

## Contamination level and ingestion dose of foodborne pathogens associated with infections

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### SUMMARY

Intake of a small dose of foodborne pathogens can cause infection. In this study, an estimation of the infectious dose of the pathogens was obtained by conducting microbiological risk assessments. The contamination levels of foodborne pathogens were analysed in 17 outbreaks of *Salmonella*, *Escherichia coli* O157, enterotoxigenic *E. coli*, *Vibrio parahaemolyticus*, and *Campylobacter jejuni* occurring in Japan between 2004 and 2006. The infectious dose was estimated in 14 of the 17 outbreaks utilizing existing data. In three outbreaks of *Salmonella* infection in which the infection rate was 89–100%, the dose of the ingested pathogens was estimated to be 259 000–14 000 000 000 c.f.u. In other outbreaks of *Salmonella* infection, the infection rate and dose of the ingested pathogens were 10–66·4% and 81–1560 c.f.u. or most probable number (MPN), respectively. The ingested *Salmonella* dose is likely to be related to the infection rate; however, storage conditions should be taken into account when making this determination. In an outbreak of *E. coli* O157 infection, the infection rate and ingestion dose were 100% and 2 to <9 c.f.u., respectively, while in an outbreak of enterotoxigenic *E. coli* infection, they were 93% and 25–1000 c.f.u., respectively. Finally, in an outbreak of *C. jejuni* infection, the infection rate and ingestion dose were 37·5% and 360 MPN, respectively. These results will be particularly valuable for risk assessment.

**Key words:** Contamination level, foodborne infections, foodborne pathogen, infectious dose.

### INTRODUCTION

Microbiological risk assessments have been developed to establish food safety priorities, strategies and practices [1]. The determination of the infectious dose of a foodborne pathogen is one of the most important and fundamental steps in evaluating outbreaks of foodborne infections. However, only a few studies have evaluated the infectious dose of foodborne

pathogens, such as *Salmonella*, *Escherichia coli* O157, enterotoxigenic *E. coli*, *Vibrio parahaemolyticus* and *Campylobacter jejuni*. In some of these studies, volunteers ingested pathogens, such as *Salmonella*, at various doses, and the ensuing symptoms were then observed [2–4]. As expected, large doses of pathogens produced overt disease. However, the level of contamination by foodborne pathogens that was determined or estimated in some actual outbreaks has been much lower. In a *Salmonella* outbreak [5], ice cream was contaminated with *Salmonella* at a concentration of <0·38 c.f.u./g. The infectious dose, based on the consumption of a single ice cream cone,

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appeared to be no more than 28 c.f.u. There is, therefore, a serious discrepancy between studies in volunteers and actual foodborne infections. Microbiological analysis performed under actual conditions of foodborne infection is required to elucidate the true infectious dose of the foodborne pathogen.

In this study, we assessed the contamination levels of foodborne pathogens in vehicle food items consumed during 17 outbreaks. We also estimated the doses of the ingested pathogens in terms of the contamination level of the pathogens in food together with the amount of food ingested by the patients. Because knowledge of the dose of ingested pathogens is very limited, the data obtained from this study will be of particular value in the development of dose-response models for risk analysis of foodborne infections.

## METHODS

The pathogen levels in outbreak-implicated vehicle food items were analysed by the prefectural governments in Japan between 2004 and 2006 (Table 1). Diluted food samples were analysed using a most probable number (MPN) method (outbreak nos. 3, 5, 8, 9, 12, 13, 16, 17) or a plating method (outbreak nos. 1, 2, 4, 6, 7, 10, 11, 14, 15). Food samples were homogenized in 9 × vol. saline.

In the MPN method, the homogenates were incubated in either three or five tubes containing enrichment broth, followed by plating onto selective agar media. To detect *Salmonella*, the homogenate was incubated in buffered peptone water (BPW, Oxoid, UK) at 35–37 °C for 20–24 h, and 1 ml of culture in BPW was transferred to 10 ml Rappaport–Vassiliadis broth (Oxoid) and incubated at 42 °C for 20–24 h. For the second enrichment, the culture was plated onto DHL (Eiken Chemical Co., Japan), MLCB (Eiken), SS (Nissui Pharmaceuticals, Japan) or XLT4 (Merck, Germany) agar media. After incubation at 35–37 °C for 20–24 h, the suspected colonies of *Salmonella* were confirmed by examining their biochemical characteristics in triple sugar iron medium (TSI, Eiken) and lysine indole motility medium (LIM, Eiken). Serotypes were tested using *Salmonella* antisera (Denka Seiken, Japan). To detect *E. coli* O157, the homogenate was incubated in modified EC broth with novobiocin at 42 °C for 20–24 h. The culture was plated onto CHROMagar O157 medium (CHROMagar, France) and sorbitol MacConkey agar with cefixim and tellurite (CT-SMAC, Eiken).

After incubation at 35–37 °C for 20–24 h, the suspected colonies of *E. coli* O157 were tested for agglutination with O157 antiserum, for the verotoxin (VT) gene by PCR assay (O157 typing set; Takara Bio, Japan), and for VT production by agglutination using a reversed passive latex agglutination kit (VTEC-RPLA, Denka Seiken). To detect *V. parahaemolyticus*, homogenates were incubated in alkaline peptone water (Nissui) at 35 °C for 18 h. The culture was plated onto TCBS agar medium. After incubation at 35 °C for 18 h, the suspected colonies of *V. parahaemolyticus* were confirmed by examining their biochemical characteristics in TSI and LIM, and on the basis of their growth in 0, 3, 7 and 10% salt, along with oxidase production. In order to detect *Campylobacter*, homogenates were incubated in Preston broth (Oxoid) at 37 °C for 48 h in a microaerobic atmosphere with BBL CampyPak (BD, USA). The culture was plated onto CCDA agar medium (Oxoid) and Skirrow's agar medium (Nissui). After incubation at 37 °C for 48 h in a microaerobic atmosphere, the suspected colonies were examined using Gram stain, oxidase test, and hippurate hydrolysis test.

In the plating method, 0.1 ml of the food homogenate and tenfold serially diluted samples were spread onto various selective agar media. To detect *Salmonella*, the following media were used for plating: XLD, DHL, SS, CHROMagar *Salmonella*, and MLCB. After incubation at 35–37 °C for 18–24 h, the suspected colonies were confirmed by examining their biochemical characteristics in TSI and LIM. Serotypes were tested using *Salmonella* antisera (Denka Seiken). To detect *E. coli* O157, the food homogenate and dilutions were plated onto CHROMagar O157 medium and CT-SMAC. After incubation at 35–37 °C for 20–24 h, the suspected colonies of *E. coli* O157 were tested for agglutination with O157 antiserum, for the VT gene by PCR assay (O157 typing set), and for VT production by agglutination using a reversed passive latex agglutination kit (VTEC-RPLA). To detect enterotoxigenic *E. coli*, the food homogenate and diluted samples were plated onto desoxycholate agar medium (Nissui). After incubation at 35 °C for 20 h, the suspected colonies were confirmed by examining their biochemical characteristics in TSI and LIM. Heat-stable enterotoxin and heat-labile enterotoxin genes were tested using a PCR assay (Primer set ELT-1&2, Primer set ESH-1&2; Takara Bio).

The numbers of patients and people involved with the food, the amount of food ingested by the patients,

Table 1. Contamination level of pathogens in food associated with outbreaks

Outbreak no.	Pathogen	Serotype (toxin producing)	Date of outbreak	Place	No. of patients	No. of persons exposed	Infection rate (%)*	Food associated with outbreak	Contamination level of pathogen in food	Food intake per person	Dose of ingested pathogen	Condition of food before test	
1	<i>Salmonella</i>	Enteritidis	Aug. 2004	Home	4	4	100.0	Puff cream from a sweet shop	39 000 c.f.u./g	90 g (per puff cream)	3 510 000 c.f.u.	48 h in refrigerator	
2			Dec. 2004	Restaurant	114	303	37.6	Raw chicken stomach	200 c.f.u./g	Unknown	Unknown	8 h at 0 °C	
3			July 2005	Nursery	19	84	22.6	Scrambled egg and soy bean cake	4 MPN/g	100 g	400 MPN	239 h at -20 °C	
4			Sep. 2005	Home	6	6	100.0	Cooked rice with red beans from catering meal	14 000 000 c.f.u./g	200–500 g	2 800 000 000–14 000 000 000 c.f.u.	24 h at 30 °C and 70.5 h in refrigerator	
5			Oct. 2005	Nursery	75	113	66.4	Dumpling with sauce of soy sauce and sugar from a sweet shop	0.9 MPN/g of dumpling (2.3 MPN/g sauce)	90 g (per serving)	81 MPN	216 h at -21 °C	
6			June 2006	Nursery	39	83	47.0	Lunch with vegetable or fruits	30 c.f.u./g	Unknown	Unknown	6 days in freezer	
7			Aug. 2006	Restaurant	29	59	49.2	Rice ball wrapped with deep-fried tofu	1 800 000 c.f.u./g	40–200 g	72 000 000–360 000 000 c.f.u.	5 days in refrigerator	
8			Cerro	Sep. 2004	School	157	1577	10.0	Bread for hamburger from a bakery	24 MPN/g	65 g	1560 MPN	72 h at -20 °C
9					Senior care facility	12	96	12.5	Salad with radish sprouts	6.6 MPN/g of salad (960 MPM/g of radish sprouts)	55 g (<1 g of radish sprouts)	363 MPN	96 h at -20 °C
10			Agona	Sep. 2006	Welfare facility	17	72	23.6	Fried soy pulp and egg	<30 c.f.u./g	50 g	<1500 c.f.u.	3 days in freezer
11	O4:H:eh,NT	Sep. 2006	Home	8	9	88.9	Sushi with fish, egg and vegetable	700 c.f.u./g	370 g	259 000 c.f.u.	360 h at <-20 °C		
12	Shiga toxin-producing <i>Escherichia coli</i>	O157:H7 (VT1 & VT2)	Feb. 2004	Home	3	6	50.0	Imported frozen hamburger beef pate	1.45 MPN/g	100 or 200 g	<108–216 MPN (estimated by an experiment with heat treatment)	<-15 °C	
13			Oct. 2004	Home	1 (carrier 2)	5	20.0	Beef	23 MPN/g	Unknown	Unknown	9 days in freezer	
14			Oct. 2006	Home	3	3	100.0	Raw beef liver	0.04–0.18 c.f.u./g	<50 g	2–<9 c.f.u.	12 days at -18 °C	
15	Enterotoxigenic <i>Escherichia coli</i>	O6:H16 (LT, ST)	Aug. 2005	Prison	401	431	93.0	Pickles of nappa with red pepper	1–40 c.f.u./g	25 g	25–1000 c.f.u.	2 days at -10 °C	
16	<i>Vibrio parahaemolyticus</i> †		Aug. 2004	Restaurant	36	50	72.0	Muscle of pen shell	240 MPN/g (total <i>V. parahaemolyticus</i> )	50 g	12 000 MPN (total <i>V. parahaemolyticus</i> )	24 h at -18 °C	
17	<i>Campylobacter jejuni</i>		June 2005	Barbecue restaurant	3	8	37.5	Raw beef liver	3.6 MPN/g	100 g	360 MPN	24 h at 10 °C	

MPN, Most probable number; VT, Verotoxin; LT, heat-labile enterotoxin; ST, heat-stable enterotoxin.

\* No. of person exposed/no. of patients × 100.

† TDH-producing *V. parahaemolyticus* O3:K6 was isolated from samples of patients. The number of total *V. parahaemolyticus* in food was measured.

and the method of food storage prior to testing are described in Table 1. The infection rate was obtained by dividing the number of patients by the number of persons exposed.

## RESULTS AND DISCUSSION

The contamination levels of food pathogens were analysed in 17 outbreaks of foodborne infections in Japan between 2004 and 2006, including 11 outbreaks of *Salmonella*, three outbreaks of *E. coli* O157, one outbreak of enterotoxigenic *E. coli*, one outbreak of *V. parahaemolyticus*, and one outbreak of *C. jejuni* (Table 1). The infectious dose specific to each outbreak was determined using existing data, except for two outbreaks of *Salmonella* infection and one outbreak of *E. coli* O157 infection.

### *Salmonella*

Seven (outbreak nos. 1–7) of the 11 outbreaks of *Salmonella* were caused by the serotype Enteritidis. The remaining four outbreaks were caused by individual strains of serotypes, i.e. Agona, Cerro, Montevideo and O4:H:eh,NT. There are some reports on the infectious dose of serotype Enteritidis [6, 7], but reports on other serotypes are limited. In this study, the ingestion doses of serotypes Montevideo (outbreak no. 9), Agona (outbreak no. 10), Cerro (outbreak no. 8) and O4:H:eh,NT (outbreak no. 11) were 363 MPN, <1500 c.f.u., 1560 MPN, and 259 000 c.f.u., respectively. The infection rate of serotype O4:H:eh,NT (88.9%) was higher than that of the serotypes Agona (23.6%), Cerro (10.0%) and Montevideo (12.5%). Because the samples from these outbreaks had been stored in a freezer, the amount of *Salmonella* in these food samples did not increase during storage.

In outbreaks nos. 1 and 4 caused by *S. Enteritidis*, the infection rates were 100%. In outbreak no. 1, the contamination level was 39 000 c.f.u./g in puff cream and the ingestion dose was 3 510 000 c.f.u.. In outbreak no. 4, the contamination level was 14 000 000 c.f.u./g in cooked rice with red beans and the ingestion dose was 14 000 000 000 c.f.u.. The food samples obtained from outbreak nos. 1 and 4 were stored for 2 days in refrigerators, and later at around 30 °C for 24 h, followed by storage in a refrigerator before testing. On the basis of the report that the *S. Enteritidis* count does not change at 10 °C for 72 h even after incubation in enrichment broth [8], we

concluded that the ingestion dose in outbreak no. 1 appears to be an actual number. However, it was expected that *Salmonella* in cooked rice with red beans, implicated in outbreak no. 4, would significantly grow at 30 °C for 24 h. The growth of *Salmonella* in food like cooked rice with red beans was not clarified, but an increase by >1000-fold is possible [8]. In outbreak no. 7, the contamination levels and the ingested *Salmonella* dose were 1 800 000 c.f.u./g and 72 000 000–360 000 000 c.f.u., respectively. The infection rate, however, was <50%.

On the other hand, the samples of outbreak nos. 2, 3, 5, 6, 8–11 were stored at 0 °C or in a freezer. Konuma [9] reported that freezing at about –15 to –25 °C either causes no decrease or causes 1 log reduction in *S. Enteritidis* population in five kinds of food, e.g. ground beef, radish sprouts, canned corn, fish meat and sweet bean paste for up to 14 days. On the basis of this report, it is considered that the *Salmonella* numbers did not increase much and decreased in these food samples prior to testing. In outbreak nos. 3, 5, and 8–10, the ingested *Salmonella* dose was <1560 MPN or c.f.u., and the infection rates were between 10.0% and 66.4%. In outbreak no. 5, the ingestion dose was 81 MPN, but the infection rate was 66.4%. It appears that the infection rate was high as most of these patients were very young children who are sensitive to pathogens. In outbreak no. 11, the dose of ingested *Salmonella* was estimated to be 259 000 c.f.u. from the *Salmonella* level in the frozen food, and the infection rate was high (89%).

### *E. coli* O157

In *E. coli* O157 outbreak no. 14, which was associated with raw beef liver, the infection rate, the contamination level, and the ingestion dose were 100% (3/3), 0.04–0.18 c.f.u./g and 2 to <9 c.f.u., respectively. The food samples had been stored in a freezer for 12 days before testing. In *E. coli* O157 outbreak no. 13, which was associated with beef, a 6-year-old child was infected and two adults were diagnosed as carriers. In *E. coli* O157 outbreak no. 12, which was associated with hamburger patties, 50% of the people who consumed this meat became infected. Hamburger patties from the same lot were cooked in the same way to measure the number of surviving pathogens. The contamination level in the cooked patties and the ingestion dose were 1.45 c.f.u./g and 108–216 c.f.u., respectively. It has been reported that the infectious dose of *E. coli* O157 is low, <100 c.f.u. or MPN

[10, 11]. The ingestion doses determined in this study are in agreement with previous reports.

### Enterotoxigenic *E. coli*

In enterotoxigenic *E. coli* outbreak no. 15, which occurred in a prison in Japan, each prisoner had ingested 25 g pickles. The infection rate, the contamination level, and the ingestion dose were 93% (401/431), 1–40 c.f.u./g, and 25–1000 c.f.u., respectively. Because there is little data available on the infectious dose of enterotoxigenic *E. coli* [12], it is not clear how to interpret these findings. Most people infected with even a small amount of *E. coli* O157 and enterotoxigenic *E. coli* exhibit symptoms. Although some people appear to be susceptible to infection with small doses of *Salmonella*, it appears that the large doses make people ill at a high rate.

### *V. parahaemolyticus*

In outbreak no. 16 of the TDH-producing *V. parahaemolyticus* O3:K6 infection, which was associated with pen shells, the total number of *V. parahaemolyticus*, but not specifically the TDH-producing *V. parahaemolyticus*, was counted. The infection rate, total *V. parahaemolyticus* contamination level, and the ingestion dose were 72%, 240 MPN/g, and 12 000 MPN, respectively. The Japanese government regulations indicate the safe level of total *V. parahaemolyticus* in seafood for raw consumption to be <100 MPN/g [13]. The number of *V. parahaemolyticus* in pen shells was higher than that suggested by the Japanese regulations.

### *Campylobacter*

In an outbreak of *C. jejuni* infection, which was associated with raw beef liver, the infection rate, contamination level, and ingestion dose were 37.5%, 3.6 MPN/g and 360 MPN, respectively. *C. jejuni* is found at high concentrations in the bile of cattle infected with the pathogen, and contaminates the liver [14]. The consumption of raw beef liver is associated with foodborne infection. The test sample was stored at 10 °C for 24 h before testing. Because *Campylobacter* is unable to grow in an aerobic atmosphere and survives under low temperature for a short time such as 24 h, the number of pathogens ingested seems accurate. The infectious dose in experimental human infection is reported to be as low as 500 bacteria [15, 16].

In this study, the ingestion dose of foodborne pathogens was estimated by using existing data on the outbreaks. Although the storage condition of the food samples before testing may have had some effects on the number of pathogens in the tested samples, it appears that relatively small doses of *Salmonella*, *E. coli* O157, enterotoxigenic *E. coli* and *C. jejuni* caused foodborne infections. In this study, the infectious doses of the pathogens were utilized in microbiological risk assessments. However, infectious doses vary by the characteristics of the exposed populations, such as age, immunity, illness, and the characteristics of the pathogens, such as virulence, resistance to environmental factors, and activities. Thus, further investigations to analyse the vehicle food items in the outbreaks are needed to identify the actual infectious dose of foodborne pathogens.

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### DECLARATION OF INTEREST

None.

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