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Investigation of norovirus occurrence in groundwater in metropolitan Seoul, Korea

Heetae Lee [a], Misoon Kim [a], Jung Eun Lee [a], MiYoung Lim [a], MinJung Kim [a], Ju-Mi Kim [a], Weon-Hwa Jheong [c], Jongmin Kim [c], GwangPyo Ko [a,b,*]

[a] Department of Environmental Health, Institute for Health and Environment, School of Public Health, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul, 151–742, Republic of Korea
[b] Institute for Microbiology, School of Life Science, Seoul National University, Seoul, 110–799, Republic of Korea
[c] Drinking Water Research Division, Water Environmental Research Department, National Institute of Environmental Research, Incheon, 404–708, Republic of Korea

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ABSTRACT

Groundwater is an important source of drinking and household water worldwide. Hence, the quality of groundwater is very important for preventing waterborne disease outbreaks and should be properly monitored. This study investigated the prevalence of waterborne viruses and fecal indicators in groundwater in metropolitan Seoul and Gyeonggi province, South Korea. A total of 116 samples of groundwater were taken using NanoCeram filters during both summer (June to August) and fall–winter seasons (October to December) in 2008. Among 71 sampling sites, 28 (48.3%) and 18 (35.3%) were positive for norovirus (NoV) from the summer and fall–winter season, respectively. The identified genotypes of NoV include GI-1, 4, 8, 9 and GII-4, 10, 11 (or 17), 13, 15 (or 16). None of fecal indicators was significantly correlated with NoV in groundwater. Among the tested fecal indicators, somatic coliphage (95.3%) showed an excellent true-negative rate of NoV occurrence. The combination of chemical, microbial and viral indicators increased the positive predictive value (50–100%). This study demonstrated a high prevalence of NoV in groundwater in metropolitan Seoul areas and characterized the positive and negative predictive values of a fecal indicator for predicting NoV prevalence.

1. Introduction

Groundwater is an important source of not only drinking water, but also industrial and household water in both developing and developed countries (Chilton, 1996). Groundwater is traditionally considered to be less at risk of contamination by waterborne pathogens due to a natural filtration mechanism by soils during gravitational movement (Quanrud et al., 2003; Yates and Gerba, 1998). It is also commonly available in many regions, relatively easy to develop, low in cost, and requires minimal treatment prior to usage (IAH, 2006; NGWA, 2010). Thus, in the United States, approximately 20% of the total withdrawal of water for use originated from groundwater in 2005 (Kenny et al., 2009). In South Korea, 11% of the total water supply originated from groundwater (K-water, 2005). However, improper management and development have led to fecal contamination of groundwater (Celico et al., 2004; Cho and Kim, 2000; Macler and Merkle, 2000). Waterborne disease outbreaks caused by groundwater contamination have been reported in many regions worldwide (Fong et al., 2007; Gallay et al., 2006; Locas et al., 2007). For example, almost 40 million people per year are estimated to be affected by waterborne outbreaks caused by groundwater contamination in the United States (Reynolds et al., 2008). Therefore, it is very important to properly monitor and maintain the quality of groundwater for public health.

The most commonly applied method for monitoring groundwater is to measure fecal indicator microorganisms, such as total coliform, *Escherichia coli*, *Enterococcus* spp., and coliphage (USEPA, 2000a). Previous studies have reported that approximately 15–86% of groundwater is contaminated with microbial indicators and other waterborne pathogens (Chae et al., 2008; USEPA, 2000b). Viruses are particularly problematic because they can efficiently penetrate soil due to their small size. Bacterial fecal indicators such as fecal coliform and *Enterococcus* spp. are the most commonly applied indicator microorganisms for water quality. However, these bacterial indicator organisms have been criticized for not properly representing viral pathogens (Leclerc et al., 2000). Thus, viral fecal indicators such as somatic and male-specific coliphage have been proposed for monitoring viral pathogens by the USEPA (USEPA, 2000a). However, it is still controversial whether these viral fecal indicators are appropriate as an indicator for viral contamination because previous studies have reported a weak correlation with viral pathogens (Jiang et al., 2007; Locas et al., 2007). Thus, it is important to evaluate whether these fecal indicators would be appropriate for waterborne viral pathogens.

In 2006, foodborne outbreaks associated with NoV occurred in the metropolitan Seoul area and affected approximately 2000 schoolchildren,

* Corresponding author at: Department of Environmental Health, Institute for Health and Environment, School of Public Health, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul, 151–742, Republic of Korea. Tel.: +82 2 880 2731; fax: +82 2 762 9105.
E-mail address: gko@snu.ac.kr (G. Ko).

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which was the largest foodborne outbreak reported in the history of South Korea (KCDC, 2010). During an epidemiological investigation, contaminated groundwater was also suspected of contaminating foods with NoV. In addition, various genotypes of NoV have been found in groundwater in South Korea (Kim et al., 2005; Lee and Kim, 2008). However, the prevalence of viral pathogens including NoV has not yet been fully evaluated in the metropolitan Seoul area in South Korea. The population of metropolitan Seoul and Gyeonggi province is over 20 million, which is almost 50% of the total population in South Korea (KOSIS, 2006). Thus, it is necessary to determine the extent of viral pathogen contamination of groundwater in these areas. The objectives of this study were to investigate the prevalence of enteric viruses including NoV in metropolitan Seoul and Gyeonggi province in South Korea and to evaluate whether any biological and/or chemical fecal indicators can be used for predicting the presence of viral contamination in groundwater.

2. Materials and methods

2.1. Sampling sites

Water was sampled from a total of 71 sites located in metropolitan Seoul and the Gyeonggi providence area in South Korea (Fig. 1). The wells were located at various facilities including schools, military facilities, restaurants, public gardens, and lodging accommodations. These sampling sites were selected because they were considered potentially risky settings for fecal contamination, or because fecal indicators were previously detected. Sampling was attempted twice: in the summer season (June to August) and the fall–winter season (October to December) in 2008. Initially, 58 sites were sampled in the summer. Among them, only 45 were re-sampled in the fall–winter season; the remaining 13 were not due to refusal of compliance. Thus, an additional 13 sampling sites in these areas were chosen and sampled in the fall–winter season. Therefore, a total of 116 samples were taken from 71 sampling for this study. Information on the characteristics of the sampling sites such as depth, age of well, and intake was also collected during sampling.

2.2. Water sampling

The sampler consisted of a flow meter, a pressure gauge, a NanoCeram cartridge filter (Cat. #: VS2.5-10, Argonide, USA), a cartridge filter housing, and tubing, which was manufactured following the USEPA manual (USEPA, 2001a). At most sites, sampling was performed through a hole directly connected to groundwater. At some sites, groundwater was temporarily stored in a tank and stored water was sampled. The flow rate of sampling was 10 liters per min (LPM). Approximately 500 L groundwater was sampled from most sites. At some sites, the water pressure was not high enough to obtain a 500-L sample and thus less was sampled. The entire sampling device was autoclaved prior to field sampling. The valves connected to the sampler were sterilized with 70% alcohol. Actual sampling was performed after 5–10 min of flow. After sampling, the cartridge filter was removed from the housing and immediately stored at 4 °C. Sample analysis such as elution and detection was performed within 12 h of sampling.

2.3. Concentration of groundwater

The virus adsorption-elution (VIRADEL) technique was used in the recovery of NoV, which was evaluated in previous study (Lee et al., 2011). Briefly, the sampled filter was subjected to elution by 1.5% beef extract and 0.05 M glycine (pH 9.5). The cartridge housing was filled with elution buffer and allowed to be in contact for 30 min. Then pressurized nitrogen gas was used to force out the eluent. The eluent was subjected to acid precipitation with 1 M HCl. The precipitant was centrifuged at 2500 *g* at 4 °C for 15 min. The pellet was completely dissolved using 20 mL 0.15 M sodium phosphate (Na_2HPO_4•$7H_2O$, pH 9.0–9.5). The suspension was centrifuged at 7000 *g* at 4 °C for 10 min and the supernatant was carefully collected using a pipette. The processed eluent was adjusted to neutral pH (7.0–7.5) with 1 M HCl. The sample was filtered through a 0.45-μm pore size syringe filter to remove non-viral organisms and stored at −70 °C until analysis.

2.4. RNA extraction and analysis of NoV and other enteroviruses by RT-PCR assay

Viral RNA extraction was performed by using the QIAamp® Viral RNA Mini Kit (Qiagen, USA) according to the manufacturer's instructions. All RT-PCR amplification steps were performed using a Qiagen OneStep RT-PCR Kit (Qiagen, USA). Two previously reported nested RT-PCR assays were used for each genogroup (GI and II) of NoV (Hafliger et al., 1997; Kim

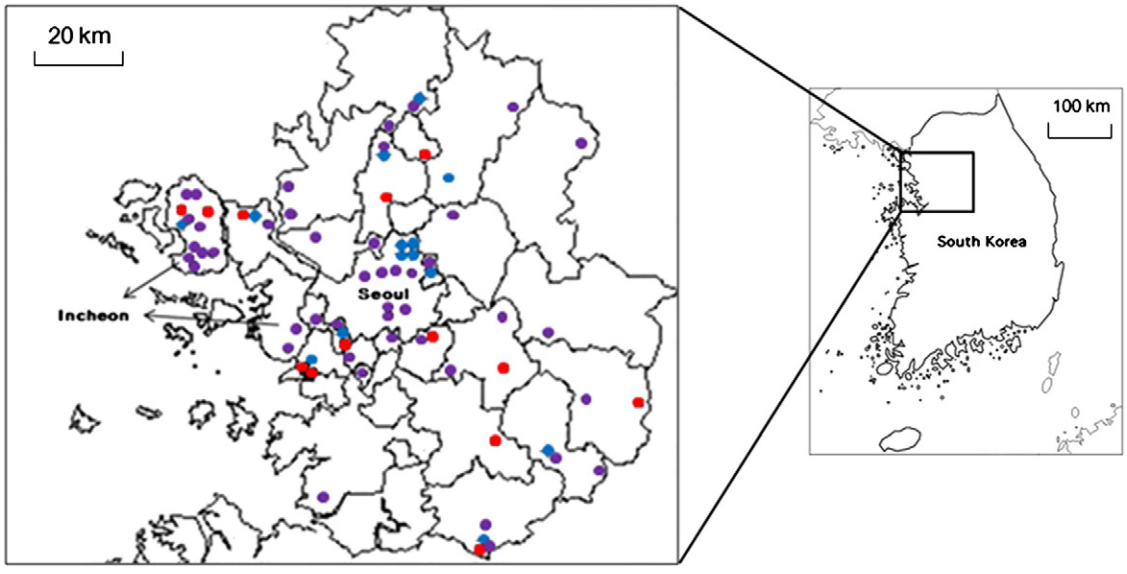

Fig. 1. Sampling sites for NoV investigation in metropolitan Seoul. ●Sites sampled only in the summer season (n = 13). ●Sites sampled only in the fall–winter season (n = 13). ●Sites sampled in both the summer and fall–winter seasons (n = 45).