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Case Report

An acute gastroenteritis outbreak caused by GII.P16-GII.2 norovirus associated with airborne transmission via the air conditioning unit in a kindergarten in Lianyungang, China

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ABSTRACT

Noroviruses are a common cause of acute gastroenteritis outbreaks in institutions including schools and kindergartens around the world. An outbreak caused by GII.P16-GII.2 norovirus in a kindergarten in Lianyungang, Jiangsu Province, China is reported here. An epidemiological investigation was conducted, and pathogen detection was performed. The descriptive analysis indicated that this outbreak in middle class 1 had a point source. Twenty cases of acute gastroenteritis occurred in this class within a period of 8.5 h; the attack rate was 52.6% (20/38). Airborne transmission via the air conditioning unit in a confined restroom could have played a critical role in this outbreak. Sequence analysis of GII-positive samples confirmed that the norovirus GII.P16-GII.2 variant was the etiological agent of this outbreak.

Introduction

Noroviruses are a common cause of acute gastroenteritis in institutions including schools and kindergartens around the world. At 8:00 on June 10, 2017, the local health bureau in the municipal city of Lianyungang was informed by Beilei kindergarten that many children in middle class 1 had become ill with vomiting and diarrhea on the afternoon of June 9, 2017 (Friday). A group of epidemiologists were sent to Beilei kindergarten to investigate this outbreak, with the aim of determining the etiology and mode of transmission, and to instigate control measures to control the epidemic over the following days.

Beilei kindergarten is a state-owned child care center that cares for 330 children in eight classes (three junior classes, three middle classes, and two senior classes) and has 34 members of staff. There are two preschool teachers and a child care teacher in each class. The child care teacher is in charge of the children' daily life in kindergarten, including taking food and drink from the kitchen to the classroom, distributing food to every child, and finally washing and disinfecting the tableware. The canteen is staffed by three food handlers, who prepare lunch for all of the children in this kindergarten. Fresh vegetables, meat, fish, and other food items are sent by a food store to the kindergarten every morning. After lunch, all children are moved to restrooms for a 2.5-h lunch break from 12:00 to 14:30. The classroom windows are opened each morning, the bathrooms are cleaned and disinfected with chlorine-containing disinfectant before class, and the children's towels are washed and dried in the sun for several hours each day, except on rainy days.

Methods

In accordance with the Chinese guidelines on outbreak investigation, prevention, and control of norovirus infection (2015), suspected cases were defined as those who had acute onset of gastroenteritis with vomiting (two or more times) and/or diarrhea (loose or watery stool three times or more in the past 24 h). Probable cases were defined as those suspected cases in clusters or outbreaks of norovirus infection, associated with epidemiologically confirmed cases. Confirmed cases were defined as those suspected cases or probable cases whose stool specimens or rectal swabs and/or vomitus specimens tested positive for norovirus by RT-PCR. Non-cases were defined as students or

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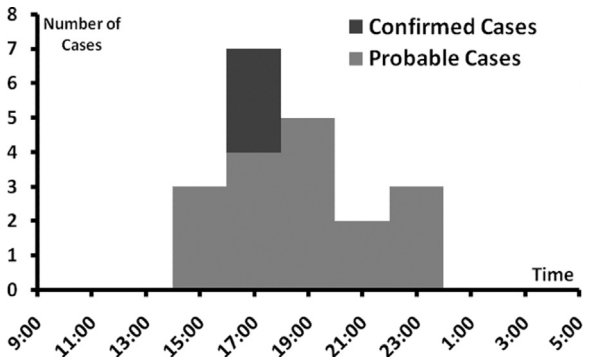

Figure 1. Distribution of norovirus cases over time from 14:30 to 23:00 on June 9, 2017.

members of staff without symptoms of diarrhea or vomiting at 72 h (the longest incubation period for norovirus).

Fecal specimens, rectal swabs, and vomitus specimens were tested for viral agents by real-time RT-PCR on an ABI 7500 system using the corresponding detection kits (Jiangsu Bioperfectus Technologies Corp., Taizhou, China).

Information was obtained from cases and non-cases using paper questionnaires. A database was set up in EpiData 3.0, and data were analyzed using Epi Info 3.5.1.

Results

At 14:30 on June 9, 2017, the first case in middle class 1 began to vomit. He was on the upper bed of a bunk bed in the restroom. The two preschool teachers used a container to catch the boy's vomitus. They wiped the boy's mouth with tissues and then put the container in the trash can outside the restroom. The second (boy) and third (girl) cases started to vomit in the classroom at 15:00. The teachers used tissues to cover the vomitus, then took this to the trash can outside the classroom and then mopped the floor clean. These three cases were quickly sent to the hospital for treatment. Class ended at 16:00, and all children went home with their guardians.

A further 20 children from middle class 1 became ill on June 9, 2017 up until 23:00, i.e. over an 8.5-h period. No cases occurred in the other seven classes over the following days. This appeared to be a point-source outbreak, due to the incubation period of norovirus infection of 12–48 h (Figure 1).

The attack rate was 52.6% (20/38) in middle class 1 (there were 41 children in this class, but three children had been absent since June 6), representing 6.1% (20/327) of the children in Beilei kindergarten. The main symptoms were vomiting (90.0%), nausea (75.0%), abdominal pain (70.0%), fever (55.0%), and diarrhea (35.0%).

According to the Surveillance Program for the Viral Diarrhea Outbreak of Jiangsu Province, a total of 28 specimens (18 rectal, nine stool, and one vomitus) were collected during the outbreak: six from the cases, two from the preschool teachers in middle class 1, one from a food handler, eight from child care teachers, and 10 from a non-case group in middle class 3. Five of these specimens tested GII-positive by RT-PCR (three cases and two preschool teachers in middle class 1).

The GII-positive samples were further genotyped by semi-nested PCR and sequencing at Jiangsu Provincial Center for Disease Control and Prevention. Phylogenetic analysis showed that the strains isolated in this outbreak were GII.P16-GII.2 strains.

The kindergarten provides lunch (but no other meal) for all children in the eight classes, all year round, which includes a meat dish, a vegetable dish, soup, and rice. All children and members of staff drink cooled boiled water kept in insulated containers in each classroom. These containers are filled by the child care teachers, who use portable kettles to transfer boiling water from the water heater in the kitchen. The insulated containers are cleaned and dried out each day after class.

The outbreak occurred only in middle class 1; no cases occurred in the other seven classes during the possible incubation period for norovirus. Thus it was assumed that the kindergarten lunch and boiled water were not risk factors in this outbreak. The time interval between the first case and the other two cases was only half an hour, and the additional 17 cases in middle class 1 children occurred within a period of 8.5 h. This incubation period for the norovirus infections, as well as laboratory testing, did not support

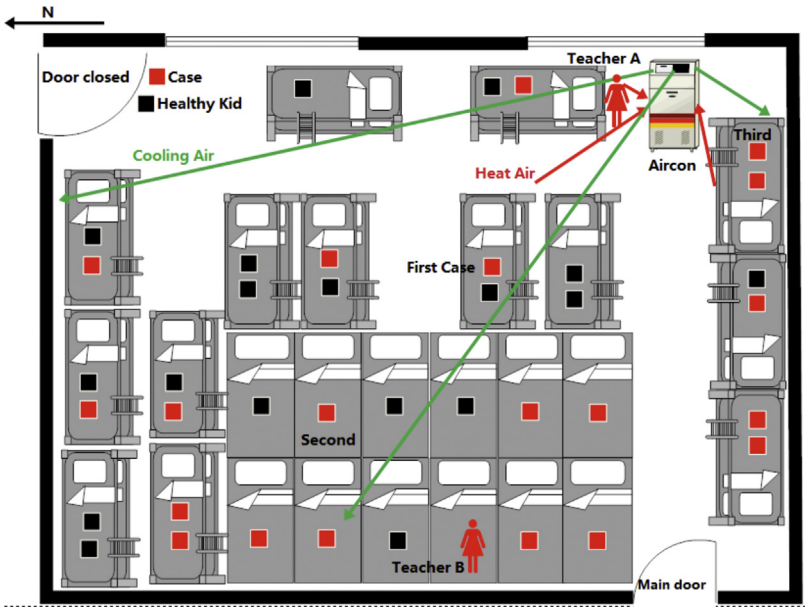

Figure 2. Twenty cases occurred in the restroom of middle class 1.

the hypothesis of airborne transmission of norovirus from the vomitus of the first three cases in the restroom and the classroom.

When the outdoor temperature is not too high, the windows and doors of the classroom and restroom are opened to provide ventilation. However, after International Children's Day (June 1), the preschool teachers in middle class 1 began to turn the air conditioning unit on in the restroom during the hottest time of the day (midday) to maintain the temperature at 27 °C, and the windows and door were closed. The restroom has 14 bunk beds and 12 single beds for the 38 children to use during their lunch break; the two preschool teachers sit on the edges of the beds while caring for the children. The restroom has two windows on the east wall and a main door in the west wall for ventilation. Seven of the cases were children who had been on the lower bunk beds (13 children total), seven cases were children who had been on single beds (11 children total), and six cases were children who had been on the upper bunk beds (14 children total) (Figure 2).

Preschool teacher A related that she had had diarrhea four to five times, with soft stool accompanied by abdominal pain, one Saturday afternoon in March 2017 (she did not remember the exact date), and had received two bottles of a liquid medicine as treatment. She had then felt better and had continued to work. On the day of the outbreak, she had sat on the edge of a lower bunk bed near the air conditioning unit. The heated air would have been drawn back in through the sides of the air conditioning unit and then the cooled air blown out of the air outlet to cover a fan-shaped area in which all of the cases occurred.

An aerosol of norovirus particles could have been spread in the restroom through the air conditioning unit, causing many children to become infected. The three children who were absent and the child care teacher who was not in the restroom did not become infected with norovirus. Hence, it is believed that the mode of transmission in this outbreak was airborne transmission via the air conditioning unit in a confined restroom. Preschool teacher A might have been the reservoir and disseminator of norovirus.

All cases, as well as the other children in all classes in Beilei kindergarten, were followed until the end of June 2017. There were no new cases at this kindergarten during this period. Fecal specimens and rectal swabs from two preschool teachers and four cases in middle class 1 tested negative for norovirus on June 26 and June 29, 2017, respectively. This outbreak of norovirus infection was then considered to have ended.

Discussion

This article reports a point-source outbreak of acute gastroenteritis caused by GII.P16-GII.2 norovirus infection in one kindergarten in the municipal city of Lianyungang, Jiangsu Province, China on June 9, 2017. Three years ago, in the same province, there was an acute outbreak of gastroenteritis caused by GII.17 norovirus in one high school in the municipal city of Wuxi (Shi et al., 2016). There was also a sharp increase in outbreaks of acute gastroenteritis caused by recombinant GII.2 noroviruses (GII.P16-GII.2) in Guangdong, China at the end of 2016 (Lu et al., 2017). The GII.P16-GII.2 virus caused 79% of the 56 outbreaks and was first detected in China in August 2016 (Ao et al., 2017). Seventy-eight percent of the GII.2 outbreaks occurred in kindergartens in Guangdong, China starting November 14, 2016. GII.P16-GII.2 was the predominant strain responsible for 66.7% of outbreaks in kindergartens in Taiwan in 2016 (Liu et al., 2017), as well as 42% of outbreaks in Germany (Niendorf et al., 2017), and 14% of outbreaks in France (Bidalot et al., 2017). Parra et al. reported that non-GII.4 genotypes seem to infect infants more frequently, because adults have built immunity to different genotypes over time (Parra et al., 2017).

Many investigations have reported the airborne transmission of norovirus due to exposure to vomitus or fomites (Repp and Keene, 2012; Marks et al., 2003; Evans et al., 2002; Marks et al., 2000). However, laboratory testing and the descriptive analysis of the outbreak reported herein did not support the hypothesis of the airborne transmission of norovirus from the vomitus of the first three cases in the restroom and classroom, nor did it support foodborne transmission via the kindergarten lunch or boiled water.

Some studies have confirmed that norovirus excretion can continue for long periods. Norovirus was detected for up to 7 days after inoculation of the virus in one volunteer study (Graham et al., 1994). Furthermore, norovirus RNA was found for up to 44.5 days following symptom onset in a naturally infected individual (Tu et al., 2008).

Environmental transmission occurs when episodes of vomiting or diarrhea contaminate surfaces with infectious virus particles that may persist for weeks (Manuel et al., 2015; Lopman et al., 2012a). Infected individuals shed a large amount of norovirus in both fecal material and vomitus, contributing to the high number of outbreaks observed annually in environments with close quarters, such as cruise ships, restaurants, long-term care facilities, and schools (Manuel et al., 2015; Lopman et al., 2012a; Hall, 2012).

Dormitories and classrooms are the main places where students study and live, which are highly centralized and relatively confined environments. Swab samples from these environments have tested positive for norovirus. After the implementation of environmental disinfection and hand washing measures, the number of cases has been shown to decrease sharply. Thus, environmental transmission is likely to have contributed to these outbreaks (Lopman et al., 2012b; Zheng et al., 2015).

It could be assumed that teacher A carried and excreted norovirus for more than 60 days from the date of falling ill with acute gastroenteritis in March to June 9, 2017, the day on which her stool sample tested positive for GII.P16-GII.2 norovirus.

The daily temperature in Lianyungang was high between March and June: the average highest daily temperature was 21.9 °C in April, 28.5 °C in May, and 29.1 °C in June (Anon, 2017). The concentration of norovirus particles in the air in the classroom and restroom with the windows and doors open would have been lowered by the natural ventilation. However, it would have been higher in the restroom when the windows and door were closed, spreading rapidly via the air conditioning unit and eventually causing half of all children and teacher B to become infected.

Hence, airborne transmission of norovirus via the air conditioning unit in a confined restroom could have played a critical role in this outbreak.

Ethical approval

This outbreak investigation was conducted by public health agencies as part of their legally authorized mandate and was therefore considered a minimal risk research and was exempted from ethical approval by the institutional review boards.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2017.10.003.

References

Anon, http://15tianqi.cn/lianyunganglishitianqi/. 2017.

Ao Yuanyun, Wang Jinjin, Ling Hua, He Yaqing, Dong Xiaogeng, Wang Xuan, et al. Norovirus GII.P16/GII.2–associated gastroenteritis, China, 2016. Emerg Infect Dis 2017;23(July (7)):1172–5, doi:http://dx.doi.org/10.3201/eid2307.170034.

Bidalot Maxime, Théry Lucie, Kaplon Jérôme, De Rougemont Alexis, Ambert-Balay Katia. Emergence of new recombinant noroviruses GII.p16-GII.4 and GII.p16-GII.2, France, winter 2016 to 2017. Euro Surveill 2017;22(April (15)):30508, doi:http://dx.doi.org/10.2807/1560-7917.ES.2017.22.15.30508.

Evans MR, Meldrum R, Lane W, Gardner D, Ribeiro CD, Gallimore CI, et al. An outbreak of viral gastroenteritis following environmental contamination at a concert hall. Epidemiol Infect 2002;129(October (2)):355–60.

Graham DY, Jiang X, Tanaka T, Opekun AR, Madore HP, Estes MK. Norwalk virus infection of volunteers: new insights based on improved assays. J Infect Dis 1994;170:34–43.

Hall AJ. Noroviruses the perfect human pathogens?. J Infect Dis 2012;205:1622–4, doi:http://dx.doi.org/10.1093/infdis/jis251.

Liu Luke Tzu-Chi, Kuo Ting-Yu, Wu Ching-Yi, Liao Wan-Ting, Hall Aron J, Wu Fang-Tzy. Recombinant GII.P16-GII.2 Norovirus, Taiwan, 2016. Emerg Infect Dis 2017;23(July (7)):1180–3, doi:http://dx.doi.org/10.3201/eid2307.170212.

Lopman B, Gastañaduy P, Park GW, Hall AJ, Parashar UD, Vinjé J. Environmental transmission of norovirus gastroenteritis. Curr Opin Virol 2012a;2:96–102, doi:http://dx.doi.org/10.1016/j.coviro.2011.11.005.

Lopman B, Gastañaduy P, Park GW, Hall AJ, Parashar UD, Vinjé J. Environmental transmission of norovirus gastroenteritis. Curr Opin Virol 2012b;2:96–102, doi:http://dx.doi.org/10.1016/j.coviro.2011.11.005.

Lu J, Fang L, Sun L, Zeng H, Li Y, Zheng H, et al. Association of GII.P16-GII.2 recombinant Norovirus strain with increased Norovirus outbreaks, Guangdong, China, 2016. Emerg Infect Dis 2017;23(July (7))1188–90, doi:http://dx.doi.org/10.3201/eid2307.170333 [Epub 2017 July 15].

Manuel CS, Moore MD, Jaykus LA. Destruction of the capsid and genome of GII.4 Human Norovirus occurs during exposure to metal alloys containing copper. Appl Environ Microbiol 2015;81:4940–6, doi:http://dx.doi.org/10.1128/AEM.00388-15.

Marks PJ, Vipond IB, Carlisle D, Deakin D, Fey RE, Caul EO. Evidence for airborne transmission of Norwalk-like virus (NLV) in a hotel restaurant. Epidemiol Infect 2000;124(June (3)):481–7.

Marks PJ, Vipond IB, Regan FM, Wedgwood K, Fey RE, Caul EO. A school outbreak of Norwalk-like virus: evidence for airborne transmission. Epidemiol Infect 2003;131(August (1)):727–36.

Niendorf S, Jacobsen S, Faber M, Eis-Hübinger AM, Hofmann J, Zimmermann O, et al. Steep rise in norovirus cases and emergence of a new recombinant strain GII.P16-GII.2, Germany, winter 2016. Euro Surveill 2017;22:30447, doi:http://dx.doi.org/10.2807/1560-7917.ES.2017.22.4.30447.

Parra GI, Squires RB, Karangwa CK, Johnson JA, Lepore CJ, Sosnovtsev SV, et al. Static and evolving norovirus genotypes: implications for epidemiology and immunity. PLoS Pathog 2017;13:e1006136, doi:http://dx.doi.org/10.1371/journal.ppat.1006136.

Repp KK, Keene WE. A point-source norovirus outbreak caused by exposure to fomites. J Infect Dis 2012;205(June (11)):1639–41, doi:http://dx.doi.org/10.1093/infdis/jis250.

Shi Chao, Feng Wei-Hong, Shi Ping, Ai Jing, Guan Hong-Xia, Sha Dan, et al. An acute gastroenteritis outbreak caused by GII.17 norovirus in Jiangsu Province, China. Int J Infect Dis 2016;49(August)30–2, doi:http://dx.doi.org/10.1016/j.ijid.2016.05.004 [Epub 2016 May 17].

Tu ETV, Bull RA, Kim MJ, McIver CJ, Heron L, Rawlinson WD, et al. Norovirus excretion in an aged-care setting. J Clin Microbiol 2008;46:2119–21.

Zheng QM, Zeng HT, Dai CW, Zhang SX, Zhang Z, Mei SJ, et al. Epidemiological investigation of a norovirus GII.4 Sydney outbreak in a China elder care facility. Jpn J Infect Dis 2015;68:70–4, doi:http://dx.doi.org/10.7883/yoken.JJID.2014.081.