

A gastroenteritis outbreak associated with drinking water in a college in northwest China

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ABSTRACT

An acute gastroenteritis outbreak occurred at a private college in June 2014 in northwest China. This outbreak involved two teachers and 629 students (range: 17–27 years, average 21.3 years). The main symptoms included non-bloody watery diarrhea, stomach ache, nausea, and vomiting, and the duration of illness ranged from 1 to 7 days. Eight of 18 water samples were disqualified. Thirty-four norovirus (NoV) RNA-positive samples were identified from 48 stool-related samples (genotyping results: 13 GII, 13 GI and 8 GI + GII mixture). Fourteen NoV samples were successfully characterized for genotype, including two GII.6, five GI.6, four GI.3, and three GI.1. Enteropathogenic *Escherichia coli* (EPEC) and enteroadherent *Escherichia coli* (EAEC) DNA was detected from patient stool specimens and water samples from well one; two EAEC strains and one EPEC strain were isolated from patient stool specimens. The risk ratios (RRs) associated with wells one and two were 1.66 and 1.49, respectively, and the RR associated with living in north dormitory building one was 2.59. The patients' epidemiological characteristics, symptoms, and duration of illness indicated that NoV-contaminated water might be the origin of this outbreak, and RR analysis suggested that the two wells were linked to the outbreak.

Key words | college students, early summer, norovirus strain diversity, northwest China, water-borne acute gastroenteritis

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INTRODUCTION

Acute gastroenteritis (AGE) is an important global public health issue, especially in developing countries, where the social and economic impacts are significant (Hall *et al.* 2013). Norovirus (NoV) is considered to be one of the leading causative agents of AGE among all age groups worldwide. The majority of human NoV cases can be classified into two genogroups: GI and GII (Vinje 2015). Furthermore, NoV GI and GII strains can be classified into nine and 22 genotypes, respectively (Kroneman *et al.* 2013). GI strains tend to dominate in water-related

outbreaks, but several reports have shown that mixtures of NoV strains can also cause water-borne infections (Kim *et al.* 2005; Hewitt *et al.* 2007; Hoa Tran *et al.* 2013).

Although the capability to detect norovirus nucleic acids is becoming available in China, the epidemiological data regarding NoV outbreaks and knowledge of this pathogen remain limited (Chen *et al.* 2016). More formal reports of NoV outbreaks are needed in China, especially in the north-western region, where economic growth rates have lagged behind those of other regions in China and studies

investigating outbreaks are still lacking. Here, we report a large diarrhea outbreak in college students.

MATERIALS AND METHODS

Outbreak description and epidemiological investigation

On June 11, 2014, more than 300 gastroenteritis cases in a private college in Shaanxi Province were detected by Network Monitoring of the Xi'an Center for Disease Control and Prevention (XACDC). An epidemiological survey and control measures were immediately initiated. Cases were defined as college staff members and students with an onset of diarrhea (≥ 3 times per day) or vomiting from June 1 to 20, 2014. A retrospective face-to-face questionnaire survey for all patients recorded on the outpatient log in the school hospital was administered through interviews, and the information collected included illness onset, symptoms, duration of illness, and eating and drinking history. All other teachers and students were surveyed by college staff, and students living in south dormitory buildings 4 and 5 were enrolled as controls because these students all learned on the job outside of the campus. An environmental survey was also implemented, including an examination of the local wells, buildings and cafeterias. On June 11, 18 water specimens were collected for laboratory testing. On June 11 and 12, 31 stool samples and 17 rectal swabs were collected from patients. On June 14, the school stopped supplying water from the local wells and began using water transported by truck from the municipal water network. Since July 5, the school began to improve the sanitary conditions of the well, and on July 28, eight water samples were sent to XACDC for sanitary index detection.

Bacterial detection in water and stool specimens

A total of 18 water samples including seven well water, six tap water, and five direct-drinking water samples were collected and transported to the XACDC for measurement of the hygienic index on the same day. Each water sample (500 mL) was measured for aerobic bacterial count, total coliforms, and thermotolerant coliforms according to the national standards for drinking water quality of China

(GB5750-2006). Nucleic acid detection of diarrheogenic *Escherichia coli* was also performed in 18 water samples and 31 stool samples using commercial real-time polymerase chain reaction (PCR) kits. Briefly, 20 mL of well water was concentrated by centrifugation at $40,000 \times g$ for 5 min; 1 mL of sediment remained, and 200 μL was employed for nucleic acid extraction. A 0.2 g stool sample was immersed in 1 mL of phosphate-buffered saline (PBS, pH 7.4) and centrifuged at $3,000 \times g$ for 5 min; 200 μL supernatant was used for nucleic acid extraction. Magnetic beads pre-filled from RNA/DNA extraction kits (Lot. T032, Tianlong Science and Technology Co., Xi'an, China) were employed with a nucleic acid extractor according to the manufacturer's instructions (NP968-S, Tianlong Science and Technology Co., Xi'an, China). Real-time PCR assays were employed to test for nucleic acids of diarrheogenic *E. coli* by commercial kits (DD0091-95, Shanghai Liferiver Bio-tech Co., China). *E. coli*, *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia enterocolitica* in 18 water samples and 31 stool samples were also detected through isolation methods.

Virus detection in water and fecal samples

The nucleotide acid of diarrheal viruses was also investigated in these 18 water samples, 31 stool samples mentioned above and 17 rectal swabs in XACDC. Rectal swabs or 0.2 g stool samples were immersed in 1 mL PBS and vortexed for 30 sec, and they were then clarified by centrifugation at $8,000 \times g$ for 5 min at room temperature. A 50 mL sample of water was concentrated by centrifugation at $200,000 \times g$ for 30 min, and 1 mL of sediment was used for detection. A total of 200 μL of supernatant of stool or sediment from water was used to extract viral nucleic acid using magnetic beads pre-filled from RNA/DNA extraction kits (Lot. T001, Tianlong Science and Technology Co., Xi'an, China) according to the manufacturer's instructions (NP968-S, Tianlong Science and Technology Co., Xi'an, China). Viral RNA was reverse-transcribed (RT) with a First Strand cDNA synthesis kit (Fermentas, EU). The RT step was carried out at 42°C for 1 h, followed by heating at 99°C for 5 min to inactivate the enzyme, and cooling at 4°C immediately. The group A rotaviruses, enteric adenoviruses, NoV GI/GII and astroviruses were simultaneously detected by PCR using the Diarrhea-V ACE Detection

system (Seegene, South Korea) according to the manufacturer's instructions.

NoV genotyping by nucleotide sequence analysis

The cDNA of GI from the above step were used directly for sequencing PCR using primers SKF/SKR, and region C of OFR2 was amplified using *Taq* DNA polymerase (Promega) at 95 °C for 5 min followed by 35 cycles of 94 °C 30 s, 55 °C 30 s, 72 °C 60 s, and a final extension at 72 °C for 10 min, followed by cooling at 4 °C (Kojima *et al.* 2002). The complete OFR2 was amplified from RNA of GII positive samples with primers GV305/GV308 using SuperScript™ III One-Step RT-PCR System with Platinum™ *Taq* High Fidelity DNA Polymerase (Invitrogen) at 50 °C for 30 min, 94 °C for 5 min followed by 35 cycles of 94 °C 30 s, 55 °C 30 s, 68 °C 150 s, and a final extension at 68 °C for 5 min, followed by cooling at 4 °C (Bull *et al.* 2012). The PCR products were purified by QIA quick PCR purification kit (Qiagen) and followed by bidirectional sequencing by BigDye 3.1 chemistry with an ABI PRISM 3130 DNA analyzer (Applied Biosystems Inc., CA, USA). Twelve GI and two GII RNA genotypes were amplified and successfully sequenced. The nucleotide sequencing data have been deposited in the GenBank database under accession numbers KX228750-KX228763.

Phylogenetic analysis

Analysis of nucleotide sequence differences and alignment of the sequences were performed using the BioEdit program (www.mbio.ncsu.edu/BioEdit/bioedit.html). Phylogenetic trees were generated with the Mega 6.0 software package using the neighbor-joining method with 1,000 bootstrap replicates (Tamura *et al.* 2013). NoV sequences used in the study were retrieved from GenBank (www.ncbi.nlm.nih.gov/Genbank).

Statistical analysis, ethics approval and consent to participate

A case distribution curve was generated with Excel, and relative risks (RRs) and 95% confidence intervals (CIs) were calculated using SPSS Statistics 17.0 software

(Chicago, IL, USA). This study was approved by the Ethics Checking Committee of Xi'an CDC (XACDC ECC 01-2014), and written informed consent was obtained from all patients.

RESULTS

Epidemiological data

The college where the outbreak occurred is located in the southern part of Xi'an, the capital of Shaanxi Province, along the Qinling Mountains. At the time of the outbreak, there were 998 teachers and 14,120 students in the college. The sex ratio of the students (male/female) was 2.05. The campus is separated into northern and southern campuses by a road. The two cafeterias in the northern campus are for students and the one in the southern campus is used by teachers. There are five water wells, four of which were used as sources of drinking water at the time of the outbreak. Two of the wells are located in the northern campus, and the other three are located in the southern campus. The students living in the south dormitory buildings attended classes in the north campus and drank the water from Wells 1 and 2.

The retrospective survey revealed that the first case occurred on June 1, and there were no gastroenteritis cases reported at this school after June 18. In this outbreak, 631 cases (including two teachers and 629 students) were reported, involving 440 men and 191 women. The sex ratio (male/female) was 2.30. The ages of the patients ranged from 17 to 27 years, with a median age of 21.3 years. Of the 631 cases, 537 (85.10%) reported diarrhea, 483 (76.54%) stomach ache, 446 (70.67%) nausea, 419 (66.48%) vomiting, 155 (24.58%) headache, 116 (18.43%, 38 ± 0.2 °C) fever, and 28 (4.47%) weakness. The duration of illness ranged from 1 to 7 days, with a median duration of 2 days.

This outbreak lasted for 18 days, and the daily case distribution is shown in Figure 1. The retrospective investigation showed that beginning on June 1, the college hospital began to see students with diarrhea and vomiting, and through June 11, the case number increased to 358. On June 14, the well water supply was interrupted, and

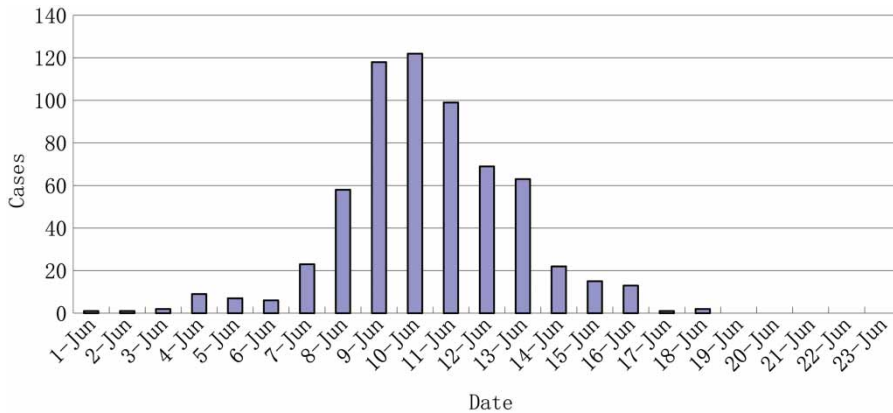


Figure 1 | Epidemiological data for the gastroenteritis outbreak on a campus in the northwest of China in early summer of 2014. Graph showing the number of cases reported every day and that the outbreak began on 1 June, peaked on 10 June, and ended on 18 June.

municipal water was transported to the college. After June 19, no additional cases were reported. The control group comprised 26 students, and the attack rate was 0.969%. Wells 1 and 2 were located in the back yard between the building and the wall around the northern campus. The sanitary conditions of Wells 1 and 2 were poor as the wells were surrounded by garbage and feces. The two wells were both below ground level, and there was a leaking trail of rain and sewage on the well wall.

Risk rate evaluation

The risk patterns for students living in different dormitory buildings and drinking water from different wells were

analyzed. RRs are listed in Table 1, and the statistical analysis implied that living in the northern part of the school and drinking water supplied from Wells 1 and 2 were risk factors.

Microbial detection

The national standards for drinking water quality of China stipulate that the limit of aerobic bacterial count is 100 colony-forming-units (CFU)/mL, and pathogenic organisms, total coliform, thermotolerant coliform and *E. coli* should be negative (GB5749-2006). Among the 18 water samples, eight samples were judged as disqualified according to the standards. The numbers of aerobic bacterial count were 260–24,000 CFU/mL in four well water, one tap water and

Table 1 | Attack rates and relative risk analysis of different dormitories and wells in the AGE outbreak on a campus in the northwest of China in early summer of 2014

| Dormitory | Living number | cases | Incidence rate (%) | RR | RR 95% CI | Water supply | Attack rate (%) | RR | RR 95% CI |
|-----------|---------------|-------|--------------------|------|-----------|--------------|-----------------|------|-----------|
| NDB2 | 2,064 | 155 | 7.51 | 1.66 | 1.40–1.97 | Well 1 | 7.51 | 1.66 | 1.40–1.97 |
| NDB1 | 880 | 103 | 11.70 | 2.59 | 2.11–3.12 | Well 2 | 6.78 | 1.49 | 1.28–1.73 |
| NDB3 | 2,247 | 109 | 4.85 | 1.07 | 0.87–1.30 | Well 2 | | | |
| SDB1 | 1,212 | 61 | 5.03 | 1.11 | 0.86–1.44 | Well 3 | 3.26 | 0.72 | 0.63–0.83 |
| SDB2 | 893 | 45 | 5.04 | 1.11 | 0.83–1.50 | Well 3 | | | |
| SDB3 | 1,282 | 43 | 3.35 | 0.74 | 0.55–1.00 | Well 3 | | | |
| SDB4 | 1,348 | 14 | 1.04 | 0.23 | 0.14–0.37 | Well 3 | | | |
| SDB5 | 1,334 | 12 | 0.90 | 0.20 | 0.12–0.33 | Well 3 | | | |
| SDB6 | 1,980 | 87 | 4.39 | 0.97 | 0.79–1.20 | Well 3 | | | |
| TDB | 715 | 2 | 0.28 | 0.06 | 0.02–0.25 | Well 4 | 0.28 | 0.06 | 0.02–0.25 |
| Total | 13,955 | 631 | 4.52 | | | | | | |

NDB, north dormitory building; SDB, south dormitory building; TDB, teacher dormitory building; RR, risk ratio; 95% CI, 95% confidence interval.

three drinking water samples. In these eight disqualified waters, 6–14 most probable number (MPN)/100 mL of total coliforms and 2–9 MPN/100 mL of thermotolerant coliforms were also detected in the one tap water and the four well water samples. EPEC nucleotides were detected in samples from Well 1, and EPEC isolates were also recovered from this sample. Among the 31 stool samples, 14 were EPEC nucleotide-positive and 10 were EAEC nucleotide-positive, whereas only two strains of EPEC and one strain of EAEC were isolated. All water and stool samples were negative for *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia enterocolitica*. Besides, rotaviruses, enteric adenoviruses, NoV GI/GII and astroviruses were not found in these 18 water samples. Among the 31 stool samples and 17 rectal swab samples, 34 NoV RNAs were detected, with 13 belonging to GI, 13 to GII and eight to GI + GII. Rotaviruses, enteric adenoviruses and astroviruses were negative in these samples.

Norovirus phylogenetic analysis

Fourteen NoV RNA fragments were successfully sequenced and used for phylogenetic analysis, three of which belonged to GI.1, four to GI.3, five to GI.6 and two to GII.6. A phylogenetic tree containing the NoV RNA identified in this study is shown in [Figure 2](#). The GI.1 strains isolated in this outbreak were grouped in one clade with strains from Brazil in May 2010 and from Nanjing, China, in April 2014. GI.3 strains were grouped in one clade with strains from Brazil in 2014. GI.6 strains were grouped in one clade with strains from Nanning, China, in 2011. GII.6 strains were grouped in the same clade with strains from the Netherlands in 2014 and from Taiwan, China, in December 2013 and March 2014 ([Figure 2](#)).

DISCUSSION

Here, we report a gastroenteritis outbreak that occurred among college students in northwest China in early summer 2014. The retrospective survey showed that the index case appeared on June 1 and the case number peaked on June 9. Environmental investigation showed that the sanitary conditions around Wells 1 and 2 were

poor. RR analysis showed that drinking water from Wells 1 and 2 and living in north dormitory building 1 had high relevance in this outbreak, and the attack rate in the students of the control group was under 1%. Sanitary indices showed that the four well water samples, as well as most of the tap water and direct drinking water samples, did not meet drinking water standards, and EPEC was detected and isolated from Well 1. Food poisoning was excluded, and the outbreak ceased 3 days after use of the water supply from the wells stopped. On June 20, this school began the summer holiday in advance. The well field was cleaned thoroughly and cemented, and the well platform was raised to 60 cm above the ground; an automated chlorinator was also installed for each well. At the end of July, the well water samples all met drinking water standards. In the first week of the new semester in August, the attack rate of gastroenteritis at this school was below the normal level. It is tempting to speculate that contaminated water was the source and that this was a water-borne AGE outbreak.

The main symptoms of the patients in this outbreak included diarrhea, stomach ache, nausea, and vomiting. NoV RNA was detected in 70.8% of patient stool samples. The epidemiological investigation and test results strongly suggested that this outbreak was due to NoV from the contaminated water. In the stool specimens from this outbreak, the NoV strains showed great diversity, and 13 GI, 13 GII and eight mixtures of both genotypes were detected in 34 samples. In the 14 successfully recovered sequences, four genotypes of NoV were found, and the strains were similar to strains that have recently been circulating globally. The diversity of NoVs was probably from different wastes of contaminated water and the microbial monitoring showed that the well water was contaminated by feces. Fecal contamination of water often involves multiple norovirus strains ([Centers for Disease and Prevention 2007](#)). NoV is present at high levels in wastewater, even after treatment processes, and is the predominant cause of viral, water-borne outbreaks of AGE, especially in viral drinking-water outbreaks ([da Silva *et al.* 2007](#); [Craun *et al.* 2010](#)). Previous studies have reported that water-borne NoV outbreaks are predominantly due to GI, but there have also been several studies reporting water-borne infections due to mixtures of NoV strains and in large water-borne outbreaks more than

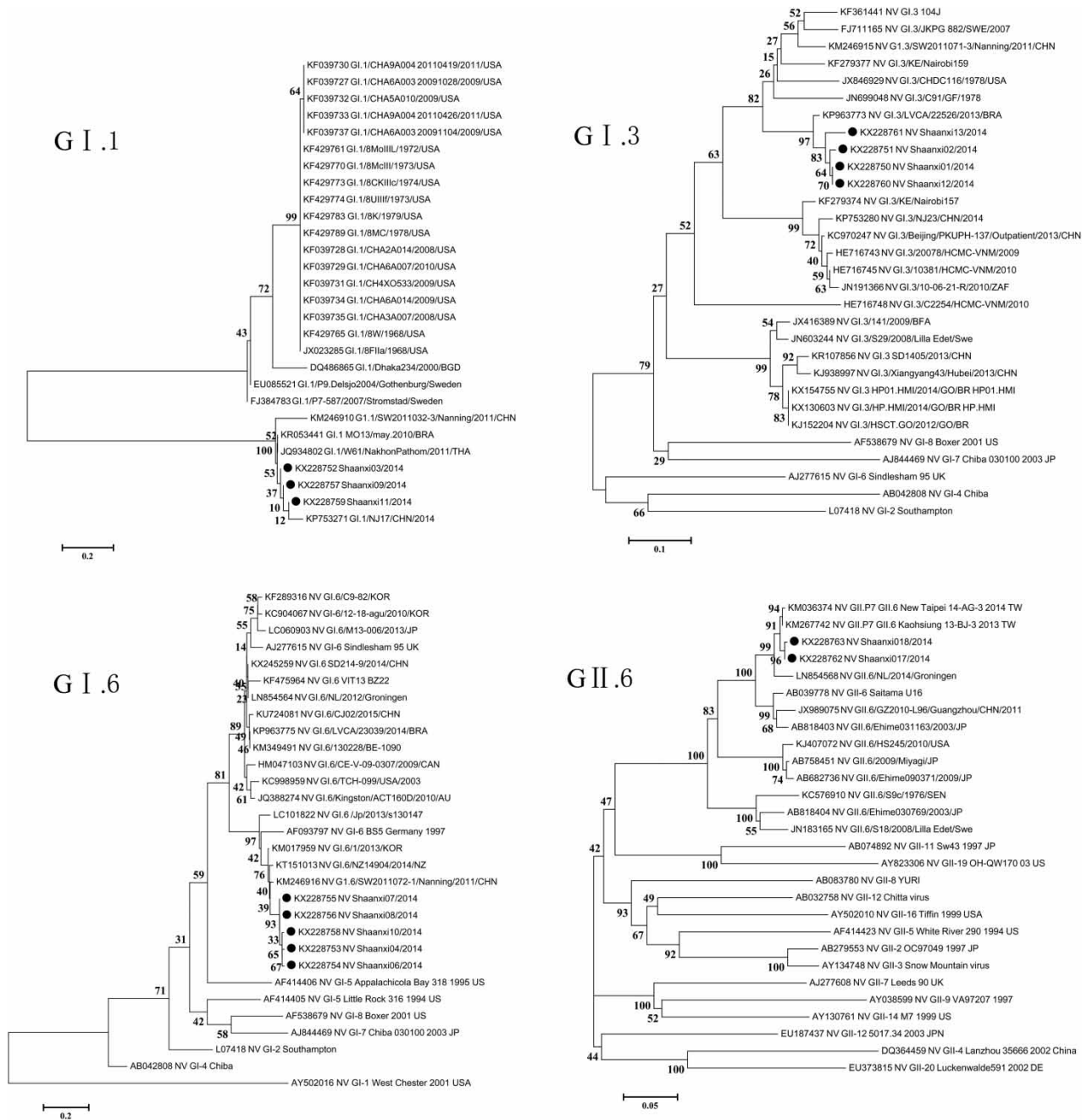


Figure 2 | Phylogenetic tree based on nucleotide sequences obtained in gastroenteritis outbreak on a campus in the northwest of China in early summer of 2014 and references strains. Phylogenetic analysis was performed with a 301 base pair sequence of 5' end of region C in OFR2 for GI and with a 1,643 bp of OFR2 for GII. Mega 6.0 software was used to construct phylogenetic trees using the neighbor-joining method with 1,000 bootstrap replicates. Sequences obtained in this study are labeled with ● and GenBank accession numbers of the sequences were listed in front.

one virus strains were more often found (Gray *et al.* 1997; Kukkula *et al.* 1999; Maunula *et al.* 2005).

According to the national standards for drinking water quality of China, microbial monitoring showed that aerobic

bacterial count of eight water samples disqualified, and total coliforms, thermotolerant coliforms and EPEC were found in these eight samples. These results indicated that the wells were not effectively disinfected, and showed the

poor cleanliness and integrity of distribution systems, and implied fecal contamination (Ashbolt *et al.* 2001). In this outbreak, bacterial pathogens including *Salmonella*, *Shigella*, *Campylobacter* and *Yersinia enterocolitica*, and viral pathogens containing rotaviruses, enteric adenoviruses and astroviruses were not found in any samples. EPEC was detected and isolated in both well 1 and the stool samples from patients, while EAEC was only found in stool of patients. EPEC is most commonly associated with acute secretory even persistent diarrhea, sometimes causing anorexia, low fever, and rapid wasting in infants less than two years old, especially in developing countries (Ochoa & Contreras 2011). EAEC was first described in 1985 and is mainly associated with persistent watery diarrhea lasting for more than 2 weeks in infants, immunosuppressive patients and travelers (Harrington *et al.* 2006; Flores & Okhuysen 2009). In this outbreak, all the patients were over 17 years old, and the duration of illness mainly lasted for 2 days, thus EPEC and EAEC were not considered as the pathogen of this outbreak.

There has historically been a consensus that NoV outbreaks mainly occur in institutional settings during the winter months, referred to as 'winter vomiting disease', but an increasing number of reports show that NoV gastroenteritis outbreaks can occur in both the spring and early summer as well (Mounts *et al.* 2000; Zhou *et al.* 2012). Therefore, it should be considered that NoV outbreaks can occur throughout the year.

The public health response was inadequate at this non-governmental college. Retrospective investigation indicated that the index case presented on June 1 and that the outbreak was raging on the monitoring system until the XACDC discovered it. Furthermore, the outbreak spread rapidly until implementation of effective control measures. This school is located in a rural area far from municipal medical facilities; therefore, almost every patient came to the school clinic for medical assistance, but this did not alert the school administrators to a possible disease outbreak. Therefore, it is essential to improve public health emergency alertness, especially in non-governmental institutions.

A limitation of this report is that we did not obtain positive results for NoV RNA from water samples because of the lack of a large-volume super-high speed centrifuge or other virus concentration devices.

CONCLUSIONS

Here we report an AGE outbreak that occurred in young adults in northwest China in early summer 2014. Our study found an association between contaminated well water and the outbreak of AGE at a college. Early identification of the potential cause of this outbreak was necessary to prevent new cases. This study implied that NoV outbreaks can occur throughout the year; thus, control measures should be adjusted accordingly.

ACKNOWLEDGEMENTS

We thank the staff from XACDC and Chang'an CDC for the epidemiological and clinical data collection.

CONFLICT OF INTEREST

No conflict of interest declared.

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First received 5 September 2017; accepted in revised form 27 February 2018. Available online 25 April 2018