

Original Article

Epidemiological Analysis of Astrovirus-Associated Diarrhea Outbreaks in Taiwan, 2012

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Abstract

Recently, viral agents have caused around 50% of reported diarrhea outbreaks in Taiwan. The reported cases were routinely screened for bacteria, norovirus and rotavirus. Noroviruses (NoVs) have been recognized as the major causal agent of outbreaks. In addition, the unknown etiological cases were further tested for sapovirus, adenovirus and astrovirus with the support of research project. There were a total of 303 reported diarrhea-associated outbreaks, in which 70 outbreaks were with unknown etiology by routine screening in 2012. In this study, we found 18 (25.7%) astrovirus-associated gastroenteritis outbreaks which affected people of 0-70 years old. Endemic peak was spring season and the incidence of astrovirus was only at first half of 2012. According to epidemiological information, the most common transmission route of astrovirus was food-borne transmission. To our knowledge, this study was the first epidemiological investigation of astrovirus-associated gastroenteritis outbreaks by detecting unknown gastroenteritis outbreaks in Taiwan.

Key word: Astrovirus, gastroenteritis outbreak, food-borne illness

Study of Correlation between Norovirus-Infected Cooks and Foodborne Disease Outbreak among X Junior High School Students

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Abstract

On January 12, 2011, we investigated the scale, transmission mode, disease pathogen, food origin, and preventive measure effectiveness of a foodborne disease outbreak which occurred among X Junior High School students after consumption of common lunch meal. Focusing on the school with the highest number of cases, the investigation results show a total of 265 students affected, with an attack rate of 39.7%. The epidemic curve and distribution of cases per class show the transmission mode to be a common source infection. Logistic regression analysis results indicated statistically significant relationship between shacha vermicelli and the outbreak (AOR=1.659, 95% CI: 1.040 ~ 2.647). The fecal samples of 26 students and 6 cooking staff were tested positive for GII.12 norovirus. The person who made the shacha vermicelli was an asymptomatic norovirus carrier, which lets us confirm that this foodborne outbreak in X school was caused by a norovirus-infected cook. After the implementation of preventive measures, such as sending the infected persons home to rest and further medical assistance, sanitizing the kitchens, restrooms, and sinks, enhancing hand-washing education, and compelling the meal company to shut down, no new cases appeared after January 17th.

Introduction

Norovirus is one of the main causes of non-bacterial gastroenteritis. It has a short incubation period (1-2 days), is highly contagious (10-100 viruses can quickly spread the disease), and is extremely active during the winter season [1-2]. It often leads to gastroenteritis outbreaks in elderly care centers, long-term care facilities, psychiatric wards, institutions for the mentally impaired, hospitals, and schools [3-8]. Norovirus can be transmitted from person to person [9-10], and also through drinking water [11-12] or foods [13]. The symptoms induced by norovirus include diarrhea, abdominal pain, nausea, vomiting, and fever. All of which are considered mild and thus infected persons can recover without medical assistance [14].

On the morning of January 14, 2011, Taiwan Food and Drug Administration reported several hundred students showing gastrointestinal symptoms of vomiting, abdominal pain, and diarrhea in several schools in Taoyuan County (C Junior High, X Junior High, T Junior High, and Y High School) on the afternoon of January 12th. Due to the large number of infected students and schools, the incident fits the epidemiology characteristics of people, place, and time; thus the need for outbreak investigation. This study shows the scale, transmission path, disease pathogen, food origin, and preventive measure effectiveness of this incident where the most gastronomical symptoms occurred in X Junior High School.

Materials and Methods

Study Subjects

According to the reported data, the numbers of students who showed gastrointestinal symptoms in each school are as following: 75 persons at C Junior High, 422 persons at X Junior High, 27 persons at T Junior High, and 17 persons at Y High School. We selected X Junior High School as the study subject as it has the highest number of cases. All classes which had 5 or more students who had consumed the lunch provided by W Meal Company on January 12th and showed gastrointestinal symptoms of vomiting, abdominal pain, and diarrhea were listed as study subjects. A total of 20 classes and 754 students were included into the study.

Case Definition

Study subjects who consumed the lunch provided by W Meal Company on January 12th and showed at least two of the following symptoms: nausea, vomiting, abdominal pain, and diarrhea are defined as cases. The rest were defined as none-cases.

Study Method

This study uses the epidemiological method of case-control study. Study subjects that fit the case definition are categorized as the case group, the rest of the none-cases are categorized as the control group.

Questionnaire Investigation

Investigators used semi-structured questionnaires as study tool. The contents of the questionnaire include basic information, the time lunch was consumed on January 12th, the dishes provided and consumed, how he/she felt, the onset of gastrointestinal or respiratory symptoms, whether medical assistance was sought or not, whether recovered or not, and time of recovery. On morning of January 17, investigators proceeded to X Junior High to conduct investigation on the students of the targeted classes. Accompanied by the school staff, investigators from Public Health Bureau of Taoyuan County Government, or local health station, or Taiwan Centers for Disease Control (Taiwan CDC) relayed the content of the questionnaires to the students in each class. Afterward, the students filled out the questionnaires and the questionnaires were immediately retracted. The questionnaires collected from each classroom were pooled together and checked individually by the

investigators at the school health center to confirm the completion and correctness of the questionnaires. Students who handed in uncompleted or incorrectly filled questionnaires were asked to re-fill the questionnaire. Those students who were absent were telephone interviewed by the school staff. After their questionnaires were all collected, the questionnaires were sent to Taiwan CDC for further processing and analysis

Cook Investigation

To investigate whether the cause of this outbreak is related to W Meal Company personnel (especially the culinary staff), we interviewed all of the employees if they have shown any gastronomical symptoms within the two weeks before January 12. In addition, to avoid any false medical history given by the employees, according to Communicable Disease Control Act and Personal Information Protection Act, we collected their names, ID, and position information. This information is used to articulate with National Health Insurance information to understand whether the employees had sought medical assistance for gastronomical symptoms before this incident. All of the cooks were required to take anal swab samples and fecal samples for testing.

Sample Collection and Laboratory Testing

Public Health Bureau of Taoyuan County Government sent investigators to X Junior High School on January 14 and collected 27 rectal swabs and 4 vomitus samples from students who still showed symptoms but haven't sought any medical assistance. In addition, 40 fecal samples were also collected from the inflicted students between January 14 and 17. At the same time, 30 rectal swabs and 30 fecal samples were collected from every employee in W Meal Company. All the samples were sent chilled to the Research and Diagnostic Center, Taiwan CDC for testing. Test items include: *Staphylococcus aureus* (including enterotoxins), *Bacillus cereus*, *Salmonella spp.*, *Vibrio parahaemolyticus*, *Shigella spp.*, *Vibrio cholerae*, *Salmonella typhi*, *S. paratyphi*, and norovirus. Test methods were conducted according to Taiwan CDC Manual for Infectious Specimen [15].

In addition, samples were taken from two lunch boxes and one soup that were kept at the school. The environmental samples taken from W Meal Company kitchen include: 1 from raw food cutting knife, 2 from cooked food cutting knives, 1 from cooked food cutting board, 1 from soup cooking pot, 1 from soup serving pot, 1 from serving platter, and 1 from the ladle. These samples were sent to the Taiwan Food and Drug Administration for testing. Test items include: *Staphylococcus aureus* (including enterotoxins), *Bacillus cereus*, *Salmonella spp.*, *Vibrio parahaemolyticus*, and *Enteropathogenic E. coli*. Tests were conducted for *Staphylococcus aureus* according to National Food Code (NFC) 0981800188 (June 9, 2009); *Bacillus cereus* NFC 0981800288 (August 13, 2009); *Salmonella spp* 0951800021 (September 4, 2006); *Vibrio parahaemolyticus* NFC 0900002815 (January 1, 2001). *Bacillus cereus* and *Vibrio parahaemolyticus* were tested using commercially available reversed latex agglutination.

Monitoring the Outbreak

After carrying out each of the preventive measures in the school and at W Meal Company, new suspect cases of gastronomic discomfort or fever among the students, faculty, or restaurant employees who had consumed the meal provided by W Meal Company [on the 12th] are registered and reported daily to assess the effectiveness of the preventive measures.

Data Management and Analysis

All collected questionnaire data were input, debugged, and filed. Afterwards, data description and analysis was conducted using SAS software. Demographic variables and onset symptoms are described using the number of persons and percentage. The ratio of the investigated class students (or subjects) that fit the case definition is defined as attack rate. The incubation period is described as the median and range. The transmission mode is shown by an epidemic curve of infected persons/day. Whether single dishes or multiple dishes were statistically related to the onset of the disease is conducted using logistic regression analysis. The relation index between each dish in the lunch meal and disease onset is represented using Odds Ratio (OR); whether it portrays statistical significance or not can be seen using 95% Confidence Intervals (CI). The related indicator of the gender adjusted divergence between each lunch dish and disease onset is calculated using Gender-Adjusted Odds Ratio (AOR). If the 95% CI does not include 1.0, it means that X dish shows statistically significant relationship to the onset of the disease. If the 95% CI includes 1.0, then X dish does not show any statistically significant association to the onset of the disease.

Results

A total of 711 questionnaires were retracted, with a response rate of 94.3%. Of these, 667 persons had consumed the lunch provided by W Meal Company on January 12th. 265 persons fit the case definition, showing an attack rate of 39.7% (265/667), with 132 males (attack rate 38.2%) and 133 females (attack rate 41.3%). Male and female attack rates show statistically significant difference ($P < 0.05$). The number of cases, number of persons who had consumed the lunch, and attack rate in each class can be seen in Table 1.

Table 1. Case numbers, number of persons who consumed lunch meal on January 12, and attack rate in each investigated class of Taoyuan X Junior High School

Class-Grade	Cases/Eaten lunch number	Attack rate %	Class-Grade	Cases/Eaten lunch number	Attack rate %
3-7	12/34	35.3	26-8	19/32	59.4
8-7	6/34	17.7	2-9	13/34	38.2
9-7	14/32	43.8	4-9	17/36	47.2
21-7	6/35	17.1	6-9	14/38	36.8
25-7	12/31	38.7	8-9	14/33	42.4
26-7	12/26	46.2	13-9	22/34	64.7
5-8	20/36	55.6	19-9	5/32	15.6
7-8	9/30	30.0	23-9	9/35	25.7
9-8	13/37	35.1	25-9	17/34	50.0
24-8	18/29	62.1	27-9	13/35	37.1

The distribution of the case symptoms are as following: vomiting 82.3% , abdominal pain 72.5%, nausea 70.2%, diarrhea 55.9%, dizziness 56.2%, headache 42.4%, weakness in limbs 33.2%, chilliness 29.4%, fever above 38°C 18.9%, and tenesmus 1.1%. The incubation period ranged from 1 to 89 hours, with a median of 28 hours. According to the epidemic curve (Figure 1) and the number of cases that occurred in each class shortly after the consumption of the lunch meal (Table 1), the transmission mode in this outbreak is most likely to be of point common source infection.

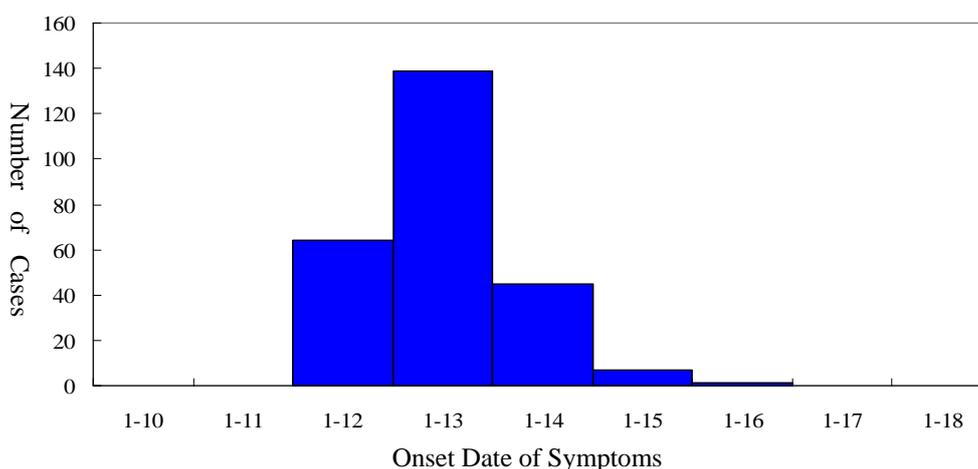


Figure 1. Distribution of symptom onset dates of food poisoning students in Taoyuan X Junior High School

After adjusted by gender difference, the simple logistic regression analysis provides AOR and 95%CI for each dish in the lunch meal on January 12: bacon and egg fried rice (AOR=3.711; 95% CI: 1.410~9.766), Japanese pork chop (AOR=2.201 ; 95% CI: 1.151~4.211), shacha vermicelli (AOR=2.118 ; 95% CI: 1.401~3.203), seasonal vegetables (AOR=1.713 ; 95% CI: 1.162~2.525), and pickled cabbage pig blood soup (AOR=1.488 ; 95% CI: 1.005~2.203). All showed statistical significance to the onset of the disease (see Table 2). The results of multiple logistic regression analysis show only shacha vermicelli (AOR=1.659 ; 95% CI: 1.040~2.647) still is significantly related to the onset of the disease (see Table 3).

Table 2. Results of the single dish analysis in the lunch meal of Taoyuan X Junior High School students consumed on January 12, 2011.

Dish	Case		Control		Odds Ratio (95% CI)	Gender-adjusted Odds Ratio (95% CI)
	Ate	Didn't eat	Ate	Didn't eat		
Bacon and egg fried rice*	260	5	375	27	3.743 (1.423~9.847)	3.711 (1.410~9.766)
Japanese pork chop*	252	13	362	40	2.142 (1.123~4.087)	2.201 (1.151~4.211)
Pork and cabbage pot stickers	245	20	357	45	1.544 (0.890~2.679)	1.564 (0.900~2.716)
Shacha vermicelli*	228	37	299	103	2.123 (1.404~3.209)	2.118 (1.401~3.203)
Seasonal vegetables*	219	46	295	105	1.727 (1.172~2.543)	1.713 (1.162~2.525)
Pickled cabbage pig blood soup*	219	46	306	96	1.494 (1.009~2.210)	1.488 (1.005~2.203)

* Shows statistical significance, 95% CL does not include 1.0.

Table 3. Results of the multiple dishes analysis in the lunch meal of Taoyuan X Junior High School students consumed on January 12, 2011.

Dish	Odds Ratio (95% CI)	Gender-adjusted Odds Ratio (95% CI)
Bacon and egg fried rice	2.292 (0.807~6.509)	2.238 (0.786~6.371)
Japanese pork chop	1.298 (0.638~2.640)	1.333 (0.653~2.724)
Shacha vermicelli*	1.662 (1.042~2.651)	1.659 (1.040~2.647)
Seasonal vegetables	1.203 (0.776~1.864)	1.193 (0.770~1.850)
Pickled cabbage pig blood soup	1.235 (0.821~1.856)	1.231 (0.819~1.851)

* Shows statistical significance, 95% CI does not include 1.0.

The laboratory test results found 26 fecal samples collected from X Junior High School students were norovirus, with a positive rate of 65.0%; 2 of the anal swabs were tested positive for *Staphylococcus aureus* and A type enterotoxin; 6 of the fecal samples collected from employees of W Meal Company were tested positive for norovirus, with a positive rate of 20.0%. No pathogens were apparent in any of the anal swabs. Additionally, the school's leftover food samples and samples from the cooking ware of W Meal Company tested negative for any pathogens.

Secondly, the 26 samples from the students were categorized for their norovirus strains, all of which were GII.12 genotypes. The 6 samples from the cooking staff were also all categorized as GII.12 genotype with one GII.2 genotype.

To prevent the highly contagious norovirus from further spreading in this outbreak, the school applied the following preventive measures under the supervision of health authorities: requesting any students who show symptoms to return home to rest and seek medical assistance; disinfect the school restrooms and sinks every morning and evening, and provide soap at each sink for students and faculty to use; enhance student's hand-washing education, especially after using the restroom and before meals. On January 16, a cleaning company was employed to conduct full-scale disinfection of the entire school grounds. In addition, on January 14, the Health Bureau compelled W Meal Company to shut down for a week for kitchen and dining area disinfection.

Discussion and Conclusion

A month before the outbreak, approximately 10 students took sick leave on every school day, with no large scale number of sick leaves (414 persons took sick leave on January 13th). Therefore, this can prove that the outbreak is a sudden occurrence. Also, the epidemic curve shows a single peak shape, which indicates common source infection as the transmission mode [16]. Most of the affected students in different classes started to show gastrointestinal symptoms in a short period of time; this is also a characteristic of common source infection. The school uses tap water. The students' symptoms were mostly gastrointestinal. On January 12th, the students all ate from their own lunch boxes, using disposable lunch boxes, with no shared containers. Combining the previous findings, we can eliminate factors of common

source infection such as air, common carrier, drinking water, and shared containers [17-20]. In other words, this incident is most likely to be relevant to the transient food-borne disease. The incubation period for norovirus is usually 21-48 hours, sometimes even 72 hours. The epidemic curve indicates as of lunchtime on January 12, patients showing incubation periods that exceed the normal period range may possibly be infected through person to person transmission [21]. Therefore, when an outbreak occurs, relative investigation and preventive measures must be conducted immediately in order to avoid further outspread of norovirus through human contact.

Twenty-six of the 40 fecal samples collected from the infected students at X Junior High School were tested positive for norovirus and 6 of the 30 fecal samples collected from the cooking staff of W Meal Company were also tested positive for norovirus. The symptom distribution and incubation period of the student cases all fit the characteristics of norovirus infection. In addition, norovirus was also tested positive in the fecal samples collected from the students in the C Junior High, T Junior High, and Y High School (non-investigated subjects) with a positive rate of 30.0% (3/10), 70.0% (7/10), and 57.1% (8/14) respectively. These can all prove that the pathogen of this food-borne disease outbreak is norovirus. Although 2 anal swabs collected from infected students were tested positive for *Staphylococcus aureus* with type A enterotoxin, its incubation period differed from most of the affected persons. Thus, *Staphylococcus aureus* is unlikely to be the main pathogen origin of this outbreak.

The employees of the W Meal Company claimed to have no gastrointestinal symptoms before the outbreak when interviewed during investigation, and their health insurance record showed no data indicating medical assistance. However, fecal samples of 6 cooking staff were tested positive for norovirus, indicating that they were all asymptomatic carriers. Barrabeig et al. stated that norovirus is found on asymptomatic cooking personnel [22]. We suspect that the norovirus they carry may be the possible cause for this outbreak. The jobs of the 6 cooking personnel are as following: 1 is in charge of washing vegetables, 1 in charge of cooking the shacha vermicelli, 1 in charge of vegetarian dishes, 1 in charge of distributing dishes, 1 in charge of transporting the meal boxes to X Junior High School, and 1 in charge of transporting the meals to another school. The fecal samples collected from the cook in charge of cooking the shacha vermicelli was tested positive for norovirus, and had the same genotype as those of the infected students: GII.12. We speculate that the shacha vermicelli is the main contaminated food source.

Usually, if the samples collected from leftover food in food-borne disease outbreaks are tested positive for the same pathogen as those found in the human samples, that particular food can be confirmed as the food source that caused the outbreak. Some studies report that norovirus can be found in the food; however, this method can only be suitable under the circumstances of a high concentration number of norovirus [23]. However, a small number of norovirus is enough to induce infection. A standardized method of testing for small amounts of norovirus in food sources is still under development [24-25]. In addition, if leftover food

samples can not be collected, we can not determine the contaminated food source. We can use epidemiological methods to discover the food origin [26]. This study uses case-control study method to discover the shacha vermicelli showed statistical significance to food-borne disease outbreak (AOR=1.619; 95% CI: 1.040 ~ 2.647). The shacha vermicelli most likely can be confirmed to be the contaminated food source that caused the outbreak.

Gould et al. study shows in the US 2009-2010, among the 7,490 food-induced outbreaks where the pathogen was identified, norovirus ranked No. 1 with 331 cases (42%) and inflicted 7,332 persons. It greatly surpassed the second pathogen, *Sallmonella spp.*, which caused 234 cases (30 %) and inflicted 7,039 persons [27]. Normally, bacteria-caused food-borne disease outbreak patients are usually much higher than virus-induced outbreaks. This is due to the fact that viruses are unable to multiply on food sources. Norovirus-caused food-borne disease outbreaks are usually relevant to the personal hygiene of cooking personnel. In this norovirus-induced incident, the cook in charge of cooking the food source was a norovirus carrier, and the virus genotype was the same as those found in the infected students. We thus suspect the norovirus-infected cook may have not washed his/her hands after using the restroom or may have washed ineffectively and “contaminated” the shacha vermicelli, and lead to the inflection of the students who consumed the food. Therefore, periodical health inspection and hand hygiene of cooking personnel is very important. In addition, the restrooms cooking personnel use must be periodically sanitized, in order to avoid the spread of norovirus.

In this norovirus outbreak, the culprit meal company was compelled to shut down for environment disinfection. After the school applied the following preventive measures: enhance the avocation of hand-washing education among students and the faculty, disinfection of the restrooms, sinks, and school grounds, and all those showing symptoms to return home to rest and seek medical assistance, Six students still took sick leave on January 17, but no further cases of vomiting or diarrhea appeared. As of the 18th, all of the patients were able to return to school. On January 20th and 21st, after 6 of the cooks were tested positive for norovirus, they were suspended from returning to work. We can confirm that these preventive measures have positive effects on the management of norovirus-induced outbreaks.

Conclusion

Diarrhea outbreaks that occur in the winter season suspect caused by norovirus should collect fecal samples from the patients and tested. Due to the low number of norovirus needed to induce its onset, pathogens may not be tested positive from leftover food samples. We propose the use of analytic epidemiologic methods to discover the possible related contaminated food source. Secondly, if norovirus is tested positive from patients and cooking staff, further comparison of genotype should be conducted in order to confirm whether or not the cause came from a singular source. There is no other way to prevent norovirus-induced

outbreaks than enhancing hand hygiene education of school faculty members and cooking staff, periodically disinfecting the kitchen, restrooms, and sink environments, and sending the infected persons for medical assistance and home to rest.

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