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Research on the contamination levels of norovirus in food facilities using groundwater in South Korea, 2015–2016

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ABSTRACT

Norovirus (NoV) is a major pathogenic virus that is responsible for foodborne and waterborne gastroenteritis outbreaks. Groundwater is an important source of drinking water and is used in agriculture and food manufacturing processes. This study investigated norovirus contamination of groundwater treatment systems at 1360 sites in seven metropolitan areas and nine provinces in 2015–2016. Temperature, pH, residual chlorine, and turbidity content were assessed to analyze the water quality. In 2015, six sites were positive for the presence of NoV (0.88%) and in 2016, two sites were positive (0.29%); in total, NoV was detected in 8 of the 1360 sample sites (0.59%) investigated. Identified genotypes of NoV in groundwater included GI.5, 9 and GII.4, 6, 13, 17, and 21. GII.17 was the most prevalent genotype in treated groundwater used in the food industry. This dominance of GII.17 was corroborated by NoV infection outbreak cases and the results of a survey of coastal waters in South Korea in 2014–2015. Although a low detection rate was observed in this study, NoV is a pathogen that can spread extensively. Therefore, it is necessary to periodically monitor levels of norovirus which is responsible for food poisoning in groundwater. This is a first report to reveal epidemic genotype shift of norovirus in groundwater treatment system of food facilities in South Korea. Our results may contribute to the enhancement of public health and sanitary conditions by providing molecular epidemiological information on groundwater NoV.

1. Introduction

Norovirus (NoV) is the leading cause of epidemic and sporadic gastroenteritis outbreaks worldwide, affecting all age groups and is responsible for approximately 90% of all viral gastroenteritis outbreaks (Lee et al., 2013b). Each year, mortality due to NoV infection is estimated at 570–800 deaths in the United States, with approximately $777 million spent in health care (Lee et al., 2015). NoV is a genus within the Caliciviridae family. The individual virus is a small non-enveloped virus with a positive-sense single-stranded RNA genome of 7.5–7.7 kb organized into three open reading frames (ORFs). ORF1 encodes six non-structural proteins, including RNA-dependent RNA polymerase (RdRP). ORF2 and ORF3 encode VP1 and VP2 capsid proteins, respectively. NoV is classified into 6 genogroups (GI–GVI) that are subdivided into at least 38 genotypes based on their cap and pol genes (Bruggink et al., 2015; Rahman et al., 2016). Strains of genogroups GI, GII, and GIV infect humans (Kim et al., 2016). Since establishing a reverse transcription-polymerase chain reaction (RT-PCR) method to target NoV in the 1990s, NoV was discovered as one of the most common causes of food and waterborne gastroenteritis outbreaks worldwide (Koh et al., 2011). Analysis of NoV genotypes is important for thorough management and control of NoV infections, as it can help in understanding how NoV circulates throughout the community (Bruggink et al., 2015). NoV tends to spread easily due to its characteristics, such as extremely low infectious doses of 18 to 1000 viral particles, great genetic diversity leading to a lack of viral cross protection, high resistance to disinfection, and strong stability against various environmental stressors (Lee et al., 2011a). Moreover, NoV is resistant to many industrial food preservation methods and can survive chilling, freezing, acidification, reduced water activity, and modified atmosphere packaging (Baert et al., 2009).

Previous studies on NoV outbreaks showed that transmission is via the fecal–oral route, consisting of direct person-to-person contact (88%), food ingestion (10%), and water intake (1.5%) (Kim et al., 2016). Particularly, the rapid and extensive spread of viral pathogens can be promoted by various aquatic environments, such as sewage, rivers, ocean water, tap water, and groundwater. Several cases of broad-range contamination by human enteric viruses have been reported in South Korean water resources (Lee et al., 2015). The United States Centers for Disease Control and Prevention (CDC) reported that

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viruses, including NoV, were the etiologic agents responsible for 44.4% of drinking water-associated gastroenteritis outbreaks from 2005 to 2006 in the United States, suggesting that viruses play a significant role in waterborne diseases (Jung et al., 2011). As the most common water quality parameter, bacterial indicators (*Enterococcus* spp. and *Escherichia coli*) are limited in properly representing viral pathogen contamination. While bacteria usually require greater quantities to be infectious, only small quantities of enteric viruses are required to disease; therefore, surveillance of environmental water for viral contamination is necessary.

Groundwater is an important source of drinking water and is used for industrial and household purposes in both developing and developed countries since it is commonly available in many regions. In USA and South Korea, 20% and 11% of the total water supply, respectively, originates from groundwater (Lee et al., 2011b). Even though natural filtration through the soil during gravitational movements reduces the risk of viral contamination, NoV was detectable in groundwater at 12 °C for 728 days by conventional PCR (Seitz et al., 2011). Moreover, numerous NoV outbreaks due to contaminated groundwater have been consistently reported in many regions worldwide (Gabrieli et al., 2009; Giammanco et al., 2014). The "Waterborne and Foodborne Viruses" consortium, organized by the Korean government, generally conducts national surveys to determine NoV levels in groundwater for food factory (Lee et al., 2014). In South Korea, the use of groundwater in food manufacturing processes has frequently led to NoV food poisoning; recent examples of large-scale outbreaks include NoV infection in 194 students in Jeju-do in May 2004 and in approximately 3000 people after eating at a food service facility in a metropolitan area in September 2006 (Lee et al., 2014). Although groundwater is a major source of NoV outbreaks in the community, little information exists on NoV contamination levels of food-catering facilities. In this study, we investigated NoV contamination of groundwater in groundwater treatment systems. This ground water was used in the food industry in South Korea in 2015–2016. In addition, we performed molecular characterizations of NoV found during the surveillance.

2. Materials and methods

2.1. Water sample collection and processing

Groundwater was sampled from a total of 1360 sites in seven metropolitan areas (Seoul, Incheon, Daejeon, Gwangju, Daegu, Ulsan, and Busan) and nine provinces (Gyeonggi-do, Gangwon-do, Chungcheognam-do, Chungcheongbuk-do, Gyeongsangbuk-do, Gyeongsangnam-do, Jeollabuk-do, Jeollanam-do, and Jeju-island) in South Korea from 2015 to 2016 (Fig. 1). Water sampling and concentration determination of the concentrations of the viral particles were conducted according to standard procedures (Lee et al., 2011b). The sampler consisted of a flow meter, pressure gauge, tubing, cartridge filter housing (3 M, Maplewood, MM, USA) and NanoCeram cartridge filter (Argonide, Sanford, FL, USA), and was assembled through a hole directly connected to groundwater supply. Approximately 1500 L of groundwater were sampled at a constant flow rate (10 L/min). The entire sampling device was autoclaved prior to usage. After sampling, the cartridge filter was removed from its housing and immediately stored at 4 °C. The sampling filter was then completely submerged into 0.5 L sterilized beef extract solution (1.5% beef extract, pH 9.5, and 0.375% glycine) and incubated for 5 min, thereby, extracting the virus into the solution. After the filter was removed, the pH of the eluate was adjusted to 3.5 ± 0.1 by 1 M HCl. The eluate was then incubated for 30 min with stirring at room temperature followed by centrifugation at 2500 ×*g* at 4 °C for 15 min. The pellet was resuspended in 20 mL of 0.15 M sodium phosphate solution (adjusted to pH 9.3), followed by incubation for 10 min at room temperature. The suspension was centrifugated at 7000 ×g and 4 °C for 10 min and filtered through a 0.45 μm syringe filter. The pH of the filtrate was adjusted to 7.3 ± 0.1, and the filtrate was stored at −70 °C.

2.2. Examination of water quality by analyzing physical-chemical parameters

Physicochemical parameters of groundwater samples were analyzed on-site (Lee et al., 2013a). Water temperature (°C) and pH were measured using portable electrode-carrying devices (pH-208; Lutron Electronic Enterprise Co. Ltd., Taipei, Taiwan). Residual chlorine and turbidity (NTU, nephelometric turbidity unit) were measured using an HI 95701C Photometer and HI 93703 Portable Microprocessor Turbidity Meter (HANNA Instruments, Woonsocket, RI, USA).

2.3. RNA extraction and nucleic acid amplification

Twenty milliliters of the eluate obtained on passing water through the NanoCeram filter was used. Viral RNA was extracted from 280 μL of the concentrate using a QIAamp viral RNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. RNA extracted (60 μL) was stored at −70 °C.

RT-PCR was conducted with a previously described primer set (GI-FIM/GI-RIM and GI-F2/GI-RIM for NoV GI; GII-FIM/GII-RIM and GII-F3M/GII-RIM for NoV GII; Table 1) (Lee et al., 2013a; Kim et al., 2016). Extracted RNA was first amplified using Verso 1-step RT-PCR ReddyMix kit (Thermo Fisher Scientific, Waltham, MA, USA) with 5 μL of template according to the following protocol: an initial RT step at 45 °C for 30 min, followed by PCR amplification at 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s, followed by a final extension step at 72 °C for 7 min. A semi-nested PCR amplification procedure was then performed using the first-round amplicon. The amplicon (2 μL) was added to 48 μL of a PCR reaction mixture containing 5 μL reaction buffer (10×; Bioneer, Daejeon, Korea), 4 μL dNTPs (10 mM; Bioneer), 2.5 μL forward and reverse primers (20 pmol), 1 μL Top DNA polymerase (Bioneer), and 33 μL distilled water. The semi-nested PCR protocol was as follows: 94 °C for 5 min, followed by 25 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s, followed by a final extension step at 72 °C for 7 min. Amplification products were confirmed by 2% agarose gel electrophoresis and further characterized by DNA sequencing. All PCR products were sequenced using an ABI Prism 3500 automatic sequencer and BigDye Terminator cycle sequencing mix (Invitrogen, Carlsbad, CA) as manufacturer described.

2.4. Phylogenetic analysis

MegAlign software (Lasergene, version 5.07; DNAstar, Madison, WI, USA) was used for phylogenetic analysis. To confirm genotypes of NoV, the CLUSTALW algorithm was used for DNA sequence alignment and construction of dendrograms (Fig. 2). In addition, the sub-clustering of GII.4 strains was performed by using Norovirus Typing Tool Version 2.0 (https://www.rivm.nl/mpf/typingtool/norovirus).

2.5. Nucleotide sequence accession number

Nucleotide sequences of NoV isolates from groundwater were submitted to the GenBank database under the following accession numbers, MF599259–MF599272 and KY982587.

3. Results

3.1. Location and seasonal pattern of NoV detection

In the present study, NoV was detected in 6 of the total 680 sites sampled in 2015 (0.88%) and 2 of the total 680 sites in 2016 (0.29%); in total, NoV was detected in 8 of the 1360 sites sampled (0.59%). In particular, Gangwon-do, located relatively north, exhibited higher NoV detection rates at 13% ($n = 40$) (Fig. 1). One sample from each south