person and subsequently to another utensil in addition to the direct transmission from a hand to touched foods. This suggests that in ordinary food preparation environments, transmission takes place dynamically between people and utensils, then most probably to cooked foods.

ACKNOWLEDGMENTS

This work was supported by Nagoya College of Nutrition, Nagoya, Japan. We are grateful to J. Noiri, M. Ootani, H. Fujiwara, and M. Iwanishi for cooking and technical assistance.

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Epidemiol. Infect. (2004), **132**, 873–879. © 2004 Cambridge University Press
DOI: 10.1017/S0950268804002778 Printed in the United Kingdom

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

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(*Accepted 12 June 2004*)

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, $P<0·001$). 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 370000), 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 34000 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*

Lunch centre	Elementary school		Junior high school	
	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)

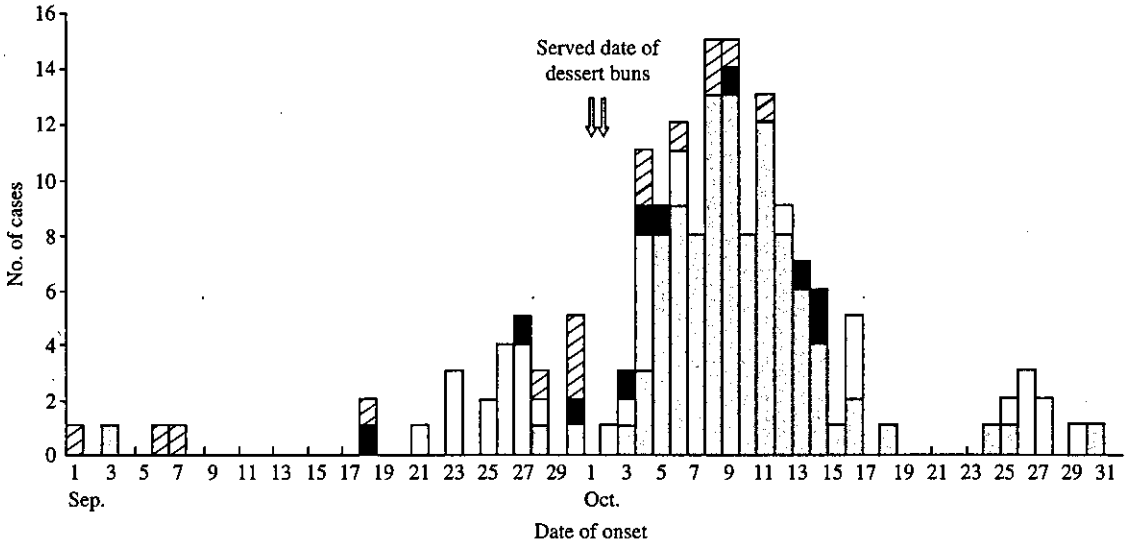

Fig. Epidemic curve of SE cases according to phage typing, Toyohashi, 2001 ($n=163$). □, PT1 ($n=102$); □, PT47 ($n=36$); ■, PT4 ($n=10$); ▨, others ($n=15$).

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

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

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, $P=0·002$; 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, $P<0·001$), 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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Journal of Food Protection, Vol. 67, No. 9, 2004, Pages 2024–2032

Archiving of Food Samples from Restaurants and Caterers—Quantitative Profiling of Outbreaks of Foodborne Salmonellosis in Japan

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MS 03-708: Received 25 August 2003/Accepted 12 March 2004

ABSTRACT

The Ministry of Health, Labor and Welfare (former MHW) of Japan issued a Directive in 1997 advising restaurants and caterers to freeze portions of both raw food and cooked dishes for at least 2 weeks. This system has been useful for determining vehicle foods at outbreaks. Enumeration of bacteria in samples of stored food provide data about pathogen concentrations in the implicated food. Data on *Salmonella* concentrations in vehicle foods associated with salmonellosis outbreaks were collected in Japan between 1989 and 1998. The 39 outbreaks that occurred during this period were categorized by the settings where the outbreaks took place, and epidemiological data from each outbreak were summarized. Characteristics of outbreak groups were analyzed and compared. The effect of new food-storage system on determination of bacterial concentration was evaluated. Freezing and nonfreezing conditions prior to microbial examination were compared in the dose-response relationship. Data from outbreaks in which implicated foods had been kept frozen suggested apparent correlation between the *Salmonella* dose ingested and the disease rate. Combined with results of epidemiological investigation, quantitative data from the ingested pathogen could provide complete dose-response data sets.

For characterization of the dose-response relationship between a foodborne pathogen and its host, epidemiological data can provide real-world information about the diversity in a population, the effect of food components, and the biological condition of the pathogen in the environment. However, quantitative data for the pathogen during outbreaks is limited primarily because it is difficult to find implicated food in microbiologically good condition at the time an outbreak is being investigated.

In March 1997, the Director General of the Environmental Sanitation Bureau of the Japanese Ministry of Health and Welfare issued a directive entitled "Control measures against foodborne outbreaks" *(7)* to all prefectural governors. In accordance with this directive, large-scale cooking facilities that prepare more than 750 meals per day or more than 300 dishes of a single menu at a time have been directed to save food for possible subsequent examination. Fifty-gram portions of each raw food material and cooked dish should be saved for more than 2 weeks at temperatures lower than −20°C. Although this directive is not mandatory, it is also applicable to small-scale kitchens in public facilities such as schools, daycare centers, hospitals, and other child-care and social-welfare facilities.

Most local governments in Japan have had regulations in place since 1972 requiring food storage for later examination, but the duration and the temperature varied among these regulations. Sometimes suspicious foods were lost under those regulatory systems, e.g., foods poosibly contaminated by pathogens with long latent periods. However, the new food-storage system has been useful primarily for incriminating food vehicles during disease outbreaks. Food stored under the new system can determine pathogen concentrations in the implicated food by enumeration of bacteria.

Foodborne *Salmonella* infections are of worldwide food safety concern. In the past decade, salmonellosis has been at the first or second most common foodborne disease in Japan in terms of both incident and patient numbers. Every year, 100 to 800 incidents of *Salmonella* infection, including outbreaks and sporadic cases with 5,000 to 17,000 patients per year, are reported. Incident numbers rapidly increased from 1996 to 1999, making salmonellosis one of the major problems of food safety in Japan. *Salmonella* Enteritidis has been the most predominant serovar in Japan since 1989 and accounted for 46 to 62% of all the isolated *Salmonella* serovars in the last 5 years *(18)*.

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In this report, epidemiological information and quantitative bacterial data concerning outbreaks of salmonellosis is summarized. The outbreaks were grouped by the setting of occurrence, and the characteristics of each exposed population are described. In addition, freezing and nonfreezing conditions of implicated foods prior to microbial examination were compared in terms of dose-response relationship, and the effect of the new food-storage system on the quality of bacterial data was evaluated.

MATERIALS AND METHODS

Individual outbreak investigations were performed by local authorities according to the Food Sanitation Law and related government ordinances, and microbiological tests were performed in local laboratories from August 1989 to September 1998. All the information was obtained from administrative foodborne outbreak reports provided by local governments. Among the reported outbreaks, those in which *Salmonella* concentration was identified in a single implicated food and those for which the infection rate in the exposed population had been determined were selected to analyze the relationship between ingested dose of bacteria per person and the infection rate.

The outbreaks were numbered and categorized by the settings where the outbreaks took place, such as schools, daycare centers, hospitals, restaurants, and confectionaries. For each outbreak, epidemiological data were compiled, i.e., serotype or phage type of *Salmonella,* food items in which the pathogen was detected, *Salmonella* concentration in those foods, food intake where possible, estimated *Salmonella* dose ingested per person, exposed population, number of patients, calculated infection rate, clinical signs, and food conditions prior to the bacterial examination. The laboratory techniques used for bacterial isolation and enumeration were also recorded. In some outbreaks, the food intake amounts were not described in the original report, although the number of bacteria per gram of cooked food was recorded. In such cases, certain assumptions concerning ingested food amounts were made after questioning local authorities, referring to cookbooks, or researching similar incidents for typical serving amounts. The methods for estimation of food intakes were also recorded. In the final analysis, the outbreaks were grouped by food conditions, i.e., whether the implicated foods had been kept frozen until subjected to microbial examination or had been stored in a refrigerator or at ambient temperature.

RESULTS

A total of 2,734 outbreaks caused by *Salmonella* were reported in Japan between 1989 and 1998 *(15),* and enumeration of implicated foods was conducted in 31 of these outbreaks. Those 31 outbreaks were numbered and categorized by the settings where the incident took place, and outbreak characteristics are summarized in Tables 1 through 5.

School lunches. Four outbreaks occurred at central kitchens of elementary schools in cities or towns (Table 1). In Japan, ages of pupils in an elementary school range from 6 to 12 years. Most schools serve the same lunch menu to all of their pupils on a single day. Most central kitchens cook a single combination of dishes each day for all schools to serve. Therefore, all the pupils in the school area are reasonably considered to be the exposed population in those outbreaks, unless uneven contamination is strongly suggested. The number of individuals exposed to the contaminated food was very large in this category, reflecting the characteristics of food distribution from these kitchens.

In outbreak data from the Japanese school lunch system, it was relatively straightforward to estimate the amounts of food taken by pupils in most cases, because the amount of each dish served to a pupil is defined based on nutritional calculations.

Daycare centers. Table 2 shows the data for the outbreaks that occurred at daycare centers. The ages of children in these facilities range from 0 to 6 years. In general, the size of the daycare center population is smaller than that of schools, and in most places each center has its own kitchen. Therefore the exposed populations were smaller than those in school outbreaks. Infection rate for outbreak 7 (daycare center) was higher than that for outbreak 4 (school) even though ingested doses were similar. This difference might reflect the susceptibility of the population.

In outbreak 6, both children and their adult nurses became ill, and the infection rate was calculated for both groups. In this particular outbreak, higher sensitivity of children was indicated.

Hospitals. In all three outbreaks that occurred at hospitals (Table 3), raw eggs were pooled and used to prepare the implicated foods without heating; both of these practices are considered risk factors for *Salmonella* Enteritidis infection.

Hospital inpatients are generally considered more susceptible to pathogens than are healthy nonhospitalized individuals. The infection rates in these outbreaks, however, were not very high compared with those in outbreaks that occurred in restaurants in which similar bacterial doses were ingested (Table 4). The influence of concurrent medications, including antibiotics, might be an important consideration.

Restaurants, hotels, caterers, and a company dormitory. Table 4 summarizes data from outbreaks associated with restaurants, hotels, caterers, and other business kitchens. Because these kitchens differ in size, the scale of each outbreak also differs considerably. The amount of ingested food in most cases was not specified in the original reports. Because small restaurants do not always need to follow the food-storage directive, food-storage conditions were also different among these outbreaks. Leftover portions of food kept in the refrigerator or at ambient temperatures were examined for a causative pathogen. In most of the outbreaks in this category, there were high infection rates even at low doses, despite the assumption that exposed people of this group were mostly healthy adults.

Confectionaries. Outbreaks associated with confectionaries and a bakery are summarized in Table 5. Similar to outbreaks associated with restaurants, the number of people involved in each outbreak is variable, from one small incident with 5 patients to a very large outbreak with 1,371 patients. In all the outbreaks summarized in this study, only one (outbreak 31) was caused by homemade mayonnaise in bread, resulting in one death.

TABLE 1. *Salmonellosis outbreaks associated with school lunches*

Outbreak no.	*Salmonella* serotype (phage type)	Vehicle	Bacterial concentration	Food intake (g)	Dose ingested (CFU/ person)	No. exposed	No. sick	Infection rate (%)
1	Enteritidis (PT 1)	Peanut dressing	<100 CFU/g[c]	80	<8,000	2,267	418	18.44
2	Enteritidis	Peanut dressing	4.3 MPN/g[d]	80[e]	344	1,320	179	13.56
3	Enteritidis (PT 22)	Beef and bean sprouts with sesame dressing	40 CFU/g[f]	22	880	10,552	967	9.16
4	Enteritidis (PT 1)	Spinach with peanut dressing	1.4 MPN/g	35	49	5,320	644	12.11

[a] D, diarrhea; S, stomachache; Fe, fever; V, vomiting; N, nausea; C, chill; Fa, fatigue; H, headache; O, others.
[b] SE, *Salmonella* Ententidis; SS, *Salmonella-Shigella* agar; EEM, *Enterobacteriaceae* enrichment mannitol; SBG, selenite brilliant green; MLCB, mannitol lysine crystal violet brilliant green agar; DHL, deoxycholate hydrogen sulfide lactose agar.
[c] Direct plate count, detection limit 100 CFU/g.
[d] MPN, most probable number.
[e] Food amount undefined in original report but assumed to be the same as that in outbreak 1.
[f] Direct plate count.

Effect of new food-storage system on determination of bacterial concentration. To evaluate the effect of the new food-storage system on the ability to determine bacterial concentration in implicated foods, all the outbreaks were divided into two groups: those in which the causative food was frozen and those in which the food was not frozen before microbial examination. Foods were frozen in 13 outbreaks at the daycare centers, schools, hospitals, and some of the restaurants; at most of these facilities the food was stored according to the new directive. However, in 18 outbreaks the foods were kept in refrigerators or the temperature condition prior to bacterial examination was unknown. Freezing and nonfreezing conditions were compared in evaluating the relationship between bacterial dose ingested and infection rate. Figure 1 includes the data from frozen materials, which suggest a correlation between bacterial dose and infection rate. However, the correlation was unclear for outbreaks in which the foods were not frozen (Fig. 2). Outbreaks in which bacterial concentration was not specified were not included (outbreaks 1, 8, and 30).

DISCUSSION

Because it is often difficult to obtain incriminated foods associated with bacterial disease outbreaks, quantitative information on these contaminated foods is limited *(1–6, 10–13, 16, 19, 20)*. In summarizing 31 outbreaks

TABLE 2. *Salmonellosis outbreaks at daycare centers*

Outbreak no.	*Salmonella* serotype	Vehicle	Bacterial concentration	Food intake (g)	Dose ingested (CFU/ person)	No. exposed	No. sick	Infection rate (%)
5	Enteritidis	Macaroni salad	1,000 CFU/g[c]	40[d]	44,000	152	52	34.21
6	Enteritidis	Chicken and eggs on rice	27 CFU/g[c,e]	150[e]	4,050	16[f] 117[g]	3[f] 50[g]	18.75[f] 42.74[g]
7	Enteritidis	Egg salad	0.78 MPN/g[h]	40	31.2	156	42	26.982

[a] For abbreviations, see Table 1, footnote *a*.
[b] For abbreviations, see Table 1, footnote *b*.
[c] Direct plate count.
[d] Food amount undefined in original report but assumed to be the same as that in outbreak 7.
[e] Mean of reported values.
[f] Adult nurses.
[g] Children under 6 years of age.
[h] MPN, most probable number.

TABLE 1. *Extended*

No. with clinical signs[a]									Food condition prior to testing	Maximum food amount analyzed (g)	Reported laboratory test methods[b]
D	S	Fe	V	N	C	Fa	H	O			
309	383	148	28	125	75		212	154	Frozen for 9 days	25 (qualitative)	10-g Sample negative for SE when 0.1 ml of 10× diluted sample plated on SS medium; SE detected from 25-g sample incubated in EEM
137	102	75	65	12	27	51	52	88	Unknown		Three-tube MPN method using SBG medium, confirmed on DHL
851	839	594	151	286	380	479	541		Refrigerated	0.01	0.1 ml of 10× diluted 10-g sample plated on MLCB
463	584	314	58	94	55	88	173		Frozen for 7 days	1.0	10-g Sample diluted to 1:10, then five-tube MPN method using EEM, SBG, SS, and DHL

caused by *Salmonella* in Japan in the last 10 years, we identified a wide range of outbreak types, from small to large, occurring at many kinds of facilities and affecting susceptible and healthy individuals. Outbreak data are valuable because they reflect events taking place in the real world. Nevertheless, compared with experimental data using animals and human volunteers, there is a high level of uncertainty associated with every step of data collection in outbreaks, such as food intake, number of exposed or sick individuals, food vehicle, food condition before it was examined for bacterial contamination, and methods and skills of laboratory technicians. Although in most of the outbreaks summarized here a single food vehicle was suspected, assumptions were needed to complete some data sets; in some original reports, food intake and therefore bacterial dose ingested were not determined. For those incidents, assumed values were selected from the literature, cookbooks, or other appropriate sources. Further refinement of epidemiological investigation techniques is needed.

In accordance with the new food-storage system being implemented in Japan, implicated foods are to be kept frozen for at least 2 weeks at lower than −20°C until they can be examined for microbial contamination. In a preliminary study, the number of *Salmonella* Enteritidis cells was not changed by freezing at −25°C for 2 weeks and was reduced by one log after 2 weeks at −20°C *(14)*. Therefore, we did not add any adjustment to the reported number of bacteria for further analysis in this report. When an aliquot of food is frozen close to the time when the other part of the food is eaten, the bacterial concentration in the frozen portion should reflect the concentration in the food actually ingested. Thus, the freezing of food aliquots should help reduce the uncertainty associated with bacterial concentration in implicated foods.

This food-storage system keeps samples of a suspected food vehicle in good condition for bacterial examination. The introduction of this system in Japan is not mandatory, and its cost is significant. Nevertheless, new freezers have been installed in many large cooking facilities and in most school kitchens to meet these sample-storage requirements,

TABLE 2. *Extended*

No. with clinical signs[a]									Food condition prior to testing	Maximum food amount analyzed (g)	Reported laboratory test methods[b]
D	S	Fe	V	N	C	Fa	H	O			
43	28	38	9		1	1	12		Frozen for 7 days	0.01	0.1 ml of 10× diluted 10-g sample plated on MLCB
49	46	6	6	6			9	10	Frozen for 7 days		Not reported
38	17	34	13						Frozen for 9 days	1.0	Five-tube MPN for the 10-fold diluted sample

TABLE 3. *Salmonellosis outbreaks in hospitals*

Outbreak no.	*Salmonella* serotype (phage type)	Vehicle	Bacterial concentration	Food intake (g)	Dose ingested (CFU/person)	No. exposed	No. sick	Infection rate (%)
8	Enteritidis (PT 1)	Tartar sauce	<100 CFU/g[c]	36	<3,600	126	36	28.57
9	Enteritidis	Natto with raw eggs	1,200,000 CFU/g[d]	50	60,000,000	191	45	23.56
10	Enteritidis (PT 4)	Grated yam diluted with soup	2,400 MPN/g[e]	60	144,000	343	75	21.87
11	Typhimurium	Grated yam diluted with soup	2,300 CFU/g[d]	60[f]	138,000	99	40	40.40
12	Typhimurium	Grated yam diluted with soup (with quail eggs)	40,000 CFU/g[d]	60[f]	2,400,000	79	39	49.37

[a] For abbreviations, see Table 1, footnote *a*.
[b] For abbreviations, see Table 1, footnote *b*.
[c] Direct plate count, detection limit 100 CFU/g.
[d] Direct plate count.
[e] MPN, most probable number.
[f] Food amount undefined in original report but assumed to be the same as that in outbreak 10.

TABLE 4. *Salmonellosis outbreaks associated with restaurants*

Outbreak no.	*Salmonella* serotype (phage type)	Vehicle	Bacterial concentration	Food intake (g)	Dose ingested (CFU/person)	No. exposed	No. sick
13	Enteritidis (PT 4)	Seared beef	2,000 CFU/g[c]	120	240,000	5	3
14	Enteritidis (PT 1)	Thin omelet	200 MPN/g[d]	30[e]	6,000	885	558
15	Enteritidis	Omelet	1,000 CFU/g[c]	150[e]	150,000	11	10
16	Enteritidis (PT 1)	Broiled tiger prawn with egg yolk	2,400 MPN/g	40	96,000	104	70
17	Enteritidis	Plain rolled egg	0.135 CFU/g[c]	80	11	363	198
18	Enteritidis	Scallop cream sauce	20,000 CFU/g[c]	50	1,000,000	38	30
19	Enteritidis	Natto with raw eggs	12,000[f]	60	720,000	9	9
20	Enteritidis	Grated yam diluted with soup	32,000[f]	60	1,900,000	123	113
21	Enteritidis	Spaghetti salad	120,000 CFU/g[c]	120	14,000,000	78	73
22	Enteritidis	Omelet	1,600 CFU/g[c]	150	240,000	103	57
23	Enteritidis	Bavarois	1,000[f]	100	100,000	123	100
24	Bareilly O7	Sauce for octopus pancake	2,800,000[f]	50	14,000,000	68	34
25	Oranienburg O7	Grated yam diluted with soup	50,000,000[f]	150	7,500,000,000	11	11

[a] For abbreviations, see Table 1, footnote *a*.
[b] For abbreviations, see Table 1, footnote *b*.
[c] Direct plate count.
[d] MPN, most probable number.
[e] Food amount undefined in original report but assumed to be the same amount suggested in cook books.
[f] Enumeration method not described in the original report, assumed to be CFU/g.

TABLE 3. *Extended*

No. with clinical sign[a]								Food condition prior to testing	Maximum food amount analyzed (g)	Reported laboratory test methods[b]
D	S	Fe	V	N	C	Fa	H			
35	21	27	2	7	4	3	6	Frozen for 6 days	25 (qualitative)	10-g Sample negative for SE when 0.1 ml of 10× diluted sample was plated on SS medium; SE was detected from 25-g sample that was incubated
35	17	39	1					Refrigerated for 1 day	0.01	0.1 ml of 10× diluted sample plated on MLCB and DHL
68	18	68	3	1	4	13	7	Frozen for 2 days	1.0	10-g Sample diluted to 1:10, then five-tube MPN method using EEM, SBG, SS, and DHL
31	9	34	7	2	4	1		Refrigerated for 6 days	1.0	10-g Sample diluted to 1:10, then five-tube MPN method using EEM, SBG, and DHL
34		35	5		4		4	Refrigerated for 2 days	0.01	0.1 ml of 10× diluted sample plated on DHL.

TABLE 4. *Extended*

Infection rate (%)	No. with clinical signs[a]									Food condition prior to testing	Maximum food amount analyzed (g)	Reported laboratory test methods[b]
	D	S	Fe	V	N	C	Fa	H	O			
60.00	3	3	3	1	1	3	3	1		Refrigerated for 2 days	0.005	0.05 ml of 10× diluted sample of 10 g plated on DHL
63.05	538	460	321	60	159	311	286	234		Refrigerated for 2 days	1.0	25-g Sample diluted to 1:10, then five-tube MPN method using EEM, SBG, and DHL
90.91	10	10	9	2	2	8	6	5	9	Frozen for 7 days	0.01	0.1 ml of serially diluted sample from 10 g plated on DHL
67.31	70	55	55	14	22	37	39	20		Frozen	0.01	0.1 ml of 10× diluted sample of 25 g plated on SS
54.55	173	105	72	13	32	46	76	37	131	Unknown		Positive on SGB from 10-g sample, negative from 1 or 0.1 g
78.95	28	24	28	4	12	18		15	9	Refrigerated		Diluted sample was directly plated
100.00	9	9	9	2	2	9	1	4	6	Frozen for 3 days	0.01	5-g Sample serially diluted, 0.1 ml of dilution plated on DHL
91.87	95	46	112	35	44	1		18		Refrigerated for 1 day	0.01	0.1 ml of 10× diluted sample plated on MLCB and DHL
93.59	71	58	51	18	41	44		38	20	Unknown		Directly plated on SS and DHL
55.34	55	40	51	4	2	23	10	29	22	Unknown		Directly plated on DHL
81.30	75	65	92	16	45	64	8	74		Refrigerated for 1 day	0.01	0.1 ml of 10× diluted sample of 10 g plated on DHL
50.00	34	29	27	4	12	12	23	20		Frozen at home	0.01	0.1 ml of 10× diluted sample of 10 g plated on MLCB and DHL
100.00	11	8	8	1	4	6	6	5		Refrigerated for 2 days	0.01	0.1 ml of 10× diluted sample of 10 g plated on MLCB and DHL

TABLE 5. *Salmonellosis outbreaks associated with confectioneries*

Outbreak no.	*Salmonella* serotype (phage type)	Vehicle	Bacterial concentration	Food intake (g)	Dose ingested (CFU/ person)	No. exposed	No. sick	Infection rate (%)
26	Enteritidis	Green-tea ice cream, cheese cake	19,000[c] <300[c]	200 200	3,800,000	5	5	100.00
27	Enteritidis (PT 4)	Three-layer cake	5 MPN/g[d] (sponge) 23 MPN/g (mousse)	30[e]	420[d]	5,103	1,371	26.87
28	Enteritidis	Tiramisu	1,600,000[c]	80	13,000,000	7,873	697	8.85
29	Enteritidis	Cake	6,000[c]	100	600,000	13	11	84.62
30	Enteritidis (PT 1)	Sherbet	<3,000[c]	80	Unknown	83	68	81.93
31	Enteritidis (PT 4)	Homemade mayonnaise in bread	460 MPN/g	120	55,000	2,907	498	17.13

[a] For abbreviations, see Table 1, footnote *a*.
[b] For abbreviations, see Table 1, footnote *b*.
[c] Enumeration method not described in the original report, assumed to be CFU/g.
[d] MPN, most probable number
[e] Ingestion of the same amount of sponge and mousse was assumed.

reflecting serious concerns for food safety by the staff in those facilities and by the Japanese public after the tragedy of large outbreaks caused by enterohemorrhagic *Escherichia coli* O157:H7 in 1996 *(17)*.

Additional efforts by local governments to conduct quantitative examinations on bacterial load in stored food portions during outbreaks could bring about better dose-response modeling in hazard characterization of risk assessment. Some Japanese outbreak data were used in the international *Salmonella* risk assessment conducted by FAO and WHO *(8, 9)*. An adequate food storing system, quantitative analysis of outbreaks, and complete epidemiological studies could effectively improve food safety by adding considerable information to microbiological risk assessment.

The outbreak data presented here indicate a correlation between the *Salmonella* dose ingested and the infection rate when the stored foods were frozen. In contrast, outbreaks where the foods were not frozen resulted in data points that are distributed widely in the dose range, and the relationship seemed unclear. Time and temperature histories for nonfrozen foods between the time the original food was consumed and the time when the stored sample was examined for bacteria differ greatly. In some of the foods, *Salmonella* could have grown before the examination, and the resulting bacterial concentration might overestimate the dose that was actually consumed. It is also possible that competition with coexisting flora could diminish *Salmonella* numbers during storage. Possible quantitative changes in *Salmonella* concentration during the storage period increases the uncertainty associated with bacterial dose ingested for outbreaks where samples of the implicated foods

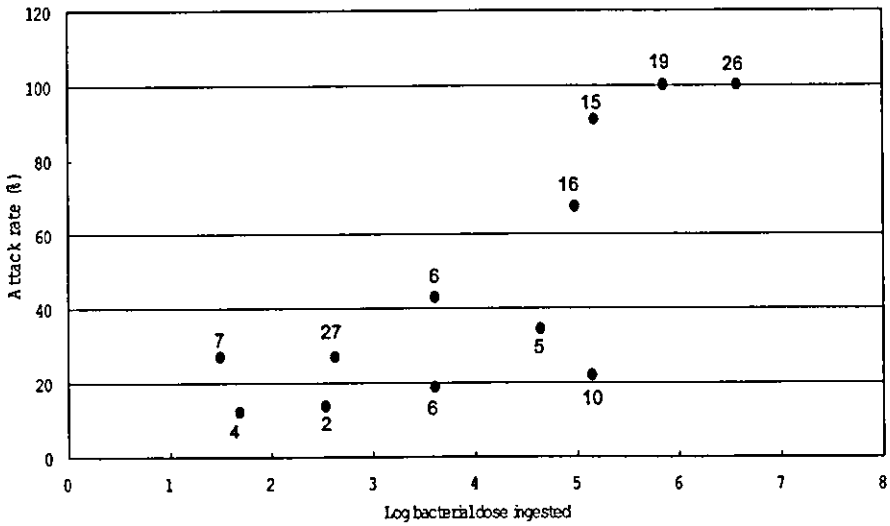

FIGURE 1. *Dose-response relationship in salmonellosis outbreaks in which food samples were kept frozen before being subjected to microbiological examination. Each dot represents data from an individual outbreak:* Salmonella *dose ingested and infection rate. Outbreak number is indicated.*

TABLE 5. *Extended*

No. with clinical signs[a]									Food condition prior to testing	Maximum food amount analyzed (g)	Reported laboratory test methods[b]
D	S	Fe	V	N	C	Fa	H	O			
5	5	4	1	1	3	5	2	7	Frozen		Unknown
923	1,035	791	285	377	548	652	1,035		Frozen		10-g Sample diluted to 1:10, then MPN method using SBG and MLCB
665	545	627	183	158	201	89	183	77	Refrigerated	0.01	0.1 ml of 10× diluted sample of 10 g plated on MLCB and DHL
10		10	4	7		3	10		Refrigerated for 2 days	0.005	0.05 ml of 10× diluted sample directly plated on DHL
65	49	63	11	12	19	23	30	14	Frozen	0.01	0.1 ml of 10× diluted sample of 25 g plated on MLCB and SS
447	375	353	83	150	135	104	152		Ambient temperature for 5 days	1.0	10-g Sample diluted to 1:10, then MPN method using EEM, SBG, and DHL

have not been frozen. Even when time and temperature conditions are accurately recorded, it is very difficult to predict changes in bacterial concentration and to estimate the concentration prior to storage, i.e., at the time of consumption.

It was not the purpose of this study to develop a new dose-response model for *Salmonella* infection or to evaluate appropriate curve-fitting functions using these data points. The data presented here are meant to be a source for the development of a new model or for improvment of existing models. However, some cautions for further statistical analysis are warranted by careful examination of the epidemiological data. For such categories as number of exposed individuals, the data differ among real outbreaks. Here, infection rates were simply calculated as the proportion of sick individuals in the exposed population. Therefore, the individual data points in Figures 1 and 2 do not indicate the numbers of people involved. In developing a dose-response curve from these data sets, we must consider the difference between the information provided from outbreaks with, for example, 5 exposed people and from those with 3,000 exposed people, even if the same infection rates are observed. One possible solution to this problem might be estimating the likelihood of infection using the number of exposed and number of sick individuals instead of a simple calculation of the proportion of sick people. This approach would allow inclusion of information derived from outbreaks of different sizes in the extrapolated dose-response curve.

When attempting an international or regional risk assessment, data often are collected from various countries where food consumption patterns or food types are sometimes quite different. Many Japanese outbreak data were used in the international *Salmonella* risk assessment. Although western cuisine is popular in Japan, there are still many traditional foods and some of them have been involved in the outbreaks. In the process of hazard characterization, food materials should be taken into account because some of them can inhibit or enhance the survival of pathogens in the food or even in the intestinal tract of the host. Unique food materials or production-to-serving processes in some countries could influence the output when

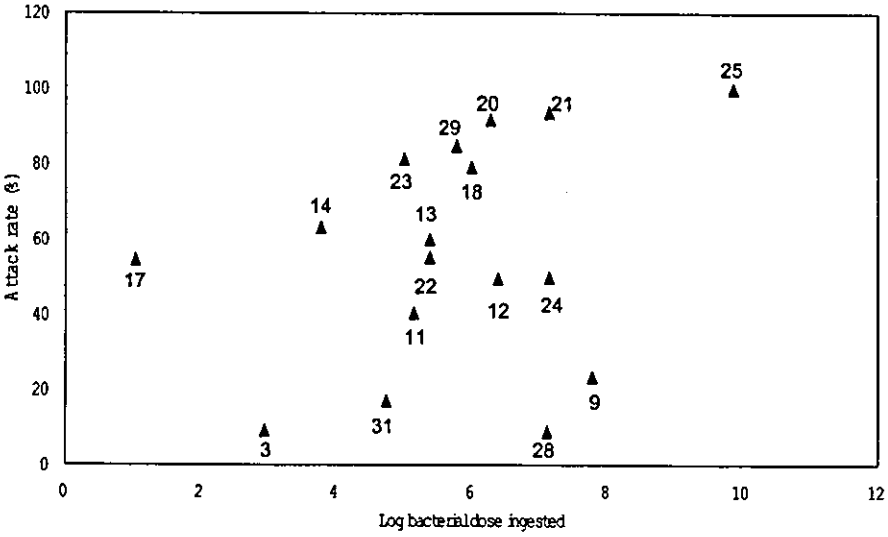

FIGURE 2. *Dose-response relationship in salmonellosis outbreaks in which food samples were not frozen before being subjected to microbiological examination. Each triangle represents data from an individual outbreak:* Salmonella *dose ingested and infection rate. Outbreak number is indicated.*

global data are combined for a risk assessment. During the Food and Agriculture Organization/World Health Organization risk assessment, further analysis into the effects of food ingredients on dose-response assessment were not conducted. However, such in-depth investigation into the identity of implicated foods is necessary in international or regional risk assessments.

The information presented here can be subjected to further analysis from different viewpoints in various research fields. Summaries of Japanese outbreak data relating to other pathogens such as enterohemorrhagic *E. coli, Staphylococcus aureus, Bacillus cereus, Clostridium perfringens* are now in preparation.

ACKNOWLEDGMENTS

This study was supported by Grants for Health Science from the Ministry of Health and Welfare, Japan. The authors thank authorities in every local government and laboratory for kindly providing information in administrative documents and for permission to summarize those data for scientific publication. The authors also thank Anna M. Lammerding and Aamir Fazil (Health Canada) and Eric D. Ebel (U.S. Department of Agriculture) for their valuable comments.

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日本食品微生物学会雑誌 Jpn. J. Food Microbiol., 21(3), 193-200, 2004

＝原 著＝

調理施設から採取された黄色ブドウ球菌のRAPD-PCR, BSFGEおよびPFGEによる遺伝子多型解析

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(受付 平成15年12月12日)
(受理 平成16年 6月28日)

Genotyping of *Staphylococcus aureus* Collected from Food Preparation Facilities Using Random Amplified Polymorphic DNA Analysis, Biased Sinusoidal Field Gel Electrophoresis and Pulsed-Field Gel Electrophoresis

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Sixty-four *Staphylococcus aureus* isolates from the hands of study participants, foodstuff, prepared food, cooking utensils, and cooking equipment at food preparation facilities were genotyped by Randomly Amplified Polymorphic DNA (RAPD) analysis, Biased Sinusoidal Field Gel Electrophoresis (BSFGE) and Pulsed-Field Gel Electrophoresis (PFGE).

The results of the genotyping revealed that diffusion of *S. aureus* had occurred. *S. aureus* originating from the hands of a study participant was transmitted to a cooking knife and to prepared food (salad). Furthermore, another transmission was found by an unknown person between a frying pan and a faucet.

The strains were divided into 11 different genetic types by RAPD analysis based on two primers, and 12 different genetic types by BSFGE. The PFGE types were completely consistent with the BSFGE types. The types obtained by both techniques were similar for all strains examined. However, in RAPD typing, 8 types (73%) were consistent with the PFGE types, but the other 3 types were not consistent. RAPD provided less discrimination than PFGE.

Key words: *Staphylococcus aureus*, RAPD-PCR, BSFGE, Food preparation facilities, Cross contamination

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