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Short communication

Recovery of structurally intact norovirus from food-contact surfaces

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ABSTRACT

Human noroviruses (NoVs) are the most common cause of gastroenteritis and are responsible for at least 50% of all gastroenteritis outbreaks worldwide. NoV can be transmitted directly via person-to-person contact and contaminated fomites or indirectly via contaminated raw and ready-to-eat foods. However, there have not been enough studies that examine the detection and persistence of NoV on various food-contact surfaces, which may provide information regarding the transmission of NoV in public places, such as restaurants, hospitals, and nursing homes. In order to determine the persistence of NoV, the ability of NoV GII.4 to persist on six kinds of surfaces was investigated for up to 28 d post-inoculation, using an immuno-magnetic separation (IMS) procedure combined with quantitative real-time RT-PCR (qRT-PCR). NoV GII.4 was detected in all test samples, even after 672 h (28 d).

1. Introduction

In recent years, norovirus (NoV), recognized as the most important foodborne virus, is the most frequent cause of acute viral gastroenteritis worldwide (Lai et al., 2013; Partridge, Evans, Raza, Kudesia, & Parsons, 2012; Shin & Sobsey, 2008; Tsang et al., 2008). According to the Centers for Disease Control and Prevention (CDC) in the USA, more than an estimated 5.5 million cases of NoV infection occur annually, representing approximately 55% of all viral outbreaks in 2010 (Scallan et al., 2011). In 2007, 97 NoV-associated outbreaks affected 2345 patients. A total of 32 (2009) and 30 outbreaks (2010) affecting 568 and 1985 patients, respectively, were reported to the Korean Food & Drug Administration (KFDA). Over the last few years, the number of cases has continued to increase, and these outbreaks now represent 30% of all foodborne outbreaks in Korea (Kim et al., 2012).

NoVs are transferred through the fecal–oral route or direct person-to-person transmission. Virus- related food borne infection may be spread by foods as a result of direct or indirect contamination of the foods and water with fecal material, which may reside on contaminated surfaces (Anderson et al., 2001; Berg, Kohn, Farley, & McFarland, 2000; Clark, Barrett, Rogers, & Stapleton, 2006; Green et al., 1998; Koopmans & Duizer, 2004). Environmental transmission and contamination of NoV in hospitals, bistros, and schools has been reported and associated with contaminated work surfaces, door handles, floors, equipment, switches, and television consoles (Gallimore et al., 2005, 2006; Green et al., 1998). Several groups have monitored viral environmental contamination in various settings, including catering companies (Boxman et al., 2011), pediatric units (Soule et al., 1999), hospitals (Cheesbrough, Barkness-Jones, & Brown, 1997), day-care facilities (Butz, Fosarelli, Dick, Cusack, & Yolken, 1993), and hotels (Cheesbrough, Green, Gallimore, Wright, & Brown, 2000). Although several groups have examined environmental settings by monitoring enteric viruses in public places, there have been few reports that examine NoV recovery rates on various material surfaces.

The objective of the present study was to detect the NoV GII.4 on six material surfaces—rubber, stainless steel, glass, ceramic tile, wood, and polyvinyl chloride (PVC)—that were artificially inoculated with NoV and investigate NoV recovery rates using an immuno-magnetic separation (IMS) procedure combined with quantitative real-time RT-PCR (qRT-PCR).

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2. Materials and methods

2.1. Virus origin

NoV-positive stool samples, representing genotype GII.4, were provided by Kim Laboratories Inc. (Rantoul, IL., USA). NoV GII.4 was confirmed by qRT-PCR and sequencing. Stool samples were diluted in RNAse-free water (Quanta Biosciences, Gaithersburg, MD, USA) to obtain a 40% suspension, vortexed briefly, and clarified by centrifugation at 550 × *g* for 2 min to remove solids, and the supernatant was stored in 1 mL aliquots at −80 °C.

2.2. Preparation of various surface materials

This study included various food-contact surfaces. Rubber (KOMAX Industrial Co., Ltd., Seoul, Korea), glass (GukjeYuri), stainless steel (Posco Co., Ltd., SUS ANSI 304SS 2B), PVC (HDPE; Daesung Industry Co.), wood (Heilongjiang Zhongji IMP & EXP), and ceramic tile (Hankook Chinaware Co., Ltd.) were selected as representative materials used by the food industry. The six material-types were cut into 2 × 2 cm pieces. The rubber and wood coupons were made from household rubber gloves and timber cutting board respectively. The stainless steel used in this study for surface preparation was from an ANSI 304SS 2B stainless steel plate sheet. All the coupons were decontaminated by wiping thoroughly with 40,000 ppm hypochlorite and 70% ethanol, cleansed in Ultrasonic Cleaning Solution (Fisher Scientific, Pittsburgh, PA, USA) for 10 min, and rinsed in deionized water. The surfaces were then soaked for 30 min in 95% ethanol, air dried in a laminar flow hood for 30 min, and then exposed to 30 Watt pressure and a 257 nm UV lamp (Sankyo Ultraviolet Co., Seoul, Korea) for 10 min. Each dried material was then wrapped in clean UV-disinfected aluminum foil, placed in a sealed bottle, and autoclaved at 121 °C prior to use in the experiment.

2.3. Viral inoculation

Ten microliters of 40% NoV GII.4 stool stock samples (3.66 $\log_{10}$ genomic copies/10 μL) was inoculated on the six surface types. We made a mixture with 10 uL of GII.4 stool sample and 190 uL phosphate buffered saline (PBS) and used the 200 ul of NoV solution in order to ease to spread. To allow absorption of spiked viruses on surfaces, all prepared test samples were incubated at 18–20 °C in a laminar flow hood for 5 min. The recovery rates of NoV GII.4 were measured at predetermined times, with measurements taken at 0.5, 1, 3, 5, 8, 12, 24, 72, 120, 168, 240, 336, 504, and 672 h, after inoculation on each surface coupons.

2.4. Immunomagnetic separation (IMS) Beads

Nano-magnetic beads (5 g/mL stock suspension, Carboxyl-Adembeads 200 nm, ADEMTECH, Pessac, France) were coated with antibodies against NoV GII.4 (monoclonal antibodies #Cat MABG22, Kim Laboratories Inc.). Naked beads were used to conjugate each antibody and were prewashed 3 times with activation buffer, according to the manufacturer's instructions. The final concentration of IMS beads was 10 mg/mL in the provided storage buffer. Carboxyl-Adembeads can be activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, MW 191.7), which reacts with carboxylic acid groups to form an amine-reactive intermediate. The first step of activation involved washing the carboxyl-Adembeads with activation buffer (1 ×, diluted in distilled water). EDC solution (4 mg/mL) was mixed with activation buffer (1 ×, diluted in distilled water), with 80 μL of EDC solution per milligram of beads. The mixtures were incubated for 10 min at 37 °C while shaking. The activated beads were then ready for coating with ligand. The second step of antibody immobilization involved adding 50 μg of NoV GII.4 strain antibodies per milligram of activated particles. After incubation for 2 h at 37 °C while shaking, 200 μL of bovine serum albumin (BSA) solution in activation buffer (0.5 mg/mL) was added to 100 μL of GII.4 strain antibody-coated beads. This mixture was incubated for 30 min at 37 °C while shaking. Finally, the beads were washed twice with storage buffer (1 ×, diluted in distilled water), resuspended with storage buffer to a final solution of 10 mg/mL, and stored at 2 °C.

2.5. Virus concentration

Inoculated viruses were eluted from the each of the six surfaces, immediately after the NoV GII.4 suspensions were dried for 5 min at room temperature in a laminar flow hood as a positive control, by using the 0.05 M glycine-0.14 M NaCl (pH 7.5) elution buffer (Baert, Uyttendaele, & Debevere, 2008). Each spiked sample was soaked in 20 mL of elution buffer for 30 min at room temperature with constant shaking (approximately 60 rpm). The NoV GII.4 was eluted by repeated pipetting (50 times) of the inoculated area with 20 mL of 0.05 M glycine and 0.14 M NaCl (pH 7.5) for each surface, and each eluate was placed into a 50 mL conical tube. This secondary suspension was added to the primary elution suspension, and the entire 40 mL eluate was used for the IMS technique, capturing the NoV GII.4 by using the IMS beads and constantly shaking for 1 h at room temperature. The selected beads with captured NoV GII.4 were collected by the PolyATtract® System 1000 (Promega, Madison, WI, USA) and washed with PBS. The beads were then suspended in 140 μL of 1 × PBS and transferred to a 50 mL sterile conical tube.

2.6. Viral RNA extraction

Viral RNA was purified by adding AVL extraction buffer containing carrier RNA and 96–100% ethanol and applying the sample to a QIAamp spin column (Qiagen, Hilden, Germany). After 2 washes with buffers AW1 and AW2, the RNA was eluted with 60 μL of elution buffer (AVE) and stored at −80 °C until further use, while 5 μL of each test sample was used directly in qRT-PCR.

2.7. Quantitative real-time -PCR

One-step qRT-PCR amplification was performed in triplicate for each coupon using a 7500 Fast Real Time PCR System (Applied Biosystems, Foster City, CA, USA) and the Quantitect Probe RT-PCR kit (Qiagen). To quantify NoV in the stool samples, an external standard curve was derived from a 10-fold series of dilution from 1.0E + 2 copies to 1.0E + 7 copies using a 917-bp DNA fragment containing the ORF1–ORF2 junction region that was produced from the NoV GII cluster four by PCR using primers N1/Mon383. At ten-fold serial dilutions of GII.4 standard RNA transcripts, qRT-PCR standard curves were linear, with a slope of −3.31, and the coefficient of determination (R^2) was >0.9972 (Fig. 1). One-step qRT-PCR was performed using 5 μL of RNA eluate in a total volume of 20 μL on an Applied Biosystems 7500 PCR system with the following cycling parameters: 50 °C for 10 min, denaturation at 95 °C for 5 min, and then 40 cycles of amplification with denaturation at 95 °C for 10 s and combined annealing and extension at 60 °C for 30 s. NoV GII.4 primer sequences (10 μM each) were JJV2F: 5′-CAA GAG TCA ATG TTT AGG TGG ATG AG-3′ and COG2R: 5′-TCG ACG CCA TCT TCA TTC ACA-3′, which were used to amplify a 122-bp fragment of the NoV GII.4 polymerase gene (Gentry, Vinje, & Lipp, 2009). The TaqMan probe (Ring2, 10 μM) was FAM: 5′-TGG GAG GGC GAT CGC AAT CT-3′. BHQ was also used. One step qRT-PCR amplification data