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An Investigation of a Norovirus Outbreak Linked to Contaminated Vegetables in Mueang District, Chanthaburi Province, Thailand, December 2021–January 2022

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Abstract

A norovirus outbreak in Mueang District, Chanthaburi Province, was notified on 29 Dec 2021. This study's objectives were to describe epidemiological characteristics of the outbreak and identify possible sources of the ongoing outbreak. A descriptive study was performed by reviewing diarrhea cases and laboratory results from 1 Dec 2021 to 5 Jan 2022. Interviews were conducted with recent diarrheal cases who had onset during 3 to 5 Jan 2022. Polymerase chain reaction and genetic sequencing were used on patients and environmental specimens to identify genetic linkage. In the retrospective cohort analysis, a medical student cluster was investigated. A total of 675 diarrheal cases were found in Mueang District. Tha Chang Subdistrict had the highest morbidity rate at 0.95%. Out of 77 patient specimens, 30% (23/77) were confirmed norovirus. The median age of cases was 26 years old. Common symptoms included diarrhea (89%), nausea (67%), abdominal pain (67%), and fever (56%). No severe cases were reported. The interviews of recent diarrheal cases revealed that they visited four markets before symptom onset. Seventy percent (21/30) of the markets' vegetable samples tested positive for norovirus. However, the samples of tap/drinking water, ice, and seafood tested negative. Patient and vegetable genotypes were matched as GII.4[P7]. Contaminated vegetables were suspected sources of the outbreak. The recommendation for early case detection and pre-cool season preventive measures are ensuring adequate residual chlorine level, promoting handwashing, and washing vegetables before consumption.

Keywords: norovirus, outbreak, acute diarrhea, foodborne, Chanthaburi, Thailand

Introduction

Norovirus is the primary cause of acute gastroenteritis.[1] As of 2019, norovirus was classified into 10 genogroups (GI–GX) and 48 genotypes.[2] During 2015–2020, genotype GII.4 was the most common cause of norovirus illnesses worldwide and Thailand.[1,3,4] There is no specific antiviral medication for the virus. Norovirus can be detected in patients (stool, vomitus, and serum), food, water, and the environment by the polymerase chain reaction technique.

Globally, from 2008–2020, norovirus was estimated to be a cause of acute diarrhea in approximately 685 million cases annually, of which 200 million cases were among children under five. The overall detection rate

of norovirus was 18% among acute gastroenteritis patients.[1,5] Norovirus outbreaks are usually detected in healthcare facilities, schools, cruise ships, and restaurants.[6] Previous studies showed that leafy greens (such as lettuce), fresh fruits, and shellfish (such as oysters) were commonly identified as implicated foods for norovirus outbreaks.[7,8]

During 2015–2018, a surveillance conducted in Thailand for acute viral gastroenteritis revealed that the positive rate of norovirus was 12% among patients with acute gastroenteritis, with the predominance of norovirus GII.4.[4,7] Between 2017 and 2021, there were a total of nine norovirus outbreaks in Thailand. The outbreaks usually occurred during cool season, between November and February. Seventy-seven percent of those outbreaks occurred in school settings, while 11% were in prisons and 11% among travelers. The most commonly affected age group was children between 6–12 years old, followed by 13–18 years and more than 18 years.[9]

On 29 Dec 2021, the Department of Disease Control received a notification of the large number of acute diarrheal cases across Mueang District, Chanthaburi Province. This province is located on the eastern coast of Thailand, and consisted of 536,557 population and was divided into 10 districts. The number of cases were still higher than the 5-year median even though the local team had investigated for seven days. This study's objective was to describe epidemiological characteristics of the outbreak and identify possible source of the ongoing outbreak.

Methods

A descriptive study was conducted on diarrheal patients in Mueang District diagnosed with ICD-10 acute diarrhea and food poisoning at Hospital A, Private Hospital B, and Private Hospital C between 1 Dec 2021 and 5 Jan 2022. Data were gathered from two sources. The first source was the national communicable disease surveillance system (R506), for defined cases in Mueang District, Chanthaburi Province. This study compared cases during the study period with the past 5 years for trends. The second source was hospital data from the three Mueang District hospitals, where most patients in Mueang District went for treatment. Diarrheal cases were defined as patients from Mueang District diagnosed with ICD-10 acute diarrhea (codes A02.0, A04.0–A04.9, A08.0–A08.9, A09.0, A09.9) and food poisoning (codes A05.0, A05.2–A05.4, A05.8–A05.9). Viral gastrointestinal pathogen screening test and bacteria culture results from 1 Dec 2021, to 5 Jan 2022 were also reviewed.

To get information of food exposure among the recent diarrheal cases at the time of investigation, this study selected the diarrheal cases who had onset during 3–5 Jan 2022. The recent diarrheal case was identified as a patient who had visited any of the three hospitals who had symptoms onset between 3–5 Jan 2022 meeting at least one of the following clinical criteria: 1) experiencing diarrhea or vomiting at least once, or 2) having abdominal pain along with at least one of the following symptoms: fever, nausea or headache, and having a history of either residing in Mueang District or visiting within three days before the onset of symptoms. Phone interviews were done among the recent diarrheal cases to get history of food consumption and the sources of food.

This study obtained stool samples or rectal swabs from recent diarrheal cases admitted to the three hospitals. Laboratory testing involved real-time reverse-transcription polymerase chain reaction and sequencing of the norovirus gene, performed at the Center of Excellence in Clinical Virology (CECV). Seafood was collected from a Chanthaburi beach and a Mueang District market, both associated with recent cases. Regarding vegetables, this study acquired samples from four Mueang District markets, specifically selecting those suitable for raw consumption. This study also traced vegetables in Chanthaburi Province to a Pathum Thani market, selecting the varieties positive for norovirus in Chanthaburi Province. CECV analyzed both seafood and vegetables using the same method. This study gathered tap water samples from seven Mueang District locations, covering Mueang District communities. Furthermore, this study collected tap water and used water from three Mueang District markets, Chanthaburi Province, and a Pathum Thani market. Ice and pure water samples were taken from four large Mueang District ice factories and three small ice stores near the market and Chanthaburi Provincial Public Health Office. Water and ice samples underwent coliform bacteria testing, bacterial culture, and viral pathogen testing at Thai Red Cross Emerging Infectious Disease Health Science Centre, National Institute of Health, Department of Medical Sciences, and CECV.

This study conducted retrospective cohort only among the recent diarrheal cluster identified during the period of study to identify possible source of exposure. The cluster occurred among medical students who joined a workshop in hospital A between 23 and 25 Dec 2021. This study identified history of food consumption during 23–25 Dec 2021 by medical students and staff who joined the workshop by face-to-face interview.

Then the cases were defined as medical students and staffs with diarrheal symptoms as described previously. Relative risk and 95% confidence interval were calculated using univariate analysis. Fresh stool from medical students who met the case definition were sent for viral pathogen testing by real-time PCR method at the Thai Red Cross Emerging Infectious Disease Health Science Centre.

Results

The number of acute diarrhea cases during December 2021–January 2022 in Chanthaburi Province from R506 and ICD-10 code exceeded the 5-year median since 23 Dec 2021. The local team was aware of an abnormal event through social media on 28 Dec 2021 and immediately began investigation during 29–30 Dec 2021 (Figure 1). The result showed most cases were found in Mueang District. During the period of 16–29 Dec 2021, stool examination in 93 cases of acute diarrhea at private hospitals B and C revealed that 19.2% (5/26) of the cases were caused by norovirus, 6.8% (2/29) were rotavirus and 10.5% (4/38) were bacteria. A total of 675 cases were reported in Mueang District, and Tha Chang Subdistrict had the highest morbidity rate at 0.9% (186/19,482). The most affected age group was 5–14 years, followed by 0–4 years.

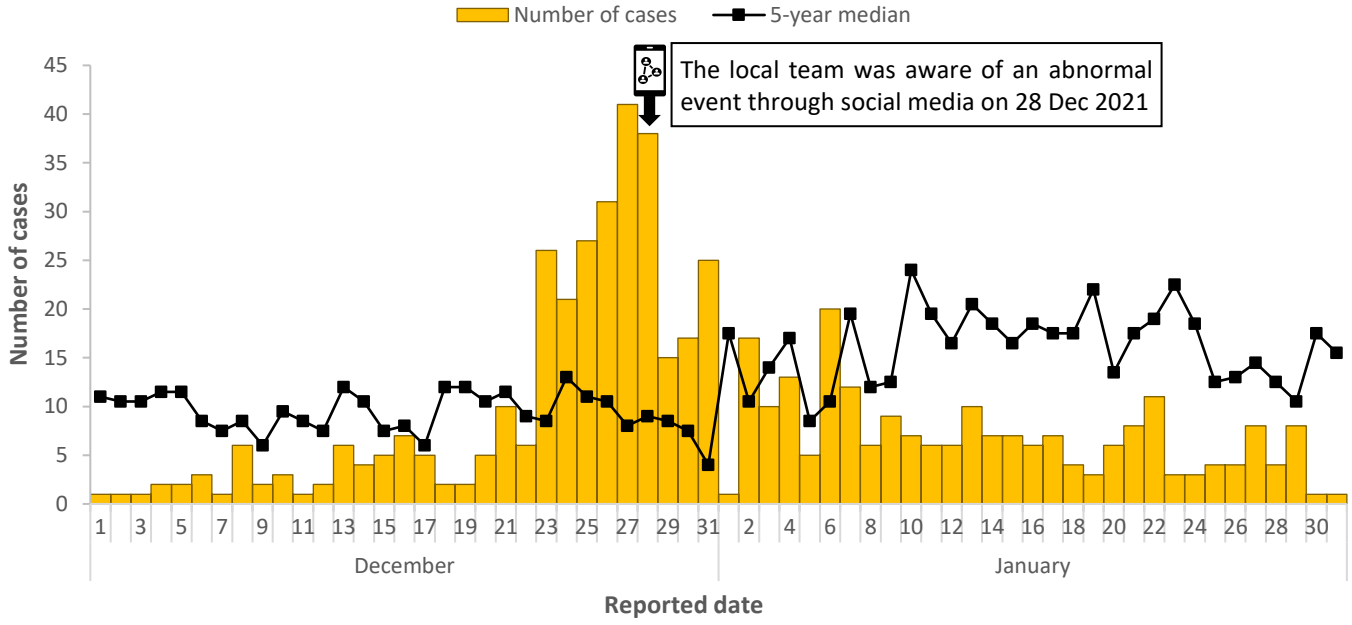

Figure 1. Number of acute diarrheal cases in Mueang District, Chanthaburi Province from R506 and ICD-10 code by reported date during December 2021–January 2022 compared with 5-year median (n=535)

As of 5 Jan 2022, the number of diarrhea cases in the district remained higher than the 5-year median. This study investigated 55 recent diarrheal cases visiting three hospitals in Mueang District, Chanthaburi Province during 3–5 Jan 2022. Male to female ratio was 1: 1.5, and median age (Q1, Q3) was 26 years (10, 35). Eighty-nine percent of the cases had diarrhea followed by nausea (67%), and abdominal pain (67%). There was no dead case, with 34% (19/55) of cases were inpatients while the rest were outpatients. The interviews revealed the food consumed by the cases was from sources around their homes and workplaces. Additionally, 43% of the cases reported a history of visiting four large markets in Mueang District within 3 days prior to the onset of acute diarrhea.

Retrospective Cohort Study in a Cluster

During the period of the investigation, one cluster of norovirus outbreak among medical students at Hospital A was detected. The cluster occurred following a workshop held between 23 and 25 Dec 2021, which was attended by 36 medical students and five staff. The overall attack rate was 46% (19/41) with four confirmed cases. All cases were medical students. Male to female ratio was 1: 1.1. The median age was 23 years old, and they were treated as outpatients. The most clinical symptoms were diarrhea (100%) followed by vomiting (57%) and nausea (47%). The first case had onset at 8:00 AM on 24 Dec 2021, and most cases had onset during 6:00 PM–12:00 AM on the same day (Figure 2). The incubation period for norovirus is between 12–48 hours. When comparing incubation period with the onset time of symptoms, it was suspected the exposure occurred between midday to midnight on 23 Dec 2021. There were four possible meals to be the source. The retrospective cohort showed no food item was a statistically significant risk factor (Table 1).

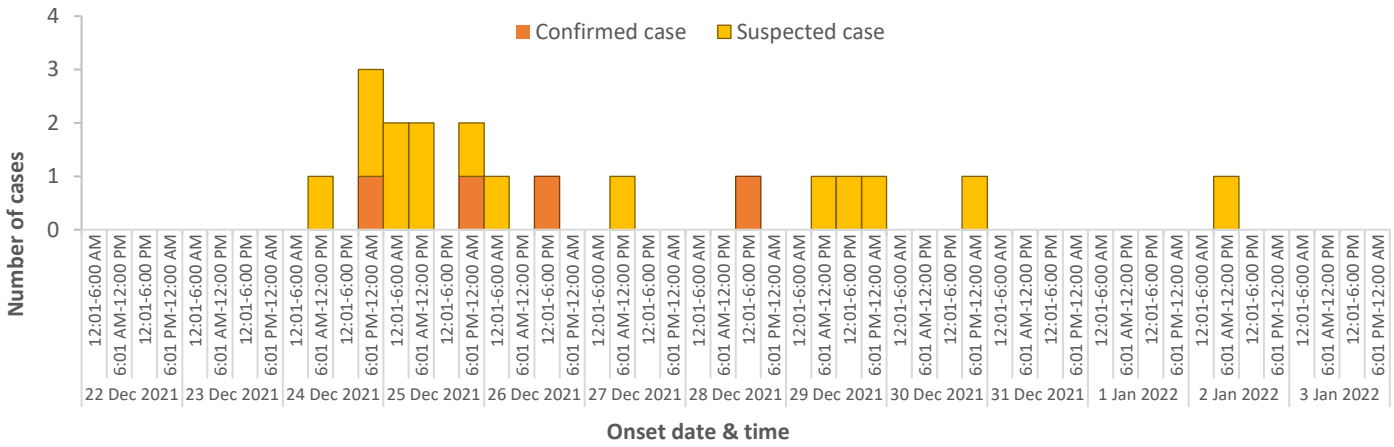

Figure 2. Epidemic curve of the suspected and confirmed cases in the medical student cluster during 23–25 Dec 2021 (n=19)

Table 1. Relative risk of four probable source meals and menus in the workshop in hospital A during 12 PM–12 AM on 23 Dec 2021

Meal	Exposed		Unexposed		Relative risk	95% confidence interval
	Case	Non-Case	Case	Non-Case		
Lunch	20	22	2	1	1.42	0.27–7.30
Bamboo lining soup	18	21	3	3	0.92	0.38–2.20
Crab roe chili	17	19	4	5	1.06	0.47–2.38
Vegetable	19	21	2	3	1.18	0.38–3.64
Fried chicken	18	18	3	6	1.5	0.56–3.99
Cooked rice	17	21	4	3	0.78	0.37–1.62
Lod chong with coconut milk	18	20	3	4	1.10	0.44–2.76
Afternoon break	20	22	2	1	1.42	0.27–7.30
Soft drink	18	22	3	2	0.75	0.33–1.65
Curry puff	17	22	4	2	0.65	0.33–1.27
Dinner	19	21	3	2	1.18	0.38–3.64
Spicy chicken soup	18	19	3	5	1.29	0.49–3.36
Roast pork	17	19	4	5	1.06	0.47–2.38
Minced shrimp omelet	17	19	4	5	1.06	0.47–2.38
Cooked rice	17	19	4	5	1.06	0.47–2.38
Orange	16	17	3	5	1.29	0.49–3.38
Night break	18	19	5	3	1.29	0.49–3.36
Orange juice	15	18	4	4	0.91	0.44–1.87
Bread	15	18	4	4	0.91	0.44–1.87

The food preparation process began at 6:00 AM, starting with the purchase of raw food and ice from Market E and Ice B factory. The food was prepared and cooked 2–4 hours before serving. The cooked food was packed in boxes 1–2 hours before serving and kept at room temperature before being served.

Laboratory Investigation

A total 77 stool specimens were examined, of which 30% (23/77) tested positive for norovirus. The analysis occurred in two phases: in December 2021, the local team detected norovirus in 10% (6/55) of the tested specimens. Additionally, the investigation team found increased positive norovirus to 77% (17/22) of samples from recent diarrheal cases visiting three hospitals during 3–5 Jan 2022 (Table 2). This study collected ice products and pure water from various ice factories. Most of the ice and pure water were found to contain coliform bacteria, and were tested positive for various bacteria such as *Bacillus cereus*, *Aeromonas* spp., from bacterial cultures. However, norovirus was not detected (Table 3). Seventy percent (21/30) of vegetable samples from the four markets were positive for norovirus. The other water and food samples in Mueang District, Chanthaburi Province were negative for norovirus, while tap water in Pathum Thani Province was positive.

Norovirus sequencing identified three genotypes from the recent patients. The predominant genotype identified in food samples was Norovirus GII.6[P7], and it was traced back to vegetables originating from Market F in Pathum Thani Province, which supplies products to Market D. Market D, is known as the major

fresh vegetable market in Mueang District. On 5 Jan 2022, this study found genotype GII.4[P7] in vegetables from market D and a patient from Tha Chang Subdistrict who had a history of travel around three subdistricts, although the patient did not report history of visiting to Market D.

Table 2. Laboratory result of food, water and ice samples related to the outbreak from rectal swab and fresh stool samples from patients in three hospitals during 3–5 Jan 2022

Hospital	Test	Result	Number of cases	Organism
Hospital A	Real-time PCR	Positive	12	Norovirus GII
		Negative	5	
Private hospital B	Real-time RT-PCR	Positive	4	Norovirus GII
		Negative	0	
Private hospital C	Real-time RT-PCR	Positive	1	Norovirus GII
		Negative	0	

PCR: polymerase chain reaction. RT-PCR: reverse-transcription polymerase chain reaction.

Table 3. Laboratory of food and ice samples related the outbreak during 29 Dec 2021–23 Feb 2022

Collected date	Samples	Source	Detected samples / total samples		
			Bacteria	Norovirus	Norovirus sequencing
Water and ice					
29 Dec 2021	Ice	Factory A, B, C, D	Coliform+ (2/4) *Bacillus cereus* (3/4)	0/4	-
8 Jan 2022	Ice	Factory A, B, E, F, G	-	0/4	-
17 Jan 2022	Ice	Factory A, B, C, D	*Aeromonas* spp., Biovar sobria (2/4)	0/4	-
	Pure water	Factory A, B, C, D	*Aeromonas* spp., Biovar sobria, *Bacillus cereus*, *Escherichia coli* (4/4)	0/4	
18 Jan 2022	Tap water	Market C, D, E	-	0/3	-
	Used water	Market C, D, E	-	0/3	-
23 Feb 2022	Tap water	Market F	-	1/1	GII.X[P7]
Seafood					
5 Jan 2022	Squid, shrimp	Market A	-	0/2	-
6 Jan 2022	Oyster, shrimp	Chao Lao Beach	-	0/5	-
Vegetables					
5 Jan 2022	Lettuce, Chinese cabbage, basil	Market D	-	2/3	GII.6[P7] GII.4[P7]
14 Jan 2022	Chinese cabbage, cabbage, morning glory, celery, basil, sweet basil, lettuce, tomato, radish, cucumber	Market D	-	9/11	GII.6[P25] GII.6[P7] GII.6[P31] GII.3[P7] GII.3[PX] GII.21[P31] GII.21[PX]
18 Jan 2022	Morning glory, lettuce, coriander, dill, Chinese cabbage	Market C	-	4/5	GII.X[P7] GII.X[PX] GII.6[P17]
	Morning glory, kale, bok choy, paco fern, dill	Market E	-	3/5	GII.17[P17] GII.4[P17] GII.6[P7]
23 Feb 2022	Lettuce, Chinese cabbage, kale, morning glory, coriander, basil	Market F	-	3/6	GII.X[P7] GII.X[P31]

Discussion

This study detected the diarrhea outbreak in Mueang District, Chanthaburi Province, from 23 Dec 2021 to 5 Jan 2022 due to a large number of diarrhea cases that exceeded the 5-year median, based on the communicable disease surveillance system. Norovirus was the most predominant pathogen of this outbreak. This outbreak occurred during cool season of Thailand similar to the previously reported global situations.[7] Norovirus can cause acute gastroenteritis in all age groups, but typically affects young children under five years of age.[1,10]

Several recent diarrheal cases have exposed large food markets in Mueang District. Since this study did not find positive norovirus from water or ice, whereas several vegetables specimens showed positive for the virus, this study suspected the source was likely to be food, especially vegetables. Vegetables are commonly reported as the source of norovirus infection including vegetables, salads, berries, and fruits.[1] This study detected norovirus contamination in various vegetables, such as Chinese cabbage, lettuce, cabbage, morning glory, and dill, obtained from four markets in Mueang District. Upon tracing back to the origins of the contaminated vegetables, norovirus was also detected in both the vegetables and tap water. However, the market authorities did not permit an investigation into the source of the contaminated tap water. This lack of access to explore the source could be linked to inadequate chlorine levels within the water system.[7] However, this study did not detect norovirus in shellfish and seafood, which were commonly identified as sources of other norovirus outbreaks. Thus, vegetables were highly possible to be one source of infection in this outbreak.

Although norovirus was detected from the human and environmental specimens, this study did not find a clear linkage between recent diarrheal cases and vegetables. However, it is possible that patients may have contracted norovirus from fresh vegetables consumed at restaurants they visited. Additionally, it was possible that the vegetables might have been contaminated with norovirus from contaminated water (tap water in market F) during cultivation or processing.

The norovirus outbreak among the medical student cluster was the only cluster captured by an event-based surveillance in the area. Although it had been 13 days since the onset, this study still found norovirus positive from stools. According to the epidemic curve, a point common source was most probable. The exposure time was suspected to be on 23 Dec 2022. However, this study did not find any food item that was a statistically significant risk factor causing sickness. The most suspected dish was fresh vegetable served in lunch boxes since this study found positive norovirus in vegetable samples in Market E later on.

Limitations

Due to the delayed investigation, this study did not have specimens from patients and vegetables during the early phase of the outbreak. Thus, this study could not conclude that these vegetables were also responsible as the only source of the outbreak during that period. Since this study's main objective was to control the ongoing outbreak, this study tried to find the source of infections by focusing on the recent cases visiting the major hospitals in the district and tried to identify common exposure among them. Memory bias could occur when this study asked about food exposure three days before the onset. Recall bias might occur among cases in the analytic study, although this study used the menu on each day of the workshop to help interviewees recognize food items they have eaten. This study could not get the specimen to test for norovirus from asymptomatic chefs, assistants and staff who prepared and served food in the workshop.

Recommendations

R506 should have an alert function for early warning when the number of cases is higher than the 5-year median, so that local epidemiologist could detect the outbreak and perform investigation early. Close cooperation with local public health organization and Provincial Waterworks Authority is required to ensure the quality of tap water, focusing on maintaining residual chlorine levels at the required standard. Risk communication for norovirus prevention should be done to the public before cool season by promoting hand hygiene, and cleaning vegetables with running water before eating or cooking.

Conclusion

There was a norovirus outbreak in Mueang District, Chanthaburi Province from 23 Dec 2021 to 6 Jan 2022. The majority of cases were aged below 15 years and lived in Tha Chang Subdistrict. There were no severe or fatal cases. This study investigation identified various genotypes of norovirus, but only GII.4[P7] was found to be matched between patients and vegetables. Although this study did not find any direct epidemiological linkage between the patient and the contaminated vegetables, this study identified leafy vegetables as potential sources of infection for the cases in early January 2022. This study did not find evidence of other potential sources of infection for the

entire outbreak. To prevent future outbreaks, this study recommends strengthening early detection of acute gastroenteritis cases, risk communication of diarrhea before and during cool season, especially avoiding eating fresh vegetables from unsafe sources, and washing vegetables thoroughly with running water before eating or cooking.

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Suggested Citation

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