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First Molecular Detection of Group A Rotaviruses in Drinking Water Sources in Beijing, China

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Abstract The most prevalent group A rotavirus found in the diarrheic children was also determined in drinking water sources including raw water, treated water and tap water in Beijing, and then the possible contamination contributions to tap water for human consumption were discussed in this study. A total of 26 raw water samples, 77 treated water samples and 143 tap water samples in Beijing were collected for analysis of group A rotavirus from April 2006 to August 2007. According to the results, it was shown that group A rotaviruses occurred in 9 raw water samples (34.6%), 9 treated water samples (11.7%) and 32 tap water samples (22.4%) during the sampling period, and low disinfectant residuals or a vulnerability of the distribution system to pressure transients, in addition to raw water, may account for the group A rotaviruses contamination to tap water. The rotavirus contamination observed in this study may highlight a potential public health risk and illustrate the importance of including routine virological analysis of drinking water supplies during winter time in Beijing.

Keywords Group A rotavirus · Drinking water sources · Raw water · Treated drinking water · Tap water · Beijing

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Group A rotaviruses are the major etiological cause of acute viral gastroenteritis in infants and young children worldwide (Wang et al. 2007). This may result in a significant disease burden and economic effect of direct medical costs, loss of work, quality of life and mortality. In recent years, several studies have evaluated the role of viral agents systematically in childhood diarrhea in Beijing and also reported that rotavirus was detected in nearly 60% of all diarrheal patients (Liu et al. 2006). Group A rotaviruses have been demonstrated to be a significant cause of sporadic and epidemic pediatric gastroenteritis in Beijing, China.

After replication in the gastrointestinal tract, rotaviruses are excreted in high numbers and may be dispersed into the environment water. The stability of rotaviruses in environmental water and their resistance to the physicochemical treatment processes may facilitate their transmission through water. Many studies have confirmed the presence of group A rotaviruses in sewage and treated effluents (Lodder and de Roda Husman 2005; Pusch et al. 2005). Rotaviruses can enter source water through various ways, including sewage overflows or sewage systems that are not working properly. In parallel with inter-human contamination, drinking water might play an important role in the occurrence of sporadic cases.

However, the quality evaluation of drinking water does not require testing for rotavirus as a parameter for water quality in China. There is no data reported group A rotaviruses in contaminated water using the molecular detection up to now. The possible source or reservoir of rotavirus infection would be obtained by the detection of group A rotaviruses in the aquatic environment comparing to published data with clinical infections from the same regions.

In this paper, a primer pair selected from published rotavirus gene 9 nucleic acid sequences encoding the

serotype-specific antigen VP7 (GenBank accession number K02033) were used for the amplification of viral RNA in water samples by RT-nested PCR. We have to determine whether or not the most prevalent rotavirus found in children diarrheic samples were also present in drinking water sources including raw water, treated water and tap water and discuss the possible contamination contributions to tap water for human consumption.

Materials and Methods

In this study, 26 raw water samples, 77 treated drinking water samples as well as 143 tap water samples were collected from April 2006 to August 2007. The raw waters serving the water works in Beijing were taken from acceptable quality reservoirs, lakes and groundwater sources which conform to national specifications for the production of safe tap water. After the processes of conventional treatment and advanced treatment which consists of chemical feed, coagulation, flocculation, sedimentation, rapid gravity filtration, activated carbon adsorption and chlorination, treated water was collected from several water works before it was sent to the extensive distribution network in Beijing. Tap water from different water works was taken systematically in a public area chosen for its easy access (school or city hall) within the city limits. At the same month there was a 10 days' interval between collecting three kinds of water samples.

Two liter of water samples in a sterile glass container were concentrated 4,000 times to about 0.5 mL by tangential ultrafiltration (Minitan device, followed by Centriprep 100; Millipore Corporation, Bedford, Mass.) before analysis as described by Soule et al. (2000) with minor modification.

RNA was extracted using the Qiagen viral RNA extraction kit (QIAgen, Germany) strictly by following the manufacturer's instruction. The 50 μL RNA eluates were stored at −20°C until amplification of nucleic acid was performed.

After denaturation at 97°C for 5 min, RNA was immediately cooled on ice. A single-step RT-PCR reaction for rotavirus was performed with the following reaction condition: 10 mM Tris-HCl (pH 8.3) (Amresco), 50 mM KCl (Amresco), 1.5 mM $MgCl_2$ (Amresco), 0.2 mM dNTPs (Promega), 200U M-MLV RT (Promega), 2.5U Ex-Taq DNA polymerase (TaKaRa), 20 U RNasin (Promega) and 1 μM each of primers Beg9 and End9 by (Gouvea et al. 1990) which were used for the amplification of sequences from the VP7 gene of group A rotaviruses. The RT-PCR reactions were incubated for 30 min at 37°C followed by 30 cycles of 1 min at 94°C, 2 min at 55°C and 1 min at 72°C,with a final extension of 7 min at 72°C. Nested PCR was performed with 5 μL of the RT-PCR amplification product under the following conditions: 1× Ex-Taq buffer (Mg^{2+}) (TaKaRa), 0.1 mM of each dNTPs (Promega), 1U Ex-Taq DNA polymerase (TaKaRa), 1 μM each of primers R3 and Rp (Baggi and Peduzzi 2000). Initial denaturation for 2 min at 94°C was following by 3 cycles of 30 s at 94°C, 30 s at 50°C and 30 s at 72°C and then by 27 cycles of 15 s at 94°C, 15 s at 50°C and 20 s at 72°C with a final extension of 7 min at 72°C. A positive control (human Wa rotavirus strain, obtained from Institute for Virus Disease Control and Prevention, China CDC) was included for each PCR assay. A 5–10 μL volume of nested PCR products (189 bp) was analyzed by electrophoresis on a 1.8% agarose gel in 1× TAE buffer along with a 100 bp MW ladder as a standard marker. The gels were stained with ethidium bromide and visualized through an UV transilluminator.

Standard precautions were applied in all the manipulations to reduce the possibility of sample contamination by amplified DNA molecules. Separate laboratories were used for reagents, treatment of samples and manipulation of amplified fragments. Negative controls for RNA extraction, RT-PCR and nested PCR were included in each test.

Nested PCR products were sequenced at the Chinese National Human Genome Center, Beijing using the same reverse Rp primer. The sequences were aligned and searched for nearly identical sequences with the rotavirus sequence using the Basic Local Alignment Search Tool (blast) program available on the NCBI network server.

Total coliform (TC), faecal coliform (FC) and *Streptococcus faecalis* were counted by using a membrane filtration method following standard protocols (Jiang and Chu 2004). Commercially available m-Endo, m-FC and KFC medium will be used for cultivation of TC, FC and *Streptococcus faecalis*, respectively. The pH of the water collected was measured on site with a portable pH meter. Residual chlorine was measured with a colorimetric kit.

Results and Discussion

In this study, rotaviruses were detected in 9 of 26 (34.6%) samples of raw water drawn from the source water serving the water works (Table 1). Nine of seventy-seven (11.7%) of treated water samples and 32 of 143 (22.4%) tap water samples from the distribution network in a geographically different region in Beijing were also detected (Table 1). All positive samples gave the correct VP7 sequence after using blast on NCBI.

The 9 positive results in raw water (Fig. 1) and treated water samples (Fig. 2) collected in October 2006 were detected, respectively. Group A rotaviruses were also detected in all the 11 tap water samples (Fig. 3) which were collected in the same time. However, the other 21

Table 1 Water samples analyzed for the presence of rotavirus

Type of sample (region)	Sampling period (month/year)	Source of samples	Total no. of samples	No. of positive samples
Raw water	4/2006, 10/2006, 1/2007, 5/2007	Reservoirs, lakes and groundwater	26	9
Treated water	4/2006, 10/2006, 1/2007, 5/2007	Water works	77	9
Tap water	9/2006–8/2007	Public areas	143	32

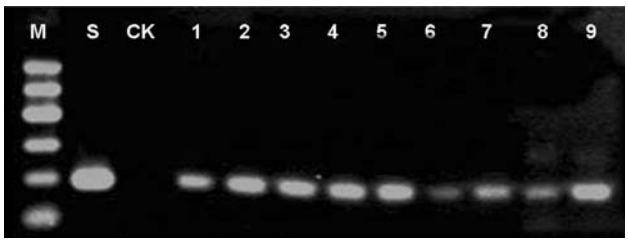

Fig. 1 Detection of rotavirus in raw water samples by RT-nested-PCR in October 2006. *Lanes*: *M* DNA ladder (100–600 bp), *S* positive control, *CK* negative control, 1–9 water samples. The positive band denotes 189 bp products

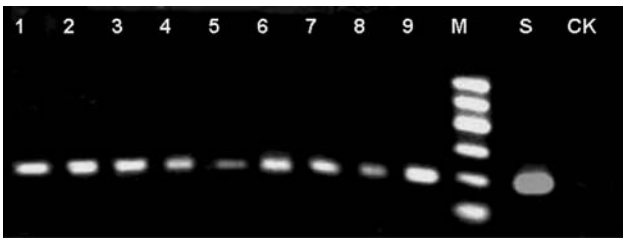

Fig. 2 Detection of rotavirus in treated water samples from water-works by RT-nested-PCR in October 2006. *Lanes*: *M* DNA ladder (100–600 bp), *S* positive control, *CK* negative control, 1–9 water samples. The positive band denotes 189 bp products

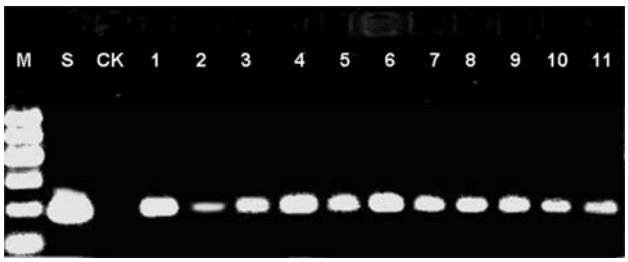

Fig. 3 Detection of rotavirus in tap water samples by RT-nested-PCR in October 2006. *Lanes*: *M* DNA ladder (100–600 bp), *S* positive control, *CK* negative control, 1–11 water samples. The positive band denotes 189 bp products

positive samples in tap water were taken from November 2006 to January 2007. In addition, no group A rotaviruses were detected in any of the raw and treated drinking water samples collected in January 2007, but interestingly group A rotaviruses were still found from site 4 to 8 in tap water (Fig. 4). These results corresponded with the reported

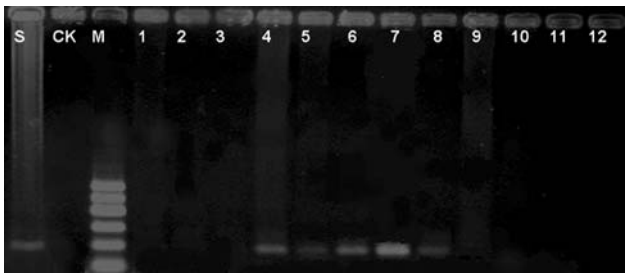

Fig. 4 Detection of rotavirus in tap water samples by RT-nested-PCR in January 2007. *Lanes*: *M* DNA ladder (100–600 bp), *S* positive control, *CK* negative control, 1–12 water samples. The positive band denotes 189 bp products

occurrence of greatest rotavirus infection during the winter months in Beijing (Orenstein et al. 2007).

The characteristics of the water samples are summarized in Table 2. The pH value of all the drinking water samples were between 6.5 and 8.5, and only one raw water sample over 8.0. The concentration level of residual chlorine in treated drinking water was accorded with regulations ($\geq$0.3 mg L^{-1}) by the National Standards for Drinking Water in China and that in tap water samples from distribution system was a little higher than or equal to 0.05 mg L^{-1} which was the minimum required in the Standards. All the tap water had heterotrophic plate counts of <80 CFU mL^{-1}, and TC, FC as well as *Streptococcus faecalis* counts of 0 CFU $(100\ mL)^{-1}$, which indicated an acceptable microbiological tap water quality.

In this study, molecular methods were applied to the detection of group A rotaviruses in selected water in Beijing, China. The specificity and sensitivity of the RT-nested PCR method that we used for detection of rotavirus from water is previously reported (He et al. 2008). Since unprotected RNA is relatively unstable and short-lived it would be altered during the concentration and purification procedures, it could be reasonably concluded that an RNA detected by RT-nested-PCR corresponds to the presence of complete viral particles in the original sample (Orenstein et al. 2007). The volume of water tested was chosen 2 L because this quantity could easily be absorbed by an individual in 1 day (Orenstein et al. 2007). Previous study indicated the minimum infectious dose is low for group A rotaviruses (close to 1 $TCID_{50}$) while the sensitivity of this

Table 2 Physical characteristics of raw water, treated drinking water and tap water samples

Measurement	Raw water	Treated drinking water		Tap water	
	pH	pH	Residual chlorine (mg L^{-1})	pH	Residual chlorine (mg L^{-1})
Minimum	7.05	7.22	0.3	7.13	0.05
Maximum	8.15	7.70	1.0	7.52	0.10
Mean	7.56	7.47	0.5	7.34	0.06
Median	7.62	7.48	0.6	7.35	0.07

technique is approximately 1 $TCID_{50}$ (Soule et al. 2000). Therefore, the viral load present in the positive samples might be sufficient to cause an infection, namely human contamination from such samples would be likely.

Rotaviruses have been recognized as the most important cause of acute infectious gastroenteritis among infants and children worldwide since their discovery in the 1970s (He et al. 2008). But there were little data about the occurrence and viability of rotavirus in drinking water supplies and limited knowledge on the efficacy of drinking water treatment processes and pathway from raw water to tap water in the world. The virus has only been found in drinking water source such as groundwater that have been contaminated with the feces from infected humans in USA (Borchardt et al. 2003). A few studies have mentioned the presence of rotaviruses in drinking water (Gratacap-Cavallier et al. 2000) or the occurrence of epidemics originating from contaminated water (Hung et al. 1984; Keswick et al. 1984). Our results provide new information on rotaviruses in water and identify the potential risk of waterborne transmission in Beijing. In fact, viruses can infiltrate the ground, penetrate to depths greater than 67 m and remain latent there for several months, as long as at low temperature and environmental humid (Gutierrez et al. 2007). This preliminary study has highlighted the need for further surveillance of aquatic environments to identify the rotavirus strains circulating in the community.

This study has furthermore provide valuable information regarding the prevalence of group A rotaviruses in water sources, which meant conventional treatment even advanced treatment in drinking water plants have limited virus removal efficacy. The high positive prevalence of rotavirus in tap water could result from distribution of treated drinking water into this water body. In addition, the presence of group A rotaviruses in drinking water underlines shortcomings in quality specifications. It confirmed that present water quality assessments, which relied on the use of the classic bacteriological parameters bacterial indicators, did not sufficiently reflect the occurrence of group A rotaviruses.

The result of samples collected in January 2007 suggested that there might be other unidentified sources in addition to raw water were responsible for the contamination to tap water. Similar study in Wisconsin, USA reported that enteric viruses including group A rotaviruses were found in all drinking-water well regardless of the level of surface water contributions (Borchardt et al. 2004). The expansive nature of the distribution system makes it vulnerable to contamination. Pipe breaks, particularly during the fall and winter when temperature changes place added stresses on the distribution system pipelines. It was confirmed that low or negative pressure transients events occur in distribution systems which provide a potential portal for entry of groundwater, faecal indicators and viruses in the soil and water exterior to the distribution system (LeChevallier et al. 2003). The data in Table 2 indicated that despite disinfectant residuals maintained in the distribution network, it was at very low levels at the ends of the system. One study showed that people who lived in zones far away from the treatment plant had the highest risk of gastroenteritis (Payment et al. 1997). Low disinfectant residuals or a vulnerability of the distribution system to pressure transients may account for the viral etiology of the illnesses observed (LeChevallier et al. 2003; Karim et al. 2003).

Our results further suggested that low disinfectant residuals or a vulnerability of the distribution system to pressure transients, could be responsible for the group A rotaviruses contamination to tap water, which might increase levels of waterborne gastrointestinal illnesses caused by group A rotaviruses in infants and young children in Beijing. This study confirmed that the classic bacteriological parameters did not sufficiently reflect the occurrence of group A rotaviruses in aquatic environment. The presence of even a few enteric viruses like group A rotaviruses in a large volume of tap water poses a threat to public health. Early detection of viruses in drinking water system will enable effective management of public water supplies and the implementation of appropriate preventive control measures.

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