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An outbreak of food-borne salmonellosis linked to a bread takeaway shop in Ben Tre City, Vietnam



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SUMMARY

Objectives: To identify the vehicle, source, and causative agent of a community-wide food-borne outbreak of gastroenteritis.

Methods: We conducted a case–control study. Cases were city residents diagnosed with gastroenteritis and hospitalized in Ben Tre City from 22 to 25 May 2013; 41 cases were selected randomly from a list of hospitalized patients. Controls were age- and gender-matched healthy neighbours of cases. Participants were interviewed using a standard questionnaire. Samples from patients and food were tested at reference laboratories. We used conditional logistic regression to calculate matched odds ratios (mORs) for the association of gastroenteritis with food items consumed.

Results: Of the 41 cases enrolled in the study, 61% were males and the median age was 33 years; cases resided in 12 wards of the City. Of 13 food items consumed by the cases, only stuffed bread was significantly associated with gastroenteritis (mOR 21.3, 95% confidence interval 6.3–71.8). Among the 29 cases who ate stuffed bread, the median time to illness onset was 9 h. Patient stool samples and bread samples were positive for *Salmonella* species.

Conclusions: Stuffed bread was the likely vehicle of the outbreak. The laboratory testing capacity for serotypes of *Salmonella* should be strengthened in Vietnam. Food-handler training in basic food safety measures should be improved.

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1. Introduction

Food safety is an important public health concern in Vietnam. In 2013, the media repeatedly reported that dozens to hundreds of people had been hospitalized with acute gastroenteritis due to the consumption of stuffed bread across the country.¹

On May 23, 2013, two cases of acute gastroenteritis occurring shortly after the consumption of stuffed bread from a food stand were reported by a provincial hospital of Ben Tre City to the Ben Tre Food Safety Agency (FSA). The number of cases admitted to the hospital increased sharply to more than 150 by May 24, 2013. After preliminary investigations, the FSA suspected that stuffed bread

sold at the food stand was associated with the outbreak. As the food stand sold more than 1000 bread items daily, the FSA requested that the business close pending further investigations.

On May 27, 2013 the FSA requested further technical support from the regional Institute of Hygiene and Public Health to investigate the outbreak. We conducted an investigation to identify the vehicle, source, causative agents, and risk factors associated with the outbreak, and to make recommendations to prevent similar outbreaks.

2. Methods

According to baseline surveillance of notifiable diseases, 7–12 cases of gastroenteritis per month had been attended to at health centres and/or hospitals in the city during the 3 months prior to the

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outbreak. Initial interviews with 12 patients hospitalized with fever, diarrhoea, abdominal pain, vomiting and/or nausea at Ben Tre City suggested that the bread sold at the food stand may have been linked with the illness. To confirm the association and to identify the vehicle and risk factors linked to the outbreak, we conducted a matched case–control study.

Case finding: Local health authorities informed the communities in Ben Tre City and advised those with symptoms of gastroenteritis to visit a health centre or hospital. All health centres/hospitals were requested to report any patients with these symptoms immediately to the FSA by telephone and written report. A list of 173 patients (no fatal cases) attending six health centres/hospitals from 22 to 25 May 2013 was obtained from the FSA.

Case definition: A case was a resident of Ben Tre City who had attended a local health centre/hospital with fever, diarrhoea, and abdominal pain, and was diagnosed with gastroenteritis by a clinician between 22 and 25 May 2013.

Neighbourhood controls were used: one healthy control subject (no symptoms of gastroenteritis in the past 7 days), matched for gender and age (± 1 year), who lived in the same community as the case. Neighbourhood controls were identified by staff at the local health centre of the case.

Among 163 cases who met the case definition, 41 were selected randomly from the list of gastroenteritis patients admitted to one of the six hospitals using random numbers created by R software. Cases and controls were interviewed face-to-face using a standard questionnaire collecting details of 13 food items consumed by the cases in the 24 h before illness onset. Clinical, diagnosis, and treatment information was obtained from the hospital records. Data on food items consumed by controls were collected for the same time period as for the matched case. Informed consent was obtained from all study participants. As this was a public health response to an outbreak, no institutional review was required.

Data were analyzed using R software (Epi and survival packages). We used conditional logistic regression to calculate matched odds ratios (mORs) and 95% confidence intervals (CIs) for the consumption of the 13 food items.

Food samples, including ingredients of the bread, were taken by the FSA from the food stand on May 23, 2013, put into sterile boxes, and kept at 4 °C. Sixteen stool samples were collected from hospitalized patients. All samples were shipped to the provincial and regional reference laboratories for testing of the following: *Salmonella* species, *Escherichia coli*, *Bacillus cereus*, *Clostridium perfringens*, and *Staphylococcus aureus*. The laboratory methods were based on the Vietnam Food Safety Standards (approved by ISO/IEC 17025) and the Association of Official Analytical Chemists international standards for food items.^{2,3} Cultures were obtained using Hektoen enteric medium, xylose lysine deoxycholate agar, and eosin methylene blue agar.

We inspected the sanitary conditions at the three sites where the bread ingredients were processed, including the kitchen layout, restroom conditions, and hygiene and storage conditions for raw and cooked foods. The origin of food products and the methods of food preparation and storage were recorded. We collected rectal swabs from the food-handlers and reviewed their health check-up and training on food safety records. They were also interviewed regarding knowledge and were observed for personal hygiene practices.

3. Results

3.1. Descriptive epidemiology of the outbreak

Of the 41 cases enrolled in the study, 25 (61%) were males and the median age was 33 years (range 3–57 years). **Figure 1** shows the 41 cases by time of onset of acute gastroenteritis. The cases resided in 12 wards of Ben Tre City, distributed around the food stand. Twenty-nine (71%) cases who had consumed bread from the food stand had onset of symptoms within 5–23 h (median 9 h).

All cases were previously healthy and had not had any symptoms of gastrointestinal disorder within 7 days prior to hospitalization. Thirty-four (83%) cases had abdominal pain as the first symptom. Symptoms upon hospitalization were fever (100%), diarrhoea (100%), abdominal pain (100%), vomiting (76%), nausea (68%), headache (61%), myalgia (20%), and bloody diarrhoea (17%). Vital signs of all patients were within the normal ranges except for fever. The white blood cell count was elevated in most cases, and the median percentage of neutrophils was 83% (interquartile range 79–88%). All patients recovered after treatment. By 8:00 a.m. on May 30, 2013, all cases had been discharged uneventfully after 1–5 days of hospitalization (median 3 days).

3.2. Case–control study

Cases had consumed 13 food items including two drinks. Of the 13 food items consumed by the study subjects, only the stuffed bread was significantly associated with gastroenteritis in the univariate analysis: 70.7% of the cases compared with 9.8% of controls reported eating stuffed bread (mOR 21.3, 95% CI 6.3–71.8). The 12 other food items were not associated with gastroenteritis (**Table 1**).

3.3. Environmental and laboratory investigation

Bread ingredients included pork bologna, pork pate, salted and dried pork, and raw egg mayonnaise. Pork products were prepared at food preparation sites 1 and 2 (**Figure 2**). At food preparation site 3, raw chicken egg yolk was used to make mayonnaise.

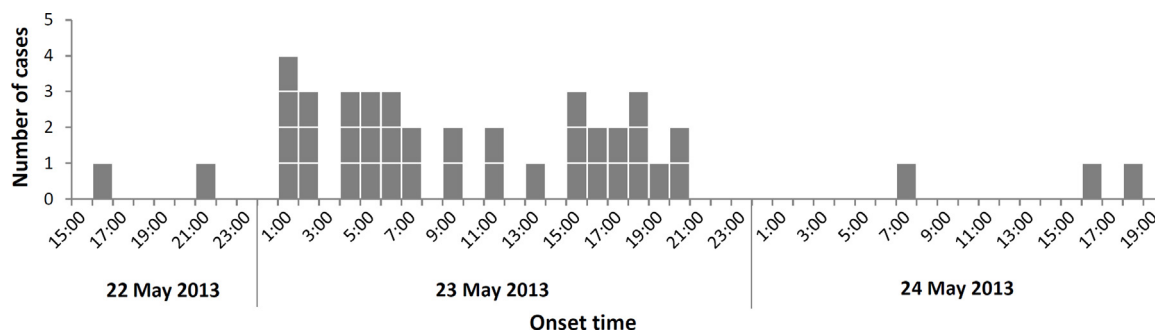


Figure 1. Cases ($n = 41$) with acute gastroenteritis by time of onset, May 2013, Ben Tre, Vietnam.

Table 1

Univariate matched odds ratios for the association between food items and gastroenteritis, May 2013, Ben Tre, Vietnam

Food items eaten	Cases (n=41)	Controls (n=41)	mOR	95% CI
Stuffed bread from food stand X	29 (70.7%)	4 (9.8%)	21.3	6.3–71.8
Iced-tea	8 (19.5%)	7 (17.1%)	1.2	0.4–3.6
Brined pork	17 (41.5%)	16 (39.0)	1.1	0.5–2.7
Brined goby	7 (17.1%)	7 (17.1%)	1.0	0.3–3.1
Sweet bread	1 (2.4%)	1 (2.4%)	1.0	0.1–16.3
Noodle without meats	1 (2.4%)	1 (2.4%)	1.0	0.1–16.3
Soya milk	3 (7.3%)	3 (7.3%)	1.0	0.2–5.2
Fresh vegetables	7 (17.1%)	10 (24.4%)	0.6	0.2–1.9
Vegetable soup with pork	14 (34.1%)	19 (43.6%)	0.6	0.3–1.5
Fried pork	3 (7.3%)	5 (12.2%)	0.6	0.1–2.6
Brined duck egg and pork	4 (9.8%)	8 (19.5%)	0.5	0.1–1.6
Noodle with meats	1 (2.4%)	9 (22.0%)	0.1	0.01–0.8
Coconut soup	1 (2.4%)	0 (0%)	-	-

mOR, matched odds ratio; CI, confidence interval.

Of the collected bread ingredients, 200 g each of pork bologna, pork pate, salted and dried pork, and raw egg mayonnaise all grew *Salmonella* species and *E. coli*. Of the 16 patient specimens collected, seven were positive for *Salmonella* species, but none for *E. coli*. Because the eggs used to prepare the mayonnaise were bought from many vendors in wet markets of the city, their origins could not be traced.

We observed poor hygiene practices at the three food preparation sites: insects were found and the kitchenware was not separated for cooked foods and raw foods. No food samples were stored after cooking at the preparation sites.

After bread ingredients were prepared, cooked foods were brought to preparation site 3 to make the bread and to sell to consumers. Cooked foods were kept at room temperature during serving and leftovers were stored at the end of each working section.

Eight food-handlers were involved in preparing the ingredients and the bread. Their rectal swabs were culture-negative for enteric bacterial pathogens. Six did not have any valid health certificates, and the health certificates of two had expired. No food-handler had a valid certificate for training in food safety, and their knowledge and practices of food safety, personal hygiene principles, and safe temperatures for cooked food were

poor. No food-handler had had symptoms of food-borne illness a week before the outbreak.

On May 24, the food stand was closed until the poor hygiene conditions and preparation processes were addressed. After closing, no new cases related to the stuffed bread were reported. Food-handlers were trained in food safety, personal hygiene principles, and safe temperatures for cooked food.

4. Discussion

The results of our epidemiological investigation support the hypothesis that bread from the food stand was responsible for this outbreak. In addition, cases consuming the bread had clinical symptoms and laboratory findings compatible with salmonellosis. Seven out of 16 stool specimens as well as all four specimens of bread ingredients grew *Salmonella* species. Taken together, these findings suggest that *Salmonella* species was the likely aetiology of the outbreak.^{4–6}

Food handlers did not have any symptoms of gastroenteritis and their rectal swabs were negative for *Salmonella*. Hence, the source of *Salmonella* could have been the food ingredients. Although the raw egg mayonnaise was positive for *Salmonella*, eggs were no longer available during the investigation of the food preparation sites and we could not trace the origin of the eggs. We could therefore not investigate whether the eggs were contaminated by *Salmonella*, but it is plausible that the source of *Salmonella* species was eggs.^{4,7} Although there is no direct evidence, the preparation procedures for the raw egg mayonnaise (Figure 2) and poor personal hygiene practices of the food-handlers during bread preparation, suggest that the mayonnaise may have been contaminated by *Salmonella* species from raw eggs and subsequently cross-contaminated the other ingredients of the bread.^{4,7}

Salmonella can survive the food preparation process⁸ and even in mayonnaise kept at 4 °C.⁹ In addition, *Salmonella* can grow faster at room temperature.⁴ The fact that mayonnaise is a high fat product, which could reduce the infecting dose of *Salmonella*,⁴ could explain the sharp increase in cases in a short time. *E. coli* bacteria found in the ingredients were likely a contaminant due to the poor personal hygiene of the food-handlers during bread preparation.

All case-control studies are susceptible to selection and information biases. To minimize these biases, we employed

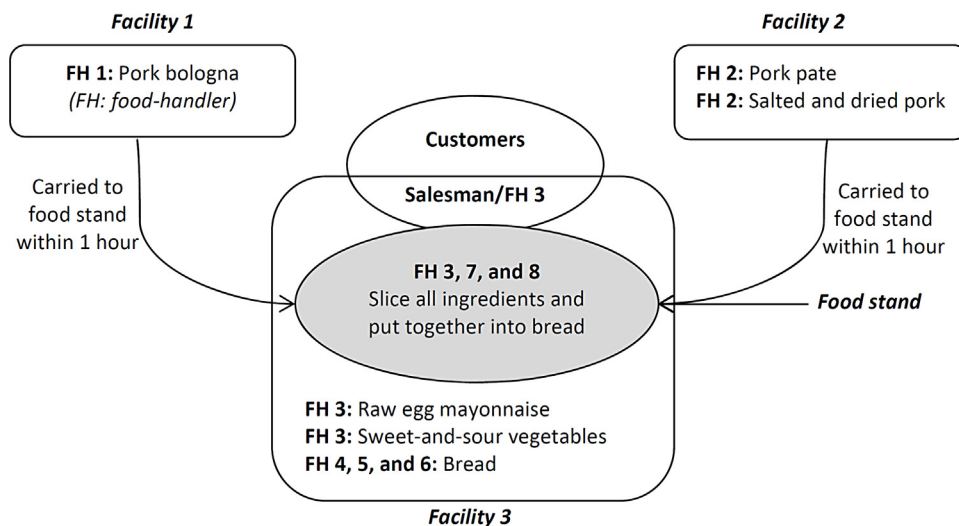


Figure 2. Flow chart of preparation of stuffed bread at food stand X by food-handlers (FH).

unambiguous case and control definitions, trained the interviewers, and pre-tested the questionnaire. Also, food items consumed by controls were ascertained for the same time period as for the matched case. A major limitation of the study, however, was a delay in initiating the investigation, resulting in delayed identification of the vehicle and testing of suspected food items, including raw eggs. Another limitation was that serotyping of bacteria was not performed by laboratories because of the limited capabilities of provincial laboratories as well as a lack of knowledge and skills of local health workers in specimen collection during the investigation. Training in sound epidemiological investigation methods and laboratory capacities for health personnel are therefore critical in resource-poor settings.

Many outbreaks of food-borne illness from stuffed bread have occurred throughout Vietnam in recent years.¹ However, these outbreaks were investigated only by testing food items in the laboratory without epidemiological data to support the testing results. Our investigation highlights the application of standard approaches for outbreak investigation combining several lines of investigation (including epidemiological, clinical, laboratory, and environmental) to provide more comprehensive evidence.

This outbreak also illustrates the substantial economic losses to business resulting from food handlers' poor knowledge of hygiene and food safety practices. Therefore, health education for food-handlers and the enforcement of safe food-handling practices are critical for the prevention of similar outbreaks and to avoid the loss of income resulting from business closure and the need to compensate those affected for the consequences of food poisoning.¹⁰

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Conflict of interest: No conflict of interest to declare.

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