An Acute Gastroenteritis Outbreak Caused by Astrovirus Serotype 4 in a School of Shenzhen City, China, 2017

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Abstract

Background: In November of 2017, a school outbreak of acute gastroenteritis was reported from Shenzhen City. An investigation was conducted to identify the cause, exposure mode and recommend control measures.

Methods: A suspect case was defined as anyone in the school who developed symptoms of ≥ 1 vomitus or ≥ 3 loose stools/24 hours from October 23- November 17. A confirmed case was a suspect case with a positive astrovirus PCR (+) stool or rectal swab specimen. We reviewed absenteeism records and interviewed students and teachers to identify cases; collected stool or rectal specimens from ill students, food handlers and sanitation staff and tested them for common viral or bacterial pathogens. 5 classes with highest Attack Rates (AR) were chosen as a study cohort to identify the mode of transmission.

Results: 98 student-cases (6 confirmed) were identified from October 23- November 14. The main symptoms included vomiting (69%), abdominal pain (59%), nausea (47%), diarrhea (43%) and cough (38%) with the median duration of illness of 2 days (range: 1-8 days). The AR was 3.2% (98/3053) and involved 63% (31/49) of the classes with the median age of 10 years (range 6-15 years). Based on epidemiological investigation, the food and water supplied in the school were not associated with this outbreak (P > 0.05). The multivariate analysis showed that touching with ill students (RR = 3.95, 95% CI = 2.18-5.58) and the handles of faucet (RR = 4.18, 95% CI = 1.50-8.37) were associated with illness. The cleaning staff used the same cleaning cloth and gloves to sweep water faucet, toilets and waste bins. Astrovirus serotype 4 was detected in 6 ill students and 1 asymptomatic cleaning staff.

Conclusions: This outbreak was caused by astrovirus serotype 4 and the main exposure mode was likely person to person and touching a contaminated water faucet. Recommendations included immediate isolation of cases, proper handling of vomits, with environmental disinfectants, and training in good hand washing for students and teachers. Moreover, the drinking water faucet should be disinfected daily.


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Keywords
Astrovirus, Outbreak, Person-person, Environment exposure

Introduction

Human astrovirus (HAstV) was first detected by Appleton and Higgins in 1975 in association with an outbreak of acute gastroenteritis in a maternity ward in England, and the same year named by Madeley and Cosgrove because of its star-like appearance [1]. Astrovirus along with rotavirus, norovirus and sapovirus, is recognized as a common cause of viral acute gastroenteritis in adults and children. Similarly, with other enteric pathogens, the main clinical symptoms of astrovirus are vomiting, diarrhea, abdominal cramps and nausea [2]. However, incubation period varies within those common enteric viruses and astrovirus is longest among them, for example, the median incubation period for astrovirus is 4.5 days, 1.2 days for norovirus genogroups I and II, 1.7 days for sapovirus, and 2.0 days for rotavirus [3]. Direct contact with vomits or feces of infected persons, sharing food, water, utensils, and contact with a contaminated environment are all possible routes of astrovirus transmission [4]. Among the 8 serotypes of astrovirus identified, serotype 1 is the predominant strain worldwide. In addition, the detection rate of astrovirus types 2 to 8 has increased by using newly developed assays [5].

Shenzhen is a coastal city in Guangdong province, southern China. As a fast-developing city, many migrant populations and their kids are living in Shenzhen. Since October 23, 2017, more than 20 students developed a sudden onset of vomiting and diarrhea from a private school. In order to identify the source of contamination and infection route, Shenzhen Bao’an Center for Disease Control and Prevention (CDC), jointly with Chinese field epidemiology training program (CFETP) conducted a retrospective cohort study to investigate the risk factors and implement control measures associated with this acute gastroenteritis outbreak.

Methods

Study setting

The school, located at Bao’an district of Shenzhen city, was a private school from the 1st to 9th grades with a total of 49 classes, 3053 students and 151 staff, including teachers, kitchen staff and cleaning staff. The school supplied meals and bottled drinking water for every staff that lived on the school campus. All the students lived nearby so no accommodation offered for them. The school had only 1 cafeteria in which 84 students had breakfast and 1292 students had lunch, and all staff had 3 meals. Epidemiologists visited the premises after the outbreak to review the kitchen facilities and preparation procedures. All the meals were cooked in the kitchen of the school cafeteria. The cafeteria provided freshly prepared and completely cooked meals every day for part of students and all teachers. The Shenzhen Food and Drug Administration (Shenzhen FDA) requires that school cafeterias keep a food sample for 48 hours in one fixed refrigerator for each meal and that raw foods be stored in another refrigerator in a separate room. Students and all staff had breakfast in the cafeteria, while lunch for students were delivered to their own classrooms. All foods were served as usual from October 23 to November 17. The school provided direct drinking water for all students, which located at the playground. A total of 13 classes consumed commercial bottled water manufactured by 7 commercial brands, while teachers consumed bottled water offered by school.

Epidemiological investigation

We designed a retrospective cohort study to investigate and analyze the potential transmission mode. In this outbreak, cases were classified as probable cases and laboratory-confirmed cases. Probable case was defined as the person who had ≥ 1 vomits or ≥ 3 loose stools within 24 hours in this school between October 23 and November 17, 2017 without laboratory confirmation of astrovirus infection. Confirmed case was defined as the persons with identified astrovirus in his or her stool or rectal swabs among probable cases. Carriers are those who have astrovirus in their stool or rectal swab specimen without presenting any symptoms during the study period. We searched for cases among all students and school staff based on gastrointestinal symptoms. A questionnaire was used to collect information for cases on demographic characteristics, symptoms, meals in the school cafeteria, sources of drinking water, history of contact with persons who had diarrhea and/or vomiting, exposure to environmental fomite and personal hygiene habits. The study was conducted according to the principles and guidelines of the Declaration of Helsinki and was approved by the Research Ethics Committee at the Bao’an CDC.

Specimen collection

Stool specimens were collected from 25 patients within 3 days post symptoms onset, because stool shed more virus load than other specimen. Due to the potential role of cleaning staff in transmitting the intestinal virus, we collected 2 rectal swabs of cleaning staff who were mainly engaged in sweeping the toilet and environment during the field investigation, even though none of them had reported any similar symptoms. All samples were placed on ice and transported to Beijing CDC laboratory via bio-safety cycle boxes and transfer vehicles.

Laboratory examination

The common intestinal bacteria including Escherichia coli...
coli, Salmonella, Shigella, Yersinia enterocolitica, Cholerae, Vibrio parahaemolyticus, Aeromonas hydrophila and Plesiomonas shigelloides were identified via bacterial culture according to the technical procedures of diarrheal pathogenic spectrum surveillance formulated by the China Center for Disease Control and Prevention. The intestinal viruses including Rotavirus, Enteric adenovirus, Norovirus, Sapovirus and Astrovirus were detected using the real time reverse transcription PCR detection kits (Bioperfectus Ltd., Taizhou, CHN) according to the manufacturer’s protocol. The QIAGEN One-Step RT-PCR Kit (QIAGEN, Hilden, Germany) was used to amplify the partial ORF2 gene of HAsTV with primers Mon269 and Mon270 [6]. The PCR products were purified and sequenced directly on an ABI 3730xl DNA Analyzer using a BigDye Terminator v3.1 Cycle Sequencing Kit (ABI, Austin, TX, USA). The sequence was compared with reference strains in GenBank to determine the genotype using BLAST.

**Statistical analysis**

The distribution of major symptoms in students was summarized by frequency and percent. A retrospective cohort study was conducted to investigate possible risk factors for acute gastroenteritis among five classes with highest Attack Rates (AR). Overall attack rate was calculated using the number of cases divided by the total number of students in the school. To evaluate the risk factor on the route of transmission, relative risks (RRs), with the corresponding 95% confidence intervals (CIs), and Fisher’s exact test were calculated. A P-value of < 0.05 was considered statistically significant.

**Results**

**Descriptive epidemiology**

A total of 98 cases developed gastrointestinal symptoms during this outbreak, and the attack rate was 3.2%. All cases were students and distributed across 9 grades and 31 classes (Figure 1), including 61 boys and 37 girls (male-to-female ratio = 1.65:1). The attack rate in students from each grade (elementary and middle school) was not statistically different (P = 0.07). The average age was 10-years-old (range 6-15 years-old). The main symptoms were vomiting (69%), abdominal cramps (59%), nausea (47%), diar-

<table>
<thead>
<tr>
<th>Symptom/sign</th>
<th>No. of cases (n = 98)</th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vomiting</td>
<td>68</td>
<td>69</td>
</tr>
<tr>
<td>1 time</td>
<td>28</td>
<td>41</td>
</tr>
<tr>
<td>2 times</td>
<td>25</td>
<td>37</td>
</tr>
<tr>
<td>≥ 3 times</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>41</td>
<td>43</td>
</tr>
<tr>
<td>Bellyache</td>
<td>58</td>
<td>59</td>
</tr>
<tr>
<td>Nausea</td>
<td>46</td>
<td>47</td>
</tr>
<tr>
<td>Cough</td>
<td>37</td>
<td>38</td>
</tr>
<tr>
<td>Fever</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Ventosity</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

Note: The red dot represents for one case; the room number with yellow color means this classroom has commercial bottled drinking water.

**Figure 1**: Cases distribution among all classrooms in school compound.
drinking water was adopting a physical filter which can exclude the macromolecule substance when water passed by. As we investigated, only 13% of cases consumed direct drinking water in this school. Moreover, student guardians from 13 classes invested separately to buy commercially-bottled water in the market for their kids; however, the brands were manufactured by as more as 7 companies. In conclusion, the attack rate among those classes who were supplied with barreled purified water had no difference with those who did not have in the classroom (p = 0.96).

Retrospective cohort study

Altogether, 5 classes with higher attack rate were selected for the retrospective cohort study. A total of 269 pupils have completed the questionnaire, among which there were 23 cases and 249 non-cases. Table 2 illustrated the univariate and multivariate analysis results. Astrovirus outbreak rates were found to differ: Drinking water from home was found to be related to decreased rates, while having breakfast outside of school, contacting with school cases, touching direct drinking water faucet were found to have an elevated astrovirus outbreak rate. Going to washroom more than 3 times per day was found to be marginally related to rates of outbreak. No difference was found in term of seeing vomits nearby, having cleaned vomits before outbreak occurrence, washing hands thoroughly and commuting to school by school bus. In the multivariate analysis, contacting with school cases and touching direct drinking water faucet was still significantly related to increased astrovirus outbreak rates after adjusting for potential confounding factors (RR = 3.95, 95% CI: 2.18-5.58) (RR = 4.18, 95% CI: 1.50-8.37). And having Breakfast outside and drinking water from home were not statistically significant after adjusting for potential confounding factors.

Cafeteria and drinking water investigation

We inspected the school’s kitchen, pantry and dining hall and canteen. This school had 1 cafeteria providing various food for students and teachers, in which 13 employees working as kitchen staff. The environment at the canteen looked clean. The cafeteria was completely independent in both staff and food raw materials. No employee presented any symptom during this epidemic. The attack rate among those who took breakfast in the school canteen was not significantly different with those who took breakfast out of school (p = 0.3), while the pupils who took lunch in the school was less likely to get infected than those who took lunch out of school (p = 0.01).

We inspected their sources of cooking water and drinking water. Both were a public water source and were supplied as usual, during the epidemic, no community cases were reported. The mechanism of direct drinking water was adopting a physical filter which can exclude the macromolecule substance when water passed by. As we investigated, only 13% of cases consumed direct drinking water in this school. Moreover, student guardians from 13 classes invested separately to buy commercially-bottled water in the market for their kids; however, the brands were manufactured by as more as 7 companies. In conclusion, the attack rate among those classes who were supplied with barreled purified water had no difference with those who did not have in the classroom (p = 0.96).

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A sample from a school case was detected sapovirus positive as Table 3 shows. All specimens were tested negative for other bacterial and viral pathogens. Furthermore, the causing pathogen associated with this outbreak was found to be astrovirus type 4 strain which was rarely reported worldwide. Days between resolution to taking sample were ranged from 1 to 12 days, while the first laboratory-confirmed case was the person who shedding virus as long as 12 days since all the symptoms released.

**Control measures**

To control the outbreak, the Bao’an CDC used immediate control measures as soon as the epidemiological survey initiated on Nov 7, including environment disinfection, case isolation, school bus closure and hand hygiene enforcement. Since then, the classrooms and public areas were disinfected by local health department every day until outbreak suspended. Ill students were excluded at least 3 days since the date of onset, and chemical disinfection for hand hygiene were available in classrooms and washrooms, which was a central approach to interrupt the chain of astrovirus transmission. All those comprehensive control measures worked efficiently as no more case reported after Nov 14, 2017.

**Environmental survey**

An environmental survey of the school compound was conducted to assess the general sanitation of the school. There were only 2 cleaning staff responsible for the sanitation and hygiene of school public environment including the washrooms, corridors, playground, stairs, direct drinking water and waste bins. A total of 14 washrooms were provided for boys or girls on the school. Each washroom merely had one water faucet for washing hand, thus the lack of hand washing facilities and washrooms was a serious problem in this school. Based on the observation, cleaning staff used same towel and gloves to sweep and disinfect toilet surface, waste bin and direct drinking water faucet. Sometimes, the cleaning staff swept the toilet floor with classroom floorcloth which was nearby the toilet without washing and disinfection.

**Laboratory tests**

A total of 7 samples were tested positive for astrovirus using real time RT-PCR including 2 rectal swabs and 5 stool specimens. Of the 7 positive rectal swabs and stool specimens, 6 were from student cases and 1 from asymptomatic cleaning staff who did not report any clinical symptoms. In addition, another 1 stool sample from a school case was detected sapovirus positive as Table 3 shows. All specimens were tested negative for other bacterial and viral pathogens. Furthermore, the causing pathogen associated with this outbreak was found to be astrovirus type 4 strain which was rarely reported worldwide. Days between resolution to taking sample were ranged from 1 to 12 days, while the first laboratory-confirmed case was the person who shedding virus as long as 12 days since all the symptoms released.

**Table 2: Univariate and multivariate analysis of the relative risk of astrovirus during a school outbreak.**

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Exposed total</th>
<th>Exposed cases</th>
<th>Exposed AR (%)</th>
<th>Unexposed total</th>
<th>Unexposed cases</th>
<th>Unexposed AR (%)</th>
<th>Univariate analysis RR (95% CI)</th>
<th>Multivariate analysis RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contacting with school cases</td>
<td>32</td>
<td>9</td>
<td>28.1</td>
<td>237</td>
<td>14</td>
<td>5.9</td>
<td>6.23 (2.43-15.97)</td>
<td>3.95 (2.18-5.58)</td>
</tr>
<tr>
<td>Touching direct drinking water faucet</td>
<td>21</td>
<td>5</td>
<td>23.8</td>
<td>248</td>
<td>18</td>
<td>7.3</td>
<td>3.99 (1.31-12.15)</td>
<td>4.18 (1.50-8.37)</td>
</tr>
<tr>
<td>Having Breakfast from vendors</td>
<td>128</td>
<td>16</td>
<td>12.5</td>
<td>141</td>
<td>7</td>
<td>5.0</td>
<td>2.74 (1.09-6.89)</td>
<td>2.03 (0.81-4.58)</td>
</tr>
<tr>
<td>Water from home</td>
<td>189</td>
<td>12</td>
<td>6.3</td>
<td>80</td>
<td>11</td>
<td>13.8</td>
<td>0.43 (0.18-1.01)</td>
<td>0.43 (0.16-1.11)</td>
</tr>
<tr>
<td>Seeing vomits</td>
<td>36</td>
<td>5</td>
<td>13.9</td>
<td>233</td>
<td>18</td>
<td>7.7</td>
<td>1.92 (0.67-5.66)</td>
<td></td>
</tr>
<tr>
<td>Cleaning vomits</td>
<td>4</td>
<td>1</td>
<td>25.0</td>
<td>264</td>
<td>21</td>
<td>8.0</td>
<td>3.86 (0.38-38.73)</td>
<td></td>
</tr>
<tr>
<td>Went to toilet ≥ 3</td>
<td>74</td>
<td>10</td>
<td>13.5</td>
<td>192</td>
<td>13</td>
<td>6.8</td>
<td>2.15 (0.89-5.15)</td>
<td></td>
</tr>
<tr>
<td>Washing hand thoroughly</td>
<td>116</td>
<td>9</td>
<td>7.8</td>
<td>150</td>
<td>14</td>
<td>9.3</td>
<td>0.82 (0.34-1.96)</td>
<td></td>
</tr>
<tr>
<td>Catching school bus</td>
<td>47</td>
<td>3</td>
<td>6.4</td>
<td>221</td>
<td>20</td>
<td>9.0</td>
<td>0.69 (0.20-2.41)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3: Results of samples collection and detection.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of samples</th>
<th>Samples origination</th>
<th>Date of onset</th>
<th>Date of resolution</th>
<th>Date of collecting sample</th>
<th>Duration of illness</th>
<th>Days between resolution to taking sample</th>
<th>Detection result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stool</td>
<td>school cases</td>
<td>10/27</td>
<td>11/4</td>
<td>11/16</td>
<td>8</td>
<td>12</td>
<td>astrovirus</td>
</tr>
<tr>
<td>2</td>
<td>Stool</td>
<td>school cases</td>
<td>11/14</td>
<td>11/15</td>
<td>11/16</td>
<td>1</td>
<td>1</td>
<td>astrovirus</td>
</tr>
<tr>
<td>3</td>
<td>Stool</td>
<td>school cases</td>
<td>11/3</td>
<td>11/5</td>
<td>11/16</td>
<td>2</td>
<td>11</td>
<td>astrovirus</td>
</tr>
<tr>
<td>4</td>
<td>Stool</td>
<td>school cases</td>
<td>11/2</td>
<td>11/5</td>
<td>11/10</td>
<td>3</td>
<td>5</td>
<td>astrovirus</td>
</tr>
<tr>
<td>5</td>
<td>Stool</td>
<td>school cases</td>
<td>11/3</td>
<td>11/7</td>
<td>11/16</td>
<td>4</td>
<td>9</td>
<td>astrovirus</td>
</tr>
<tr>
<td>6</td>
<td>Rectal swab</td>
<td>school cases</td>
<td>11/5</td>
<td>11/8</td>
<td>11/10</td>
<td>3</td>
<td>2</td>
<td>astrovirus</td>
</tr>
<tr>
<td>7</td>
<td>Rectal swab</td>
<td>asymptomatic staff</td>
<td>-</td>
<td>11/17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>astrovirus</td>
</tr>
<tr>
<td>8</td>
<td>Stool</td>
<td>school cases</td>
<td>11/4</td>
<td>11/8</td>
<td>11/10</td>
<td>4</td>
<td>2</td>
<td>sapovirus</td>
</tr>
</tbody>
</table>

*Clean staff is asymptomatic, date of illness is not applicable.*
Discussion

Acute gastroenteritis is an important public health issue in child worldwide [7]. Recent studies have shown that among pathogens causing viral gastroenteritis, astrovirus was the third most common viral cause of acute gastroenteritis outbreaks in North Carolina from 2005-2007 and of sporadic cases in children in China from 1998-2005, just after rotavirus and calicivirus [8]. Thirty-five percent of children experienced astrovirus infections and the prevalence of astrovirus in diarrheal stools was 5.6% according to a multiple-country study [9]. As previous research revealed, the classic human astrovirus is highly diverse and consists of eight genotypes (genotype 1-8), while genotype 1 was the most prevalent genotype globally. However, astrovirus induced outbreaks are rarely reported in China so far. According to published literature, only 2 kindergartens, 1 school and 1 hospital from 4 provinces have reported astrovirus outbreaks in mainland China before 2017. Of those 4 outbreaks, 2 of them were caused by astrovirus genotype 1 based on further sequencing [10-13].

On the basis of symptom profiles, illness incubation and duration periods, and positive stool/rectal swab specimens, astrovirus (a human pathogen) was the most likely etiology of this outbreak, and the laboratory detection confirmed that a less common strain, genotype 4, was identified as the causative etiology for this outbreak. The research also found one case shed virus as long as 12 days since his symptoms free. According to a retrospective study conducted in USA, a subset of cases is associated with long-term virus shedding (range 17–183 days), which is consistent with our finding [14]. In conclusion, the long shedding period of astrovirus is a big challenge of prevention and control for outbreaks.

Moreover, this school did not provide enough hand washing facilities and direct drinking water vessels for students. It was not safe that the main waste bins were near the direct drinking water which is not accordance with the principle that waste bins should be in a secure area away from water and play areas. In addition, small number of cleaning staff and low level of environment hygiene requirements posed this school a big threat to enteric disease spreading. Because of intense labor, two cleaning staff dealt with their work roughly, more specifically, they cleaned the washroom not frequently and use same towels and gloves to sweep all infrastructure and environment surface based on our observation. In accordance with school hygiene regulation, multiple-use towels are not recommended due to increased risk of re-contamination and cross infection, and if non-disposable hand towels are used, they should be identified as only for drying hands and washed at least daily. It is also recommended that a schedule is in place listing each area and items to be cleaned, how often they are to be cleaned and who is responsible, and that a record of cleaning is kept signed and dated, as well as the importance of complying with basic guidelines for environment disinfection. Frequently-touched environmental surfaces are cleaned and disinfected more frequently than the daily cleaning schedule normally recommended. This outbreak highlights that the appropriate number of sinks and toilets should be installed in a school facility as well.

Our study has several limitations. First, the outbreak characteristics were highly presumptive of contaminated environment transmission, but this was not confirmed through detection of astrovirus in implicated high-touch items, for example direct drinking water faucets, door handles, gloves or towels. Second, recall bias was difficult to avoid in this research since the young pupils were lack of communication and understanding ability, for example, they hardly remember the details about duration of onset, risk factors and behavior history when the investigator asked them questions.

Conclusions

This outbreak was caused by astrovirus which was rarely reported before and the main exposure mode was likely person to person and touching a contaminated water faucet. Recommendations included immediate isolation of cases, proper handling of vomits, with environmental disinfectants, and training in good hand washing for students and teachers. Moreover, the drinking water faucet should be disinfected daily. Continuous surveillance is needed to monitor the circulating strains of astrovirus outbreak in China.

Potential Conflicts of Interest

We declare that we have no conflicts of interest.
References