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Gastroenteritis Outbreak of Rotavirus G3P[8] in a Secondary School in Pathum Thani Province, Thailand, 2022

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Abstract

On 14 Sep 2022, the Division of Epidemiology was notified of a cluster of food poisoning in a secondary school. We conducted an investigation to describe the epidemiological characteristics of the outbreak, identify the causative agent, source of illness, and possible risks, and provide control measures. An electronic-based questionnaire was distributed to school members. Students and staff were interviewed. Inspection of the school canteen, water supply system, toilets, and hand-washing facilities as well as observation of personal and food hygiene practices among food handlers were done. A retrospective cohort study was conducted. The overall attack rate was 40.4% (684/1,695). Most cases were students (95.9%). Thirteen out of twenty-eight rectal swabs were positive for rotavirus, with two being identified as genotype G3P[8]. Being exposed to foods or drinks from the school canteen was a significant risk factor (adjusted odds ratio (AOR) 2.35, 95% CI 1.23–4.52), and bringing a drink to school was protective (AOR 0.67, 95% CI 0.50–0.88). Although rotavirus was not detected in the environment, contamination of groundwater used for cooking and drinking was evident. We recommend routine water quality testing and installation of groundwater treatment to ensure the safety of the water supply.

Keywords: rotavirus, food poisoning outbreak, school, groundwater

Introduction

Group A rotavirus infection is one of the leading etiologies of acute gastroenteritis worldwide. The World Health Organization reported that every year rotavirus infection causes more than 200,000 fatalities in children under the age of five years with 85% of deaths occurring in low-income countries in Asia and Africa.[1,2] In Southeast Asia, more than half of all diarrhea deaths were associated with rotavirus infection.[3]

Rotavirus is a non-enveloped, double-stranded virus belonging to the *Reoviridae* family. They can be classified into G-genotypes and P-genotypes based on capsid proteins.[4] About 90% of human group A rotavirus infections are caused by various combinations of five rotavirus genotypes (G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8]).[5,6] Only rotavirus genotype G9 is associated with increased severity of diarrheal disease but the evidence remains controversial.[7]

Two live-attenuated oral vaccines for rotavirus are available, namely Rotarix™ and RotaTeq™ and, since their introduction in 2006 and 2008, respectively, the prevalence of rotavirus-associated diarrhea has gradually declined worldwide.[8,9] Rotavirus vaccines, available for infants aged 6-32 weeks, have been part of Thailand's Expanded Program on Immunization since 2020.[10]

Classic presentations of rotavirus infection include fever, vomiting, and watery diarrhea. Children aged less than 5 years are at risk of severe dehydration.[10] In adults, however, symptoms and severity of rotavirus

diarrhea varies. Asymptomatic carriers comprise up to almost 80% of infected adults.[11,12]

On 14 Sep 2022, the Division of Epidemiology was notified of a suspected food poisoning cluster of approximately 400 students in a secondary school in the Khlong Si Subdistrict, Khlong Luang District, Pathum Thani Province. A joint investigation was conducted during 15–16 Sep 2022 to identify the causative agent, source of illness, and possible risk factors, describe the epidemiological characteristics of the outbreak, and provide control and prevention measures.

Methods

Descriptive Study

We performed an active case finding and a descriptive study by distributing an electronic-based, self-administered questionnaire to all students and staff of the affected school. A list of hospitalized students and staff was obtained and in-depth interviews via telephone were conducted. Medical records of students and staff and infirmary records were reviewed. Information retrieved included demographic characteristics, clinical data, history of food and beverage consumption, and sanitation behaviors.

Our case definitions were as follows: suspected cases were students or staff who developed at least one of the following symptoms during 27 Aug to 21 Sep 2022: diarrhea, mucus in stool, stomachache, nausea, and vomiting. Confirmed cases were suspected cases who tested positive for gastrointestinal pathogens in stool or vomitus samples via bacterial culture or reverse transcriptase polymerase chain reaction.

Laboratory Study

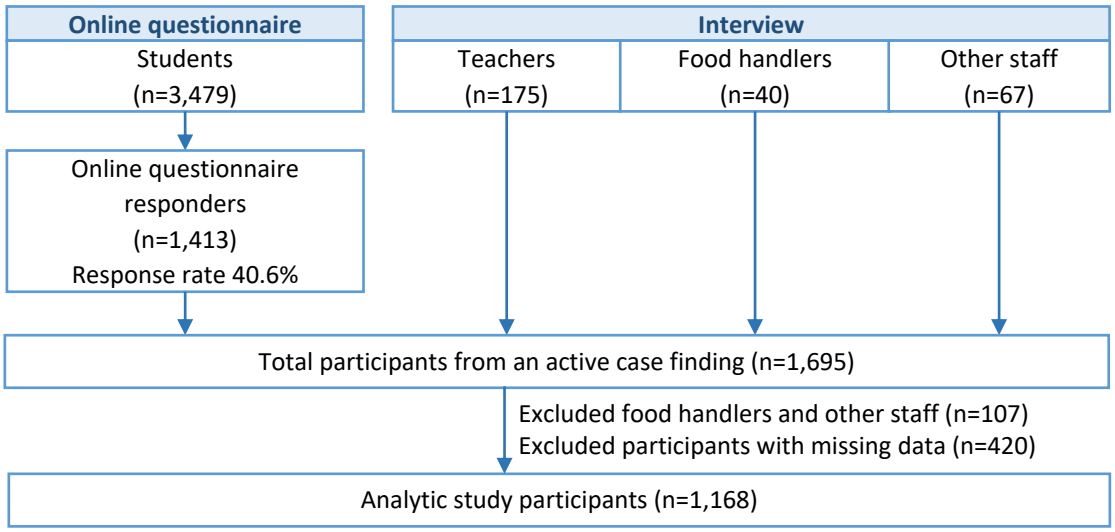
A total of 56 rectal swabs and fresh stool samples were randomly obtained from 26 suspected cases who were symptomatic on the days of specimen collection, as well as two food handlers who reported having diarrhea within the last month. Hand swabs of food handlers and swabs from canteen equipment were also obtained. Cary Blair transport media and universal transport media were used for bacterial and viral testing, respectively. In addition, we collected samples of water from a drinking fountain, bottled drinking water, cooking water used in the school canteen, water from storage tanks and groundwater wells, and ice for consumption and for food storage. All specimens were sent for enteropathogenic bacterial culture and reverse transcriptase polymerase chain reaction for norovirus and rotavirus at the National Institute of Health of Thailand. Some of the positive human specimens were then sent for genotype identification by the Sanger sequencing method at the National Institute of Health of Thailand.[13]

Environmental Study

The dining and cooking areas of the school canteen, the school water supply system, toilets, and hand-washing facilities were inspected. We observed the food preparation and serving processes, food and raw material storage in the canteen, and student's eating and hygiene habits of students. Food handlers were asked about their history of illness in the past month, source of raw ingredients, and cooking processes. The canteen manager, canteen janitors, and the school premises manager were also interviewed. Hands of food workers, selected food items, and kitchen surfaces were tested for coliform bacteria using an SI-2 test kit.

Analytic Study

A retrospective cohort study was conducted. We employed the census method of data collection. The main exposure of interest was a history of food or beverage consumption in the canteen. The non-exposure group included persons who did not buy food or drink from the canteen from 27 Aug 2022 to the investigation day. Covariates included gender, age, occupation, and sanitation behaviors. Cases were defined as either suspected or confirmed based on our descriptive study and non-cases were participants who did not meet the criteria for either. We recruited all students and teachers in the school into the study cohort. Those who failed to respond to the questionnaire or had missing data on the history of food and beverage consumption or sanitation behaviors were excluded. Sample size calculation was performed using the formula for comparing two proportions for a cohort study.[14,15] The following parameters were applied; type I error=5%, power=80%, probability of an outcome in the exposed group=41%, probability of an outcome in the unexposed group=18.7%, and relative risk (RR)=2.18 based on a previous study.[16] The required number of study subjects was 65 in the exposed group and 65 in the unexposed group. Univariable and multivariable logistic regression models were used to determine factors associated with being a case. Known risk factors from the literature and variables with a *p*-value less than 0.2 from the univariable analysis were included in the multivariable analysis. Crude RR, adjusted odds ratio (AOR), *p*-value, 95% confidence interval (CI), and population attributable fraction (PAF) for stores and food items were calculated. *P*-values less than 0.05 were considered statistically significant. We used R version 4.2.1 for statistical analysis.



Some teachers were respondents of the online questionnaire as well as being interviewees.

Figure 1. Recruitment of participants in a food poisoning outbreak in a secondary school, September 2022

Results

Setting

The affected school is located in the Khlong Si Subdistrict, Khlong Luang District, Pathum Thani Province. It comprises 3,479 students, 175 teachers, 40 food handlers, and 67 non-teaching staff. There is one school canteen for everyone in the school, which is open during lunch time.

Descriptive Study

Of all school members, 1,413 students and 282 staff were either interviewed or responded to our questionnaire. The response rate was 40.6% among students (Figure 1). A total of 684 cases (651 suspected cases and 33 confirmed cases) were identified, given an overall attack rate of 40.4% (684/1695). Of these, 656 (95.9%) were students and 294 (43.0%) were male. The median (interquartile range) age of cases was 15 (14–17) years. The attack rate was highest among students (46.4%) (Table 1). Cases were closely distributed across student grades and classes. The grade-specific attack rates ranged from 37.4–52.8% (Table 2). Most cases lived in Pathum Thani Province (92.8%) and about half resided in Khlong Luang District (48.7%).

Table 1. Attack rates of the food poisoning outbreak in a secondary school during September 2022, by occupation (n=1,695)

Occupation	Total population	Suspected case	Confirmed case	Attack rate (%)
Student	1,413	636	20	46.4
Teacher	175	14	7	12.0
Food handler	40	-	2	5.0
Others	67	1	4	7.5
Total	**1,695**	**651**	**33**	**40.4**

Table 2. Attack rates of the food poisoning outbreak among students of a secondary school during September 2022, by grade (n=1,393)

Grade	Case no./total no.	Attack rate (%)
7	94/197	47.7
8	93/206	45.1
9	102/273	37.4
10	116/221	52.5
11	86/163	52.8
12	145/333	43.5

The grade data of twenty students were missing.

Twenty-four percent of the cases went to hospital and 25 were hospitalized. All cases had mild-to-moderate dehydration and there were no serious complications or deaths. The most common symptoms were diarrhea (85.0%), stomachache (79.0%), vomiting (64.5%), fever (57.1%), and nausea (49.9%) (Figure 2).

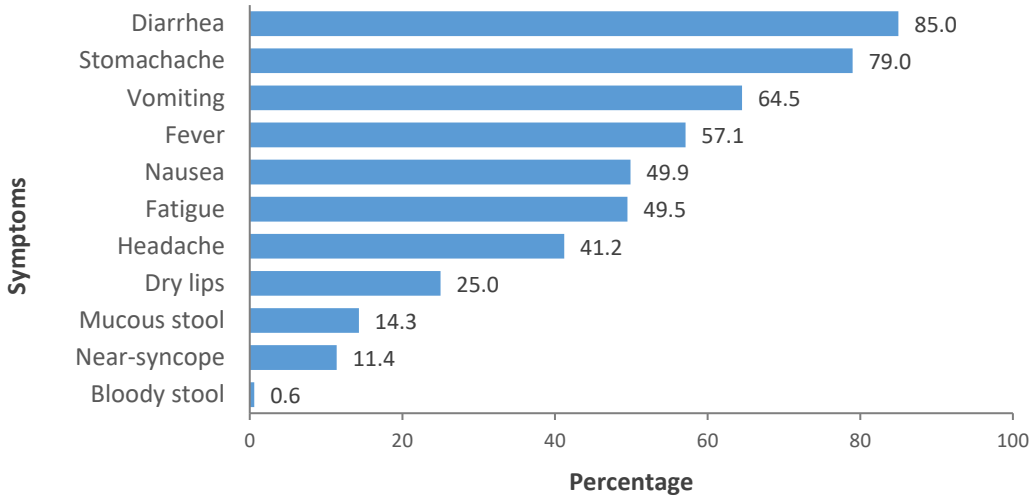


Figure 2. Frequency of symptoms among food poisoning cases in a secondary school, September 2022 (n=684)

Figure 3 shows the epidemic curve, which indicated a mixed source pattern. Out of 684 cases, 42.11% (288/684) of the data was missing due to the absence of onset time. A few cases developed symptoms on 1 Sep 2022. The number of cases increased rapidly on 11 September and peaked on 11 Sep 2022. The onset of the last known case was 17 Sep 2022. No common school events or sports activities were held before the outbreak. However, there was heavy rain on the afternoon of 8 Sep 2022 and parts of the Khlong Luang District were flooded. A school examination was held from 12–19 Sep 2022, during which half the students came to school each day. A school holiday occurred from 20 Sep to 27 Oct 2022.

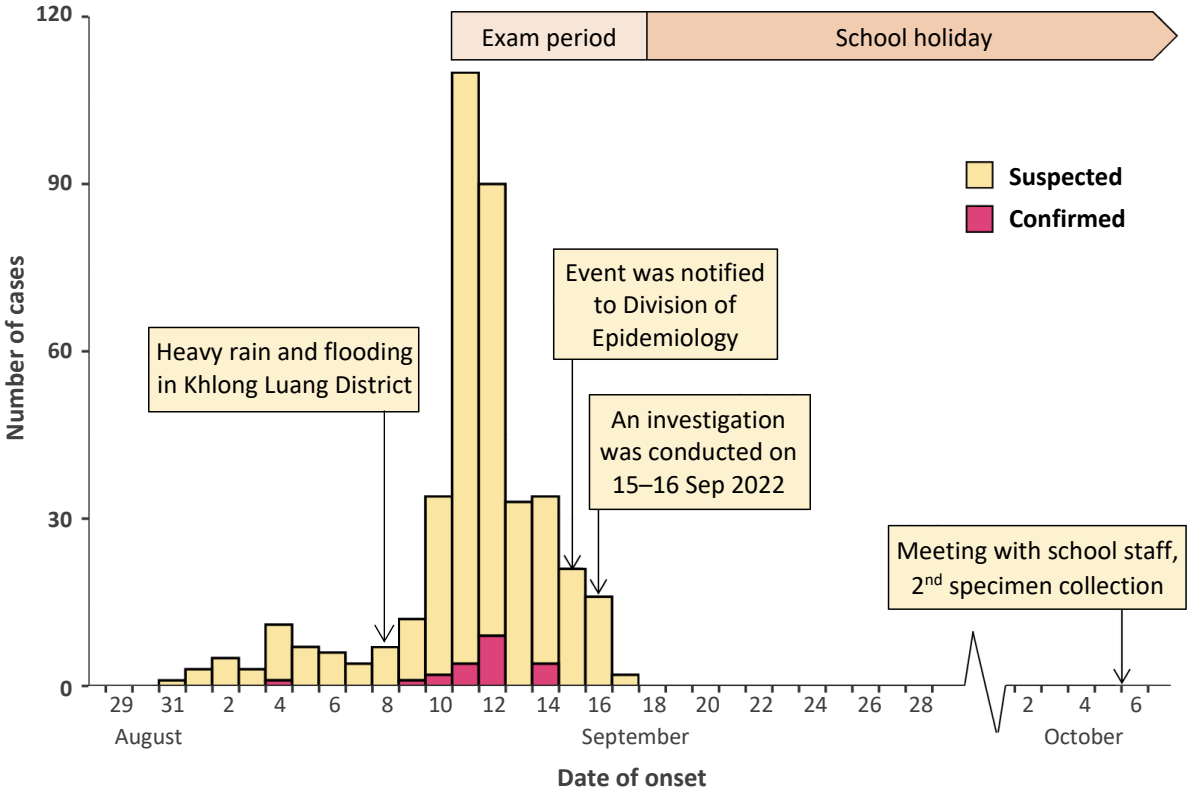


Figure 3. Epidemic curve of a food poisoning outbreak in a secondary school, September 2022 (n=396)

Analytic Study

A total of 1,168 participants (1,137 students and 31 teachers) were included in the analysis. From univariable analysis, the relative risks and PAF by stores (21 stores in total with varying types of food sold in each store, such as noodle soup and rice) and by food items were very similar, ranging from 0.83–1.31 and 0–16.34%, respectively. With this reason, we then hypothesized that there was a common exposure in the school canteen. Therefore, we grouped all stores into one variable, namely "overall exposure to foods or drinks from the canteen" between 27 Aug 2022 and 16 Sep 2022. Overall exposure to foods or drinks in the

canteen was a significant risk factor with an AOR of 2.35 (95% CI 1.23–4.52), PAF of 55.48% (95% CI 17.51–76.46), and the attributable number of cases was 379. Exposure to any foods and to any drinks in the canteen had AOR of 2.38 (95% CI 1.29–4.38) and 1.26 (95% CI 0.95–1.67), respectively. Bringing one's own lunch, bringing one's own drink, and bringing one's own utensils were significant protective factors from the univariable analysis. After adjusting for covariates, bringing one's own drink (AOR 0.67, 95% CI 0.50–0.88) remained the only significant protective factor (Table 3).

Table 3. Univariable and multivariable analysis of factors associated with food poisoning among students and teachers in a secondary school during September 2022 (n=1,168)

Variables	Crude RR (95% CI)	Adjusted OR (95% CI)	*P*-value	% PAF[a] (95% CI)
Male (vs. female)	0.97 (0.82-1.13)	0.99 (0.76–1.29)	0.942	NA (NA–10.63)
Age (cont. var)	-	1.02 (0.97–1.06)	0.519	-
Student (vs. teacher)	0.98 (0.61-1.59)	1.51 (0.36–6.37)	0.573	NA (NA–83.94)
Overall exposure to foods or drinks from the canteen	2.54 (1.52-4.22)	2.35 (1.23–4.52)	0.010	55.48 (17.51–76.46)
Washing hands before eating	0.88 (0.75-1.05)	0.92 (0.71–1.20)	0.549	NA (NA–9.16)
Bringing one's own lunch	0.37 (0.20-0.68)	0.55 (0.23–1.31)	0.174	NA (NA–1.96)
Bringing one's own drink	0.74 (0.62-0.87)	0.67 (0.50–0.88)	0.004	NA (NA–NA)
Bringing one's own utensils	0.58 (0.44-0.77)	0.67 (0.42–1.07)	0.096	NA (NA–1.22)

RR: Relative risk, OR: Odds ratio, PAF: Population attributable fraction, CI: Confidence interval
[a]Negative values are denoted as NA.

Laboratory Study

Thirteen out of twenty-eight stool specimens from suspected cases (11 from students and teachers, and 2 from symptomatic food handlers) were positive for rotavirus. An additional 14 suspected cases who visited hospitals also reported that they tested positive for rotavirus. Rotavirus G3P[8] was confirmed in the suspected cases and food handlers. Other pathogens found in fecal matters of the cases included norovirus, *Staphylococcus aureus, Bacillus cereus, Vibrio cholera non-O1, Plesiomonas shigelloides,* and *Aeromonas* spp. (Table 4). Similar enteropathogenic bacteria were presented in hand swabs of food handlers, water from various sources, ice specimens, and some environmental swabs. To re-assess water contamination, we collected groundwater specimens from the school three weeks after the initial investigation. The pathogens identified were comparable to the ones obtained from the samples collected initially.

Table 4. Gastrointestinal pathogens isolated from food poisoning among students and teachers, food handlers, water, ice, and environmental specimens in the school, September 2022

Source	Bacterial culture		Viral RT-PCR	
	No. of isolates (samples)	Pathogen (n)	No. of isolates (samples)	Pathogen (n)
Students and teachers (stool samples and rectal swabs)	6 (26)	*S. aureus* (3) *B. cereus* (2) *V. cholera* non-O1 (1) *P. shigelloides* (1) *Aeromonas hydrophila* (1) *Aeromonas veroii* (1)	11 (26)	Rotavirus G3P[8] (1) Rotavirus, untyped (10) Norovirus
Food handlers[a] (stool samples)	1 (2)	*P. shigelloides* (1)	2 (2)	Rotavirus G3P[8] (1) Rotavirus, untyped (1)
Food handlers (hand swabs)	2 (3)	*B. cereus* (1) *Aeromonas* spp. (1)	0 (3)	Tested negative
Water for cooking[b]	1 (1)	*Aeromonas* spp. (1)	0 (1)	Tested negative
Water from storage well	1 (1)	*B. cereus* (1) *Aeromonas veroii* (1)	0 (1)	Tested negative
	1 (1)[d]	*B. cereus* (1) *Aeromonas hydrophilla (1)*	0 (1)	Tested negative

Table 4. Gastrointestinal pathogens isolated from food poisoning among students and teachers, food handlers, water, ice, and environmental specimens in the school, September 2022 (cont.)

Source	Bacterial culture		Viral RT-PCR	
	No. of isolates (samples)	Pathogen (n)	No. of isolates (samples)	Pathogen (n)
Groundwater well no.1[c]	1 (1)	*B. cereus* (1)	0 (1)	Tested negative
	1 (1)[d]	*B. cereus* (1) *Aeromonas caviae* (1) *Aeromonas veronii* (1)	0 (1)	Tested negative
Groundwater well no.2	1 (1)[d]	*Aeromonas veronii* (1)	0 (1)	Tested negative
Drinking fountain	1 (2)	*B. cereus* (1)	0 (2)	Tested negative
Bottled water	0 (1)	-	0 (1)	Tested negative
Ice for food storage	2 (2)	*Salmonella* spp. (1) *E. coli* (1) *B. cereus* (1) *P. shigelloides* (1) *Aeromonas hydrophila* (2) *Aeromonas veroii* (2)	Not sent	Tested negative
Ice for consumption	Not sent	-	0 (2)	Tested negative
Utensil swabs	0 (2)	-	0 (2)	Tested negative
Dish swabs	0 (2)	-	0 (2)	Tested negative
Swabs from cooler boxes	2 (2)	*Aeromonas veroii* (1) *Aeromonas caviae* (1) *Aeromonas* spp. (1)	0 (2)	Tested negative
Swabs of canteen water tap	1 (1)	*Aeromonas caviae* (1)	0 (2)	Tested negative
Swabs of drinking fountain	0 (1)	-	0 (1)	Tested negative

[a]*Collected from symptomatic food handlers only*
[b]*Collected from water tap in school canteen*
[c]*Groundwater well which supplied school canteen*
[d]*Results from specimen collection on 6 Oct 2022*

Environmental Study

The physical structure of the canteen complied with hygiene standards issued by the Thai Department of Health, Ministry of Public Health. We observed no insects or pests upon investigation. Handwashing facilities (a sink with tap water and soap provided) were present. However, based on our interview, soap was not always available, and some students were not aware of the handwashing area in the school canteen. The annual inspection of the canteen by the local public health authorities was interrupted due to the COVID-19 pandemic during the last two years.

Some food sold in the canteen was pre-cooked by food handlers at their homes and brought to school each morning. Raw ingredients were bought from nearby local markets twice a week and stored at the homes of the food handlers. Water from a faucet in the canteen was used directly for cooking and preparing beverages. Some raw materials were stored in the same container where ice for consumption was kept. From the SI-2 test, 73.5% (25/34) of hand swabs from food handlers, 50.0% (8/16) of food items, and 20.0% (1/5) of utensils and dish swabs tested positive for coliform bacteria.

Most food handlers wore a mask and hair covering at all times. Some food handlers used their bare hands to prepare and serve food. Two of the food handlers from the same noodle shop reported a recent history of diarrhea and both came to work on the days of illness. All food handlers were required to submit a health check-up certificate to the canteen manager every year; however, we were unable to verify this on the days of inspection.

Groundwater was the main water supply in the school. There was no groundwater treatment system in place. The groundwater well, which provided water supply for the school canteen, is located near a cesspool and sewer pipes. Gross contamination of water was observed in the water tanks. Most students drank bottled water sold by the school, although there were drinking fountains where water was supplied from the provincial waterwork. The free residual chlorine level in pre-filtered water was 0.04 parts per million, which

was lower than the recommended standard.[17] Ice for all stores in the canteen is supplied daily from an ice factory in the province.

Actions Taken

A meeting with school staff was held and a school renovation plan was developed. Groundwater wells were immediately shut down. The water supply in the school canteen was replaced by water from a drinking water factory. Symptomatic food handlers were not permitted to work until they tested negative for rotavirus. Daily case monitoring was done by school infirmary staff. Active surveillance of food poisoning and diarrhea clusters in high-risk spots (e.g., daycare centers and kindergartens) was conducted by the local authority. After school re-opening, the daily number of cases who developed food poisoning or acute diarrhea did not exceed two.

Discussion

We report a food poisoning outbreak in a secondary school in Pathum Thani Province, Thailand. From 1 Jan to 4 Nov 2022, 39 other food poisoning outbreaks were reported in Thailand, of which 18 (46.2%) occurred in the school setting. The overall attack rate in this outbreak was 40.4%, which was higher than the median attack rate of foodborne events reported in schools in Thailand in 2022 (27.7%, range 3.1–56.3%).[18] This event was one of the largest food poisoning outbreaks in Thai educational institutions.

The most likely causative agent of this outbreak was rotavirus G3P[8]. Though rotavirus vaccines have been added into routine immunization, most students were born prior to its availability. Studies showed genotype G3P[8] to be the most frequently detected strain of rotavirus in Thailand during 2016–2019 in both children and adults.[19,20] Although rotavirus is more prevalent among children, it is not uncommon for adults to be infected with the pathogen.[6,11] Clinical manifestations of rotavirus infection in adults can vary. The most common symptoms in a prior study were diarrhea, abdominal pain, and nausea, consistent with our findings.[21] Although other bacteria and viruses identified from human specimens were known to cause foodborne infection, they were less likely to be the main contributors of this outbreak as these pathogens were found in only a few cases and no clear evidence of their epidemiological linkage was identified. Co-infection was a possibility despite the very small number of cases (n=3) showing mixed organisms (rotavirus and other organisms).

Groundwater supplying the school canteen was the most probable source of this outbreak. Every store in the canteen used this water for cooking, which resulted in cross-contamination. Results of the analytic study showed that exposure to foods or drinks from the canteen was a significant risk factor. Attack rates and relative risks of food items and canteen stores were homogenous. Multiple enteropathogenic bacteria were detected in water specimens from groundwater tanks, a water storage well, and water taps in the canteen. These bacterial species were consistent with species found in the stool samples of cases. It was likely that the water was contaminated with human feces. Leakage of the cesspool or deterioration of sewage pipes is plausible. Previous rotavirus gastroenteritis outbreaks due to contamination of the water supply system of a hotel have been reported in Thailand.[12] Similar to our study, the hotel where the outbreaks occurred used unchlorinated groundwater from a well near a sewage pond for cooking and drinking.

Rotavirus can survive in freshwater for up to 10 days at 20°C and has a very small infectious dose (10–100 viral particles).[4,22] While groundwater use is not common nowadays in urban areas due to strict control by the government, in rural areas groundwater is often used as a primary source of drinking water.[23] This study highlighted the public health importance of routine quality testing and a treatment system for groundwater.

This outbreak was preceded by a period of heavy rain which resulted in flooding in the Khlong Luang District. During September–October 2022 Thailand suffered from its worst flood in many years.[24] Pathum Thani Province was also affected. The flooding may have contributed to the contamination of the water supply in the school. Floodwater was found to be associated with a higher concentration of enteric pathogens, specifically *Escherichia coli* and rotavirus group A.[25] Moreover, flooding is found to be a predisposing factor for rotavirus outbreaks, even in surrounding regions that might not be directly affected by the flood.[26]

Limitations

First, we were unable to obtain a full list of names and contact information of students due to privacy issues. Also, students were not available for interviews during the school examination period. We distributed an electronic-based questionnaire to all students and obtained an overall response rate of only 40.6%. Analytic results should be interpreted with caution as they might be subjected to non-response bias. Second, memory bias was possible as a nature of a retrospective study. Third, we were unable to detect rotavirus from water samples, probably because of the higher detection limit of the conventional PCR method. Fourth, the attack rate reported herewith was subject

to overestimation as symptomatic cases were likely to respond to the survey than those with mild or no symptoms, not to mention the no-show school members. Fitfh, no leftover food was available for microbial testing on the investigation days. Lastly, most food handlers were unwilling to provide information about their history of illness within the past month and refused to provide consent for specimen collection.

Recommendations

We recommended that the school should cease using the groundwater supply until appropriate treatment systems are in place. The canteen manager should ensure that food handlers follow standard personal and food hygiene practices, e.g., storing raw materials and cooked food separately and separating ice for consumption from ice for food storage. Moreover, food handlers should always wear protective masks, hair covers, and gloves while handling food, and that they refrain from work while having an illness. Handwashing with soap, especially before and after eating and after using the toilet, should be promoted among all students and staff. Local health authorities should closely monitor acute gastroenteritis events, especially during and after floods. Routine food sanitation surveillance and hygiene training sessions for food handlers should be resumed immediately.

Conclusion

A food poisoning outbreak occurred in a secondary school with an attack rate of 40.4%. Rotavirus G3P[8] was the most likely pathogen responsible since it was detected in the majority of cases. The most affected group were students. No severe case or death was reported. No cluster of food poisoning or acute diarrhea was detected in the local community during the same period. Exposure to foods or drinks from the school canteen was a risk factor. While we were unable to detect rotavirus in the environment, our analytic and environmental results suggested contamination of the school water supply as the most likely source of the outbreak. The groundwater well located close to a cesspool and the lack of a water treatment system were two issues that need to be addressed. Our investigation demonstrated that contaminated drinking water is a key public health risk. To ensure the safety of the water supply, we recommended routine water quality testing and the installation of a groundwater treatment system.

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Suggested Citation

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