



Epidemiological Investigation of an Outbreak of Salmonellosis in Gyeongju, Korea

Seok-Ju Yoo, Hyun-Sul Lim, Kwan Lee

Department of Preventive Medicine, Dongguk University College of Medicine, Gyeongju, Korea

Objectives: A salmonellosis outbreak occurred within a community of Gyeongju residents who ingested catered food from a wedding in June 2009. We aimed to epidemiologically investigate the probable vehicle of the infection.

Methods: We conducted a retrospective cohort study on 34 local residents who ingested the wedding food.

Results: Among the 34 residents, 31 (91.2%) reported symptoms of infection after eating the food. Among all of the wedding foods, pan-fried foods were highly associated with the diarrheal attack rate. On bacteriological examination, *Salmonella* species were detected in the pan-fried foods among the leftover foods and in 17 of the 31 stool specimens from the cases. There were five different types of pan-fried foods, but the onset of symptoms was independent of the ingredients used. We found that the pan-fried food was prepared at a food store in Seoul and that eggs were a common ingredient.

Conclusions: The major cause of the salmonellosis in this population was presumed to be the pan-fried food prepared with contaminated eggs. These food items might have been partially undercooked because of their irregular shape, which allowed the *Salmonella* species to survive and multiply before ingestion.

Key words: Epidemiologic studies, *Salmonella* infections, Egg

INTRODUCTION

Salmonellosis is an etiologic term used to describe a various conditions that affect humans and many animal species [1]. Non-typhoidal salmonellosis is an invasive intestinal disease contracted after ingesting food contaminated with *Salmonella enterica* [2]. The *S. enterica* serovars Typhimurium and Enteritidis are most commonly associated with food-borne illnesses in humans [3]. *Salmonella* serotype Enteritidis infections have been more frequently linked to shell egg consumption, whereas

Salmonella serotype Typhimurium infections are more frequently associated with the consumption of contaminated chicken meat [4]. The incidence of *S. enterica* serotype Enteritidis infection has increased worldwide [5,6] and also is the most frequent type in the Korea [7]. On June 23, 2009, the Gyeongju Health Center was informed of an outbreak of gastroenteritis among the local residents of Gyeongju. In response to this outbreak, the Communicable Disease Control Team, which consists of the Department of Preventive Medicine of Dongguk University College of Medicine and the Gyeongju Health Center, convened to identify the probable vehicle of infection.

METHODS

Epidemiological Investigation

On June 23, 2009, the same day the outbreak was reported, the Communicable Disease Control Team immediately visited the location of the outbreak. We found that all of the affected

Received: March 15, 2014; **Accepted:** April 9, 2014

Corresponding author: Hyun-Sul Lim, MD, PhD

123 Dongdae-ro, Gyeongju 780-714, Korea

Tel: +82-54-770-2401, **Fax:** +82-54-770-2438

E-mail: wisewine@dongguk.ac.kr

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

residents had eaten wedding foods in the village hall or at their own houses between June 21 and 22, 2009. The wedding foods were provided by the parents of the bridegroom after the wedding ceremony. Thus, we visited the house of the bridegroom's parents and conducted face-to-face interviews with his family to investigate the types of wedding foods served and their transportation routes.

Based on the initial, basic investigation, we created a structured questionnaire and visited the village again the following day to collect data using face-to-face interviews. In total, 34 people who had eaten the wedding foods were interviewed. The questionnaire collected data on individual characteristics such as age, gender, and symptoms (onset, type, frequency, period, and severity) as well as the list of wedding foods (pan-fried foods, rice cakes, melons, and yellow melons) each person consumed.

On June 27, 2009, we visited the food store where the pan-fried foods were made in Seoul. The common ingredients used to make the pan-fried foods included eggs, wheat flour, salt, and pepper. Eggs used by this food store are supplied every morning by two chicken farms. We also investigated the common cooking methods and found that all of the primary ingredients (beef, Alaska pollock, skewered foods, shiitake mushrooms, and shrimp) were battered in a mixture of wheat flour and 30 cracked raw eggs then fried with oil in a pan.

Laboratory Tests

On the day of the outbreak, we collected samples of any leftover wedding foods and the drinking water. We also collected stool samples from 32 people over 2 days from June 24 until June 25, 2009. Stools from all infected people were included; however, two out of three people who did not have symptoms declined laboratory testing. Stool and food samples were sent to the Gyeongju Health Center and Gyeongsangbukdo Government Public Institute of Health and Environment for laboratory testing. On June 27, 2009, we also collected stool samples from the three employees of the food store who prepared and cooked the pan-fried foods. These samples were sent to the Seoul Metropolitan Government Research Institute of Public Health and Environment. Ten species of bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Bacillus cereus*, and *Clostridium perfringens*), five species of viruses (rotavirus, astrovirus, norovirus, adenoviruses, and sapovirus), and one species of

protozoa (*Cryptosporidium*) were tested for in all of the samples.

Case Definition

We defined a case as probable if a person had diarrhea more than twice in one day within 48 hours after eating the wedding foods from June 21 until June 24, 2009. We defined a case as definitive if a pathogen was detected in the stool.

Statistical Analysis

Data from this retrospective cohort of 34 local residents were entered into the SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) for analysis. Fisher's exact test was used to compare the attack rates between people who ate and did not eat any of the tested foods. A p -value <0.05 was considered statistically significant. All surveys were administered after obtaining informed consent.

RESULTS

Epidemiological Findings

The wedding ceremony was held in Seoul on June 21, 2009. After the wedding ceremony, the wedding foods, including pan-fried foods, rice cakes, melons, and yellow melons were transported from Seoul to Gyeongju by bus, which took approximately 4 hours. The temperature and humidity in Seoul that day were 25.1°C and 77.8%, respectively. In Gyeongju, the records indicated 27.8°C and 74%, respectively.

The bridegroom's father and older brother ate the transported foods the evening of the wedding, after arriving at

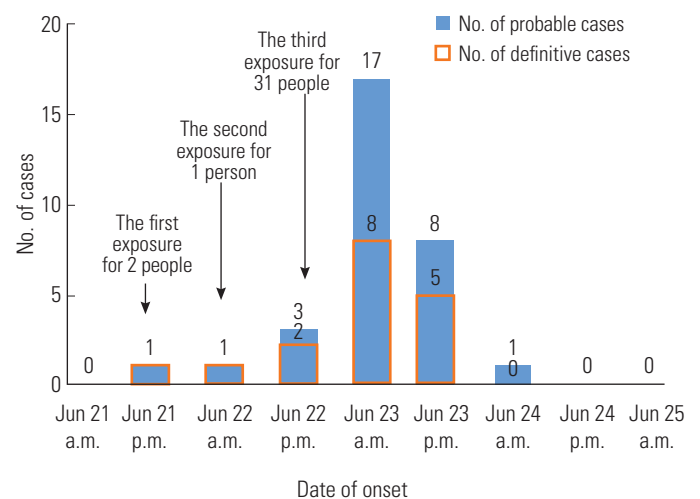


Figure 1. Distribution of cases by the date of onset.

their home in Gyeongju. They reported experiencing symptoms that evening and until the morning the following day.

The morning after the wedding, the sister-in-law of the bridegroom also ate the transported foods and experienced symptoms the following afternoon (29 hours later). At approximately 5:00 p.m. the day after the wedding, the bridegroom's mother and 30 neighbors ate the transported foods in the village hall. Of these 31 people, 28 experienced symptoms from that evening until the morning of June 24, 2009. The mean incubation period for all those affected was 15 hours (range, 1 to 33 hours). After June 25, 2009, no one else was affected (Figure 1).

Thirty-one of the 34 people who ate the transported wedding foods reported symptoms after eating. All of the affected individuals had diarrhea. Other symptoms included vomiting (n=18), fever (n=16), abdominal pain (n=15), chills (n=10), nausea (n=8), and headaches (n=6). Thirty-one of the 34 cases were probable cases with an attack rate of 91.2%. According to the definitive case definition, 17 of the 34 cases (50%) were confirmed.

Attack Rate Among Probable and Definitive Cases

The attack rate between people who did and did not eat the wedding foods was calculated for the probable and definitive cases. Among the probable cases who ate the pan-fried foods, the attack rate was significantly higher among those who ate the foods than it was among those who did not eat the foods. However, no significant difference was observed for the attack rates among those who ate the other wedding foods (rice cakes, melons, and yellow melons) compared to those who did not eat these foods. Among the definitive cases, no significant difference was observed for the attack rates of any of the wedding foods for the comparison between the subjects who

ate the foods versus those who did not eat the wedding foods (Table 1).

According to the collected data, five different pan-fried foods were served that included shrimp, beef, Alaska pollock, or shiitake mushrooms, but the onset of symptoms were independent of the type of pan-fried food eaten.

Results of the Laboratory Tests

Salmonella species were detected in 17 of the 31 stool specimens among probable cases as well as in the pan-fried food samples. However, no pathogens were detected in the other wedding foods (rice cakes, melons, and yellow melons), the water in the village hall, or the stool specimens from the three people who worked at the food store in Seoul.

DISCUSSION

The main symptoms of the probable cases included diarrhea, vomiting, and fever, and the average incubation period was 15 hours. These symptoms correspond with the classic symptoms and incubation period of salmonellosis (12 to 48 hours) [1]. According to the results of the laboratory tests and statistical analyses, it was apparent that the pan-fried foods were contaminated with *Salmonella* species. There are several ways in which the pan-fried foods could have become contaminated by *Salmonella* species. First, the people who cooked or shared the pan-fried foods could have contaminated the food if they were carriers of the infection. Previous reports have described food handler-associated [8,9] and person-to-person transmissions [10] of *S. enteritidis* infections. However, in our investigation, the three people who cooked the pan-fried foods had no symptoms and no evidence of bacteria/vi-

Table 1. Attack rate by the type of food ingested

Food	Ingestion	Probable cases			Confirmed cases		
		Positive	Negative	p-value ¹	Positive	Negative	p-value ¹
Pan-fried foods	Yes (n=30)	30 (100.0)	0 (0.0)	0.001	16 (53.3)	14 (46.7)	0.60
	No (n=4)	1 (25.0)	3 (75.0)		1 (25.0)	3 (75.0)	
Rice cakes	Yes (n=16)	13 (81.3)	3 (18.7)	0.09	6 (37.5)	10 (62.5)	0.30
	No (n=18)	18 (100.0)	0 (0.0)		11 (61.1)	7 (38.9)	
Melon	Yes (n=10)	10 (100.0)	0 (0.0)	0.54	4 (40.0)	6 (60.0)	0.71
	No (n=24)	21 (87.5)	3 (12.5)		13 (54.2)	11 (45.8)	
Yellow melon	Yes (n=10)	10 (100.0)	0 (0.0)	0.54	6 (60.0)	4 (40.0)	0.71
	No (n=24)	21 (87.5)	3 (12.5)		11 (45.8)	13 (54.2)	

Values are presented as number (%).

¹Two-tailed p-value tested by the Fisher's exact test.

ruses in their stool samples. In addition, the cooks wore cotton and vinyl gloves to protect against contamination. Moreover, the bride's family also ate the pan-fried foods which were cooked by the same people the day before the wedding ceremony; however, none of the members of the bride's family reported symptoms of infection. Thus, the possibility that the cooks contaminated the pan-fried foods is low.

A second possible means of contamination may have been due to the use of contaminated ingredients to make each type of pan-fried food (beef, Alaska Pollack, shiitake mushrooms, and shrimp). Previous reports have indicated that the use of multiple, raw produce items can increase the risk of a salmonellosis outbreak [11]. However, in our study, symptom onset was independent of the type of pan-fried food ingested. The presence of one contaminated material could contaminate other types of pan-fried foods during the cooking process; however, the probability that all types of pan-fried foods could have become contaminated in this way is low.

Finally, eggs are a common source of contamination. Many cases of *Salmonella*-contaminated eggs have been reported [6,12,13], and people could manifest symptoms of infection, unless the eggs are cooked safely [14,15]. In this case, one contaminated egg may have polluted all of the pan-fried wedding foods because they were soaked in the same mixture of 30 cracked raw eggs. Combining a large mixture of eggs can be a risk factor of a *Salmonella* outbreak because the mixture of raw eggs increases the risk of infection from a single, contaminated egg [16].

Pan-fried foods are irregularly shaped, with many prominences and depressions; therefore, they could have been partially undercooked. *Salmonella* species would then survive in the depressions and multiply prior to ingestion. One previous case of contaminated eggs was reported among people who ate *Chiles rellenos* [17]. *C. rellenos* are a type of Mexican food and are prepared in a very similar manner to that of pan-fried Korean foods. Ultimately, dishes containing eggs have the highest potential to contaminate prepared foods.

This study has several limitations. First, the samples of pan-fried foods were clustered, so the individual type of pan-fried food was not separately tested in the laboratory. Second, the cooking utensils could have contaminated the food [18]. However, we were not able to examine the cooking utensils because they had already been washed by the time we visited the food store. The likely source of infection may have occurred at the chicken farms [19]. Unfortunately, we were not

able to check the eggs and chickens from the two chicken farms that supplied the eggs to the food store on June 21 because they refused to cooperate with our investigation. Last, pulsed-field gel electrophoresis was not performed; thus, it is unknown whether clonal relationships between the stool specimens from the subjects and leftover food samples existed.

In conclusion, the eggs used to make the pan-fried foods may have been contaminated with *Salmonella* species, and the bacteria might have multiplied due to incomplete cooking. Thus, the government and related institutions should monitor and manage farm egg production to prevent *S. enteritidis* contamination through careful checks of the egg distribution and storage processes. In addition, the development of a vaccination program could reduce the risk of infection [20]. Above all, eggs should be fully cooked and careful attention should be paid to the overall process of cooking eggs to prevent *S. enteritidis* infections.

ACKNOWLEDGMENTS

We thank the officials of Gyeongju Health center for their assistance in the overall investigation. We also appreciate the officials of Gyeongsangbuk-do Government Public Institute of Health and Environment and the Seoul Metropolitan Government Research Institute of Public Health and Environment for performing the laboratory tests.

CONFLICT OF INTEREST

The authors have no conflicts of interest with the material presented in this paper.

REFERENCES

1. Painter JA, Perch M, Voetsch AC. Nontyphoidal salmonellosis. In: Wallace RB, editor. Maxcy-Rosenau-Last public health & preventive medicine. 15th ed. New York: McGraw-Hill; 2007, p. 437-440.
2. Day JB, Basavanna U, Sharma SK. Development of a cell culture method to isolate and enrich *Salmonella enterica* serotype enteritidis from shell eggs for subsequent detection by real-time PCR. *Appl Environ Microbiol* 2009;75(16):5321-5327.
3. Centers for Disease Control and Prevention (CDC). *Salmonella* surveillance: annual summary, 2006. Atlanta: US Department of Health and Human Services; 2008, p.1.

4. Clavijo RI, Loui C, Andersen GL, Riley LW, Lu S. Identification of genes associated with survival of *Salmonella enterica* serovar Enteritidis in chicken egg albumen. *Appl Environ Microbiol* 2006;72(2):1055-1064.
5. Laupland KB, Schonheyder HC, Kennedy KJ, Lyytikäinen O, Valiquette L, Galbraith J, et al. *Salmonella enterica* bacteremia: a multi-national population-based cohort study. *BMC Infect Dis* 2010;10:95.
6. Braden CR. *Salmonella enterica* serotype Enteritidis and eggs: a national epidemic in the United States. *Clin Infect Dis* 2006; 43(4):512-517.
7. Park HM, Lee DY. Prevalence and characteristics of *Salmonella* spp. in Korea, 2012. *Public Health Wkly Rep* 2013;6(6):105-110 (Korean).
8. Hedberg CW, White KE, Johnson JA, Edmonson LM, Soler JT, Korlath JA, et al. An outbreak of *Salmonella enteritidis* infection at a fast-food restaurant: implications for foodhandler-associated transmission. *J Infect Dis* 1991;164(6):1135-1140.
9. Khuri-Bulos NA, Abu Khalaf M, Shehabi A, Shami K. Foodhandler-associated *Salmonella* outbreak in a university hospital despite routine surveillance cultures of kitchen employees. *Infect Control Hosp Epidemiol* 1994;15(5):311-314.
10. Delarocque-Astagneau E, Desenclos JC, Bouvet P, Grimont PA. Risk factors for the occurrence of sporadic *Salmonella enterica* serotype enteritidis infections in children in France: a national case-control study. *Epidemiol Infect* 1998;121(3):561-567.
11. Centers for Disease Control and Prevention (CDC). Outbreak of *Salmonella* serotype Saintpaul infections associated with multiple raw produce items: United States, 2008. *MMWR Morb Mortal Wkly Rep* 2008;57(34):929-934.
12. De Reu K, Grijspeerdt K, Messens W, Heyndrickx M, Uyttendaele M, Debevere J, et al. Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including *Salmonella enteritidis*. *Int J Food Microbiol* 2006;112(3):253-260.
13. Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, Gast R, Humphrey TJ, et al. Mechanisms of egg contamination by *Salmonella enteritidis*. *FEMS Microbiol Rev* 2009;33(4):718-738.
14. Calvert N, Murphy L, Smith A, Copeland D. A hotel-based outbreak of *Salmonella enterica* subsp. *Enterica* serovar Enteritidis (*Salmonella enteritidis*) in the United Kingdom, 2006. *Euro Surveill* 2007;12(3):222.
15. Marcus R, Varma JK, Medus C, Boothe EJ, Anderson BJ, Crume T, et al. Re-assessment of risk factors for sporadic *Salmonella* serotype Enteritidis infections: a case-control study in five FoodNet Sites, 2002-2003. *Epidemiol Infect* 2007;135(1):84-92.
16. Sobel J, Hirshfeld AB, McTigue K, Burnett CL, Altekruze S, Brenner F, et al. The pandemic of *Salmonella enteritidis* phage type 4 reaches Utah: a complex investigation confirms the need for continuing rigorous control measures. *Epidemiol Infect* 2000;125(1):1-8.
17. Centers for Disease Control and Prevention (CDC). Outbreaks of *Salmonella* serotype enteritidis infection associated with eating raw or undercooked shell eggs: United States, 1996-1998. *MMWR Morb Mortal Wkly Rep* 2000;49(4):73-79.
18. Humphrey TJ, Martin KW, Whitehead A. Contamination of hands and work surfaces with *Salmonella enteritidis* PT4 during the preparation of egg dishes. *Epidemiol Infect* 1994;113(3): 403-409.
19. Berghold C, Kornschober C, Weber S. A regional outbreak of *S. enteritidis* phage type 5, traced back to the flocks of an egg producer, Austria. *Euro Surveill* 2003;8(10):195-198.
20. Toyota-Hanatani Y, Ekawa T, Ohta H, Igimi S, Hara-Kudo Y, Sasaki K, et al. Public health assessment of *Salmonella enterica* serovar enteritidis inactivated-vaccine treatment in layer flocks. *Appl Environ Microbiol* 2009;75(4):1005-1010.