

***Salmonella enterica* Serovar London Infections Associated with Consumption of Infant Formula**

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Epidemiologic studies were conducted on 31 cases of *Salmonella* group E infection detected in 2000 through a laboratory-based pathogen surveillance in Gangwon Province, Korea. Data were collected on the environmental exposures and the patients' foods, including the brand(s) of milk consumed before the onset of diarrhea. The patients' medical records were also reviewed. All of the patients were infants under 10 months of age except one 7-year old child.

Surprisingly, all of the infants were fed with infant formulas from Company A, although two infants were fed with infant formulas from both Company A and Company B. Antimicrobial susceptibility test and pulsed-field gel electrophoresis (PFGE) were performed in 25 out of 31 isolates from the patients and in 1 isolate from an opened packet of infant formula collected from the home of an infected infant. All of the 26 isolates were *Salmonella enterica* serovar London. They showed a single PFGE pattern, and all of the isolates were susceptible to the 18 antibiotics tested. The causative agent of the salmonella outbreaks in the Gangwon Province and its surrounding areas was *Salmonella* London, and the highly likely source of the infection was infant formula from Company A.

Key Words: Salmonellosis, infant, formula, Korea

INTRODUCTION

For many centuries, fluid milk has been evapo-

rated to provide an easily portable and readily reconstituted food, and large-scale commercial drying of milk has been practiced for about one hundred years. The potential for dried milk to be a vehicle for staphylococcal and salmonella food-poisoning has been recognized.^{1,2} Rowe et al.² reported that raw milk is often contaminated with *Salmonella* strains through the spray drying process with insufficient heat treatment or inappropriate evaporation process. *Salmonella* infections through infant formula have been reported since 1950 in many countries including England, the US, Canada, and Spain, and an outbreak due to *Salmonella* Anatum was reported in England and France in recent years.³ In studies carried out by the US Food and Drug Administration, the US Department of Agriculture, and various state health departments, several *Salmonella* serotypes have been isolated from the products and environments of dry-milk establishments in many states, suggesting that dried milk may be an important factor in the salmonella problem.⁴

Through a laboratory-based pathogen surveillance in Gangwon Province, Korea, 31 cases of *Salmonella* group E infection were detected between April and December, 2000. All but one of these cases occurred in infants under 10 months of age who resided in different geographical parts of the Province and its surrounding areas. The ages of these patients, in particular, suggested the involvement of a baby food product. In the present study, we describe the epidemiologic and

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the laboratory studies that led to the recognition of *Salmonella enterica* serovar London infections in association with an infant formula in Korea.

MATERIALS AND METHODS

Epidemiologic investigation

Epidemiologic studies were conducted on 31 *Salmonella* group E-infected cases (a total of 29 patients with 2 patients being infected twice). Information was obtained about the date of onset, combined symptoms such as fever, diarrhea, and vomiting, duration and severity of illness, illness of other families contacted, environmental exposures, and the patients' foods, including the brand(s) of milk and dairy products consumed before the onset of diarrhea. Structured questionnaires were completed by interviewing the infants' parents personally or by telephone. Medical records of the patients were also reviewed.

Isolation and identification of *Salmonella* London

Twenty-five out of 31 *Salmonella* group E isolates from the cases were gathered. *Salmonella* strains were finally confirmed using an API 20E kit (bioMerieux, Marcy-l'Etoile, France) and antisera (Difco, Detroit Michigan, USA). Twenty-one samples of the infant formulas purchased from local retail stores and 2 samples of milk powder in an opened can of infant formula from the home of infected infants were gathered. The pre-enrichment and direct enrichment methods were applied to isolate outbreak strains of *Salmonella*.

For the pre-enrichment method, 25 grams of powdered sample was obtained aseptically and placed in a sterile bag containing 225 ml of buffered peptone water (pH 7.2). After mixing in a stomacher completely, the mixture was incubated at 35°C for 12 hours and 10 ml of the culture was placed in 90 ml of Tetrathionate broth (Difco) supplemented with brilliant green (10 ppm), and then incubated at 41.5°C^{5,6} for 18 hours. Then the culture was streaked on *Salmonella*-*Shigella* Agar medium (Difco) and Xylose Lysine Brilliant Green Agar medium (Difco) to isolate a single colony.⁷

Salmonella suspected colonies were streaked on Kligler Iron Agar medium (Difco). *Salmonella* strains were finally confirmed using an API 20E kit (bioMerieux) and the serological test. The direct enrichment method was done by suspending 10g of each powdered sample of infant formula directly into 90ml of tetrathionate broth (+ BG 10ppm), followed by the same procedures as the pre-enrichment method.

Antibiogram and molecular typing

Antimicrobial susceptibility tests and pulsed-field gel electrophoresis (PFGE) were done with 26 *Salmonella* London isolates (25 out of 31 isolates from the cases and 1 from the powder in an opened can of infant formula from the home of case #10). Antimicrobial susceptibility test was done by the NCCLS disc diffusion method.⁸

The following kinds of disc were used: amikacin, ampicillin, amoxicillin/clavulanic acid, ampicillin/sulbactam, carbenicillin, chloramphenicol, ceftriaxone, cefotaxime, cephalothin, ceftiofloxacin, ciprofloxacin, gentamicin, kanamycin, streptomycin, sulfamethoxazole/trimethoprim, nalidixic acid, tetracycline, and ticarcillin. For the PFGE pattern analysis, genomic DNA of the *Salmonella* strains was digested with *Xba* I (New England Biolabs, MA, USA), and the fragments were separated with a CHEF MAPPER (Bio-Rad, Hercules, CA, USA) under the following conditions: initial switch time of 2.2 sec, final switch time of 63.8 sec, and run time of 16 hours. After ethidium bromide staining and photographing, the patterns were visually compared.

RESULTS

Salmonella London enteritis occurred over a nine-month period beginning in April, reaching a peak in July, and then declining, with the last case being reported in December 2000 (Fig. 1). Among the total 31 cases under the investigation, all of the patients were infants under 10 months of age, except one 7-year old child (Table 1). Infections were more prevalent in girls than in boys and the outbreak cases occurred at diverse geographical locations, with 26 cases occurring in different

parts of Gangwon Province (one patient was actually a non-resident but was infected after visiting her grandmother's home in Gangwon Province) and 5 cases developing in the surrounding areas of Gangwon Province (Fig. 2). All patients experienced the characteristic symptoms of salmonella enteritis, including fever, diarrhea and vomiting. Bloody diarrhea was also developed in 14 cases but there were no deaths. The interval from the time of onset to hospitalization ranged from 0 to 17 days, with a median of 2 days. The period of hospitalization ranged from 1 to 10 days, with a median of 5 days. In a single case, a patient aged 7 years had a history of contacting an unconfirmed patient with diarrhea. The feeding method for the infected infants was recorded, and breast-feeding was used in 5 cases with supplementary bottle-feeding. In most cases, infants were fed solely with a bottle (25 out of 30 cases). Surprisingly, all of the infants who developed diarrhea were fed with the infant formula manufactured by Company A (only two cases were fed with infant formulas from both Company A and Company B) (Table 2). The 7 year-old girl was not fed any infant formula but she consumed carton-packaged liquid milk from several dairy companies.

All of the 26 isolates were *Salmonella* London. They showed a single PFGE pattern (Fig. 3), and all of the isolates were susceptible to the 18 antibiotics tested. No *Salmonella* organism was isolated in the 21 samples of infant formulas

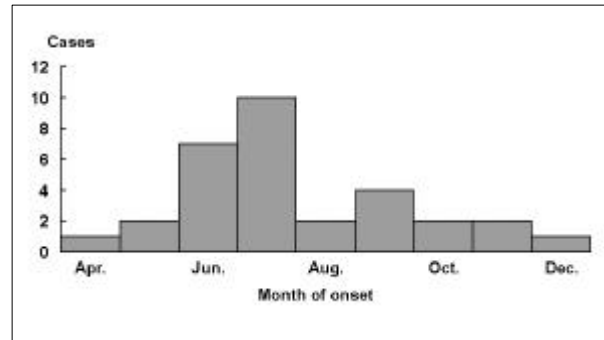


Fig. 1. Monthly distribution of *Salmonella* London cases by illness onset time.



Fig. 2. Geographical distribution of *Salmonella* London cases in the Gangwon Province, Korea.

Table 1. Age and Sex Distribution of Patients in *Salmonella* London Cases

Age (Months)	Cases			%
	Male	Female	Total	
<1	-	1	1	3.2
1	4	4	8	25.8
2	4	1	5	16.1
3	1	4	5	16.1
4	-	5	5	16.1
5	-	4	4	12.9
6	1	-	1	3.2
9	1	-	1	3.2
85	-	1	1	3.2
Total	11	20	31	100.0

Table 2. Distribution of *Salmonella* London Cases by Infant Formula Brands

Brand	Cases*	No. of tests with infant formula purchased at retail stores
Total	30	21
A	30	9
B	2	4
C	-	4
D	-	4

*Two cases were fed with formula from two companies; A and B.

purchased from retail stores within the inflicted area.

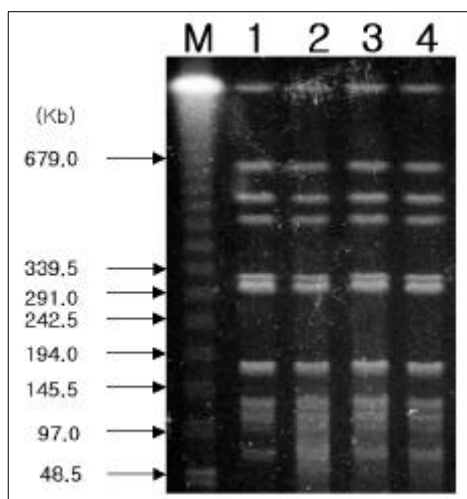


Fig. 3. PFGE profiles of *Xba* I-digested genomic DNA from isolates of *Salmonella* London. Lane M, lambda DNA ladder as a molecular size marker; Lanes 1 to 3, isolated from patients; Lane 4, isolated from infant formula consumed by a patient.

DISCUSSION

Following the confirmation of one case of *Salmonella* E infection in a 3-month-old infant, 31 cases of *Salmonella* London infection were reported at different geographical locations in Gangwon Province and its surrounding areas between April and December, 2000. A particular feature of this outbreak was that all of these patients were aged younger than 10 months, except one 7-year old child. This epidemiologic pattern of cases occurring with the notable

involvement of infants under 10 months of age pointed toward some contaminated vehicle infection to which infants were exposed preferentially. Furthermore, the long duration of the outbreak strongly suggested either that the vehicle of infection had a long shelf life or that continued contamination was occurring. The relatively small number of cases suggested a slight or sporadic contamination of the product.

The investigation into what kind of foods might have been involved was complicated by the long intervals between the dates of onset and the start of the investigation, which ranged from 3 weeks to 4 months. Itemized dietary histories and important information such as lot numbers of the infant formulas consumed were impossible to obtain. A request was made for the brand names of infant formula used in the families of the infant cases. They all were fed with the formulas from Company A (two infant patients were fed with products manufactured by both Company A and B), although there were 4 products commercially available in the Korean market. The results of serologic analysis showed that all of the 26 isolates were *Salmonella* London. They showed a single PFGE pattern, and all of the isolates were susceptible to the 18 antibiotics tested. Considering the epidemiologic and microbiologic results, this outbreak was evoked by a common vehicle, probably the infant formula products of Company A.

It was difficult to determine the time of contamination to the infant formula consumed by the infected infants (whether during the manufacturing process or after opening the package for consumption). However, the infant formula might have been contaminated during the process at the factory, because the *Salmonella* strain isolated

from the infant formula was grown only in the selective medium after pre-enrichment, but not by the direct enrichment method. Normally, sublethally injured organisms need the pre-enrichment procedure to multiply to the level of cells needed for secondary selective culture.⁹

No *Salmonella* organism was isolated from the 21 samples of the intact product of infant formula purchased from the local retail stores. The very low number of *Salmonella* London organisms, if they were present in the milk powder, may explain the difficulty experienced in isolating the bacteria from the limited number of retail samples (a total of 9 samples from Company A). According to an earlier study, recovery of coliform organisms from dried milk decreased with increasing storage time and organisms were not recoverable after 9 months.¹⁰

Furthermore, some other studies have shown that there is a slight reduction in *Salmonella* surviving after 15 weeks in dried milk stored at 4°C, because the rate of destruction of the organisms increases in powder stored at higher temperatures.^{11,12} In a survey of 200 milk-drying plants in the United States, 1% of 3,315 product samples and 8.2% of 1,475 environmental specimens were reported to be contaminated with *Salmonella*.²

The number of cases implicated, including the cases not detected in the Gangwon Province, is probably much higher than the number of confirmed cases in this study, since most clinical laboratories in Gangwon Province do not routinely investigate pathogenic bacteria including *Salmonella* spp. from patient's stool. To our knowledge, this is the first study on salmonellosis that has been strongly linked to infant formula in Korea.

We faced several limitations in this investigation. Particularly, the time delay between the beginning of the illnesses and initiation of the investigation may have contributed to recall bias. However, it is unlikely that the parents of the patients who answered us could not remember the brand name of the infant formula. According to data from the KNIH and Provincial Institutes of Health & Environment, a total of 61 strains of *Salmonella* London were isolated in Korea in 2000 and they were identified predominantly in the

stool of infants. In conjunction with the salmonella outbreaks, we heard from one staff of Company A that the Korea Food and Drug Agency investigated the manufacturing lines of Company A in August 2001. However, no information is available on this matter.

The causative agent of the salmonella outbreaks in Gangwon Province and its surrounding areas in 2000 was *Salmonella* London, and the highly likely source of the infection was infant formula from Company A. We believe that the cases of this study were a part of a *Salmonella* London epidemic in Korea.

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