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Full Length Research Paper

Development of a method to detect infectious rotavirus and astrovirus by reverse transcriptase polymerase chain reaction (RT-PCR) with propidium monoazide (PMA)

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Rotavirus and astrovirus are two kinds of water-borne pathogens that can cause severe diarrhea in children, infants and immunocompromised humans. Both can be present in untreated and inadequately treated water. Molecular methods, such as the reverse transcriptase polymerase chain reaction (RT-PCR), have been used to detect two viral genomes in a few hours, but they cannot distinguish between infectious and noninfectious rotavirus and astrovirus. In our study, we developed a propidium monoazide-reverse transcriptase polymerase-chain reaction (PMA-RT-PCR) assay to determine the infectivity of these two RNA viruses. Rotavirus and astrovirus stool samples were detected by RT-PCR in conjunction with PMA, respectively. Stool samples inactivated with heat treatment (95°C) were processed at the meantime as controls. The result showed that infectious virus samples gave positive results, while noninfectious virus samples presented negative results. To determine the viability of rotavirus and astrovirus in source water, a total of five source water samples were also collected from different reservoirs in July, 2012. One sample showed a positive result, which meant that PMA-RT-PCR method can be successfully applied to the viral detection in water samples. Data obtained in this study suggest that pretreatment of viruses with PMA prior to RT-PCR is a reliable method for distinguishing between infectious and noninfectious rotavirus and astrovirus. To our knowledge, this is the first report of application of this technique to rotavirus and astrovirus.

Key words: Rotavirus, astrovirus, propidium monoazide (PMA), source water.

INTRODUCTION

Rotavirus and astrovirus are the main pathogens that can cause severe infection in the immunocompromised humans (eg. children, old, patient). Infection always occurs following ingestion of contaminated food, drinking

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water, or recreational water. Traditionally, PCR method was designed to detect rotavirus and astrovirus for drinking water. Recent research showed that only viable pathogens are likely to pose a threat to humans (Nocker and Camper, 2009; Nocker et al., 2006). However, polymerase chain reaction (PCR) method could not distinguish the infectious from noninfectious virus. This drawback has greatly limit the application of PCR.

In recent times, propidium monoazide-polymerase-chain reaction PMA-PCR was reported to be an easy-to-use alternative to PCR distinction between viable and nonviable cells (Nocker et al., 2006; Pan and Breidt, 2007). Propidium monoazide, a DNA-intercalating dye, will enter only dead cells with compromised cell capsid and then covalently bind to DNA, or attach to extracellular DNA upon exposure to bright light (Nocker et al., 2006) and PMA-PCR have successfully discriminated between viable (or intact) and dead Bacteroidales cells (Bae and Wuertz, 2009), enteric virus (Parshionikar et al., 2010). While as far as we know, there is no report on rotavirus and astrovirus with PMA-PCR detection (Belliot et al., 1997; Dubios et al., 1997).

In this study, we developed a PMA-reverse transcriptase PCR (RT-PCR) assay in respect to enteric RNA viruses. This method was also used to determine the viability of rotavirus and astrovirus in source water. The results suggested that pretreatment of viruses with PMA prior to RT-PCR was a reliable method for distinguishing between infectious and noninfectious viruses that were inactivated by treatment at 95°C. To our knowledge, this is the first report of application of this technique to rotavirus and astrovirus.

MATERIALS AND METHODS

Virus strain

Rotavirus and astrovirus stool sample were kindly provided by Professor Duan Zhaojun, Chinese Center for Disease Control and Prevention, which were detected as positive samples.

Thermal inactivation of viruses

1.5 ml PBS buffer (pH 7.2~7.4) was added into 100 µl stool specimen. Subsequently, vortex the mixture and leave it for 10 min followed by centrifuging at 8000 rpm for 5 min. Remove 200 µl supernatant to a clear and dry EP tube for dead treatment. The suspension was incubated at 95°C for 20 min.

Sampling and pretreatment

The pilot distribution system used in this study comprised of five source water reservoirs. 500 ml water was sampled by a sterile bottle and store at 4°C for less than 2 h before virus concentration. Enrichment for rotavirus and astrovirus are given in the following report (Haramoto et al., 2005).

PMA preparation

PMA {Phenanthridium, 3-amino-8-azido-5-[3-(diethylmethylammonio) propyl]-6-phenyl dichloride; Biotium, Inc.,Hayward, California} was reconstituted with 20% dimethyl sulfoxide to create a stock concentration of 1 mg/ml and stored at − 20°C in the dark.

PMA processing

Samples were treated as described by Parshionikar et al. (2010) with slight modifications. In the dark room, 50 µl PMA was added into 200 µl heat-treated and non-treated aliquot to a final concentration of 250 µg/ml. All the tubes were then placed on to a rocker for 5 min. After mixing, the tubes was placed to ice to prevent overheat and exposed to a 500 W halogen light at a distance of 20 cm for 5 min.

Viral RNA extraction

Once the exposure was completed, the viral RNA was extracted using a viral DNA/RNA extract kit (TIANGEN, Beijing) according to the manufacturer's instruction. After the RNA was eluted into a 1.5 ml EP tube, it was stored at -20°C, and subsequently thawed and analyzed by RT-PCR.

RT-PCR

Conventional RT-PCR was performed as the follows conditions. Primer used in this study are shown in Table 1. Briefly a single-step RT-PCR reaction for viruses was performed with the following reaction condition: 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Promega, Beijing, China), 200U M-MLV RT (Promega, Beijing, China), 2.5 U Ex-Taq DNA polymerase (TaKaRa, Dalian, China), 20U RNasin (Promega, Beijing, China), and 1 M each of primers. The cycling condition of rotavirus were as follows: an initial denaturation at 94°C for 3 min followed by 35 cycles of 1 min at 94°C, 1 min at 53°C and 1 min at 72°C and final extension 5 min at 72°C. And the cycling condition of Astrovirus were as follows: an initial denaturation at 94°C for 3 min followed by 30 cycles of 40 s at 94°C, 1 min at 55°C and 40 s at 72°C and final extension 10 min at 72°C. Negative and positive control samples were included. The PCR product was analyzed by gel electrophoresis with Goldview nucleic acid stain.

RESULTS

Rotavirus and astrovirus stool samples were inactivated thermally at 95°C for 20 min. The viable and inactivated viruses were then treated with PMA. RNA was extracted, and RT-PCR was performed. Figure 1 shows the results of rotavirus after PMA treatment. Inactivated rotavirus with PMA in line 3 showed no objective stripe in electrophoretogram, which suggested that PMA had successfully inhibited PCR reaction by cross linking with cDNA. However, viable rotavirus with PMA in line 5 revealed that PMA had no effect on PCR reaction, as PMA could not penetrate into the intact capsid. Meanwhile, the contrast group in line 4 and line 5 indicated that inactivated and viable rotavirus without

Table 1. Gene targets and primers used for relative of genomic RNA from the virus strains.

Strain	Gene target	Primer name and sequence (5′–3′)	Product length	References
Rotavirus	VP7	Beg9 GGCTTTAAAAGAGA GAATTTCCGTCTGG VP7-1 GATCCTGTTGGCCATCC	392 bp	Dubios et al., 1997
Astrovirus	ORF 1a	Mon340 CGTCATTGTTTGTTGTCATACT Mon348 ACATGTGCTGCTGTTACTATG	289 bp	Belliot et al., 1997

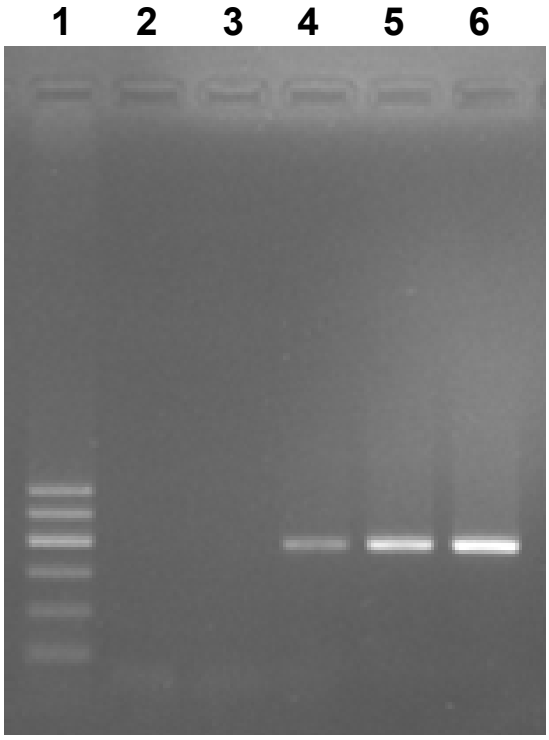

Figure 1. PMA-PCR Detection for rotavirus. (1. Marker; 2. Negative control; 3. Noninfectious rotavirus with PMA treatment; 4. Noninfectious rotavirus without PMA treatment; 5. Infectious rotavirus with PMA treatment; 6. Infectious rotavirus without PMA treatment).

PMA work as positive result as usual. The contrast demonstrated that only PMA could distinguish the viable rotavirus from inactivated one. Figure 2 shows the results of astrovirus after PMA treatment. Line 3, which represented for the inactivated astrovirus with PMA, displayed no objective stripe in electrophoretogram. It was probably because thermal treatment at 95°C for 20 min could inactivate whole astrovirus in the tube. None astrovirus survived from the heating condition. Thus this part of astrovirus could not impede PMA permeating into the capsid. Nevertheless, lines 4 to 6, which revealed positive results, indicated that viable astrovirus could get a positive result in PMA-PCR

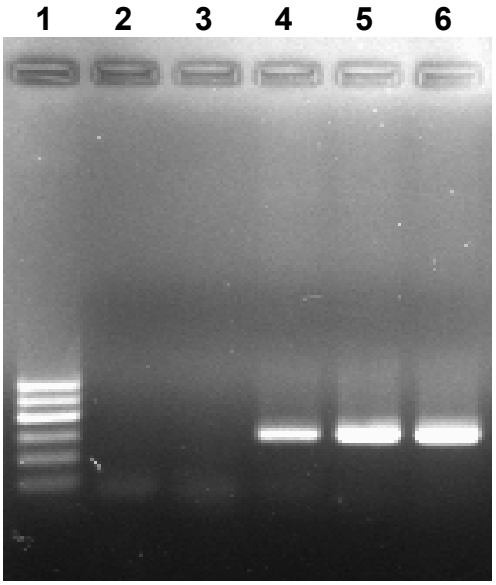

Figure 2. PMA-PCR detection for astrovirus, (1. Marker; 2. Negative control; 3. Noninfectious astrovirus virus with PMA treatment; Noninfectious astrovirus without PMA treatment; 5. Infectious astrovirus with PMA treatment; 6. Infectious astrovirus without PMA treatment).

detection. PMA-RT-PCR method was used to detect the enteric virus of water reservoirs in our study. Table 2 shows that reservoir No. 2 suggested a positive results, which meant that PMA-PCR method successfully applied to the viral detection in water samples.

Table 2. The results of five water reservoirs enteric viral detection by PMA-PCR method.

Water reservoir No.	Rotavirus/Astrovirus
1	-/-
2	+/-
3	-/-
4	-/-
5	-/-

"+"positive, "-"negative.

DISCUSSION

Rotavirus and astrovirus are typical waterborne virus that can cause acute diarrhea in children or infants (Prüsset al., 2002). There had been reported that enteric viruses caused a large waterborne outbreak of acute gastroenteritis in Finland (Maunula et al., 2008). The aim of this study was to validate the applicability of a PMA-PCR live-dead distinction method to determine the viability of rotavirus and astrovirus. PMA is a high affinity photoreaction DNA binding dye, which is cell membrane-impermeable, and thus can be selectively used to modify only exposed DNA from dead cells while leaving DNA from viable cells intact. Propidium monoazide has been successfully used to differentiate viable and non-viable bacteria in conjunction with qPCR (Nocker et al., 2006). This report provided more evident support on this aspect.

In our study, PMA was added as an identifying agent which distinguished viable virus from nonviable virus. Rotavirus and astrovirus got an ideal result by PMA-PCR detection. The negative results of inactivated samples with PMA treatment demonstrated that PMA penetrated into the capsid and bounded to RNA which inhibited the following reaction, and the viable aliquot suggested PMA was unavailable to the intact capsid.

Five different reservoirs are the main ones for domestic water. The viral contamination which is easily transported by water will affect the public health. Thus it is necessary to detect viral level in source water. However, traditional PCR method is not able to distinguish the survival status of viruses. In our research we used PMA-RT-PCR method to deal with this problem. The results suggested that PMA-PCR method could detect viable rotavirus at reservoir No. 2, which also revealed the contamination situation of this reservoir.

Conclusion

In conclusion, we developed a PMA-RT-PCR assay to determine the infectivity of these two RNA viruses. One sample showed a positive result, which meant that PMA-RT-PCR method could be successfully applied to the viral detection in water samples. Data obtained in this study suggested that pretreatment of viruses with PMA prior to RT-PCR was a reliable method for distinguishing between infectious and noninfectious rotavirus and astrovirus. It could provide more information to relevant administrator, and this technology will attribute to more fields in near future.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

Bae S, Wuertz S (2009). Rapid decay of host-specific fecal Bacteroidales cells in seawater as measured by quantitative PCR with propidium monoazide. Water Res. 43(19):4850-4859. http://dx.doi.org/10.1016/j.watres.2009.06.053

Belliot G, Laveran H, Monroe S (1997). Detection and genetic differentiation of human astrovirues:phylogenetic grouping varies by coding region. Arch. Virol. 142:1323-1334. http://dx.doi.org/10.1007/s007050050163

Dubios E, Le Guyader F, Haugarreau LK, Cormier H, Pommepuy M (1997). Molecular Epidemiological Survey of Rotaviruses in Sewage by Reverse Transcriptase Seminested PCR and Restriction Fragment Length Polymorphism Assay. Appl. Environ. Microbiol. 63(5):1794-1800.

Haramoto E, Katayama H, Oguma K, Ohgaki S (2005). Application of cation-coated filter method to detection of noroviruses, enteroviruses, adenoviruses, and torque teno viruses in the Tamagawa River in Japan. Appl. Environ. Microbiol. 71(5):2403-2411. http://dx.doi.org/10.1128/AEM.71.5.2403-2411.2005

Maunula L, Klemola P, Kauppinen A, Söderberg K, Nguyen T, Pitkänen T, Kaijalainen S, Simonen ML, Miettinen IT, Lappalainen M, Laine J, Vuento R, Kuusi M, Roivainen M (2008). Enteric Viruses in a Large Waterborne Outbreak of Acute Gastroenteritis in Finland, Food Environ. Virol. 1(1):31-36. http://dx.doi.org/10.1007/s12560-008-9004-3

Nocker A, Camper AK (2009). Novel approaches toward preferential detection of viable cells using nucleic acid amplification techniques. FEMS Microbiol. Lett. 291(2):137-142. http://dx.doi.org/10.1111/j.1574-6968.2008.01429.x

Nocker A, Cheung CY, Camper AK (2006). Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs dead bacteria by selective removal of DNA from dead cells", J. Microbiol. Methods. 67(2):310-320. http://dx.doi.org/10.1016/j.mimet.2006.04.015

Pan Y, Breidt F Jr (2007). Enumeration of viable Listeria monocytogenes cells by real-time PCR with propidium monoazide and ethidium monoazide in the presence of dead cells. Appl. Environ. Microbiol. 73(24):8028-8031. http://dx.doi.org/10.1128/AEM.01198-07

Parshionikar S, Laseke I, Fout GS (20101). Use of propidium monoazide in reverse transcriptase PCR to distinguish between infectious and noninfectious enteric viruses in water samples. Appl. Environ. Microbiol. 76(13):4318-4326. http://dx.doi.org/10.1128/AEM.02800-09

Prüss A, Kay, D, Fewtrell L, Bartram J (2002). Estimating the burden of disease from water, sanitation, and hygiene at a global level. Environ. Health Perspectives. 110(5):537-542. http://dx.doi.org/10.1289/ehp.02110537