

A waterborne outbreak of epidemic diarrhea due to group A rotavirus in Malatya, Turkey

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SUMMARY

We characterized an outbreak of acute diarrheal disease caused by group A rotavirus that occurred during the Autumn of 2005 in Malatya City, Turkey. A total 9907 patients between 0 to 91 years old (mean age: 25.05±19.67) were included in the epidemic. The patients' data were prospectively collected and statistically analyzed. Microbiologic analyses were performed to determine the etiologic agent. Rapid onset diarrhea (98.36%), abdominal cramps (69%), fever (44.4%) and vomiting (69.6%) were the most common symptoms observed in patients. Rotavirus antigen was detected in 52.7% of the studied patients. RT-PCR analysis led to identification of Group A rotavirus as the causative agent of this epidemic. Simultaneous measurements of the drinking water samples yielded very low chlorine levels; as low as 0 to 0.05 mg/L. The outbreak investigation team indicated possible contamination of a large water depository from a water well, which supplies drinking water to two major districts of the city. Effective chlorination and blockage of the passage between the well and the water depository stopped the outbreak. This outbreak shows the high epidemic potency of rotavirus in large human populations, including all age groups, and underlines the importance of water safety in pipeline systems.

KEY WORDS: Rotavirus, Outbreak, Waterborne, Diarrhea, Chlorination

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INTRODUCTION

Rotavirus is a non-enveloped, triple-layered icosahedral virus that is classified as a genus in the Reoviridae family. The virus has a double-stranded RNA genome made up of 11 segments that encode six viral proteins (VP) and six non-structural proteins (NSP). The virus possesses at least three important antigenic specificities based on VP6, VP7 (glycoprotein designated G) and VP4

(protease-sensitive protein P) (Fischer *et al.*, 2004). Seven different rotavirus groups (named A to G) have been characterized in many animal species. Group A rotavirus is the primary human pathogen world-wide, and is the most common cause of rotavirus epidemics and sporadic cases (Parashar *et al.*, 2005). On the other hand, group B and C rotaviruses, also known as swine rotaviruses (Parashar *et al.*, 2005), have been reported in limited geographic regions (Cunliffe *et al.*, 2005; Bern *et al.*, 1992; Fischer *et al.*, 2005). Rotavirus is one of the most frequently reported etiologic agents of severe and dehydrating gastroenteritis. Approximately one-third of all diarrheal pediatric hospital admissions can be attributed to this pathogen, and an estimated

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475,000-580,000 children around the world die of rotavirus infections each year (Carraturo *et al.*, 2008; Parashar *et al.*, 2004). All children will have rotavirus-associated diarrhea at least once during the first five years of life (Matsumoto *et al.*, 1989). The clinical manifestation of a rotavirus infection varies from an asymptomatic to mild infection to severe and sometimes life-threatening disease (Barman *et al.*, 2006).

Although the detailed mechanism of protective immunity against rotavirus is not understood, serogroup-specific immunity is thought to play a major role (Offit *et al.*, 1996). The main strategy for severe rotavirus disease control is vaccination because rotavirus infections show similar frequency patterns throughout the world, regardless of hygiene and development standards (Fischer *et al.*, 2004; Kapikian *et al.*, 2001). Therefore, the value of rotavirus strain surveillance in a community has been currently increasing over time (O'Mahony *et al.*, 1993). Additionally, characterization of rotavirus outbreaks will help to reveal the current magnitude of the rotavirus-mediated health threat to a community, and will guide the development of appropriate policies of prevention and control. This paper presents the clinical and epidemiological characteristics of a large outbreak of waterborne acute diarrheal disease cause by group A rotavirus in Malatya City during the Autumn of 2005. According to our knowledge, this is the largest reported rotavirus outbreak in Turkey.

MATERIALS AND METHODS

Outbreak recognition

On 21 November 2005, two state hospitals, one university hospital, and several primary care centers in Malatya City reported an unusual increase in acute diarrheal disease among the admitted patients coming from two large districts of Malatya. These patients presented with diarrhea (three or more liquid stools over a 24-h period), abdominal pain, fever and vomiting. The number of patients with this set of symptoms progressively increased; approximately 10000 patients with similar clinical symptoms applied to the health care centers by the end of the 14-day period. The Communicable Diseases Department of the Turkish Ministry of Health confirmed this report.

The investigation team, consisting of clinical microbiologists, epidemiologists, infectious disease residents, public health specialists and an environmental engineer was established with the collaboration of Turkey's Health Ministry, Ankara Refik Saydam Reference Laboratory, Inonu University and Malatya Governor and Municipality to investigate the outbreak.

Outbreak area

Malatya City is located on East Anatolian region of Turkey, with an estimated 400,000 urban population. Almost all city inhabitants utilize piped fresh municipal water, which is supplied from a large spring water source in the nearest mountain. This source is located about 15 km from the city center. Despite the high quality drinking-water certificate for the source, the water is treated at the source before distribution to six major water depositories located in different parts of the city. The water is then distributed from these depositories to districts and to the homes with the pipeline system. Additionally, there is one water well (Kernek Water Well) in the south side of the city settlement, which provides water to a major water depository (Inderesi Water Depository) that supplies fresh water to two main districts in the city center, and to three student hostels in the Inonu University Campus settlement.

Data collection

The Health Department collected data from the diarrheic patients, including age, sex, localization, major symptoms, duration of illness and travel history, who applied to any health institute between 21 November and 04 December 2005. The patients whose diarrheic symptoms were explained by infection with a usual pathogen, including invasive or toxigenic *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter*, *Clostridium difficile*, *Vibrio cholerae*, *Giardia intestinalis* and *Entamoeba histolytica*, were excluded from the outbreak.

Laboratory study

Water analysis

The chemical and microbiologic quality controls of the drinking water in the pipeline system are routinely performed in Public Health Laboratory weekly.

During the outbreak, an average of 100 pipeline

water samples were collected per day from different districts of the city and subjected to analysis. Water samples were cultured in brilliant-green broth with 2% bile and lactose broth (Oxoid, UK) to assess the presence of coliform bacillus. Chemical parameters, including the Free Chlorine Concentration (FCC) of the water samples, were analyzed in the Public Health Laboratory. Since we did not have laboratory facilities to concentrate rotavirus in pipeline water, rotavirus could not be searched for in the water samples. During the outbreak, collected water samples were transported to Refik Saydam Reference Laboratory for further chemical analysis and confirmatory bacteriological tests. But they did not do any test for detection rotavirus in water samples.

Stool samples

Approximately 3250 stool samples (one sample per patient) were studied in different health institutes during the outbreak. In addition to bacteriologic cultures, these samples were also microscopically examined by the direct wet mount method to evaluate the presence of diarrhea-causing parasites. Trichrome and acid-fast staining were also performed in ambiguous cases.

Rotavirus antigen detection

On 27 November 2005, the Health Ministry supplied chromatographic immunoassay rotavirus antigen detection kits. CerTest Rotavirus (CT BioTec SL, Spain) and Vikia Rota-Adeno Combo (Bio-Mérieux, France) kits were used in the various health institutes. The procedures were performed according to the directions of the kit manufacturers. The fecal samples with detectable rotavirus antigen were stored at -40°C until they were submitted to molecular analysis.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Fecal samples collected from the outbreak patients were subjected to reverse transcription-polymerase chain reaction assay for amplification of the VP9 gene of group A rotavirus. Viral RNA was extracted from the samples by the methods described by Watanabe (Watanabe *et al.*, 2001) The RT-PCR assay was carried out by the methods described by Gouvea (Gouvea *et al.*, 1990). The amplification conditions were as fol-

lows: an initial 30-min step at 42°C , followed by 25 cycles at 94°C for 60 s, 42°C for 120 s, and 72°C for 60 s with an additional 7 min as a final extension step at 72°C . The amplification product was electrophoresed through a 2% agarose gel, stained with ethidium bromide, and visualized under UV light. A 1062-base pair product which is specific for group A rotavirus was observed.

Statistical analysis

Full data were collected for 3329 patients. These data were statistically analyzed with SPSS 15.0 (SPSS, US) computer software. Pearson's chi-square method was used to compare the frequencies of the disease symptoms between the patients under 5 years of age and those older than 5 years. A $P < 0.05$ was accepted as indicating statistical significance.

RESULTS

Outbreak characteristics

From 21 November to 04 December 2005, 9907 patients applied to a health center due to acute diarrheic illness. In addition to the initial cases, most of the following patients were reported in student hostels located in the Inonu University's Campus, and from two large districts, each consisting of a large human population and several office buildings with many employees. Regarding the city locations, 3770 patients came from these two districts and 350 student patients from the hostels. Additionally, the highest attack rates were recorded in these districts, 1/46 and 1/66, respectively, and the hostels, 1/5. The attack rate distribution of the epidemic with respect to the districts of Malatya City is illustrated in Figure 1.

Regarding the previous year, 2004, official data showed that 369 diarrheic patients were recorded from 21 November to 04 December. Figure 2 compares the daily variation of patients who admitted to a health institute with acute diarrhea during corresponding times in 2004 and in the outbreak year, 2005.

Very low FCC levels may have facilitated the epidemic spread of the pathogen until 26 November 2005. After this date, FCC levels markedly increased in the pipeline water and the incidence of the outbreak progressively decreased to the expected normal level by 04 December 2005.

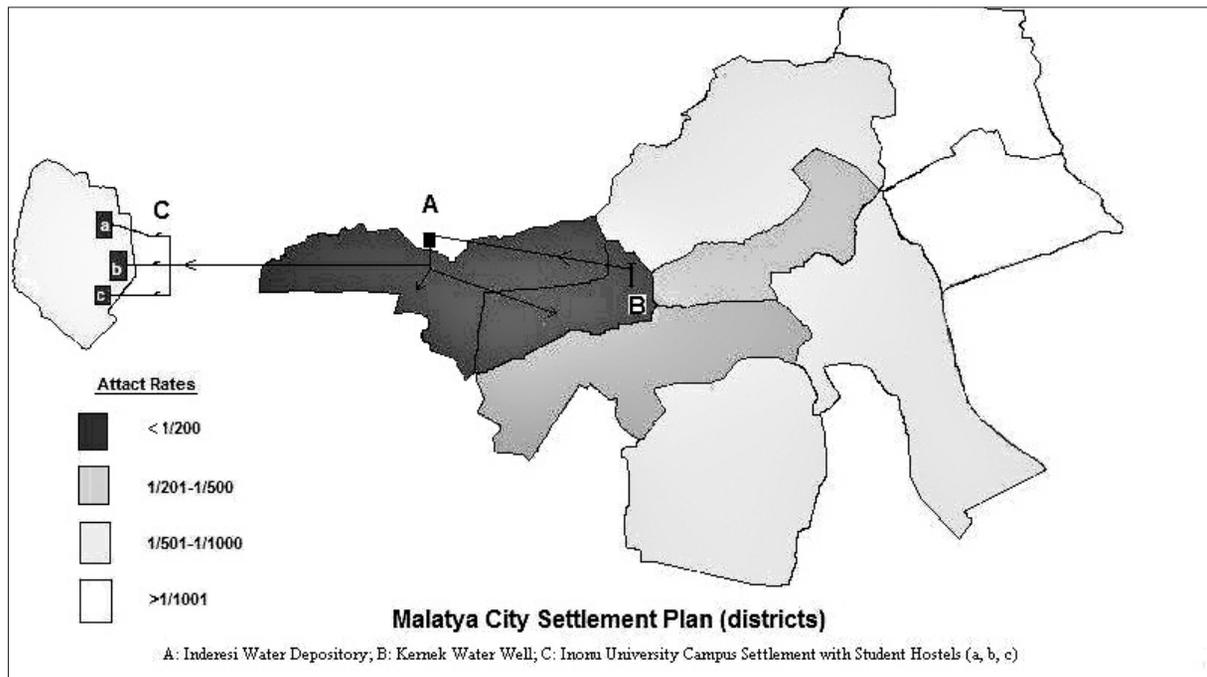


FIGURE 1 - Attack rate distribution of the epidemic according to the major districts of Malatya. The highest rates were recorded from the supply area of the Inderesi Water Depository. A relatively lower rate was obtained from the Inonu University Campus, which has its own water supply system, except for three student hostels that are supplied by the Inderesi Water Depository.

Patients' characteristics

Regarding the patients' ages, a relatively lower number of young children (2702/27.2%) were included in the epidemic. The mean age of the outbreak patients was 25.05 ± 19.67 years. The major

clinical symptoms were diarrhea (98.36%), abdominal cramps (69%), fever (44.4%), vomiting (69.6%) and headache (for adults) (64.7%). The disease was significantly more symptomatic in young children under 5 years of age. Table 1

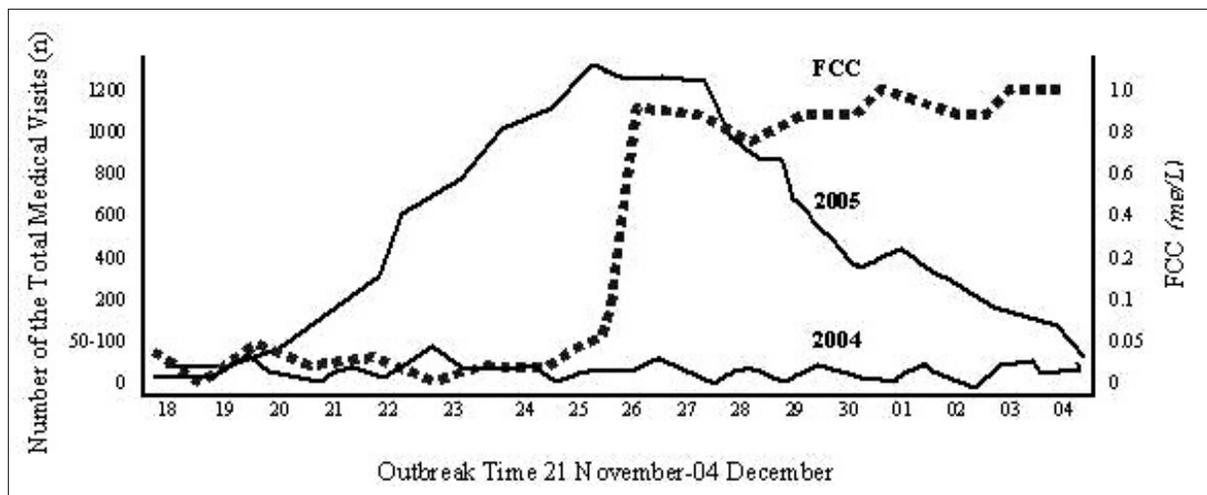


FIGURE 2 - Comparison of the incidence of diarrhetic patients between the epidemic year, 2005, with the previous year, and the daily variation of Free Chlorine Concentration (FCC) levels of the pipeline water during the outbreak period.

TABLE 1 - Characteristics of the outbreak patients and comparison of disease symptoms across patient age groups.

Mean Age (n=9907)	25.05±19.67		
Sex (F/M)	%51.4 / %48.6		
<i>Distribution of patients' characteristics according to age group (n=3329)</i>			
Age Groups	≤5 (n=593 / %)	>5 (n=2736 / %)	P* (<0.05)
Dehydration	140/23.6	103/3.8	<0.05
Fever	516/87	965/35.3	<0.05
Vomiting	506/85.3	1814/66.3	<0.05
Abdominal cramps	485/81.8	1813/66.3	<0.05
Headache	-	1771/64.7	-
Hospitalization	193/32.5	83/3	<0.05
Positive stool test	493/83.1	1432/53.3	<0.05

shows the characteristics of the outbreak patients and a comparison of the disease symptoms across patient age groups.

Water analysis

A total of 2931 water samples were received and analyzed in the Public Health Laboratory during the outbreak. Coliform bacilli growth were detected in only 26 water samples collected from different locations, which were not included the 2 affected districts, in the city settlements. However, certain intestinal pathogens, including *Salmonella*, *Shigella*, *Vibrio cholerae*, *Campylobacter* and toxigenic *E. coli*, did not grow from any of the samples. Chemical measurements of the water samples yielded normal ranges for many parameters, with the exception of FCC, which was as low as 0 to 0.05 mg/L in most districts (Figure 2).

Laboratory analysis of the stool samples

A total of 3250 stool samples were studied in the microbiology laboratories of various health institutes. Bacteriologic cultures were not indicative of a specific diagnosis. Stained and direct wet mount preparations were also negative for common pathogens. Erythrocytes were seen in 105

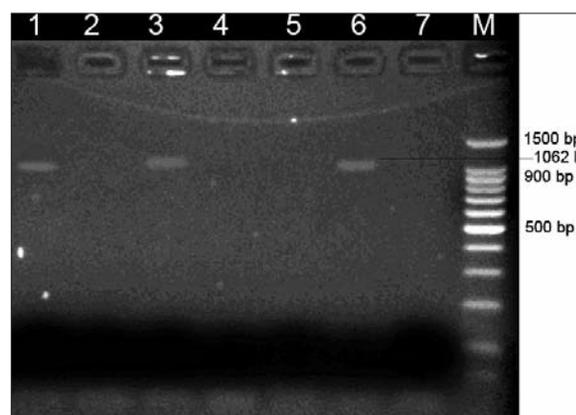


FIGURE 3 - An example of positive RT-PCR assay. Line 1 positive control, line 2 negative control, lines 3 and 6 positive stool specimens, lines 4, 5, and 7 negative stool specimens. M molecular weight marker.

samples and a few neutrophils were reported in samples obtained from 49 patients.

A total of 1714 (52.7%) of the 3250 screened diarrheic stool samples were found to be positive for rotavirus antigen in a chromatographic rotavirus antigen immunoassay. Furthermore, RT-PCR resulted in amplification of a 1062-bp DNA band that is indicative of Group A rotavirus in 33 of the 38 tested fecal samples. The PCR product photographed under UV light is shown in Figure 3.

DISCUSSION

Group A rotavirus is the leading causative agent of viral gastroenteritis in young children all over the world. Group A is detected by most commercial antigen detection kits. The virus is mainly transmitted via the fecal-oral route. Its very low concentration in secretions may be adequate to cause infection. After ingestion, rotavirus particles reach the small intestine and infect the intestinal epithelium. Following lytic replication in mature enterocytes, newly formed rotavirus particles can infect distal portions of the small intestine or be excreted in the feces (Anderson *et al.*, 2004). The virus can remain stable in environmental conditions for a long time. Fischer (Fischer *et al.*, 2002) reported that storage in ambient tropical temperatures (30°C), rotavirus particles can survive for more than 2 months and can maintain infectivity for more than 32 months

at a relatively low temperature, i.e., 10°C. Additionally, Caballero (Caballero *et al.*, 2004) reported that no significant decrease in the activity of infectious rotavirus was detected following storage for one month in seawater at 20°C. As the virus decay rate significantly increases at higher temperatures, the great majority of rotavirus infections occur in cold seasons (Kapikian *et al.*, 2001). In agreement with these data, the present outbreak was observed in the autumn-winter season, which generally has weather temperatures lower than 10°C in the daytime.

One of the largest group A rotavirus outbreaks was reported from Nicaragua in the spring 2005, affecting more than 64000 people and causing at least 56 deaths. This epidemic was caused by a mutant G4P[8] strain (Bucardo *et al.*, 2005). In Europe, a large waterborne epidemic caused by multiple emerging group A rotavirus genotypes was reported in Tirana, Albania. In total, 2722 children were seen in Tirana Hospital and 982 required hospital treatment for acute gastroenteritis (Villena *et al.*, 2003). In the present outbreak, we estimate that at least 20000 people were affected by the epidemic pathogen. In this estimation, we assume that the patients with less symptomatic disease did not apply to any health-care center, and that those who traveled from Malatya during the outbreak were treated outside the city.

Environmental transmission of rotavirus occurs mainly through shellfish grown in polluted waters and contaminated drinking water. Therefore, disinfection is an important infection control strategy for consumers, but current virus disinfection and removal practices often do not achieve adequate elimination of pathogenic viruses (Caballero *et al.*, 2004). According to a retrospective cohort study in France, a multi-pathogen waterborne outbreak following fecal contamination of groundwater occurred because of a chlorination system failure. In this French epidemic, a total 345 patients were affected and group A rotavirus was identified to be responsible for 71% of all infections (Gallay *et al.*, 2006). It has been further reported by Caballero (Caballero *et al.*, 2004) that chlorination and UV irradiation can effectively eliminate infectious rotavirus particles with sufficient contact time. More specifically, 30 min of contact with 0.2 mg/L free chlorine or 20 sec exposure to 200-220 mJ/cm² UV irradiation

yields 99.99% elimination of infective rotaviruses in the water samples.

Since the outbreak reported in this study showed a rapid and wide distribution pattern, the investigation team indicated that the outbreak was most likely caused by a waterborne pathogen. Therefore, the water distribution program of the water department was reviewed for one month retrospectively. According to the obtained data, water interruption was frequent at the main water source because of intense substructure work in the west side of the city.

To supply required water to the east side settlements, the municipality's water department authorized use of a water pump at the well of the water depository. In this way, approximately 60 L/s of untreated water was offered for utilization from 18 November 2005. Furthermore, undetectable low-level FCCs were measured in the drinking water samples during the outbreak. Therefore, the epidemic pathogen was easily spread in the piped municipality water system without exposure to any elimination process. Regarding environmental hygiene and public health, these results underscore the need for sufficient and appropriate application of the disinfection methods to prevent the dissemination and storage of virus particles in the environment.

In the present outbreak, most of the patients with severe disease were under 5 years of age. Almost 70% of the patients who required hospital treatment were infants. Furthermore, approximately one-fourth of the young children developed mild to moderate dehydration due to liquid loss because of severe diarrhea associated with vomiting. Fortunately, no death occurred. The classic target population of group A rotavirus is young children. However, more than half (5804/58.5%) of the epidemic patients were over 12 years of age. Although many physicians presume that healthy adults are not victims and that rotavirus infection will confer life-long immunity, many investigations show that re-infection can occur (Anderson *et al.*, 2004; Griffin *et al.*, 2002; Velazquez *et al.*, 1996). Although almost all adults have antibodies to rotavirus, they might still be susceptible to infection (Anderson *et al.*, 2004). Recurrent rotavirus infections have been reported to occur due to a number of reasons, depending on the viral particle and the host. There are many groups, subgroups and serotypes of the

virus, and the preliminary antibody response to disease is serotype-specific with little cross-protection (Jiang *et al.*, 2002). Second, rotaviruses have extensive antigenic and genomic diversity (Barman *et al.*, 2006), and epidemiologic shift of group A rotavirus over the years has been reported (Iturriza-Gomara *et al.*, 1995). Additionally, mutant strains of rotavirus have been shown to cause large outbreaks (Bucardo *et al.*, 2007). Some host factors have been reported to result in recurrent adult infections, such as a short-lived rotavirus-specific immune response (Anderson *et al.*, 2004), low rotavirus-specific secretory IgA levels in feces (undetectable levels as early as 1 year after infection) (Coulson *et al.*, 1992), and significantly decreased circulating levels of protective antibody (undetectable levels by the age 70 years) (Elias *et al.*, 1977).

At the beginning of the outbreak, some investigators hypothesized that the epidemic was possibly due to enteric bacteria, since coliform bacillus growth was detected in a few water samples. However, the investigation team declared that the coliform growth in the water samples might be considered an indicator of fecal contamination, as the locations of the positive cultures showed a non-specific distribution pattern over the city. Additionally, the clinical characteristics of the outbreak patients and the laboratory findings were irrelevant. Furthermore, these positive cultures were expected due to the very low FCC level in the water. Similarly, some researchers suggested that the outbreak was most likely due to amebic spread, as erythrocytes were present in a few stool samples. However, in addition to the clinical manifestation of the disease, no positive test supported this suggestion.

During the outbreak, almost all prospective bacterial and parasitic pathogens were studied, but few viral pathogens were investigated. In addition to rotavirus, only adenovirus was investigated during the outbreak period. Adenovirus was not detected in the epidemic patient samples. Rotavirus antigen was detected in 52.7% of the patients' stool samples by use of chromatographic immunoassay rotavirus antigen detection kits. Although enzyme immunoassay methods have been routinely used for rotavirus detection in stool specimens, it was indicated that immunochromatographic tests used in our study can also be useful for rotavirus detection in stool specimens

(Bon *et al.*, 2007). In addition to antigen positivity, group-A rotavirus specific RT-PCR also confirmed this waterborne outbreak. However, we could not detect rotavirus RNA in five of 38 samples analyzed with RT-PCR. We think that these PCR-negative samples were most likely due to viral elimination during more than two years of storage in the freezer, without adding any preservative. Additionally, because of no available laboratory equipment for concentration of virus, no analysis of rotavirus was performed for piped and well water samples.

In conclusion, this paper reports a large waterborne rotavirus epidemic. To our knowledge, this is the largest rotavirus outbreak in Turkey as well as in Europe. Although the majority of the patients were adults, the disease symptoms were relatively more severe in infants. The investigation team suggested that the ineffective chlorination of water and subsequent consumption of the untreated contaminated water caused this outbreak. This study shows the ability of rotavirus to cause waterborne acute diarrhea epidemics affecting large human populations and underlines the importance of water hygiene in the pipeline system.

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