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An Outbreak of Shigellosis in a Remote Village of Mongar District, Bhutan from March to April 2011

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Abstract

On 26 Mar 2011, staff at Mongar Hospital in Bhutan was notified of an outbreak of bloody diarrhea in a remote village. An investigation was conducted to determine the magnitude of outbreak and the source of infection. A case was defined as an individual in the village who developed diarrhea between 18 Mar 2011 and 3 Apr 2011. Active case finding and an environmental survey were conducted in the village. Laboratory investigations of fecal and environmental samples were carried out. Of 94 people residing in the village, 38 (40.4%) met the case definition. One case died, giving the case fatality ratio of 2.6%. The attack rate among females (51.1%) was significantly higher than males (30.6%). The secondary attack rate within households was 25.0%. *Shigella flexneri* was isolated from two of 10 stool samples. Poor sanitation and inadequate hygiene were observed in the village. Water samples were found to have fecal contamination. By multiple logistic regression analysis, significant risk factors for contracting the disease included being female, having no latrine as well as visiting and eating food in a sick neighbor's house. *Shigella flexneri* was the probable cause of the bloody diarrhea. Contaminated water might be the primary source of this enteric pathogen. Decontamination of water and improvement in hygiene might curtail future spread of the infection.

Key words: shigellosis outbreak, *Shigella flexneri*, contaminated water, Bhutan

Introduction

Shigellosis, commonly manifested by bloody diarrhea, is caused by *Shigella sonnei*, *Shigella flexneri*, *Shigella boydii* or *Shigella dysenteriae*. Among the four species, *Shigella flexneri* is the main cause of shigellosis in most developing countries.[1-2] Outbreaks of shigellosis continue to occur in many parts of the world with inadequate water supply, food safety, sanitation and hygiene.[1] The bacteria is shed in the feces of people infected with shigellosis and spread to others when they ingest food or water contaminated with the bacteria. Person-to-person transmission may also occur. Flies also transmit the organism from feces to uncovered food items and, when eaten, may cause infection.[1-2]

Diarrheal diseases remain among the top ten most prevalent diseases in Bhutan. The overall incidence of diarrheal diseases in Bhutan during 2010 was 126.6 per 1,000 people. There have been no representative data on shigellosis; however, it has been a notifiable disease in Bhutan since May 2010.[3] In Mongar District, diarrhea was among the top five causes of morbidity.[4] The incidence in Mongar during 2010 was 103.4 per 1,000 people and was higher among children under five years old (276.2 per 1,000). Dysentery contributed about 30% of diarrheal morbidity in Mongar. In 2010, the laboratory in Mongar Hospital cultured *Salmonella* and *Shigella* species from 250 stool samples from patients with dysentery. Of which, *S. flexneri* was the most common organism isolated (3.2%).

On 26 Mar 2011, an unusual rise of bloody diarrhea with death of a child was reported to Mongar Hospital by people of the Dak Village. Dak is a small village located in a remote part of Mongar District, eastern Bhutan (Figure 1). It had approximately 100 inhabitants who were mainly farmers living in 27 households. It was linked by a mule track that passed through a jungle and mountains. The nearest health care centre is in Silambe, which is situated about two-hours walking distance from the Dak Village. The

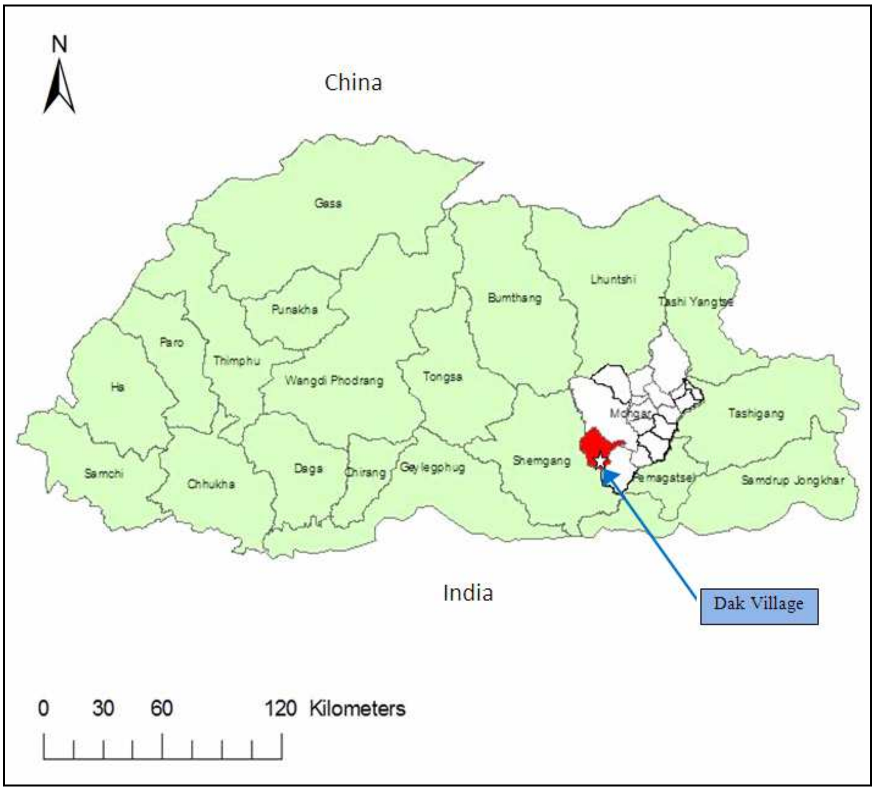

Figure 1. Map of Bhutan showing location of Dak Village in Mongar District

investigation team from Mongar Hospital and local health workers from Silambe Health Centre investigated the outbreak from 27 Mar to 1 Apr 2011. The investigation was conducted to confirm the diagnosis, describe epidemiological characteristics of the outbreak, identify risk factors, locate source of infection and provide recommendations for prevention and control of the outbreak.

Methods

Descriptive Study

The investigation team reviewed the monthly reports for diarrhea and dysentery from the Silambe Health Centre and Mongar District Health Office from January 2006 to April 2011. In Bhutan, diarrhea was defined as passing three or more loose stool over 24 hours with or without dehydration while blood dysentery was defined as diarrhea with visible blood in the stool. Active case finding was conducted by visiting all the houses and interviewing the people lived in Dak Village during the study period. A suspected case was defined as an individual living in Dak Village who developed diarrhea between 18 Mar and 3 Apr 2011. A confirmed case was a suspected case with bacteriological confirmation of a causative organism by culture or serological test.

To describe the transmission among household contacts, we evaluated the secondary attack rate using the following definitions[5]. A household contact was defined as a person living in the same house as the index patient in the household. An index case for the household was a suspected or confirmed case that had the earliest onset in a household in Dak Village between 18 Mar and 3 Apr 2011. A secondary case was a suspected or confirmed case that had onset of illness within one to seven days after the symptom onset of an index case in the same household.

Laboratory Study

Stool samples were collected for microscopic examination and culture. Stool samples for culture and drug sensitivity testing were inoculated into Xylose Lysine Desoxycholate (XLD) medium and processed immediately. The medium was packed in a cold box (vaccine carrier) which maintained temperature between 4-8°C and transported to the laboratory in Mongar Hospital. *Shigella* strains were sub-cultured on XLD and confirmed by serological testing. Suspected colonies were selected after incubation at 35°C for 24 hours. Antibiotic susceptibility was determined by the disk diffusion method.[6] Total eight antibiotics were tested, including amoxicillin, tetracycline, amikacin, gentamicin, trimethoprim/sulfamethoxazole, cefotaxime, nalidixic acid and ciprofloxacin.

Environmental Study

During household visit in the village for active case finding, we surveyed for environmental hygiene including availability of latrine and waste pit, and presence of flies in domestic and peri-domestic area of houses. Information on hand washing practice, habits related to drinking water, visiting to sick neighbors, attending the gathering event and travel to other villages was collected. We inspected the water system of the village, including drinking water sources, tanks, pipes, taps and water storage in the houses. We collected water samples from source, tank, taps and water containers, and tested for fecal coliform bacteria by the Millipore membrane filtration method in the field.

Analytical Epidemiology

The cohort study was conducted from 27 Mar to 1 Apr 2011 to identify possible risk factors for infection among all the residents of the village. All villagers were interviewed face-to-face using the questionnaire. Cases were suspected and confirmed cases identified from the descriptive study. Non-cases were those who did not report diarrhea during the study period.

Statistical Analysis

Percentage, attack rate, median and range were used for descriptive statistics of the study persons. The chi-square test was used for comparison of proportions. Multiple logistic regression analysis (unconditional method) was used to assess the effect of risk factors, after adjusting for other co-variables. Adjusted Odds Ratios (OR) and the 95% confidence intervals were calculated to indicate the strength of association. All statistical analyses were performed using Epi Info version 3.5.3.

Results

Descriptive Study

From 18 Mar to 1 Apr 2011, total 38 cases met the case definition. This yielded an attack rate of 40.4%. The first case was a 65-year-old female who had onset of illness on 18 Mar 2011. She reported that she did not travel out of the village prior to her illness. Subsequent cases had onset between 22 Mar and 1 Apr 2011 (Figure 2). A five-year-old child with onset on 24 Mar 2011 died after a day of diarrhea and vomiting. Of these patients, 14 (36.8%) were secondary cases within the households. The outbreak affected 22 of the 27 households (81.5%) in the village. The most common symptoms were diarrhea (100.0%), abdominal pain (90.0%), bloody diarrhea (71.1%), vomiting (65.8%), fever (60.5%) and dehydration (31.6%).

Among 38 case-patients, 23 (60.5%) were females and 15 (39.5%) were males. The attack rate among females was 51.1%, which was significantly higher than that of males with 30.6% (p-value 0.04). The outbreak affected all age groups in the village. However, the highest attack rate was among females of 65 years or more (Table 1). The majority of the patients were farmers (68.0%), followed by students (16.0%) and pre-school children (16.0%).

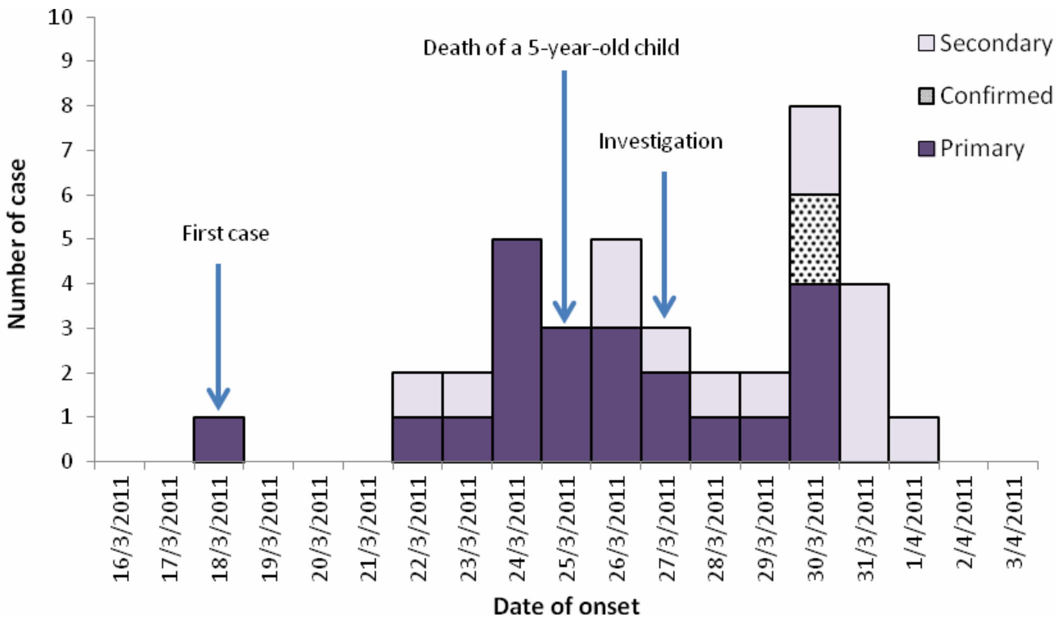

Figure 2. Epidemic curve of diarrhea cases by date of onset and primary or secondary transmission, Dak Village, Mongar, Bhutan, 17 Mar to 3 Apr 2011

Table 1. Attack rate of diarrhea cases by age group and gender in Dak Village, Mongar, Bhutan, 18 Mar to 1 Apr 2011

Age group (year)	Total population		Number of case		Attack rate (%)	
	Male	Female	Male	Female	Male	Female
0-14	15	10	7	5	46.7	50.0
15-44	14	16	3	6	21.4	37.5
45-64	12	12	1	5	8.3	41.7
≥ 65	8	7	4	7	50.0	100.0
Total	**49**	**45**	**15**	**23**	**30.6**	**51.1**

To determine the secondary attack rate within households, we identified 56 contacts in 22 households with a suspected case; 14 subsequently developed diarrhea and thus, had secondary attack rate of 25.0% (Table 2).

Table 2. Secondary attack rate of diarrhea cases by age group of primary cases in Dak Village, Mongar, Bhutan, 18 Mar to 1 Apr 2011

Primary case		Number of population at risk	Number of secondary case	Secondary attack rate (%)
Age group (year)	Number			
0-14	8	23	5*	21.7
15-44	2	8	1	12.5
45-64	7	11	2	18.2
≥ 65	7	14	6	42.9
Total	**24**	**56**	**14**	**25.0**

P-value = 0.41

**All cases were mothers of children with diarrhea.*

Laboratory Findings

Altogether 10 stool samples (five from patients who were already on antibiotic therapy and five from patients who did not receive antibiotics) were transported to the laboratory in Mongar Hospital for culture and drug sensitivity testing. The laboratory tests were performed after five days of stool sample collection. Two of five samples taken from the patients who did not receive antibiotics were found positive for *Shigella flexneri* serotype 2b. Both isolates had identical antibiotic susceptibility pattern which was sensitive to amoxicillin, trimethoprim/ sulfamethoxazole, tetracycline, amikacin, gentamicin, cefotaxime and ciprofloxacin, but resistant to nalidixic acid.

Environmental Findings

The entire village was adequately served by a rural water supply scheme obtained from a running stream. The stream was located in the middle of the jungle. There were both domestic and wild animals found grazing near the source. There was heavy rain from 15 Mar 2011 and lasted until 28 Mar 2011. Water from this source was not treated by chlorine. The water for domestic use was stored in plastic jerry-cans or buckets, and many did not have a lid. The majority (72.3%) of villagers drank water which was not boiled, chlorinated or filtered. Fifteen water samples collected from source, tank, taps and containers showed 30, 30, 32 and 50CFU (Colony Forming Unit) per 100 ml of fecal contamination respectively. The fecal contamination in household water storage containers (50CFU/100ml) was higher than other samples (Table 3).

Table 3. Water samples tested for fecal coliform bacteria in Dak Village, Mongar, Bhutan, 18 Mar to 3 Apr 2011

Type of water sample	Total number tested	Number of fecal contamination	Fecal coliform bacteria per 100ml
Source (running stream)	1	1	30
Tank	1	1	30
Tap	10	10	30
Water container	15	15	40

The assessment of individual households revealed that only 44.4% (12/27) of the households had a latrine for defecation. All were deep pit latrines and there was little possibility of contamination the water supply by latrine waste. No separate hand washing facility was seen near the latrines, yet almost all houses had a water tap nearby. Villagers without a latrine practiced open defecation in pasture land or in the jungle. Of the villagers, 18.3% did not wash hands before eating food and 56.4% did not wash hands after defecation. Similarly, a total of 60 individuals (63.8%) had visited a sick neighbor and of which, 30 (50.0%) got the infection. The infection rate among people who drank unboiled water was 48.4% and that of those who did not have a latrine was 53.7% (Table 4).

Table 4. Potential risk factor for diarrhea in Dak Village, Mongar, Bhutan, 18 Mar to 3 Apr 2011

Risk factor	Exposed		Non-exposed		Risk ratio	95% CI
	Total number	% infected	Total number	% infected		
No latrine at residence	54	53.7	40	22.5	2.4	1.3 - 4.5
Visiting a neighbor with active diarrhea case	60	50.0	34	23.5	2.1	1.1 - 4.1
Drinking unboiled water	66	48.4	28	21.4	2.2	1.1 - 5.1
No washing hands before eating food	17	47.1	77	39.0	1.2	0.7 - 2.1
No washing hands after defecation	53	50.9	41	23.5	1.9	1.1 - 3.4
Attended gathering/funeral	33	45.5	61	37.7	1.2	0.7 - 1.9

Analytic Results

All 94 inhabitants living in Dak Village during the study period were interviewed about hygienic practices. The exposed groups were those who did not have latrine for defecation, drank unboiled water, did not wash their hands before eating and visited the ill. They were compared with the control group who had a latrine, drank boiled water, washed their hands before eating and did not visit people with diarrhea. From the univariate analysis, we found that the risk of contracting the disease among people who did not have latrine was 2.4 times higher than those having latrine for defecation (Table 4). Other risk factors were drinking unboiled water, and visiting and eating food in a sick neighbor's house with diarrhea.

Table 5. Association between potential risk factors and diarrhea by multiple logistic regression analysis, Dak Village, Mongar, Bhutan, 18 Mar to 3 Apr 2011

Risk factor	Adjusted OR*	95% CI	P-value
Age	0.99	1.0 - 1.0	0.40
Gender	3.18	1.1 - 9.0	0.03
Drinking unboiled water	2.41	0.8 - 7.8	0.10
No latrine at residence	3.47	1.2 - 10.1	0.02
No washing hands before eating food	0.37	0.1 - 1.5	0.16
Visiting a neighbor with active diarrhea case	3.90	1.2 - 12.4	0.02

**Adjusted for all variables in the table.*

Multiple logistic regression analysis was used to identify the possible risk factors for infection in this outbreak, after adjusting for other co-variables (Table 5). The significant risk factors included being female, having no latrine as well as visiting and eating food in a sick neighbor's house.

Control Measures

Control measures included active case finding in Dak Village, prompt antibiotic treatment with amoxicillin or trimethoprim/sulfamethoxazole, improvement of hygiene and sanitation in the village including surroundings, latrines and compulsory hand washing before eating and feeding children. The water supply was chlorinated and people were advised to boil all drinking water. The occurrence of new cases had subsequently stopped after five days of implementing the control measures.

Discussion

The diarrhea outbreak occurred during March 2011 in a remote village of Mongar District, Bhutan was probably caused by *Shigella flexneri*. Most of the cases (71.1%) presented with bloody diarrhea while shigellosis is the most common cause of bloody diarrhea in the world.[1] Laboratory analysis of stool cultures confirmed *S. flexneri* in two of 10 patients. Negative stool cultures in five patients were probably due to the antibiotics taken before stool was collected for laboratory analysis. A study in India showed that 83.4% of stool collected from patients after antibiotics therapy had no growth on all cultured media.[7] However, *S. flexneri* was the most common organism isolated from dysentery patients in the laboratory of Mongar Hospital.[4] This organism was reported to be a predominant species in Asian countries.[8-12]

In this outbreak, children and elderly women were predominately affected. Similar age-specific attack rates were reported by a multicentre study of *Shigella* diarrhea in six Asian countries in 2006,[13] a study in a rural village of China in 2005[14] and in other countries[15]. The higher attack rate among young and elderly females might be because females, particularly the elderly, were primarily involved in taking care of the ill family member and might have higher exposure to *Shigella*-containing feces. Another reason for higher susceptibility to shigellosis may be due to less effective immune response of young and elderly people, which could be caused by poor nutritional status and low immunity prior to infection. Poor hygienic practices also favor transmission.

Shigella spreads by eating contaminated food, drinking contaminated water or direct contact with an infected person. The heavy rain in Mongar started in the second week of March 2011, lasted until last week of March and preceded the outbreak. It was quite unusual to have continuous rainfall and a diarrheal outbreak at this period. Normally, the rainy season in Bhutan as well as in Mongar starts in June and lasts till the end of August.[16] The trend of diarrheal diseases also increases correspondingly to the rainy season.[16] The presence of coliform bacteria in water and lack in chlorination were further evidences supporting the contamination of water supply. Fecal contamination could have resulted from human or animal feces, or surface organisms washing into the stream and into open water sources by the rainfall, which is common in mountainous areas.[17] In that report, fecal contaminated water sources in mountainous areas were common because of inadequate source protection, lack of water treatment and poor sanitary practices and resulted in frequent waterborne outbreaks.[17] The appearance of the cases after the rainfall had added our suspicion towards water though it was not statistically significant. On the other hand, poor sanitation and hygiene observed in the village were the clear evidence supporting that the outbreak was related to poor hygiene and sanitary practices. Similar shigellosis outbreaks related to water and poor sanitary practices were also reported in India[18], Thailand[19], Taiwan[20], Greece[21], and Spain[22].

The coliform level in the household water containers was substantially higher than other water sources. This indicated that the water contamination had increased as it reached to the consumption point. Generally, people collected water and stored it in jerry-cans or buckets for several days before refilling. Studies in India during 2008[23] and Bolivia during 2010[24] on quality of drinking water at source and point of consumption found that the practice of water collection, storage, handling, choice of storage container and hygiene significantly affected the water quality in the households.

Person-to-person spread is also the main mode of *Shigella* transmission. The high attack rate of 40.4% among the residents, high secondary attack rate of 25.0% and rapid spread covering 81.4% (22/27) of households in a short period of time suggested that nature of the outbreak was highly contagious. Transmission of infection from person-to-person could have been facilitated by inadequate hand hygiene and frequent contact with a sick person. In this outbreak, having no latrine was one of the significant risk factors. The transmission could have been increased by practice of open defecation that was likely to be the risk for food and water contamination by various disease vectors such as flies, rats and pet animals. The flies and lack in hand washing could have played a vital role in transmitting the organism via the fecal-oral route. Evidence of person-to-person transmission in this study could be further explained by a high secondary attack rate which was higher than reports of studies in Crete[25] and Belgium[26]. Furthermore, *Shigella* may survive up to 8-24 hours in water[27] and in soil at room temperature for 9-12 days[28].

Several limitations of the study were faced because of remoteness of the outbreak village. Firstly, the prevalence of diarrheal diseases before and after the outbreak could not be ascertained due to lack of data and difficulty in returning to the village. Secondly, the number of cases was limited and thus, there was limited detailed analysis of risk factors. Thirdly, we assumed that all secondary cases acquired the infection at home. Alternatively, they might have been infected during school attendance or by visiting sick friends and relatives. Fourthly, we could culture few stool samples as it was difficult in transporting samples due to small capacity of transport facility and long distance to hospital. Lastly, as water source was not treated, we did not test for residual chlorine level in drinking water.

In conclusion, this was the first documented outbreak of a diarrheal disease in the Dak Village, Bhutan. The findings from this study suggested that the poor sanitation and hygiene practice were the main factors attributed to this outbreak. This could be the result of poor living condition of villagers because of low risk perception, low literacy and lack in awareness towards hygiene practice to avert the health crisis. This study highlighted the need of having a pit latrine for defecation in every household and improvement of hygiene as effective measures for controlling future outbreaks. Interventions aimed in improving the living condition of people should go hand-in-hand with sustained health education programs in communities to reduce health risks. Safe water handling, storage practices in households and decontamination of water by boiling need to be emphasized at all levels. The supply of a kit for testing chlorine level of water in district hospitals would greatly benefit in monitoring the quality of drinking water.

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<http://www.osirjournal.net/issue.php?id=35>.

References

1. World Health Organization. Guidelines for the control of shigellosis, including epidemics due to *Shigella dysenteriae* type 1. Geneva: World Health Organization; 2005.
2. Heymann DL, editor. Control of communicable diseases manual. 19th ed. Washington, DC: American Public Health Association; 2008. p. 556-60.
3. Bhutan. Ministry of Health. Annual health bulletin, 2010. Thimphu: Bhutan Ministry of Health; 2011.
4. National Statistics Bureau. Annual Dzongkhag statistics, 2010. Thimphu: National Statistics Bureau; 2011.
5. Park K. Park's textbook of preventive and social medicine. 19th ed. Jabalpur: M/s Banarasidas Bhanot Publishers; 2007. p. 567-89.
6. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; nineteenth informational supplement. Wayne: Clinical and Laboratory Standards Institute; 2009.
7. Chitkara YK. Limited value of routine stool cultures in patients receiving antibiotic therapy. Am J Clin Pathol. 2005 Jan;123(1):92-5.
8. Dutta S, Dutta P, Matsuchita S, Bhattacharya SK, Yoshida S. *Shigella dysenteriae* serotype 1, Kolkata, India. Emerg Infect Dis. 2003 Nov;9(11):1471-4.
9. Taneja N. Changing epidemiology of shigellosis and emergence of ciprofloxacin-resistant *Shigellae* in India. J Clin Microbiol. 2007 Feb;45(2):678-9. Epub 2006 Nov 22.
10. Kansakar P, Malla S, Ghimire GR. *Shigella* isolates of Nepal: changes in the incidence of *Shigella* subgroups and trends of antimicrobial susceptibility pattern. Kathmandu Univ Med J (KUMJ). 2007 Jan-Mar;5(1):32-7.
11. Chompook P, Samosornsuk S, Von SL, Jitsanguansuk S, Sirima N, Sudjai S, et al. Estimating the burden of shigellosis in Thailand: 36 month population-based surveillance study. Bull World Health Organ. 2005 Oct;83(10):739-46. Epub 2005 Nov 10.
12. Ranjbar R, Hosseini MJ, Kaffashian AR, Farshad S. An outbreak of shigellosis due to *Shigella fexneri* serotype 3a in a prison in Iran. Arch Iran Med. 2010 Sep;13(5):413-6.
13. Von SL, Kim DR, Ali M, Lee H, Wang X, Thiem VD, et al. A multicentre study of *Shigella* diarrhoea in six Asian countries: disease burden, clinical manifestations, and microbiology. PLoS Med. 2006 Sep;3(9):e353.
14. Wang XY, Du L, Von SL, Xu ZY, Zhang YL, Hao ZY, et al. Occurrence of shigellosis in the young elderly in rural China: results of a 12-month population-based surveillance study. Am J Trop Med Hyg. 2005 Aug;73(2):416-22.
15. Kotloff KL, Winickoff JP, Ivanoff B, Clemens JD, Swerdlow DL, Sansonetti PJ, et al. Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. Bull World Health Organ. 1999;77(8):651-66.
16. Tshering D, Sithey G. Climate change and health in Bhutan. Thimpu: Royal Society for Protection of Nature; 2008.
17. Rai SK, Ono K, Yanagida JI, Kurokawa M, Rai CK. Status of drinking water contamination in Mountain Region, Nepal. Nepal Med Coll J. 2009 Dec;11(4):281-3.
18. Saha T, Murhekar M, Hutin YJ, Ramamurthy T. An urban, water-borne outbreak of diarrhea and shigellosis in a district town in

eastern India. Natl Med J India. 2009 Sep-Oct;22(5):237-9.

19. Chompook P, Todd J, Wheeler JG, Von SL, Clemens J, Chaicumpa W, et al. Risk factors for shigellosis in Thailand. Int J Infect Dis. 2006 Nov;10(6):425-33. Epub 2006 Sep 25.

20. Chao YN, Huang ASE, Chiou CS, Tsai SH, Lin CH, Lee PH, et al. A waterborne shigellosis outbreak in a primary school, Tai-Chung City, November 2007. Taiwan Epidemiol Bull. 2008;24:740-52.

21. Koutsotoli AD, Papassava ME, Maipa VE, Alamanos YP. Comparing *Shigella* waterborne outbreaks in four different areas in Greece: common features and differences. Epidemiol Infect. 2006 Feb;134(1):157-62.

22. Arias C, Sala MR, Domínguez A, Bartolomé R, Benavente A, Veciana P, et al. Waterborne epidemic outbreak of *Shigella sonnei* gastroenteritis in Santa Maria de Palautordera, Catalonia, Spain. Epidemiol Infect. 2006 Jun;134(3):598-604. Epub 2005 Sep 30.

23. Tombekhar DH, Gulane SR, Jaisingkhar RS, Manikhar MS, Vangiwar YS, Mogarekar MR. Household water management: a systemic study of bacteriological contamination between source and point of use. American-Eurasion J Agric & Environ Sci. 2008; 3(2): 241-6.

24. Rufener S, Mäusezahl D, Mosler H, Weingartner R. Quality of drinking-water at source and point-of-consumption: a field study in Bolivia. J Health Popul Nutr. 2010 Feb;28(1):34-41.

25. Samonis G, Elting L, Skoulika E, Maraki S, Tselentis Y. An outbreak of diarrhoeal disease attributed to *Shigella sonnei*. Epidemiol Infect. 1994 Apr;112(2):235-45.

26. De Schrijver K, Bertrand S, Gutiérrez Garitano I, Van den Branden D, Van Schaeren J. Outbreak of *Shigella sonnei* infections in the Orthodox Jewish, community of Antwerp, Belgium, April to August 2008. Euro Surveill. 2011 Apr 7;16(14). pii: 19838.

27. Wu FM, Beuchat LR, Doyle MP, Mintz FD, Wells JG, Swaminathan B. Survival and growth of *Shigella flexneri*, *Salmonella enterica* serovar enteritidis, and *Vibrio cholerae* O1 in reconstituted infant formula. Am J Trop Med Hyg. 2002 Jun;66(6):782-6.

28. Centers for Disease Control and Prevention. Guidance on microbial contamination in previously flooded outdoor areas. Atlanta: National Center for Environmental Health, Centers for Disease Control and Prevention; 2011.

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Bacillus cereus Food Poisoning Outbreak in a Kindergarten School, Bangkok, Thailand, December 2009

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Abstract

On 18 Dec 2009, the Bureau of Epidemiology was notified that 20 students from a private kindergarten school were treated for vomiting and diarrhea. An investigation was conducted to verify the diagnosis, identify source of the outbreak, and implement prevention and control measures. We conducted a descriptive and retrospective cohort study. Medical records at the hospital were reviewed. We also interviewed students, teachers and cooks at the school. A case was a student in this school who developed vomiting with at least one of the followings: fever, diarrhea or abdominal pain from 18 to 22 Dec 2009. Twenty three clinical specimens (vomitus and rectal swabs) and food samples were collected, and sent to National Institute of Health for bacterial culture. Logistic regression was used to determine the food items associated with illness. Symptoms included vomiting (100%), abdominal pain (59%), diarrhea (31%) and fever (26%). *Bacillus cereus* was isolated from three out of six vomitus specimens as well as the sweet stewed egg and pork served for school lunch on 18 Dec 2009. Thus, this outbreak was due to *Bacillus cereus* (emetic form) and the common source was likely to be the sweet stewed egg and pork (adjusted OR 2.1, 95% CI 1.0-4.4). To prevent similar outbreaks in the future, people involved in food preparation and serving should emphasize on personal hygiene and sanitary food handling practices. School administrators should exclude symptomatic cooks and food handlers from cooking.

Key words: Bacillus cereus, food poisoning, school meals

Background

Food poisoning is a serious public health problem throughout the world.[1] In Thailand, more than 50 food poisoning outbreaks are reported every year; most are associated with schools.[2] Only 17% of these outbreaks had a specific pathogen identified. The most common pathogens identified were *Vibrio parahemolyticus*, *Salmonella spp.* and *Bacillus cereus*.[3]

Bacillus cereus is widespread in nature and frequently isolated from soil and growing plants. It is also well adapted for growth in the intestinal tract of mammals[4] and causes toxin-mediated food poisoning.[5] The bacteria is associated with two distinct types of illness: emetic syndrome caused by a heat-stable toxin and diarrhea syndrome caused by a heat-labile toxin.[6] *B. cereus* has been established as an etiologic agent of food poisoning in Europe since 1950 and in the United States since 1968.[7,8]

On 18 Dec 2009, the Bureau of Epidemiology (BOE) received a notification from a Health Center (HC) in Bangkok that 20 students from School A, a private kindergarten in Laksi District of Bangkok, were treated at a private hospital due to vomiting within an hour after eating the school lunch. On 19-22 Dec 2009, a joint BOE and HC team conducted an outbreak investigation to determine the diagnosis, confirm the outbreak, describe characteristics of the outbreak, identify possible source(s) of infection and implement effective control and prevention measures.

Methods

Epidemiologic Investigation

We began our investigation by reviewing the national disease surveillance records on diarrhea and food poisoning in Laksi District of Bangkok, and the medical records of students treated at the hospital on 18-22 Dec 2009. We also conducted active case finding by interviewing all students, teachers and cooks who were present at the school during our visit. In addition, information from each student was validated by interview with the child's homeroom teacher. Information included sex, age, time of the lunch eaten, type and estimated amount of food eaten, onset time of signs and symptoms, and treatment. A suspected case was defined as a person who ate the lunch served in School A on 18 Dec 2009 and developed vomiting with at least one of the following symptoms: fever, diarrhea or abdominal pain from 18 to 22 Dec 2009. A confirmed case was a suspected case with laboratory confirmation of vomitus for a pathogenic agent. We analyzed descriptive data using percentage, median, range and attack rate.

A retrospective cohort study was used to determine the risk factor(s) for illness. Cohorts were students of School A who went to the school on 18 Dec 2009. A case was a student in the cohort group who had vomiting with at least one of the following symptoms: fever, diarrhea or abdominal pain. Data was analyzed using Epi Info version 3.5.1 (US CDC). Univariate and multivariate analyses were conducted, including logistic regression to control the confounding factors. Variables included in the analyses were sex, school grade and all food items served for school lunch on 18 Dec 2009. We compared food-specific attack rates and odds ratios with 95% confidence intervals.

Laboratory Investigation

Clinical specimens were collected, including vomitus and rectal swabs from students, rectal swabs from teachers, and hand and rectal swabs from cooks. All specimens were sent to the Thailand National Institute of Health (NIH) for bacterial culture. Food remnants from the lunch served on 18 Dec 2009 were also obtained and sent to NIH for testing.

Environmental Investigation

We surveyed the school kitchen, refrigerators, water supply system and toilets. In addition, we interviewed cooks and observed food preparation such as cooking, serving and cleaning. Hand washing and eating habits of the students were also evaluated. Five samples of drinking water and pipe water were collected to measure the residual chlorine.

Results

Epidemiologic Results

The surveillance data from Laksi District showed that number of diarrhea illness and number of food poisoning were higher in December 2009 than that of the five-year median (Figure 1 and 2).

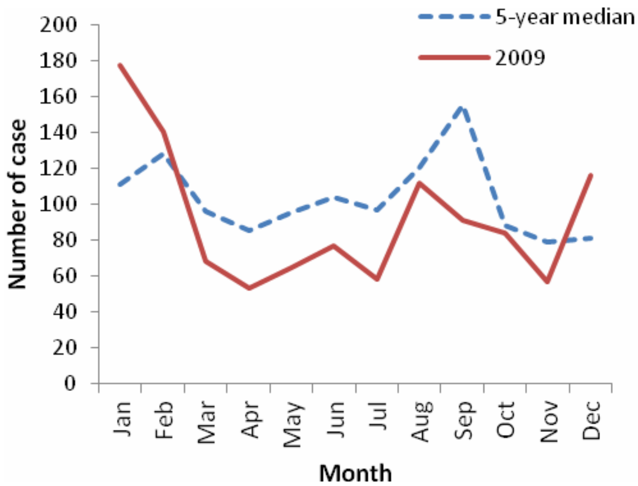

Figure 1. Number of diarrhea cases by month of onset compared with five-year median, Laksi District, Bangkok, Thailand, 2009

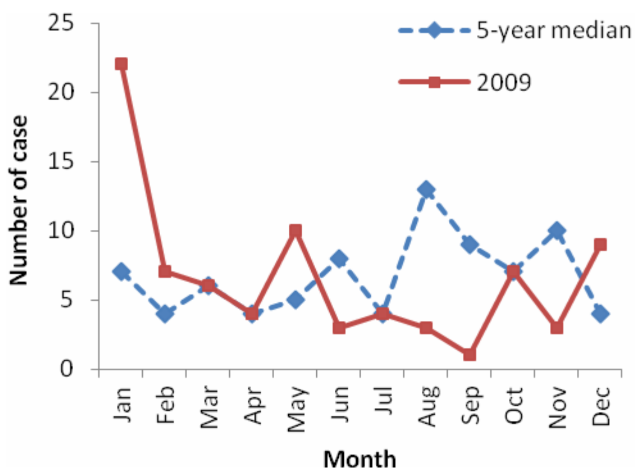

Figure 2. Number of food poisoning cases by month of onset compared with five-year median, Laksi District, Bangkok, Thailand, 2009

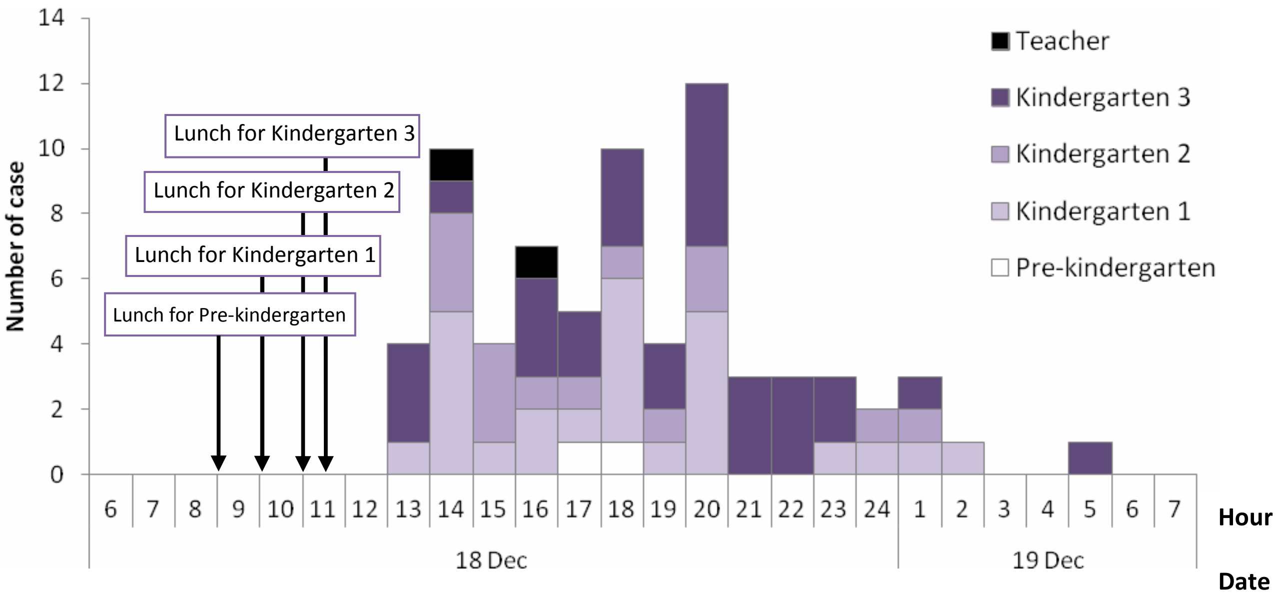

Figure 3. Number of food poisoning cases by occupation, grade and date and time of onset in School A, Bangkok, Thailand, 18-19 Dec 2009 (n=72)

The School A is a private kindergarten school with 265 students (133 males and 132 females), 36 teachers and five cooks. The overall attack rate was 27% (72/268). There were 70 cases (67 suspected and three confirmed) among students and two suspected cases among teachers. Sixty-four ill students received treatment at a hospital and 30 (47%) of them were hospitalized. Median age of the ill persons was four years, ranged from three to 35 years. The male to female ratio was 1:1. The highest attack rate was observed among students in Kindergarten 3 (Table 1).

Table 1. Number and attack rate of food poisoning cases by occupation and grade in School A, Bangkok, Thailand, 18-19 Dec 2009

Occupation	Number of case	Attack rate (%)
Student		
Pre-kindergarten	21	15
Kindergarten 1	101	28
Kindergarten 2	70	21
Kindergarten 3	73	40
Teacher	36	9

Clinical symptoms of illness included vomiting (100%), abdominal pain (59%), diarrhea (31%), fever (26%) and fatigue (19%). The epidemic curve is consistent with a common source outbreak (Figure 3). The first case had onset of symptoms at 1 pm on 18 Dec 2009 while the last case at 5 am on 19 Dec 2009. The school lunch served on 18 Dec 2009 was highly suspected to be the source of the outbreak. The median incubation period was seven hours (inter-quartile range 5.0 to 9.5 hours).

Two suspected cases among teachers were identified from active case finding. Both teachers had the same school lunch as the students on 18 Dec 2009. No other teacher ate the school lunch or became ill. The first ill teacher was a 35-year-old woman, who ate the school lunch at noon on 18 Dec 2009. She experienced abdominal pain and vomiting at 2:30 pm on that day. Her clinical symptoms improved without treatment. The second ill teacher was a 33-year-old woman, who had lunch with the first ill teacher, yet her abdominal pain and vomiting began at 4:30 pm on that day. She took Norfloxacin for one day and her symptoms improved.

Interviews indicated that two cooks had been ill before the outbreak occurred. One was a 60-year-old woman who had abdominal pain and diarrhea on 13 Dec 2009. She took an anti-diarrheal drug and her symptoms improved. The second cook was a 40-year-old woman who had vomiting and abdominal pain on 15 Dec 2009. Her symptoms improved after taking Norfloxacin for one day. Neither of these cooks were absent from work while they were ill.

In addition, the school provided soy milk to all students every morning and lunch. Lunch times were set by grade levels (Figure 3). Lunch on 18 Dec 2009 included rice, sweet stewed egg and pork, and watermelon. The amount of food served was different by grade levels. Students in pre-kindergarten, Kindergarten 1 and Kindergarten 2 received a bowl of rice (about three tablespoons), two pieces of sweet stewed egg (one egg divided into six pieces), a tablespoon of chopped pork and four pieces of pitted watermelon (1 cm^3 per piece). The amount of food for students in Kindergarten 3 was double than that of students in lower grade levels. Watermelon served for Kindergarten 3 students were not pitted.

A total of 241 students were interviewed, including 70 ill students. Fourteen students (20%) who asked for more rice, sweet stewed egg and pork, and 19 students (28%) who asked for more watermelon during lunch on 18 Dec 2009 became ill later. The attack rates by level of exposure were shown in Table 2.

Table 2. Attack rates by amount of food served during lunch in School A, Bangkok, Thailand, 18 Dec 2009

Food item	Quantity of food	Attack rate (%)	Chi-square	P-value
Soy milk (glass)	0 1 2	10 24 43	10.7	0.005
Rice (bowl)	1 2 3	23 35 78	6.7	0.10
Sweet stewed egg (piece)	1 2 3 4 8	29 21 40 37 88	11.3	0.001
Pork (teaspoon)	0 1 2 4	100 22 100 75	14.4	0.001
Watermelon (1 cm^3 piece)	1 2 3 4 5	0 20 17 22 20	9.1	0.003
Bread (piece)	0 1 2 3	50 23 41 100	10.0	0.007

Table 3. Analysis of food served during lunch in School A, Bangkok, Thailand, 18 Dec 2009

Food item	Crude OR* (95% CI)	Adjusted OR** (95% CI)
Soy milk (glass)	2.2 (1.3-3.7)	1.7 (0.4-6.8)
Rice (bowl)	2.0 (1.3-3.0)	0.7 (0.2-2.5)
Sweet stewed egg (piece)	1.6 (1.2-1.9)	2.1 (1.0-4.4)
Pork (teaspoon)	2.1 (1.4-3.2)	0.8 (0.2-4.0)
Watermelon (1 cm^3 piece)	1.2 (1.1-1.4)	1.1 (0.8-1.3)
Bread (piece)	2.0 (1.2-3.5)	0.5 (0.1-2.1)

** Logistic regression*

*** Adjusted for sex, grade and all food items*

Univariate analysis showed that all the food items served during the lunch were associated with illness. However, after adjusting for sex, grade and all food items, eating more than one piece of egg had an adjusted odds ratio of 2.1 (Table 3).

Laboratory Results

Six vomitus and two rectal swab specimens from six ill students admitted to the hospital were obtained on 19 Dec 2009. Five hand swab and five rectal swab specimesn were obtained from all five cooks. Two rectal swab specimens were obtained from two ill teachers. Three specimens of leftover food (soup of sweet stewed egg and pork, watermelon and bread) from the lunch on 18 Dec 2009 were also tested.

Three vomitus specimens from ill students and the soup of sweet stewed egg and pork were tested positive for *B. cereus*. All other specimens were negative for bacteria.

Environmental Results

There were five cooks (A-E) in the School A. The Cooks D and E had their fixed job descriptions; D was a cook's helper and E prepared only soy milk. However, the duties of Cooks A, B and C were changed every day. The Cook D was ill on 13 Dec 2009 and B on 15 Dec 2009. The preparation process for the lunch served on 18 Dec 2009 began in the evening of 17 Dec 2009 (Table 4).

Table 4. Food handling processes for lunch served on 18 Dec 2009 in School A, Bangkok, Thailand

Date and time	Food handling processes	Food handler*
17 Dec 2009		
16:00-17:30	- Purchased food at market.	B
	- Prepared sweet stewed eggs, put into soup and allowed standing at room temperature for 2 hours before refrigerated it.	C, D
18 Dec 2009		
06:00-09:30	- Reheated sweet stewed eggs and cut eggs into pieces.	B, C, D
	- Boiled the chopped pork.	D
	- Prepared watermelon.	C, D
	- Combined all ingredients into children's bowls for lunch.	A, D
09:30-10:30	- Placed lunch bowls on serving trolley and covered with cloths.	A, D
10:30-11:00	- Served lunch to pre-kindergarten.	A, D
11:00-11:30	- Served lunch to Kindergarten 1 and 2.	A, D
11:30 to noon	- Served lunch to Kindergarten 3.	A, D

** Food handler B had diarrhea on 15 Dec 2009.*
Food handler D had vomiting and diarrhea on 13 Dec 2009.

The ingredients used for the sweet stewed egg and pork were water, sugar, garlic, star anise seed, coriander roots, cinnamon and dark soy sauce. The water was boiled with all other ingredients. The boiled eggs were divided into little pieces, with one egg into six pieces. The pork was chopped and boiled in another pot. The soup, the chopped pork and egg pieces were mixed together and served.

Our survey showed that there were two kitchens; one for making only soy milk and the other for cooking. Cooked and raw foods were kept in the same refrigerator which was opened frequently. Dishes and utensils were cleaned by hands, and were put on a table outside the kitchen to dry. We observed that many leaves and dust fell onto that area (Figure 4).

Figure 4. Table outside the kitchen to dry dishes and other utensils in School A, Bangkok, Thailand, 18 Dec 2009

Usually, all students eat lunch in the school cafeteria. However, on 18 Dec 2009, the cafeteria was used to prepare for a Christmas party. Thus, only pre-kindergarten students had lunch there on that day. All other students ate lunch in their classrooms. Cooks used the same trolley to carry food from the cafeteria to every classroom, passing through the playground. Students did not wash their hands before eating. Students could ask for more food if they want.

The school used tap water for washing dishes and hand washing. Drinking water was filtered. There was a toilet in every classroom and soap was provided. The residual chlorine level of two tap water samples (kitchen and toilet) and three drinking water samples (kitchen, cafeteria and classroom) were less than 0.2 ppm, which was lower than the standard level.

Discussion

In the past two years, three outbreaks of *B. cereus* have been reported to the Thailand Bureau of Epidemiology, including two outbreaks in 2009 and one in 2008. All of these outbreaks occurred in schools.[9] Common problems associated with all three foodborne outbreaks were long standing time before serving, inadequate reheating and not excluding cooks with gastroenteritis symptoms from handling food. Suspected foods of those outbreaks were fried rice, noodle and fish balls.

In this outbreak, our findings were consistent with *B. cereus* (emetic form) infection.[6,10] The median incubation period was short, and *B. cereus* was isolated from patients and the soup of sweet stewed egg and pork. In addition, the egg was identified as a risk factor by statistical association. Thus, the most likely source of infection in this outbreak was the sweet stewed egg and pork. It had been a long interval from preparation of food until serving. The food items could be contaminated at any point of several preparation processes because the cooks used bare hands and same equipment for handling of raw and cooked food, and left food at ambient temperature after being thoroughly cooked.

The fact that students in Kindergarten 3 had the highest attack rate might reflect the delay in serving their lunch (about two hours between reheating or cooking and serving) as well as larger portion of food than those given to the lower grades. The longer time interval from food preparation to serving could have provided more opportunity for the bacteria to multiply.

Limitations

We identified several limitations in this outbreak investigation. Since the outbreak occurred among young children, the information we collected from them might not be accurate because they might not remember the food items or understand the questions. Some of them could not describe all of their symptoms. Furthermore, it was less likely that the teachers could observe and remember what each student had eaten.

Exposure misclassification might reflect recall bias that teachers could remember the food items eaten by the ill children more than that of the other children. There might have been other confounders that we did not identify.

Since 1971, more than 40 incidents of *B. cereus* food poisoning associated with consumption of cooked rice have been reported,[11] yet in our investigation, none of the rice that was served at lunch on 18 Dec 2009 was available for testing.

Conclusion

A common source food poisoning outbreak occurred in a kindergarten school in Bangkok, Thailand during

December 2009. *B. cereus* was the causative organism. The sweet stewed egg and pork served for school lunch on 18 Dec 2009 was the likely source of infection.

Actions Taken

During our investigation, we provided health education to students, teachers and cooks about food poisoning and general care for persons with gastrointestinal symptoms. A special surveillance was launched for one day after the outbreak to detect more patients with gastrointestinal symptoms among students, teachers and cooks. No additional case of food poisoning was reported in this school since 19 Dec 2009.

Recommendations

Food hygiene and sanitation should be emphasized among all cooks and other food handlers in schools to wash their hands before handling of food, wear gloves while handling of food, use separate equipment for handling raw and cooked food such as cutting board, store raw and cooked food separately, and assure adequate refrigeration for safe food storage. In addition, they should not allow food to stand at ambient temperature after being thoroughly cooked. Students should always wash their hands before eating and after using toilet. School administrators should exclude symptomatic cooks and food handlers from cooking and provide medical check-up at least once a year. All water supplies and drinking water should be monitored for adequate chlorine level at all times.

Acknowledgements

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References

1. World Health Organization. Food safety and foodborne illness. [cited 2012 Jun 1]. <http://www.who.int/mediacentre/factsheets/fs237/en/>
2. Thaikruea L, Pataraarechachai J, Savanpunyalert P, Naluponjiragul U. An unusual outbreak of food poisoning. Southeast Asian J Trop Med Public Health. 1995 Mar;26(1):78-85.
3. Thailand. Bureau of Epidemiology. Department of Disease Control. Ministry of Public Health. Food poisoning in 2008. [cited 2012 Jun 1]. <http://www.boe.moph.go.th/boedb/surdata/disease.php?dcontent=old&ds=03>
4. Stenfors Arnesen LP, Fagerlund A, Granum PE. From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol Rev. 2008 Jul;32(4):579-606. Epub 2008 Apr 15. [cited 2010 Dec 1]. <http://www.ncbi.nlm.nih.gov/pubmed/18422617>
5. US Food and Drug Administration. Bad bug book: *Bacillus cereus* and other *Bacillus spp.* [cited 2012 Jun 11]. <http://www.fda.gov/food/foodsafety/foodborneillness/Foodborneillnessfoodbornepathogensnaturaltoxins/badbugbook/ucm070492.htm>
6. Centers for Disease Control and Prevention. Guide to confirming a diagnosis in foodborne disease. [cited 2012 Jun 11]. <http://www.cdc.gov/outbreaknet/references_resources/guide_confirming_diagnosis.html>
7. US Department of Health, Education and Welfare, National Communicable Disease Center, Atlanta, Georgia. Foodborne outbreaks. MMWR annual summary. 1968: 32.
8. Midura T, Gerber M, Wood R, Leonard AR. Outbreak of food poisoning caused by *Bacillus cereus*. Public Health Rep. 1970 January; 85(1): 45-48.
9. Thailand. Bureau of Epidemiology. Department of Disease Control. Ministry of Public Health. Food poisoning in 2009. [cited 2010 Oct 15]. <http://www.boe.moph.go.th/boedb/surdata/disease.php?ds=03>

10. Beuchat LR, Ryu JH. Produce handling and processing practices. Emerg Infect Dis. 1997 Oct-Dec; 3(4): 459-465. [cited 2010 Oct 30]. <http://www.cdc.gov/ncidod/EID/vol3no4/beuchat.htm>

11. Taylor AJ, Gilbert RJ. *Bacillus cereus* food poisoning: a provisional serotyping scheme. [cited 2010 Nov 5]. <http://jmm.sgmjournals.org/cgi/reprint/8/4/543.pdf>

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Salmonella Food Poisoning in an Army Camp, Northern Thailand, October 2009

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Abstract

On 16 Oct 2009, a provincial health officer notified to the Thailand Bureau of Epidemiology that 50 Army Reserve Force Students (ARFS) from a two-week training camp in a northern province received treatment at a hospital for diarrhea in the past two days. An outbreak investigation was initiated to verify diagnosis, identify risk factors and recommend control measures. We reviewed medical records and interviewed all camp participants to identify ARFS with diarrhea. A retrospective cohort study was conducted to identify risk factors. A total of 257 diarrhea cases were identified from 470 people at the camp, including 256 ARFS (AR=57%) and one trainer (AR=17%). Common symptoms included abdominal pain (85%), loose stool (83%), fever (63%) and watery diarrhea (59%). Green chicken curry in coconut milk served at dinner on 12 Oct 2009 might be a risk factor (Adjusted odds ratio=4.5, 95% confidence interval=0.5, 42.1). No food or raw materials of the suspected meal was left for laboratory testing. Rectal swabs from seven patients and four food handlers, including the cook who prepared the suspected meal, were tested positive for *Salmonella* serogroup B. The outbreak suggested a common source. Food sanitation, particularly health screening for food handlers, should be emphasized for mass gathering.

Key words: Salmonella, food poisoning, Army Reserve Force Students, Thailand

Introduction

Bacterial foodborne infections are a common type of infection. Non-typhoidal *Salmonella* species are important causes of bacterial foodborne infections and public health problems worldwide.[1] In the United States during 1993-1997, over 2,700 foodborne outbreaks were reported, with majority (75%) were caused by bacterial agents.[2] In most instances, organisms are transmitted through consumption of contaminated food or water rather than through person-to-person contact.[3] Foodborne outbreaks of *Salmonella* have often been reported in institutional settings such as schools and nursing homes, and consumption of contaminated eggs or poultry were the common sources.[2] In Singapore, a *Salmonella* outbreak occurred in a military camp in January 2007. An investigation reported that the mashed potato was the most likely food associated with infection and food preparation in large quantities increased the risk of food contaminatation.[4]

In Thailand, foodborne diseases have been major problems for many years, with around 100 foodborne outbreaks reported annually.[5] Foodborne disease outbreaks associated with schools were commonly identified.[6] In 2007, two outbreaks of foodborne disease were reported from camps in Thailand: one in a scout camp and one in a military camp. Although the suspected foods were seafood soup and mushrooms, no causative organism was isolated. During 2008, more than 50 foodborne outbreaks in schools were reported to the Bureau of Epidemiology (BOE) of the Thailand Ministry of Public Health, including one outbreak in a scout camp. The suspected source of infection was green beans, yet no causative organism was tested positive. Of these foodborne outbreaks, six and three outbreaks were due to *Salmonella* infection in 2007 and 2008 respectively.[5]

On 16 Oct 2009, the BOE was notified by a provincial health officer that 50 people in an Army Reserve Force Students (ARFS) camp had been treated at a hospital for diarrhea in the past two days. The BOE team, the staff from Provincial Health Office and the local Surveillance and Rapid Response Team (SRRT) conducted an investigation on 12-23 Oct 2009 to verify the diagnosis, describe the characteristics of the outbreak, identify the source and risk factors of infection, control the outbreak and recommend appropriate prevention measures for future food poisoning outbreaks in ARFS camps.

Methods

Setting

Training in the ARFS camp began on 12 Oct 2009 and lasted for 12 days. The camp was organized at the School A located in central part of a rural district. People from five schools in the same province participated in the camp. There were 493 persons in the camp at the time of the outbreak, including 467 students (359 males and 108 females), seven military trainers, five school teachers, four cooks and 10 cooks' helpers.

Although the ARFS camp included students from three classes, students were grouped by training year. The students were not permitted to go outside during the training period. General activities for ARFS in the camp are shown in Figure 1.

Food in the camp was prepared by four cooks from the School A. They usually bought fresh food, ingredients and seasoning from a local market located near the school. Each chef took turns in preparing the meals, with two or three assistants. The first meal in the camp was started on 12 Oct 2009 and the last meal on 23 Oct 2009. Three meals were provided to school teachers, military trainers and ARFS every day. Each meal for ARFS included two food items whereas three items were prepared for school teachers and military trainers which included two items similar to the ARFS and one additional item.

Epidemiologic Investigation

We performed active case finding and a descriptive study in the camp using self-administered questionnaires and reviewing 60 medical records of ill people from the camp. Information included demographic characteristics such as age, gender, occupation, training year and school; clinical information such as signs, symptoms, date and time of onset, and treatment; risk factors such as suspected food consumed at the camp; and risk behaviors such as not washing hands before eating, drinking water from a friend's glass, eating with a friend's spoon and eating with bare hands.

We defined a suspected case as a student or staff who joined the ARFS camp during 12-22 Oct 2009 and had at least one of the following symptoms: watery diarrhea, mucous or bloody stool; or had at least three of the following symptoms: loose stool, tenesmus, abdominal pain, fever, nausea, or vomiting.

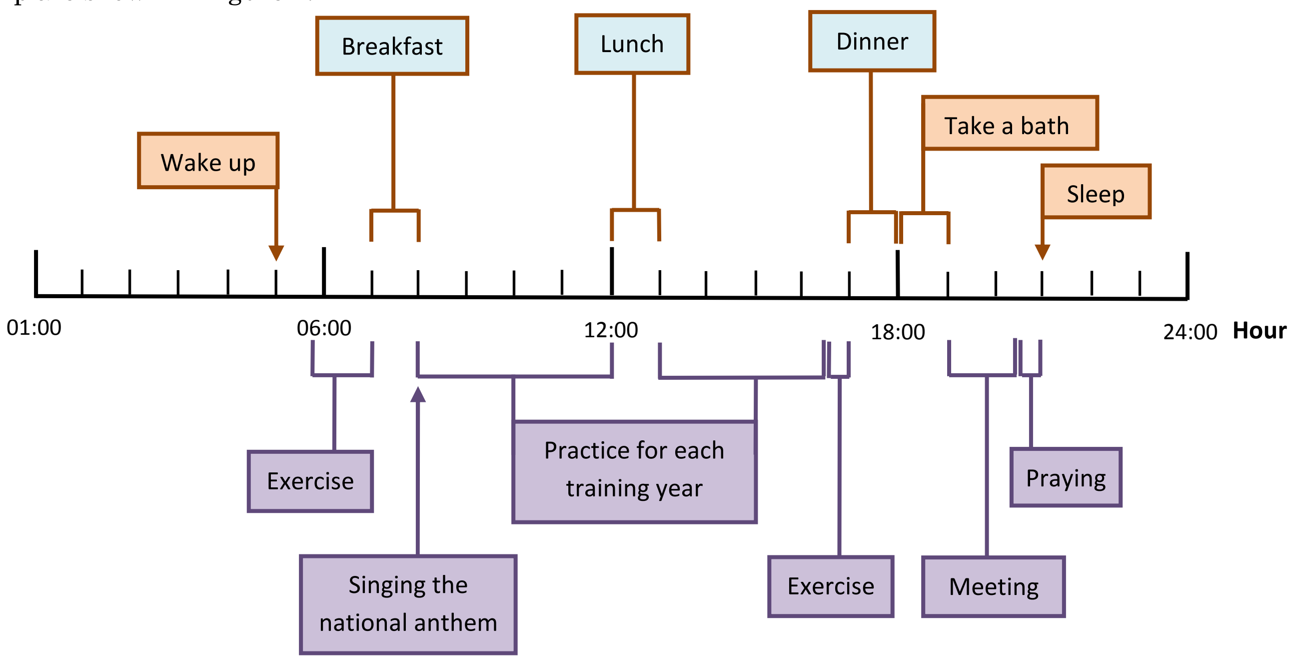

Figure 1. Daily activities in Army Reserve Force Students (ARFS) Camp, Thailand, 12-22 Oct 2009

A confirmed case was a suspected case that was tested positive for *Salmonella spp.* in stool culture while a carrier was an asymptomatic person with positive stool culture.

Environmental and Laboratory Study

We conducted an environmental study by reviewing the food menu and drinking water consumed in the camp, interviewing cooks and cooks' helpers about history of illness before the outbreak, food preparation and cooking processes, and observing the food preparation processes and eating behavior of students in the canteen during lunch. We also surveyed kitchen, cooking areas, water sources and toilets.

Rectal swabs from all ARFS with diarrheal symptoms on 16-20 Oct 2009, hand swabs and rectal swabs of cooks and cooks' helpers, and swabs from kitchen equipment were collected. In addition, samples of bottled drinking water and tap water were obtained. All specimens were sent to a laboratory in the provincial hospital for bacterial culture.

Statistical Analysis

A retrospective cohort study was conducted among students who attended the ARFS camp during 12-22 Oct 2009. Cases were either suspected or confirmed food poisoning cases identified in the descriptive study. Risk ratios were calculated for suspected risk behavior and food item, and were tested for their association with the disease by using the Chi-square, with a p-value of 0.05 or less defined as being statistically significant. Multivariable analysis (logistic regression) was conducted to calculate the adjusted odds ratio (OR) in order to identify the significant risk factors. We selected variables with a p-value 0.20 or less to be included in the adjusted model and used the backward approach to fit the model. Epi Info version 3.5.1 was used for statistical analysis (US CDC, Atlanta).

Results

Epidemiologic Investigation

During our investigation, 470 (96%) out of 493 persons in the camp returned the questionnaires. We identified 257 ill persons, corresponding to an attack rate of 55%, which included 250 suspected cases and seven confirmed cases. Seven out of 55 rectal swabs from ARFS were tested positive for *Salmonella* serogroup B by bacteria culture. Four rectal swabs from two cooks and two cooks' helpers were also cultured positive. The female to male ratio of all patients was 1:2.9 and the median age was 17 years old (Interquartile range=16-18). The attack rate for females and males was 59% and 53% respectively. Sixty people received treatment at a hospital (45 out-patients and 15 in-patients). The attack rate was the highest among the ARFS (57%). There was only one case among trainers (attack rate=17%) and no case among cooks and cooks' helpers. A few people had illness onset on 12 and 13 Oct 2009. The number of cases increased rapidly on 14 Oct and reached its peak on 15 and 16 Oct 2009 (Figure 2).

There was an unusual event on 14 Oct 2009. On that day, the ARFS were punished to mix and ate the food with their bare hands during lunch.

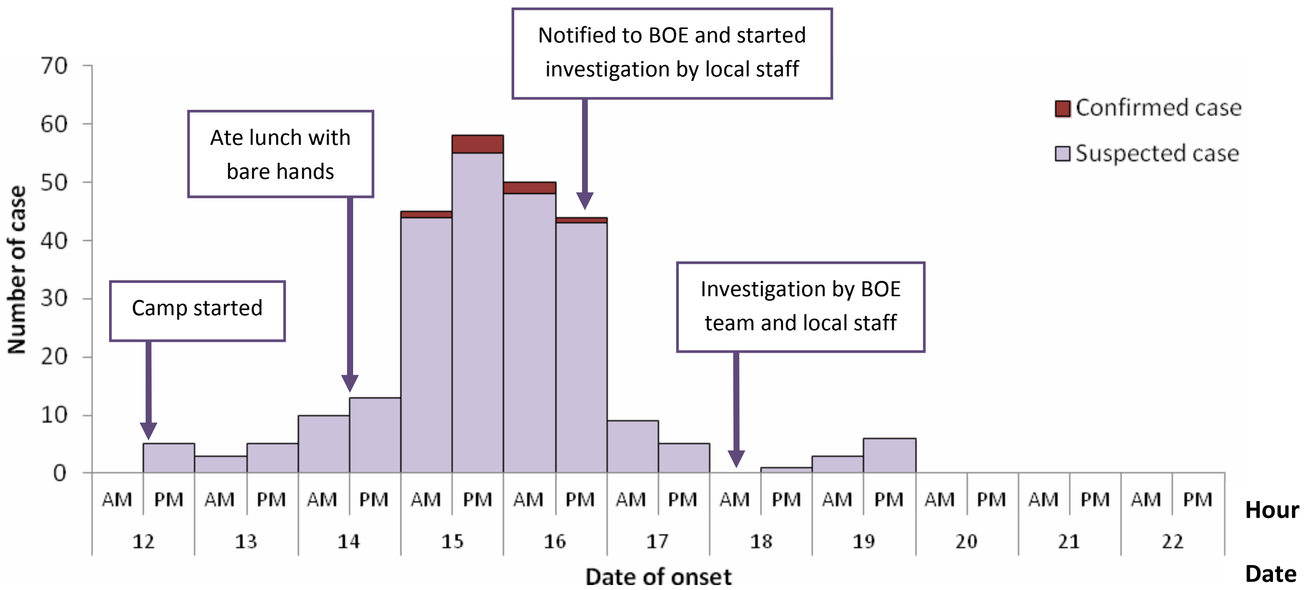

Figure 2. Number of food poisoning cases by date and time of onset in Army Reserve Force Students (ARFS) Camp, Thailand, 12-22 Oct 2009 (n=257)

The local SRRT notified the BOE about the outbreak and started the investigation on 16 Oct 2009. The joint team with BOE investigated again on 18 Oct 2009. The cases were distributed throughout all training years. The attack rate was highest among ARFS in the third training year (65%), followed by the first year (54%) and the second year (49%). Among the 257 cases (250 suspected cases and seven confirmed cases) with detailed clinical information, the most common manifestations were abdominal pain (85%), loose stool (83%) and fever (63%) (Figure 3).

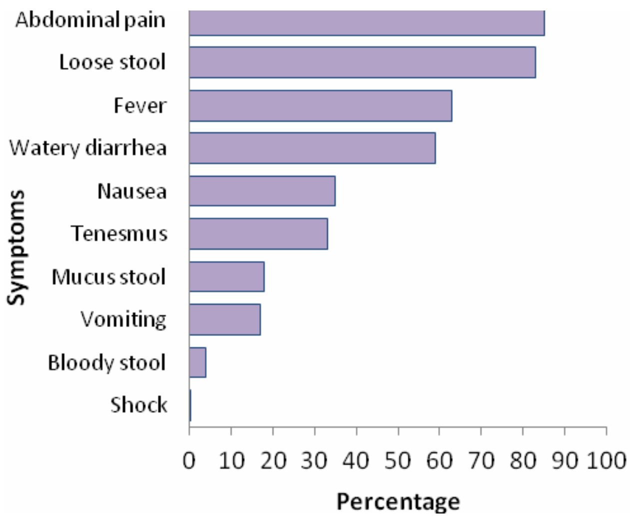

Figure 3. Symptoms of food poisoning cases in Army Reserve Force Students (ARFS) Camp, Thailand, 12-22 Oct 2009 (n=257)

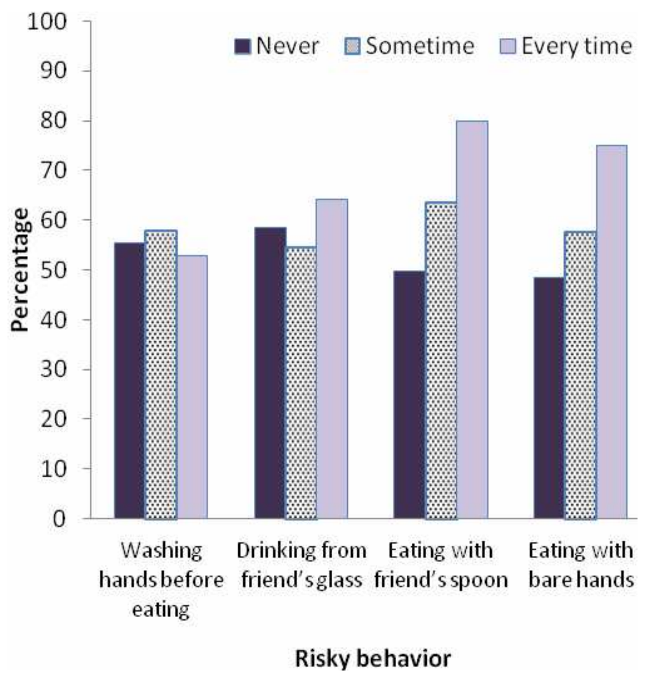

Figure 4. Attack rate by risky behavior of Army Reserve Force Students (ARFS) Camp, Thailand, 12-22 Oct 2009

We classified proportion of risk behavior into three groups: never, sometimes and every time. We found that the attack rates for hand washing before eating in each group were quite similar (55.5%, 57.9% and 52.9% respectively). However, the attack rate was higher for those who drank from a friend's glass, ate with a friend's spoon and ate with bare hands every time than that of those who behaved only sometimes or never (Figure 4).

Environmental and Laboratory Study

We surveyed the kitchen area and observed that raw and cooked foods were prepared near the canteen, and the dish washing zone was next to the preparation zone.

Cooks and cooks' helpers did not wear gloves for food preparation. Raw meat, raw vegetables and cooked food were kept together in the same cooler box. Sometimes, cooks used the same cutting board for raw meat and vegetables.

Cooking time and serving time are shown in Figure 5. Most food items contained egg, chicken and pork. Although each food item was cooked, it was served cold because cooks' helpers prepared the food, drinking water and utensils on the dining tables one to two hours before the meal time. The health screening of all cooks and cooks' helpers on 18-20 Oct 2009 did not find any person with the symptoms. However, they did not get the annual health check-up.

There was a hand washing zone in front of the canteen, but no soap was provided. The ARFS had little time to wash their hands and we found that all participants used the same toilet near the canteen. Although it appeared to be clean, there was no soap for hand washing.

We also surveyed the water supply in the camp and identified two sources of drinking water – the school water tank and the tap water tank. The school water tank was filled from a pond at the School A while the tap water tank was from the provincial waterworks authority. The camp mostly used water from the school water tank to supply canteen and toilet because water from the tap water tank was more expensive. On 18 Oct 2009, residual chlorine was measured in nine water samples. Eight samples from the school water tank and the tap water from the building and kitchen area had residual chlorine level of less than 0.2 ppm while one sample from the tap water tank had 0.5-1 ppm.

Drinking water and ice were bought from a water and ice factory which had been awarded for Good Manufacturing Practice (GMP). The ice was sent to

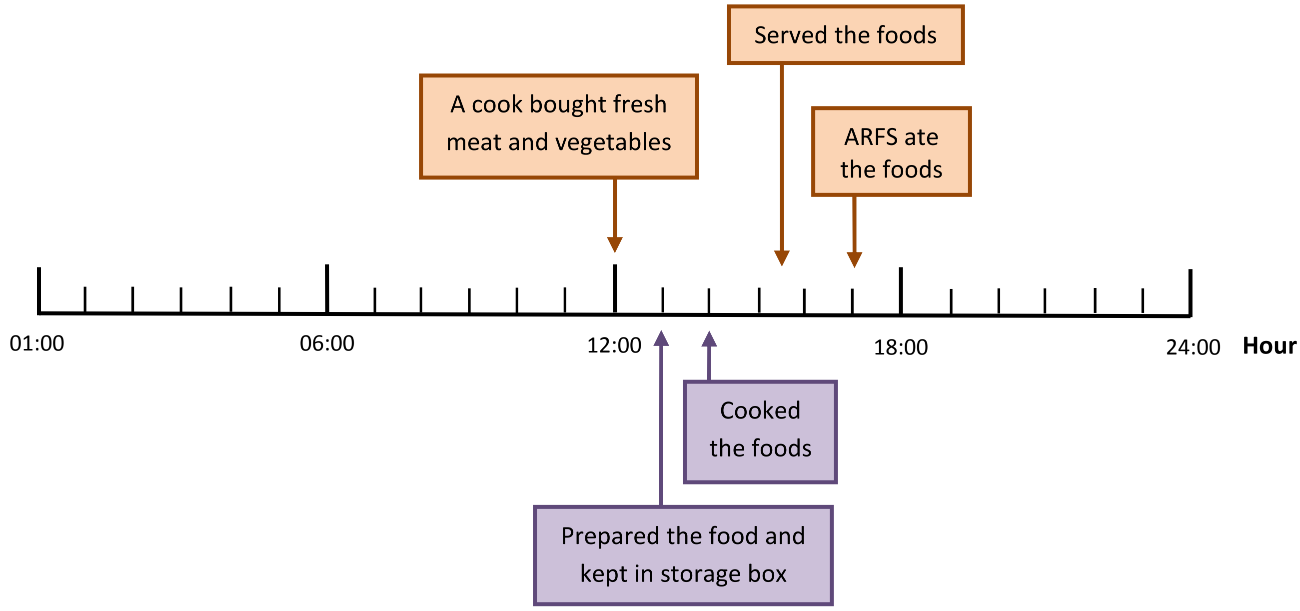

Figure 5. Timeline on food preparation for dinner in Army Reserve Force Students (ARFS) Camp, Thailand, 12-22 Oct 2009

the laboratory for testing bacteria and the results revealed negative.

The four cooks bought fresh food and ingredients from a local market located near the camp where food venders sold in the mornings and evenings. There were several kinds of food such as raw meat, vegetables, ingredients and fruits. The market was separated into four zones (cooked food zone, seasoning zone, raw meat zone and vegetable zone) and had been awarded with the "Clean Food, Good Taste" logo.

Laboratory Testing

Of 71 specimens sent for bacterial culture, 11 rectal swabs were positive for *Salmonella* serogroup B, which included samples from seven ARFS who had diarrheal symptoms and asymptomatic four cooks and cooks' helpers. Neither pathogenic bacteria nor coliform bacteria were found in hand swabs from cooks and cooks' helpers, bottled drinking water, tap water and kitchen equipment.

Statistical Results

From univariate analysis, we identified three potential risk factors: being female, being in a higher training year and eating with bare hands. The foods with a p-value of 0.2 or less included bean noodle soup, green chicken curry in coconut milk, and vegetable and pork soup.

Table 1. Univariate and multivariate analysis of risk factors and suspected foods in Army Reserve Force Students (ARFS) Camp, Thailand, 12-22 Oct 2009 (n=257)

Variable	Exposed		Non-exposed		Crude RR (95% CI)	Adjusted OR* (95% CI)
	Case	Non-case	Case	Non-case		
Female gender	65	31	191	163	1.3 (1.1-1.5)	1.7 (1.0-2.7)
Higher training year	101	54	155	140	1.2 (1.1-1.5)	1.6 (1.1-2.4)
Eating with bare hands	221	157	35	37	1.2 (0.9-1.6)	1.3 (0.8-2.2)
Bean noodle soup (lunch on 12 Oct)	250	189	1	5	3.4 (0.6-20.5)	3.0 (0.2-41.3)
Green chicken curry in coconut milk (dinner on 12 Oct)**	250	189	1	5	3.4 (0.6-20.5)	4.5 (0.5-42.1)
Vegetable and pork soup (breakfast on 14 Oct)**	218	186	3	8	2.0 (0.8-5.2)	1.5 (0.3-6.8)

** Final multiple logistic model included all cases and was adjusted for all variables in the table.*

*** The cook who prepared the meal was a carrier.*

On multiple logistic regression analysis, the adjusted OR for females was 1.7 (95% confidence interval = 1.0-2.7) and for persons in higher training years was 1.6 (95% confidence interval = 1.1-2.4). No food item was significantly associated with the illness. However, the green chicken curry in coconut milk had the highest OR of 4.5 (95% confidence interval = 0.5-42.1) (Table 1).

Discussion

Interpretation of Results

In Thailand, *Salmonella* serogroup B is the most common among patients with non-typhoidal *Salmonella* diarrhea.[7] Several *Salmonella* serogroup B outbreaks in Thailand have been reported to the BOE in recent years. In 2006, there were four outbreaks in prisons, camps of construction workers and villages. Suspected foods were raw pork, raw minced pork and raw beef respectively. In 2007, there was one outbreak reported in a village, and the suspected food was raw meat.[5] In the past, most of the suspected food items associated with the outbreak were uncooked. However, the suspected food related to this outbreak was cooked. In this outbreak, the overall attack rate (55%) was similar to those from other outbreaks reported in Thailand (30-65%).[5] The most common symptoms of non-typhoidal *Salmonella* infection are diarrhea, abdominal cramps and fever,[8] which are consistent with the clinical manifestations of the outbreak described in this report.

The epidemic curve suggests a common source outbreak and the results from our study indicated that the green chicken curry in coconut milk, which was served at dinner on 12 Oct 2009, was the likely cause of the outbreak. The cook who was a *Salmonella* carrier prepared this meal. Even though the green chicken curry in coconut milk was cooked, the contamination could have occurred during the preparation process because the cook used the same cutting board for raw meat and vegetables. After the curry was cooked, the cook garnished the curry with chili, kaffir lime leaves and sweet basil with bare hands before serving. *Salmonella* in form of biofilms could survive and resist disinfection on leafy vegetables.[1] Moreover, the food was kept for two hours at room temperature before being eaten. This could allow *Salmonella* to multiply[4] and the contamination might be likely if cooks and cooks' helpers were *Salmonella* carrier. In addition, as food sanitation was not well established in the camp, it might increase the risk of contamination.

The residual chlorine levels in some areas of the camp were below the standard level and might not be strong enough to disinfect the infectious concentration of *Salmonella* in contaminated raw food and raw vegetables. High chlorine level in water could substantially reduce *Salmonella* contamination.[1]

Action Taken

We continued active case finding and follow-up of all students until two weeks after the camp finished. We also provided health education to the ARFS, military trainers, school teachers, cooks and cooks' helpers; cleaned the kitchen area with chlorine; and recommended that the water supply should be adequately chlorinated. Food handlers in the camp, who were *Salmonella* carriers, were recommended not to handle food until they had tested negative for *Salmonella* by rectal swab culture for the second time. No additional cases of diarrheal illness were reported in the camp, and no case associated with this outbreak occurred in nearby communities. In this investigation, early detection of the outbreak by the local staff and good co-operation between participants in the camp and the investigation team might have prevented infection spreading into nearby communities.

Limitations

We experienced several limitations in this outbreak investigation because the hospital had no facility for molecular analyses and typing of *Salmonella* samples such as Pulsed Field Gel Electrophoresis (PFGE) which would conclusively show that all rectal swab cultures had the same pathogenic strain. In addition, no left-over food was tested for bacterial contamination and since our multivariate analysis was not completed in time, we could not adequately evaluate the suspected meal and the associated food while we were at the camp. Furthermore, we also found some limitations in standards for food sanitation in the camp. These limitations reflected lack of understanding of the camp staff and inadequate budget to provide a sanitary storage space for food items, clean drinking water and relevant training for managerial and cooking staff. Strict rules for training activities and extremely rigid schedules made it difficult to convince training staff that adequate time should be provided for proper hand washing. Although we surveyed the market in this outbreak, we did not collect any specimen from the market such as food and seasoning.

Recommendations

Our recommendations for food handlers in such setting included that food preparation materials for raw meat and vegetables should be completely separated, and the cooked and raw food should be separately stored, as well as for meat and vegetables. We also recommended that all food handlers should wear gloves, frequently wash their hands during food preparation and have the health check-up at least once a year. The camp or school staff must provide adequate time for hand washing during training. Soap should always be provided at the hand washing station and water supply in the camp should be adequately chlorinated. The provincial waterworks authority should provide adequate chlorinated water in this area for prevention of extensive outbreak. The officers from community hospitals and local Provincial Health Office should strengthen surveillance for diarrhea and food poisoning in hospitals and communities, and food samples should be collected and stored well, if possible, in order to identify the possible sources of outbreaks or diarrheal illness. For future investigation, after a pathogenic agent has been identified, it should be tested by molecular analysis and typing such as PFGE to determine the pathogenic strain. The suspected meal and food should be evaluated in the field to identify the source, and prevention and control measures should be applied in a timely manner.

As the outcomes after the outbreak, the carriers were not allowed handling of food as long as they shed the organism and until their rectal swabs were cultured negative in the second test. No additional case was identified in the camp and no transmission occurred in the communities.

Conclusion

The food poisoning outbreak occurred in an ARFS camp; *Salmonella* serogroup B was the causative organism. The ARFS had the highest risk. This outbreak might have been caused by a common source of food item consumed at dinner on 12 Oct 2009, yet this was not confirmed because the relevant food items were not available for testing. Major recommendations should emphasize on food sanitation, especially associated with food storage, food handling and screening for carriers among food handlers.

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References

1. Lapidot A, Romling U, Yaron S. Biofilm formation and the survival of *Salmonella Typhimurium* on parsley. Int J Food Microbiol. 2006 Jun 15;109(3):229-33. Epub 2006 Apr 17.
2. Olsen SJ, Mackinon LC, Goulding JS, Bean NH, Slutsker L. Surveillance for foodborne disease outbreaks - United States, 1993-1997. MMWR CDC Surveill Summ. 2000 Mar 17;49(1):1-62.
3. Niyogi SK. Shigellosis. J Microbiol. 2005 Apr;43(2):133-43.
4. Lee VJ, Ong AE, Auw M. An outbreak of *Salmonella* gastrointestinal illness in a military camp. Ann Acad Med Singapore. 2009 Mar;38(3):207-11.
5. Thailand. Bureau of Epidemiology. Department of Disease Control. Ministry of Public Health. Outbreak notification report 2006-2008. [cited 2010 Oct 26]. <http://www.boe.moph.go.th/boedb/d506_1/ds.php>
6. Thaikruea L, Pataraarechachai J, Savanpunyalert P, Naluponjiragul U. An unusual outbreak of food poisoning. Southeast Asian J Trop Med Public Health. 1995 Mar;26(1):78-85.
7. Moolasart P, Sangsujja J, Eampokalap B, Ratanasrithong M, Likanonsakul S. Non-typhoidal *Salmonella* diarrhea in Thai children: a study at Bamrasnaradura Hospital, Nonthaburi, Thailand. J Med Assoc Thai. 1997 Oct;80(10):613-8.
8. Heymann DL, editor. Control of communicable diseases manual. 19th ed. Washington, DC: American Public Health Association; 2008.

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