Original Article

A Food-Borne Outbreak of Gastroenteritis Caused by Different *Salmonella* Serotypes in 2 Universities in Xiamen, Fujian, China, in 2012

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SUMMARY: We investigated a diarrhea outbreak in 2 universities to identify the etiological agent responsible, the source of infection, the mode of transmission, and the risk factors. A case-controlled study was conducted using case students and asymptomatic control students who were selected randomly and frequency-matched according to class and age, and the source of food or water intake was investigated. Of the total 22,404 students at the universities, 0.25% developed *Salmonella* Infections. A total of 96% (54/56) of the case students and 30% (35/117) of the control students consumed bread products provided by the same vendor (odds ratio [OR] = 63.3; 95% confidence interval [CI], 14.9–550.7). Among the students who consumed bread, 96% (52/54) of the case students and 9% (3/35) of the control students ate egg sandwiches (OR = 277.3; 95%CI, 43.9–1,750.8). Seven strains of *Salmonella enteritidis* and 6 strains of *S. chester* were isolated from the case students or food samples. Pulsed-field gel electrophoresis typing showed the same patterns. The outbreak of gastroenteritis was caused mainly by egg sandwiches contaminated with different serotypes of *Salmonella*.

INTRODUCTION

Salmonellosis, which results from the ingestion of contaminated eggs, poultry, beef, pork, and milk (1), is a common cause of community-acquired food-borne bacterial gastroenteritis worldwide. *Salmonella* is the most common pathogenic bacteria that causes bacterial food poisoning; it accounts for 10.8% of the microbe-caused foodborne disease outbreaks in China (2). There have been numerous reports of food poisoning outbreaks associated with consumption of baked foods (3, 4).

On October 31, 2012, 11 patients developed acute diarrhea and fever in 2 universities in Jimei district, Xiamen city, China. The patients were all students enrolled in the 2 universities. Rectal swab samples from 7 patients were culture-positive for different serotypes of *Salmonella*. We surveyed the outbreak to verify the causative agent, source of infection, and mode of transmission and to recommend control measures. We also sought to investigate more general issues related to disease outbreaks caused by different *Salmonella* serotypes in China.

MATERIALS AND METHODS

We conducted this outbreak investigation immediately after the outbreak on October 31, 2012. The investigation was exempted from ethical approval and in-

formed consent because it was in response to an acute public health emergency event. Despite this contingency, we still informed every subject verbally before investigation and obtained a physically signed questionnaire. The entire investigation has been comprehensively recorded by an investigator and all the relevant documents have been filed appropriately.

Case definitions: A suspected case was defined as onset of diarrhea (≥ 3 times per day), plus at least one of the symptoms from among fever, abdominal pain, nausea, vomiting, and headache in a teacher or student of the 2 universities or residents who lived in close proximity to the 2 universities from October 28 to November 3, 2012. A confirmed case was defined as a suspected case plus a positive *Salmonella* culture from stool specimens, rectal swabs, or vomitus.

Case findings: Electronic medical records were collected from the Jimei district hospital, the 5 community health services, and 3 private clinics in Xiamen and were reviewed systematically to search for patients with vomiting, diarrhea, abdominal pain, and fever. Case definitions were applied to the review of the medical records and identify cases. The teachers and staff from the 2 universities were requested to report illnesses in the students and their families by telephone from October 28 to November 3.

Case-controlled study: All of the case students were enrolled into this case controlled study, and the controls were selected randomly from the asymptomatic students and were frequency matched according to class and age. A questionnaire was conducted by face-to-face interview in both case and control students to gather data about demographics, date of onset, symptoms and duration of illness, and history of exposure to suspected food and water items.

Laboratory investigation: Rectal swabs, stool samples, vomitus, and hand daub specimens were collected from the case students and the employees of food

Received May 30, 2014. Accepted August 18, 2014.
J-STAGE Advance Publication December 24, 2014.

DOI: 10.7883/yoken.JJID.2014.235

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production to test for the presence of *Vibrio cholerae*, *Staphylococcus aureus*, *Shigella*, and *Salmonella*. One egg sandwich and 4 raw food materials were also collected. A microbial rapid detection system (BAX[®]System Q7) and fluorescence polymerase chain reaction were adopted to detect salmonellosis according to the standard protocol of the Centers for Disease Control and Prevention. Laboratory tests were employed in conformity with standard practices.

Pulsed-field gel electrophoresis (PFGE): PFGE was implemented by following the China Pulse Net protocol. All the isolates were characterized by PFGE, which was performed using a CHEF-DR III system (Bio-Rad, Hercules, CA, USA). The digital images were analyzed using Bionumerics software (v6.6.4; Applied Mathematics, Sint-Martens-Latem, Belgium) with the Dice coefficient.

Environmental investigation: To identify the risk factors associated with the outbreak, an environmental survey was conducted, including the production environment, the bread manufacturing process, and the bread sales channels.

Statistical analysis: The chi-square test was used to compare the demographic characteristics between groups, and Fisher's exact test was used in case the expected count was less than 1. Univariate regression analysis was employed to assess the association between food consumption and case status using odds ratios (ORs) and 95% confidence intervals (CIs). Statistical analyses were performed using SPSS version 15.0; all *P*-values were 2-sided and *P* < 0.05 was considered to be statistically significant.

Ethical approval: This study was considered to be part of a continuing public health outbreak investiga-

tion by Xiamen municipal health bureau and exempt from institutional review board assessment. All data were kept confidential without patient identifiers.

RESULTS

We identified 56 cases (49 suspected and 7 confirmed) from October 30 to November 2, 2012, within 22,404 students at the 2 universities (university A, 19,978; university B, 2,426), with an attack rate (AR) of 0.25%. All cases were students, and there were no cases among the family members of the teachers and staff or the urban residents living around the 2 universities. Among the 56 case-students, the common symptoms were dizziness (91%), abdominal pain (89%), fever (84%), nausea (80%), and vomiting (43%). Eleven individuals (16%) were hospitalized, but there were no deaths. The median illness duration of the 11 hospitalized patients was 7 days (range, 4–10 days), and the median age was 20 years (range, 17–22 years).

Thirty-nine of the 56 case-students (70%) were from university A, the remaining 17 (30%) were from university B. The AR of the 7.8% (33/425) affected classes in the 2 universities ranged from 1.2% to 8.9%. The AR in male students was 1.7/1,000 (20/11,756), compared with 3.4/1,000 (36/10,648), in female students; the AR did not differ significantly between males and females in university A or university B ($\chi^2 = 0.35$, *P* = 0.55; $\chi^2 = 1.17$, *P* = 0.28, respectively).

The outbreak lasted for 72 h. It began at 5 o'clock on October 30 and had 2 peaks: the initial peak started from 12 to 24 h on October 30, and the second peak was from 12 to 24 h on October 31 (Fig. 1). The potable water at the 2 universities was provided by the urban tap

Fig. 1. Epidemic curve for reported cases of Salmonellosis.

Table 1. Age and sex of the study subjects

Type	Case (N = 56)		Control (N = 117)		P
	n	%	n	%	
Age (yr)					
17–19	24	43	40	34	0.5
20–22	31	55	75	64	
> 22	1	2	2	2	
Sex					
male	20	36	52	44	0.3
female	36	64	65	56	

Table 2. Risks of Salmonellosis by exposure in 54 cases and 35 controls among the students who consumed bread in the 2 universities in Xiamen, Fujian, China, in 2012

Bread type	Number		Exposed (%)		Odds ratio	95% confidence interval
	Case (n = 54)	Control (n = 35)	Case	Control		
Egg-sandwiches	52	3	96	9	277.3	43.9–1750.8
Toast-bread	9	9	17	26	0.6	0.2–1.6
Lon-strip-bread	4	5	7	14	0.5	0.1–1.9
Dried-meat-floss-bread	4	4	7	11	0.6	0.1–2.7
Other-bread	10	18	19	51	0.2	0.1–0.5

water network rather than barreled water. A total of 54 of the 56 case students (96%) had eaten bread produced by the same food company. The epidemiological curve of the time of bread consumption indicated 2 peaks approximately 12 h apart (Fig. 1).

All 56 case students and 117 control students were enrolled in the case-controlled study, and the distribution of subjects according to age and gender is shown in Table 1. Between October 28 and November 2, 96% of the case students consumed the bread, compared with 30% of the control students (Fisher's exact test: OR = 63.3; 95% CI, 14.9–550.7). Among the students who consumed the bread, 96% consumed egg sandwiches in the case group, compared with 9% in the control group (OR = 277.3; 95% CI, 43.9–1,750.8) (Table 2).

An environmental investigation demonstrated that the food company started to produce egg sandwiches on October 8, and 50 egg sandwiches were produced at 6 AM and 1 PM, and then distributed to 5 points of sale (POS) by a van without refrigeration facilities (Table 3). Also we found other 6 suspected cases, of which 2 ate egg sandwiches sold at POS 3, 2 ate egg sandwich sold at POS 4, 1 ate an egg sandwiches sold at POS 5, and 1 ate an egg sandwich sold at POS 1 when he visited his girlfriend at university A. All the breads were produced by the same group of workers using the same production process and the same production equipment at the food company. Records of the baking temperatures and times were not discovered during the field investigation. A number of case students reported that the omelets were soft.

Five strains of *Salmonella enteritidis* and 6 strains of *S. chester* were isolated from 8 specimens from 7 case students (Table 4), and 2 additional strains of *S. enteritidis* were isolated from an egg sandwich and sliced bread. PFGE typing confirmed the presence of the same

Table 3. the sales volume of egg sandwiches produced by the company in the 5 points of sale (POS)

date	POS 1 ¹⁾	POS 2 ²⁾	POS 3	POS 4	POS 5
October 28	30	0	4	5	5
October 29	30	0	4	5	5
October 30	30	10	5	5	5
October 31	30	10	4	5	5
total	120	20	17	20	20

¹⁾: university A.

²⁾: university B.

Table 4. Serotype among case students and foods *Salmonella* isolates

sample number	source	university	the number of bacterial strain	the serotype of bacterial strain
1	rectal swabs	university A	SZ(2012)001	<i>Salmonella Enteritidis</i>
2	stool specimens	university B	SZ(2012)004	<i>Salmonella Enteritidis</i>
3	rectal swabs	university B	SZ(2012)008	<i>Salmonella Chester</i>
		university B	SZ(2012)009	<i>Salmonella Chester</i>
4	stool specimens	university B	SZ(2012)005	<i>Salmonella Enteritidis</i>
5	stool specimens	university B	SZ(2012)007	<i>Salmonella Enteritidis</i>
		university B	SZ(2012)010	<i>Salmonella Chester</i>
6	stool specimens	university A	SZ(2012)011	<i>Salmonella Chester</i>
		university A	SZ(2012)012	<i>Salmonella Chester</i>
	vomit	university A	SZ(2012)013	<i>Salmonella Chester</i>
7	rectal swabs	university B	SZ(2012)006	<i>Salmonella Enteritidis</i>
8	egg sandwich		SZ(2012)002	<i>Salmonella Enteritidis</i>
9	sliced bread		SZ(2012)003	<i>Salmonella Enteritidis</i>

7 trains of *S. enteritidis* and 6 strains of *S. chester* (Fig. 2). Additional 3 raw food materials and 4 employees from the food production plant tested negative for *Salmonella*, *Shigella*, *V. cholerae* and *Staphylococcus aureus*.

DISCUSSION

The bacterial genus *Salmonella* is the major cause of foodborne illnesses worldwide. Native animals and wildlife have been implicated in previous community-wide outbreaks of salmonellosis (5,6). Outbreaks of *Salmonella* infections have been caused by foods of animal origin, and poultry and poultry products such as eggs are considered to be the most important food vehicles for *S. enteritidis* infections (7,8).

During the current investigation, 2 different serotypes of *Salmonella* were detected in a large outbreak linked to egg sandwiches in 2 universities in Xiamen in October 2012. According to the incubation period of *Salmonella*, the epidemic curves from the overall epidemic

Fig. 2. PFGE graph clustering analysis.

curve and the 2 universities epidemic curves suggested homologous continuous exposure, and that there might have been 2 exposure peaks. *S. enteritidis* and *S. chester* were isolated from both the case students and food samples. PFGE played a key role in identifying the outbreak-associated isolates and distinguishing them from unrelated sporadic isolates. PulseNet has greatly improved our ability to detect widely dispersed outbreaks and perform source tracking. It also allows PFGE fingerprints to be communicated rapidly with other public health laboratories that analyze bacterial foodborne pathogens to facilitate the tracking of outbreaks in other cities or provinces.

This outbreak is one of the multiple *Salmonella* serotype outbreaks caused by contaminated food (9,10). *Salmonella* accounts for 70%–80% of the total number of bacterial food poisoning outbreaks reported in the literature in China (11). Outbreaks of *Salmonella* infections and sporadic illnesses in particular have been connected to foods of animal origin (e.g., eggs, meat, and meat products) (12). Eggs are polluted via systemic poultry infections, local intestinal infection, or infection via a polluted environment (13). A previous study reported that the detection rate of *Salmonella* in egg yolks and proteins was 2.5% and 5.0%, respectively (14). In addition, the *Salmonella*-positive rate was 76.7% in fresh chicken egg yolks from a free market (15). Finally, the rates of eggshells contaminated by *Salmonella* were reported to be 15.0% and 27.5% (14,16).

The gastroenteritis outbreak was caused by infection with various *Salmonella* serotypes via contaminated egg sandwiches. It is possible that the raw eggs were contaminated, and that insufficient heating and storage at

room temperature might be risk factors for human health. Additional toppings or flavors, such as mayonnaise, eggs, and meat products, might also be contaminated with foodborne pathogens. The company failed to provide records related to the baking times and temperature records; thus, we recommend the recall and destruction of all implicated egg sandwiches. In addition, the company should stop producing egg-related products until food safety procedures are established completely. We suggest that pasteurized or irradiated egg products could be used to prepare dishes in which eggs would otherwise be pooled before cooking or when the dish containing eggs is not subsequently cooked. Adequately cooking or heat treating (including pasteurization or irradiation) animal-derived foods prepared for animal consumption should be performed to eliminate pathogens.

A major limitation of the current investigation was our inability to detect *Salmonella* in the raw eggs because only a small number of egg samples was collected. A second limitation is that we could not collect samples from the egg sandwiches consumed by the students to confirm our hypothesis.

In conclusion, this event revealed that there is a lack of Hazard Analysis Critical Control Point management and effective evaluation in some food processing enterprises. The supervisory department must strengthen the supervision and inspection of raw material source records, raw material storage times and temperatures, sterilization times and temperatures, and finished product storage times and temperatures.

Acknowledgments We are indebted to Bao-ping Zhu for his

guidance during the outbreak investigation, and his comments and suggestions on data analysis and manuscript preparation. We also thank Yidun-Zhang, Zhonglong-Chen, Peizhi-Huang and Zhimin-Qiu for their valuable contributions to this investigation and this letter. Our study was financially granted by Xiamen municipal health bureau's Medical research program entitled "Spectrum study and establishment of surveillance platform of infectious diseases in Xiamen City (WSK2010-01)."

Conflicts of interest None to declare.

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