

OUTBREAK OF NOROVIRUS GASTROENTERITIS INFECTION, THAILAND

Katie S McCarthy^{1,2}, Ratigorn Guntapong³, Aree Thattiyaphong³, Piyada Wangroongsarb³, Aron J Hall⁴, Sonja J Olsen^{2,4} and Timothy H Holtz^{2,4}

¹ASPH/CDC Allan Rosenfield Global Health Fellow, Bangkok, Thailand; ²Thailand Ministry of Public Health – United States Centers for Disease Control and Prevention Collaboration, Nonthaburi; ³National Institute of Health, Thailand Ministry of Public Health, Nonthaburi, Thailand; ⁴Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Abstract. Norovirus is a leading cause of gastrointestinal illness worldwide. We investigated an outbreak of gastrointestinal illness in Pattaya, Thailand, among participants of a course. We asked participants and family members to complete a questionnaire asking about symptoms, meals eaten, and foods consumed during the course. We collected stool samples from persons reporting illness and analyzed specimens for several viruses and enteropathogenic bacteria. We defined a case as a person having one or more episodes of diarrhea, with onset between 30 August and 1 September 2010, in a participant or family member who attended the course. Of 56 people who attended, 95% completed the questionnaire: nine met the case definition (attack rate, 17%). Common symptoms included abdominal cramps, nausea, fatigue, headache, and vomiting. Food items with elevated risk ratios included: crispy fish maw, dried squid, and cashew nut salad [risk ratio (RR) 5.1; 95% confidence interval (CI) 0.7-37]; assorted salad bar with dressing (RR 3.0; 95% CI 0.9-11); and seafood kebab (RR 5.8; 95% CI 0.8-43). Among ill persons, four (44%) provided stool samples and two (50%) were positive for norovirus. Our data suggest a foodborne outbreak of norovirus. Increased use of norovirus diagnostics as well as measures to prevent transmission may help identify additional outbreaks and improve control measures to limit the spread of outbreaks.

Keywords: norovirus, foodborne disease, disease outbreaks, Thailand

INTRODUCTION

Norovirus is the leading cause of gastrointestinal disease outbreaks throughout the world (Patel *et al*, 2009). In the United

States, norovirus is the most frequently identified cause of both sporadic disease and outbreaks caused by contaminated food (CDC, 2010b; Scallan *et al*, 2011). In Thailand, the role of norovirus among children hospitalized for acute gastroenteritis is well described, with rates of norovirus infection ranging from 6.8% to 18% (Guntapong *et al*, 2004; Hansman *et al*, 2004; Khamrin *et al*, 2007; Malasao *et al*, 2008; Khamrin *et al*, 2010). Across all age groups, a study found a norovirus

Correspondence: Timothy H Holtz, Thailand MOPH – US CDC Collaboration, DDC7 Building, 4th Floor, Ministry of Public Health, Nonthaburi, 11000, Thailand.

Tel: +66 (0) 2580 0669 ext 456; Fax: +66 (0) 2580-0712

E-mail: tkh3@cdc.gov (T. Holtz).

prevalence of 44.7% among inpatients and outpatients attending hospitals who had a clinical diagnosis of acute gastroenteritis or acute diarrhea (Kittigul *et al*, 2010). However, the role of norovirus in outbreaks of gastrointestinal illness in Thailand, especially among adults, has not been described.

In August 2010, participants attended a training course at a hotel in Pattaya, Thailand. Participants and family members shared several catered meals together during the two-day training course. Several persons became ill with moderate to severe gastrointestinal symptoms. In response, we initiated an investigation to determine the magnitude of the outbreak and the etiology of infection. Here we summarize the results of an outbreak investigation in Thailand.

MATERIALS AND METHODS

Study population and design

To identify suspected cases, we emailed all training participants, asking them to report if they or their family members experienced any gastrointestinal symptoms during or after the training course held on 29-30 August 2010. Those reporting any symptoms of gastrointestinal illness (*eg*, diarrhea, vomiting, or nausea) were interviewed regarding symptoms experienced during or after the training course. We requested stool samples from all persons reporting any illness. The information gathered from these hypothesis-generating interviews was used to modify a questionnaire from the US Centers for Disease Control and Prevention (CDC, 2010a) for the subsequent cohort study. We defined a case as one or more episodes of diarrhea, with onset between 30 August 2010 and 1 September 2010, in a participant or family member attending the course.

To identify the possible source of illness, we asked all participants of the course and family members who attended to complete a short questionnaire that asked about symptoms, meals eaten, and specific foods consumed. For many shared meals, a menu of food options was available from the establishment. Meals with menus included morning break, lunch, afternoon break, and dinner on 29 August, and morning break and lunch on 30 August. There was no menu for breakfast on either day; however, the questionnaire assessed egg, fruit, and vegetable consumption as well as other foods and drinks consumed during both breakfasts. All meals were self-serve buffets and persons consumed the foods at roughly the same times.

Specimen collection and testing

We distributed kits to collect stool samples and rectal swabs from ill persons who agreed to provide a sample within two days after completing the course. Whole stool samples were stored in cups and kept at room temperature until delivered to the laboratory within 24 hours of collection. Rectal swabs were put into tubes containing Cary Blair transport medium stored at room temperature until delivered to the laboratory within 24 hours of collection. We sent all stool samples to the National Institute of Health at the Thailand Ministry of Public Health. The laboratory tested for the presence of enteropathogenic bacteria, including: *Salmonella* species (spp), *Shigella* spp, *Escherichia coli*, *Edwardsiella tarda*, *Staphylococcus aureus*, *Vibrio* spp, *Aeromonas* spp, *Plesiomonas* spp, and *Bacillus cereus* by culture. The laboratory also tested for *Campylobacter* species by culture and biochemical analysis, and viruses, including rotavirus and norovirus, using conven-

NOROVIRUS OUTBREAK, THAILAND

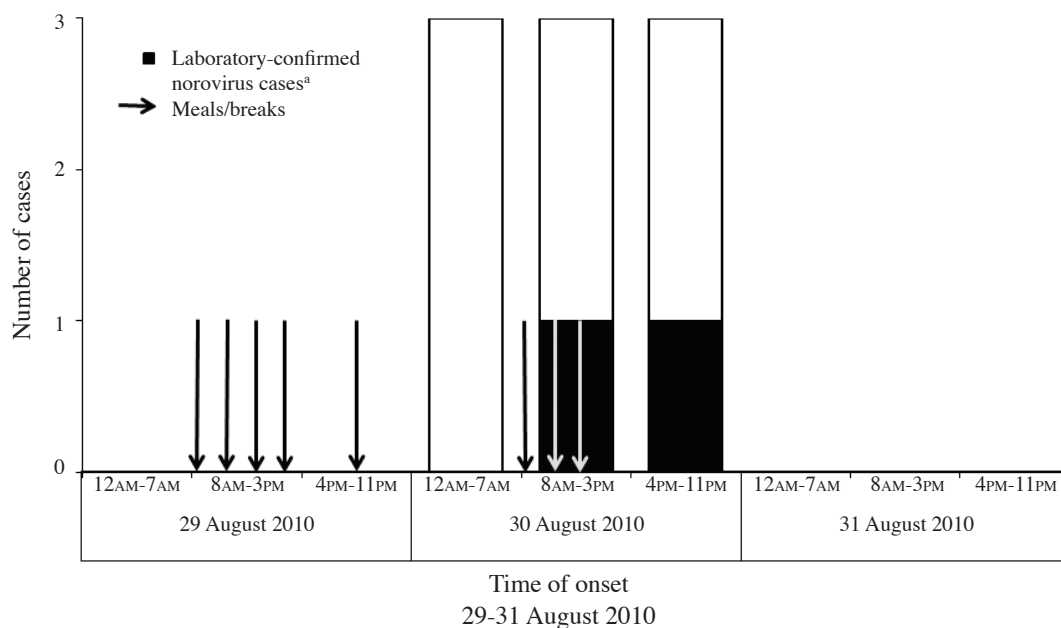


Fig 1—Cases of gastroenteritis by time of onset among participants of the training course (*Laboratory-confirmed norovirus case = whole stool specimen positive for norovirus by PCR/PAGE).

tional polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis (PAGE) of the amplified product.

We were unable to investigate food storage, preparation, and handling practices at the establishment due to the site's distance. This investigation was considered a public health response and exempted from IRB review.

Data analysis

We entered questionnaire data into a Microsoft Office Access 2007 database and analyzed the data using SPSS (version 17.0 for Windows; SPSS, Chicago, IL) and Microsoft Office Excel 2007. In univariate analysis, we calculated the Pearson χ^2 and the relative risks (RR) of disease given exposure and 95% confidence intervals (CI) for each meal overall and for individual food items within each meal.

RESULTS

There were 56 participants and family members present at the training course of whom 53 (95%) completed the questionnaire. Three persons did not complete the questionnaire: two were family members who reported not consuming any meals and did not report illness and one was a participant who was unavailable due to work and reported illness.

Eleven (21%) of 53 persons completing the questionnaire reported illness. Of these, two persons reporting illness did not meet the case definition: one reported symptoms beginning and ending on 29 August and one reported nausea and fever but did not report any other symptoms. All nine cases ($N = 9$; attack rate, 17%) reported symptom onset between 6 AM and 10 PM on 30 August (Fig 1).

Table 1
Analysis of exposures among participants of the training workshop.

Meals and food items	Proportion ill ^a		
	Exposed <i>n/N (%)</i>	Nonexposed <i>n/N (%)</i>	Relative risk (95% CI)
29 August breakfast ^b	9/47 (19)	0/6 (0)	- -
Eggs (<i>ie</i> , scrambled, fried, omelet, other)	5/33 (15)	4/14 (29)	0.5 (0.2-1.7)
Fruit (<i>ie</i> , grapes, watermelon, pineapple, other)	7/31 (23)	2/16 (13)	1.8 (0.4-7.7)
Vegetables (<i>ie</i> , corn, tomato, salad, other)	5/25 (20)	4/19 (21)	1.0 (0.3-3.1)
29 August morning break ^c	9/39 (23)	0/14 (0)	- -
Ham quiche	5/25 (20)	4/14 (29)	0.7 (0.2-2.2)
Lemon tart	6/28 (21)	3/11 (27)	0.8 (0.2-2.6)
Deep fried shrimp toast	4/25 (16)	5/14 (36)	0.4 (0.1-1.4)
Mint tea juice	4/14 (29)	5/25 (20)	1.4 (0.5-4.5)
29 August lunch	9/49 (18)	0/4 (0)	- -
Pork satay	6/28 (21)	3/21 (14)	1.5 (0.4-5.3)
Shrimp paste dip with deep fried Thai sardine	7/37 (19)	2/12 (17)	1.1 (0.3-4.7)
Crispy fish maw, dried squid, and cashew nut salad ^c	8/30 (27)	1/19 (5)	5.1 (0.7-37)
Spicy seafood soup with hot basil leaves	4/24 (17)	5/25 (20)	0.8 (0.3-2.7)
Green curry with fish ball	3/19 (16)	6/30 (20)	0.8 (0.2-2.8)
Sautéed chicken with cashew nut	3/15 (20)	6/34 (18)	1.1 (0.3-3.9)
Four kinds of vegetables in brown sauce	3/28 (11)	6/21 (29)	0.4 (0.1-1.3)
Steamed fish in banana leaf	3/22 (14)	6/27 (22)	0.6 (0.2-2.2)
Fried rice with crab meat	5/31 (16)	4/18 (22)	0.7 (0.2-2.4)
Assorted fresh fruit	7/37 (19)	2/12 (17)	1.1 (0.3-4.7)
Boiled taro in coconut milk	5/26 (19)	4/23 (17)	1.1 (0.3-3.6)
29 August afternoon break	8/41 (20)	1/12 (8)	2.3 (0.3-17)
Tuna puff	2/21 (10)	6/20 (30)	0.3 (0.1-1.4)
Marble cake	3/15 (20)	5/26 (19)	1.0 (0.3-3.8)
Finger ham sandwich	4/24 (17)	4/17 (24)	0.7 (0.2-2.4)
Chinese flower juice	5/23 (22)	3/18 (17)	1.3 (0.4-4.7)
29 August dinner	9/45 (20)	0/8 (0)	- -
Assorted cold cuts	0/2 (0)	9/43 (21)	0.0 -
Assorted salad bar with assorted dressing ^c	6/18 (33)	3/27 (11)	3.0 (0.9-11)
Tuna salad with potatoes	3/7 (43)	6/38 (16)	2.7 (0.9-8.4)
Spicy minced pork salad	3/10 (30)	6/35 (17)	1.8 (0.5-5.8)
Spicy seafood salad	4/12 (33)	5/33 (15)	2.2 (0.7-6.9)
Spicy grilled beef salad	1/7 (14)	8/38 (21)	0.7 (0.1-4.6)
French onion soup	3/11 (27)	6/34 (18)	1.5 (0.5-5.2)
Spicy seafood soup in coconut milk and galanga	3/14 (21)	6/31 (19)	1.1 (0.3-3.8)
Massaman curry with chicken	4/13 (31)	5/32 (16)	2.0 (0.6-6.2)
Poached fish with white wine cream sauce	4/21 (19)	5/24 (21)	0.9 (0.3-3.0)
Braised chicken in brown sauce	4/14 (29)	5/31 (16)	1.8 (0.6-5.6)
Deep fried fish with chili sauce	3/18 (17)	6/27 (22)	0.8 (0.2-2.6)
Sautéed squid with hot basil leaves	2/13 (15)	7/32 (22)	0.7 (0.2-2.9)
Sautéed mixed vegetables in oyster sauce	4/19 (21)	5/26 (19)	1.1 (0.3-3.5)
Fried rice with seafood	5/24 (21)	4/21 (19)	1.1 (0.3-3.5)

Table 1 (Continued).

Meals and food items	Proportion ill ^a		
	Exposed <i>n/N</i> (%)	Nonexposed <i>n/N</i> (%)	Relative risk (95% CI)
BBQ blue crab	6/30 (20)	3/15 (20)	1.0 (0.3-3.5)
BBQ prawns	8/34 (24)	1/11 (9)	2.6 (0.4-18.5)
Mini pork steak	2/17 (12)	7/28 (25)	0.5 (0.1-2.0)
Seafood kebab ^c	8/26 (31)	1/19 (5)	5.8 (0.8-43)
Assorted fresh fruit	6/27 (22)	3/18 (17)	1.3 (0.4-4.7)
Soft chocolate cake	3/7 (43)	6/38 (16)	2.7 (0.9-8.4)
Pandan caramel custard	1/8 (13)	8/37 (22)	0.6 (0.1-4.0)
Blueberry fresh cheesecake	5/15 (33)	4/30 (13)	2.5 (0.8-8.0)
Mixed fruit tart	3/16 (19)	6/29 (21)	0.9 (0.3-3.1)
Almond peach tart	2/6 (33)	7/39 (18)	1.9 (0.5-6.9)
30 August breakfast ^{b,c}	9/41 (22)	0/12 (0)	- -
Eggs (<i>ie</i> , scrambled, fried, omelet, other)	4/27 (15)	5/14 (36)	0.4 (0.1-1.3)
Fruit (<i>ie</i> , grapes, watermelon, pineapple, other)	7/23 (30)	2/18 (11)	2.7 (0.6-11.6)
Vegetables (<i>ie</i> , corn, tomato, salad, other)	4/18 (22)	5/23 (22)	1.0 (0.3-3.3)
30 August morning break ^c	4/25 (16)	5/8 (63)	0.3 (0.1-0.7)
Ham cheese croissant	2/18 (11)	2/7 (29)	0.4 (0.1-2.2)
Butter cake	1/7 (14)	3/18 (17)	0.9 (0.1-6.9)
Finger smoked pork loin sandwich	0/7 (0)	4/18 (22)	0.0 -
Lemon grass juice	3/16 (19)	1/9 (11)	1.7 (0.2-14)
30 August lunch	8/40 (20)	1/13 (8)	2.6 (0.4-19)
Spicy shrimp paste dip with prawn	4/22 (18)	4/18 (22)	0.8 (0.2-2.8)
Green papaya salad with blue crab	4/16 (25)	4/24 (17)	1.5 (0.4-5.1)
Spicy grilled eggplant salad with shrimp	5/25 (20)	3/15 (20)	1.0 (0.3-3.6)
Sour and spicy soup with fish and mixed vegetables	5/24 (21)	3/16 (19)	1.1 (0.3-4.0)
Roasted duck curry	3/22 (14)	5/18 (28)	0.5 (0.1-1.8)
Stir fried kale with oyster sauce	3/14 (21)	5/26 (19)	1.1 (0.3-4.0)
Steamed fish with chili and lemon	3/16 (19)	5/24 (21)	0.9 (0.2-3.2)
Fried rice with ham	3/20 (15)	5/20 (25)	0.6 (0.2-2.2)
Stir fried squid with curry powder	6/24 (25)	2/16 (13)	2.0 (0.5-8.7)
Assorted fresh fruit	5/23 (22)	3/17 (18)	1.2 (0.3-4.5)
Coconut iced cream	5/30 (17)	3/10 (30)	0.6 (0.2-1.9)

^a*n/N*'s are different for each food item, depending on response; ^bNo menu available; ^cPearson χ^2 , $p < 0.10$ for overall meal or individual food item; CI, confidence interval.

The mean age of case patients was 37 years (interquartile range, 32-46), one was male, and all were training participants (no significant differences between case patients and non-case patients). The median number of episodes of diarrhea per day was 3 (range: 1-8 episodes). Other symp-

toms included abdominal cramps (89%), nausea (67%), fatigue (67%), headache (67%), vomiting (67%; mean, 4 episodes in one day), body aches (56%), fever (44%), chills (11%), and constipation (1%). The mean duration of illness was 24 hours (range: 0-57 hours). Two ill persons saw

a healthcare professional for their illness; none had a stool sample tested prior to the outbreak investigation. The most commonly reported medications taken after becoming ill were oral rehydration solution (ORS, $n = 4$) and analgesics ($n = 3$).

Most people ate at all the meals [mean number of persons eating at each meal was 41 (77%), range: 25-49]. All case patients ate at all the meals on 29 August. On 30 August, only 4 had the coffee break and 1 had the lunch. Individual food items from all meals were generally not significantly associated with illness (Table 1). However, several food items from lunch and dinner on 29 August exhibited elevated risk ratios, all with p -values < 0.10 (Table 1), including crispy fish maw, dried squid, and cashew nut salad at lunch on 29 August and assorted salad bar with assorted dressing and seafood kebab at dinner on 29 August. Both salads contained raw ingredients. If lunch food items on 29 August were the source of the outbreak, the average incubation period would be 23.4 hours (range: 17.5-29.5 hours); if dinner food items on 29 August were the cause, the average incubation period would be 15.9 hours (range: 10.0–26.0 hours).

Of the 9 case patients, 4 (44%) provided a stool sample within four days of becoming ill. Whole stool samples from two persons (50%) were positive for norovirus genogroup I. One of these samples was also positive for *Aeromonas hydrophila* (25%). All four were negative for *Campylobacter* and enteropathogenic bacteria. We compared the confirmed-positive cases to their reported food consumption and found both reported consuming meals and food items with elevated risk ratios (Table 1), including on 29 August during the morning break; crispy fish maw, dried squid, and cashew nut salad at lunch on 29 August; assorted salad bar with assorted

dressing and seafood kebab at dinner on 29 August.

DISCUSSION

In this investigation, we identified nine cases of acute gastrointestinal illness among the participants and family members of a training course in Thailand (attack rate, 17%). Norovirus infection was confirmed in two of four fecal specimens collected. The symptom profile and duration of illness was consistent with point source, foodborne norovirus infection, thus we conclude it was the likely cause of the outbreak (CDC, 2011).

The cohort study did not implicate a single food item as the source of infection; however, several food items may have been contaminated. All meals were self-serve buffets provided by a single caterer, providing the opportunity for contamination of multiple food items by a food worker, hotel staff member, training participant, or family member who was shedding infectious virus. Up to 30% of norovirus infections may be asymptomatic and infected individuals shed virus on average for four weeks after symptom resolution, although the application to infectivity is unknown (Graham *et al*, 1994; Atmar *et al*, 2008). As such, an asymptomatic or previously-ill individual may have been the source of infection, potentially contaminating multiple food items at different times. In this outbreak, one person reported illness beginning and ending on 29 August, one day before cases reported onset of symptoms. This person may have been the source of the outbreak, as the person consumed shared meals on 29 August, notably, crispy fish maw, dried squid, and cashew nut salad, which was implicated as a potential source of infection. This person did not eat dinner on

29 August. While items from both lunch and dinner on 29 August were implicated as potential sources of infection and fall within a potential incubation period of 10-51 hours (Glass *et al*, 2009), the incubation period is usually 24-36 hours, making lunch on 29 August a more likely source.

Four ill persons (44%) reported using oral rehydration solution (ORS). This rate is higher than global rates of ORS use and unusual because worldwide use of ORS to control diarrhea in children has stagnated in the past decade (UNICEF/WHO, 2009). This may be a reflection of the population studied, many of whom are highly educated and have a background in healthcare.

There were several limitations to this study. First, we were unable to do a follow-up investigation of the site of the outbreak or their employees, which limited our ability to ascertain the true extent of the outbreak and the possible source of infection. Food handlers are frequently implicated in norovirus outbreaks (Lynch *et al*, 2006). In a meta-analysis, Greig *et al* (2007) found that in norovirus or probable norovirus outbreaks where a food worker was implicated, multi-ingredient foods, including various salads and produce, were the most common food categories. These outbreaks were due primarily to bare-hand contact by the food worker or food-handling by an infected person (Todd *et al*, 2007). An additional limitation was that less than half of ill persons were able to provide a stool sample for analysis and we did not assess prior illness among attendees to determine if an attendee was the source of infection. Norovirus was confirmed in only two of the four stool samples collected, possibly because of lower sensitivity of conventional PCR compared to real-time assay. Genotyping was not performed to determine if the two positive specimens were identical

viral strains, which might have provided further confirmation of norovirus as the outbreak etiology. Finally, there were a large number of food items available during meals (4 items during each break, 11 items during each lunch, and 25 items during dinner), which may have led to poor recall. However, participants could select "yes", "maybe", "no", or "don't know" for each food choice and very few selected "maybe" or "don't know". Nevertheless, misclassification of exposures was likely to be nondifferential (similar in those who did and did not get ill) and thus bias the results towards the null.

This investigation provides the first report of a laboratory-confirmed norovirus outbreak in Thailand. This outbreak underscores the need for improved surveillance and diagnostic capabilities to better characterize the role of norovirus in causing gastroenteritis outbreaks and foodborne disease in Thailand. Implementing measures to prevent norovirus transmission, such as appropriate hand hygiene, proper disinfection of surfaces, and exclusion of ill individuals, may help prevent future norovirus outbreaks (CDC, 2011).

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