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Inactivation and UV Disinfection of Murine Norovirus with TiO_2 under Various Environmental Conditions[▽]

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We studied inactivation and UV disinfection of murine norovirus (MNV) as a surrogate for human norovirus. We investigated the effects of different surface characteristics, temperatures, and NaCl concentrations on MNV survival using both a plaque assay and a real-time TaqMan reverse transcription (RT)-PCR assay. MNV survived more than 40 days on diaper material, on gauze, and in a stool suspension. Compared to inactivation at lower temperatures (−20 and 4°C), inactivation of MNV was greater at higher temperatures (18 and 30°C). On the surface of both gauze and diaper material, there was a <2-$\log_{10}$ reduction in the amount of infectious MNV in 40 days after incubation at both −20 and 4°C, compared to a >5-$\log_{10}$ reduction after incubation at 30°C in 24 days. MNV survived better in a stool suspension than on the surface of gauze or diaper material. A higher salt concentration increased the rate of inactivation of MNV. In 72 h, <0.3-, 1.5-, and 2.5-$\log_{10}$ reductions in the amount of infectious MNV occurred in distilled water and 0.5 and 1 M NaCl, respectively. We observed only minor reductions in the numbers of viral RNA copies as quantified by real-time TaqMan RT-PCR regardless of the temperature, the salt concentration, or the suspending medium. We also evaluated UV disinfection of infectious MNV with and without TiO_2. The amount of MNV was significantly reduced by 254-nm UV with and without TiO_2. When 25 mJ/cm^2 UV was used, 3.3- and 3.6-$\log_{10}$ reductions in the amounts of infectious MNV occurred with and without TiO_2, respectively. Our results demonstrate that MNV can persist in various environmental conditions and can be efficiently controlled by UV disinfection.

Noroviruses (NoVs) are water- and food-borne pathogens that cause more than 80% of nonbacterial gastroenteritis worldwide (1, 13, 15, 40). High concentrations of NoVs (~10^9 viral particles/ml) are typically present in the feces or vomit of infected patients. These viruses, released from symptomatic or asymptomatic infected people, can be transmitted through contaminated water, food, fomites, or direct contact. NoVs are highly infectious because of their low infectious dose (~10 viral particles), the lack of long-term immunity in humans, and their high levels of resistance to various environmental stresses (6, 11, 17, 19, 25, 27, 29, 37). Outbreaks caused by NoVs have been reported in a variety of settings, including cruise ships, hotels, hospitals, restaurants, and schools (2, 28, 35).

To properly control the transmission of NoVs, we must fully understand the survival and inactivation of infectious NoVs in the environment. However, due to the lack of cultivation methods for NoVs, NoV research has been limited (10). Cultivable caliciviruses, including feline calicivirus (FCV) and canine calicivirus (8, 10, 12, 18), have been commonly used as surrogates to evaluate the survival and inactivation of NoVs (21, 39). However, these surrogates may have survival characteristics different from those of human NoVs, especially some of the viruses known to be respiratory pathogens. In fact, FCV is very susceptible to low pH, unlike other enteric viruses (5). To evaluate the survival and inactivation of human NoVs, researchers have performed human challenge studies (23, 26). These studies showed that human NoVs were resistant to chlorination and could survive for more than 2 months. However, these human challenge studies failed to determine the rate of inactivation of human NoVs because of the limited number of human volunteers and high cost.

A newly identified murine norovirus (MNV) is easily cultivated in tissue cultures using the RAW 264.7 cell line (41). MNVs are typically spread by the fecal-oral route and are biologically similar to human NoVs (22). A recent study reported that MNVs were more resistant to low pH (pH 2), unlike calicivirus surrogates, such as FCV. These data suggest that MNVs are the most appropriate surrogates for human NoVs (5). Therefore, we investigated the survival and inactivation of MNVs under various environmental conditions. We determined the effects of surface characteristics, temperature, and salt concentration on the survival of MNVs. Additionally, we studied the inactivation of MNVs with 254-nm UV with and without a photocatalyst (TiO_2), which can be an effective disinfectant for human NoVs.

MATERIALS AND METHODS

MNV culture and stock. MNV1 was obtained from Herbert W. Virgin at the Washington University School of Medicine (St. Louis, MO). This virus was cultured in RAW 264.7 cells in Dulbecco's modified Eagle's medium (MEM) (Gibco, Grand Island, NY) containing 10% fetal bovine serum (Gibco), 10 mM HEPES, 10 mM sodium bicarbonate, gentamicin (50 μg/μl), and 10 mM nonessential amino acids. Viruses were inoculated and cultivated on confluent RAW 264.7 cell monolayers for 3 to 4 days. Infected cells were subjected to freezing and thawing three times to release the viruses, mixed with an equal volume of chloroform, and then centrifuged at 2,000 × *g* for 10 min at 4°C. To further concentrate the MNV, we subjected the supernatant to ultrafiltration (Amicon Ultra-15; Millipore, Billerica, MA) at 5,000 × *g* for 10 min at 4°C. The super-

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TABLE 1. Primers and probe for real-time TaqMan RT-PCR of MNV

Oligonucleotide	Sequence (5′→3′)	Location[a]
MNV1 F	ACG CCA CTC CGC ACA AA	5614–5630
MNV1 R	GCG GCC AGA GAC CAC AAA	5649–5657
MNV1 P	VIC-AGC CCG GGT GAT GAG-MGB	5632–5646

[a] Nucleic acid sequence locations based on the GenBank accession no. DQ285629 sequence.

natant from the ultrafiltration unit was recovered and stored at −80°C until it was used for experiments. The titer of MNV was determined by a plaque assay and was estimated to be approximately 10^{11} PFU/ml.

MS2 assay and stock. Bacteriophage MS2 (ATCC 15597-B1) was grown and assayed by single-agar-layer methods (39a). The virus was purified from infected cell lysates from a single-agar-layer plaque assay plate with confluent lysis by extraction with an equal volume of chloroform and centrifugation at 4,000 × *g* for 30 min. The supernatant was recovered and stored frozen at −80°C. The MS2 stock titer was determined by single-agar-layer methods, and the titer was approximately 10^{10} PFU/ml.

MNV plaque assay. RAW 264.7 cells were seeded into 60-mm plates at a density of 5×10^6 cells per plate and allowed to adhere for 6 h at 37°C in the presence of 5% CO_2. Tenfold serial dilutions of MNV were prepared on ice using supplemented Dulbecco's MEM. Cell culture medium was decanted, and cells were inoculated with 0.5-ml virus suspensions. After incubation for 1 h at 37°C in the presence of 5% CO_2, the inocula were aspirated and replaced with 3 ml of a solution containing one part 1.5% SeaPlaque agarose and 2 parts supplemented MEM, allowed to solidify, and incubated at 37°C for 36 to 48 h until plaques were visible. To better visualize the plaques, we added a neutral red solution (final concentration, 0.1%) to 3 ml of the mixture containing 1.5% SeaPlaque agarose and supplemented MEM, allowed it to solidify, and incubated it at 37°C for 6 to 8 h. The plaques on plates containing 5 to 50 plaques were counted, and the virus titer was expressed in PFU per milliliter.

RNA extraction. Viral RNA was extracted from sample suspensions using a QIAmp viral mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Briefly, 140 μl of a virus suspension was mixed with 560 μl of a guanidinium thiocyanate lysis solution, followed by RNA precipitation with an equal volume of 96 to 100% ethanol. Viral RNA was further purified by use of a QIAmp mini column (Qiagen viral RNA kit). Purified RNA samples were stored at −80°C until they were used for reverse transcription (RT)-PCR.

Quantitative real-time TaqMan RT-PCR assay. The MNV genome sequence was obtained from GenBank (accession no. DQ285629). Based on the GenBank nucleic acid sequence, the primers and probes for a real-time TaqMan RT-PCR assay were designed using Primer Express software (Applied Biosystems, Foster City, CA). Table 1 shows the oligonucleotide primers and probe designed in this study. To prepare the standard for quantification, we cloned genes of the capsid region by using the TA cloning vector (catalogue no. TK6002; Promega, Madison, WI), and the plasmid DNA constructed was purified using a plasmid DNA purification kit (Labopass no. CMP0112; Cosmo Genetech, Seoul, Korea). The concentrations of purified plasmids were determined with a Nanodrop spectrophotometer (ND-1000; Nanodrop Technologies, Wilmington, DE) at 260 nm, and serial dilutions of plasmid DNA were used to obtain a standard curve. The reaction mixture (final volume, 25 μl) contained 12.5 μl 2× one-step RT-PCR master mixture (model 7300; Applied Biosystems), 0.2 μl primer (100 mM), 0.07 μl probe (100 mM), 2.5 μl template RNA, and water. A one-step assay was performed by using the following amplification conditions: RT for 30 min, followed by an initial denaturation at 95°C for 10 min and then 45 cycles of denaturation for 10 s at 95°C, annealing for 20 s at 50°C, and elongation for 30 s at 72°C.

Survival of MNV on surfaces. Prior to characterizing the survival of NoVs, we optimized methods for viral extraction from test surfaces. Gauze and diapers (catalogue no. B414A003-7004; Yuhan-Kimberly Inc., Seoul, Korea) were purchased from a local supermarket and used as environmental surfaces. The gauze (2 by 0.1 cm) and diaper material (1 by 1 cm) were prepared, exposed to 254-nm UV for 5 min for disinfection, and stored in a petri dish for further use. Viral stocks (0.1 ml containing 10^8 PFU) were deposited in the center of the individual gauze and diaper samples and then assayed. The following six extraction methods were tested: phosphate-buffered saline (PBS) with sonication for 30 min (method 1), PBS with vortexing for 10 min (method 2), PBS with both sonication and vortexing (method 3), 0.3% beef extract buffer with sonication for 30 min (method 4), 0.3% beef extract with vortexing (method 5), and 0.3% beef extract with both sonication and vortexing (method 6). Each solution was centrifuged at 2,000 × *g* for 10 min at 4°C, and the viruses in the supernatant were serially diluted and analyzed by a plaque assay.

After optimization of the viral elution methods, MNV inactivation was measured at four temperatures (−20, 4, 18, and 30°C). The viral stock (0.1 ml containing 10^8 PFU) was deposited on either the gauze or diaper samples, and the inoculated samples were stored at −20, 4, 18, and 30°C for up to 40 days. After 0, 10, 20, 30, and 40 days, virus was recovered from the inoculated surfaces using 5 ml of 0.3% beef extract elution buffer and sonication for 30 min at 4°C. Each eluted suspension was centrifuged at 2,000 × *g* for 10 min at 4°C, and the resulting supernatant was analyzed by both the plaque assay and real-time TaqMan RT-PCR. These experiments were repeated three times.

Survival of MNV in human stool. A stool sample from a healthy adult was obtained from the Korea Center for Disease Control. A 0.1-ml stool suspension was mixed with an equal volume of virus stock (containing 10^9 PFU), mixed by vortexing for 30 s, and stored at −20, 4, 18, and 30°C for 40 days. After 0, 10, 20, 30, and 40 days, each sample was analyzed by both the plaque assay and real-time TaqMan RT-PCR. These experiments were repeated three times.

Effect of salt concentration on the survival of the MNV. The stability of the MNV in the presence of various salt concentrations (0, 0.5, and 1 M NaCl) was analyzed. We mixed 0.1-ml salt solutions (0, 1, and 2 M NaCl) with 0.1 ml of virus stock (containing 10^9 PFU); the preparations were mixed by vortexing for 30 s, and the final NaCl concentrations were 0, 0.5, and 1 M. These viral suspensions were stored at room temperature (24°C), sampled after 24, 48, and 72 h, and assayed by both the plaque assay and real-time TaqMan RT-PCR. These experiments were repeated three times.

Inactivation by UV and by UV with TiO_2. A collimated beam UV apparatus containing two low-pressure 254-nm UV lamps was used as described in previous studies (24). Viral suspensions were exposed to the emitted UV light in a petri dish (60 by 15 mm). The UV irradiance was measured with a radiometer (model IL 500; International Light, Inc., Newburyport, MA). TiO_2 particle suspensions were prepared using 1, 10, 100, and 1,000 mg and 1 liter of PBS, and each TiO_2 suspension was sonicated for 15 min and preexposed for 5 min to the UV lamp. MS2 was suspended in 10-ml TiO_2 suspensions and exposed for 522 s. After UV exposure, the virus was assayed by single-agar-layer methods. TiO_2 particles (Degussa Co., Incheon, Korea) were sonicated for 15 min before the experiment and were preexposed to UV for 5 min with OH to obtain a steady state and increase the photocatalytic efficiency. Approximately 10^7 PFU/ml MNV was suspended in 10 ml PBS and PBS containing TiO_2 and exposed to 254-nm UV at room temperature. The UV exposure times were 0, 93, 186, 232, and 276 s. The UV exposure doses (mJ/cm^2) were calculated from the product of the exposure time and the adjusted UV irradiance (μW/cm^2). The viral titers were estimated by both the plaque assay and real-time TaqMan RT-PCR. The plaque assay experiment was repeated five times, and the real-time TaqMan RT-PCR assay experiment was repeated three times.

TABLE 2. Comparison of recovery efficiencies with different methods

Material	Recovery rate (%) with[a]:					
	Method 1	Method 2	Method 3	Method 4	Method 5	Method 6
Gauze	70.1 ± 4.9	67.0 ± 5.3	46.2 ± 3.4	89.2 ± 6.2	73.4 ± 7.5	63.8 ± 6.8
Diaper	100 ± 8.7	79.6 ± 5.6	78.4 ±15.2	100 ± 2.6	100 ± 12.7	89.4 ± 10.9

[a] The elution methods are described in the text. The values are averages ± standard deviations (n = 3).

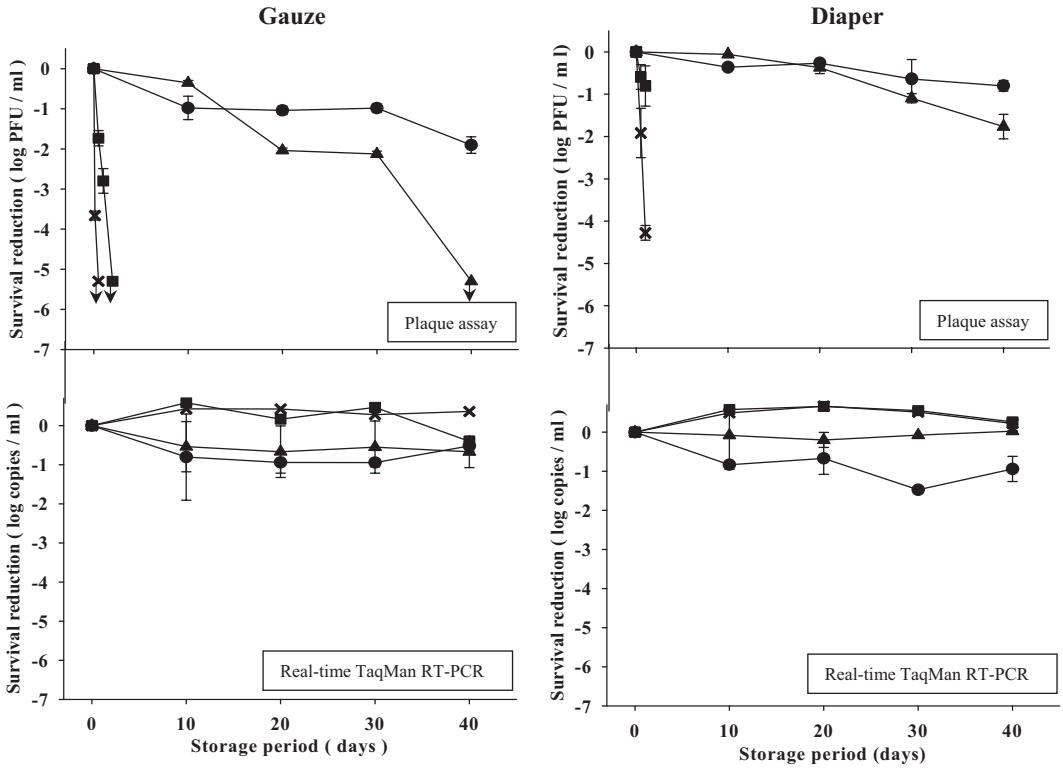

FIG. 1. Survival of MNV on surfaces at −20°C (●), 4°C (▲), 18°C (■), and 30°C (×). The arrows indicate detection limits ($n = 3$).

RESULTS

Recovery of MNV from surfaces. Table 2 shows the recovery efficiencies for various elution methods. The levels of recovery from the gauze ranged from 46.2 to 89.2%, and the levels of recovery from the diaper material ranged from 78.4 to 100%. Neither sonication nor vortexing significantly affected viral infection in the plaque assays. Of the six elution methods, use of method 4 (0.3% beef extract with sonication for 30 min) resulted in the highest recovery efficiency (89.2% recovery from the gauze and 100% recovery from the diaper material). The values obtained with elution method 4 were significantly higher than the values obtained with any other method tested for gauze ($P = 0.05$, Kruskall-Wallis test). Thus, elution method 4 was selected for determining the survival of the infectious MNV on both the gauze and diaper material.

Survival of MNV on surfaces. Figure 1 shows the survival of the MNV on both the gauze and the diaper material under the temperature conditions used (−20, 4, 18, and 30°C) for 40 days. After 40 days of incubation at −20°C, a <2-$\log_{10}$ reduction (99%) in the MNV level was observed for the gauze, and a <1-$\log_{10}$ reduction (90%) was observed for the diaper material. At 4°C an approximately 2-$\log_{10}$ reduction was observed after 30 days for the gauze, and a <2-$\log_{10}$ reduction was detected after 40 days for the diaper material. The MNV did not survive well at higher temperatures. At both 18 and 30°C, >3-$\log_{10}$ reductions were observed after 1 day for both the gauze and the diaper material. When the MNV was assayed by real-time TaqMan RT-PCR, the concentrations were consistent for 40 days regardless of the temperature or the inoculated surface.

Survival of the MNV in stool suspension. Figure 2 shows the survival of the MNV in a stool suspension incubated at −20, 4, 18, and 30°C for 40 days. The MNV in the stool suspension was more stable. A <1-$\log_{10}$ reduction was observed after 40 days of incubation at 4°C. However, a more rapid decline was observed at higher temperatures (18 and 30°C). A >5-$\log_{10}$ reduction was observed after just 24 h of incubation at 30°C. An approximately 4-$\log_{10}$ reduction was observed after 40 days of incubation at −20 and 18°C. When we performed real-time TaqMan RT-PCR assays, we found that just a small reduction in the MNV level had occurred after 40 days. A greater reduction in the level was observed with the real-time RT-PCR assay when the preparation was incubated at −20°C.

Survival of the MNV in salt solution. Figure 3 shows the survival of the MNV in 0.5 and 1 M NaCl solutions incubated for 72 h at room temperature. The results showed that the MNV survived better in the presence of lower NaCl concentrations. After 72 h, approximately <0.5-, 1.5-, and 2.5-$\log_{10}$ reductions in the numbers of viable MNV were observed when preparations were suspended in distilled water and 0.5 and 1 M NaCl, respectively. A <0.5-$\log_{10}$ reduction was observed regardless of the salt concentration when the viral suspension was assayed using the real-time TaqMan RT-PCR assay.

Determination of the concentration of TiO_2 for UV disinfection with TiO_2. To determine the optimal concentration of TiO_2 particles in the solution irradiated with 254-nm UV, we first examined the inactivation of MS2. MS2 was suspended in preparations containing various concentrations of TiO_2 (0, 1, 10, 100, and 1,000 mg/liter) and exposed to a 60-mJ/cm^2 dose (110 μW/cm^2 UV irradiance and a 552-s exposure time) of

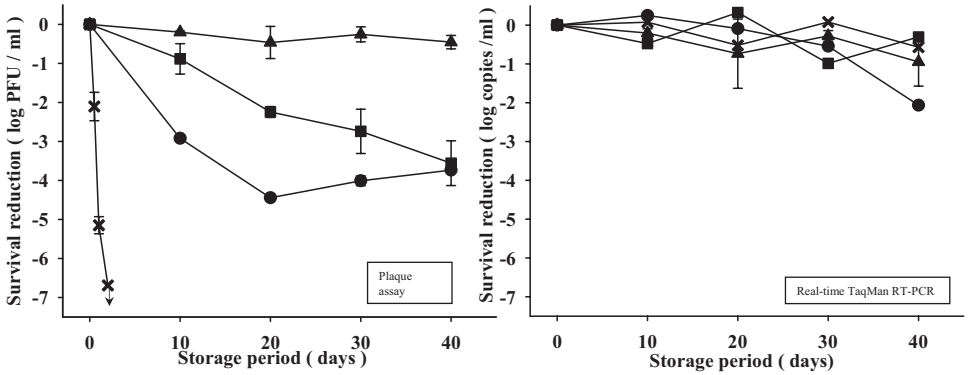

FIG. 2. Survival of MNV in a stool suspension at −20°C (●), 4°C (▲), 18°C (■), and 30°C (×). The arrows indicate detection limits ($n = 3$).

254-nm UV. A reduction in the level of MS2 due to exposure to UV with TiO_2 was observed (Fig. 4). An approximately 3-$\log_{10}$ reduction was detected for MS2 with both 1 and 10 mg/liter TiO_2, which was greater than the inactivation by other concentrations of TiO_2 particles. Therefore, we used 10 mg/liter of TiO_2 to characterize the rate of disinfection of MNV.

Inactivation by UV and by UV with TiO_2. Figure 5a shows the inactivation of MNV by 254-nm UV. The MNV was more susceptible to 254-nm UV than MS2. Only 1.6-$\log_{10}$ inactivation of MS2 occurred with 30 mJ/cm^2, whereas 3.3-$\log_{10}$ inactivation occurred with 25 mJ/cm^2. The maximum detectable reduction in the level of MNV was only 5 $\log_{10}$ due to the lower titer of the MNV viral stock. When the MNV was exposed to 30 mJ/cm^2, the maximum detectable level of reduction was observed. No evidence of tailing or flattening of the inactivation curve was observed.

Figure 5b shows the inactivation of MNV by UV with and without 10 mg/liter TiO_2. With TiO_2 particles without UV light, MNV was not inactivated at all. When MNV was exposed to UV light with TiO_2 particles (10 mg/liter), a 3.6-$\log_{10}$ reduction in the level of MNV occurred after 230 s. The inactivation of MNV by UV with TiO_2 particles was slightly greater than that by UV without TiO_2 particles. However, the inactivation with TiO_2 particles and the inactivation without TiO_2 particles were not significantly different. When we investigated this finding using real-time TaqMan RT-PCR assays, we observed no significant reductions.

DISCUSSION

Despite of the importance of NoVs, their persistence in various environmental conditions and the efficiency of disinfection by 254-nm UV and by UV with TiO_2 have not been well studied. In the present study, we investigated the survival and inactivation of an MNV as a surrogate for human NoVs. The MNV was more stable at a lower temperature (4°C) than at higher temperatures (18 and 30°C). These results indicate that NoVs can survive for a prolonged period of time, particularly at low temperatures, which is consistent with the results of a previous study (5). Similar results have been reported for other enteric viruses (3, 20). These results explain why outbreaks caused by NoVs are much more prevalent in the winter than in the summer (42). Unlike bacterial pathogens, NoVs cannot multiply once they are released from an infected human. In the environment, NoVs can be killed by various environmental stresses (for example, temperature, radicals, and solar irradiation). Our findings suggest that temperature is one of the most important factors in determining the survival of NoVs in the natural environment.

Comparisons can be made between the survival of MNV and the survival of FCV. A previous study showed that <1-$\log_{10}$ reductions in the amounts of FCV and canine calicivirus were observed after 2 weeks of incubation at 4°C, and a 3-$\log_{10}$ reduction occurred after 1 week of incubation at 20°C (12). Another study reported that FCV survived more than 1 month

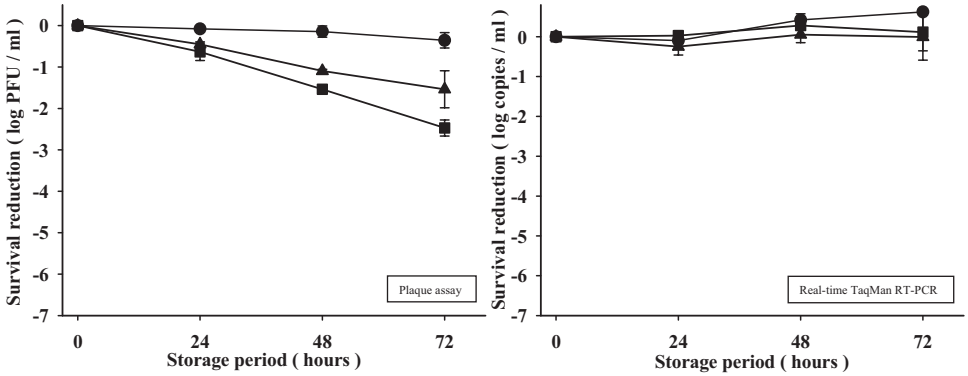

FIG. 3. Survival of MNV in distilled water (●), 0.5 M NaCl (▲), and 1 M NaCl (■) ($n = 3$).

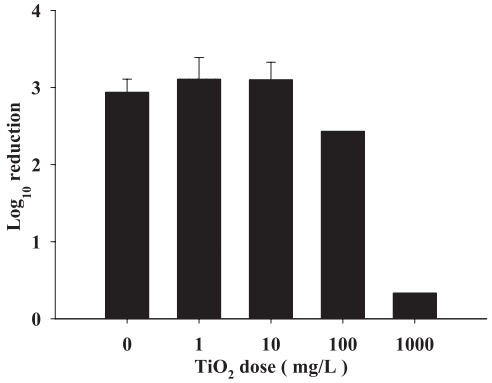

FIG. 4. Optimization of TiO_2 dose using MS2. The UV dose was 60 mJ/cm^2 ($n = 3$).

in marine water at 4°C, and only a 1-log_{10} reduction was observed after 25 days of incubation at 20°C (21). Still another study reported that FCV was stable for 14 to 21 days in suspension at room temperature, less stable at 37°C, and completely inactivated at 56°C in 1 h (10). Overall, our results are consistent with those of these studies. The results demonstrate that NoVs can survive for a long time, particularly at lower temperatures, and can be transmitted to susceptible populations through environmental media (16, 25).

Our data indicate that MNVs can survive better in a stool suspension than on the surface of gauze or a diaper. These results suggest that if the diaper had been soiled with feces, NoV would have persisted longer than it did when only it was present on the diaper. Typically, viruses survive better in liquid suspensions than on surfaces. After 30 days of incubation at 18°C, there was a 2.7-log_{10} reduction in the stool suspension, compared to more than the maximum reduction (5.3-log_{10} reduction) on the gauze and diaper surfaces. Several factors can explain the greater persistence of infectious NoVs in stool samples than on surfaces. One factor is that NoVs survive better under wet conditions. Cannon et al. compared the stability of MNV on wet and dry stainless steel coupons (5). After 7 days of incubation at 4°C, only a 1-log_{10} reduction in the amount of MNVs was observed on wet stainless steel, but a 2-log_{10} reduction was observed on dry stainless steel (5). These results indicate that NoVs survive better under wet conditions. Another explanation is that other chemical and microbiological factors present in stools protect NoVs from various environmental stresses. Previous studies have shown that reovirus is more stable in the presence of fecal material (31, 34).

To determine the survival rate of the MNV on the surface of the gauze and the diaper material, we tested several elution methods. Our results showed that a combination of 0.3% beef extract buffer and sonication was highly efficient for eluting MNV from both surfaces. Beef extract is a very efficient buffer commonly used to elute various viruses from filters, food, and other surfaces (30). A previous study showed possible inhibition of a subsequent PCR (4). However, in our study, 0.3% beef extract did not significantly inhibit our molecular assays,

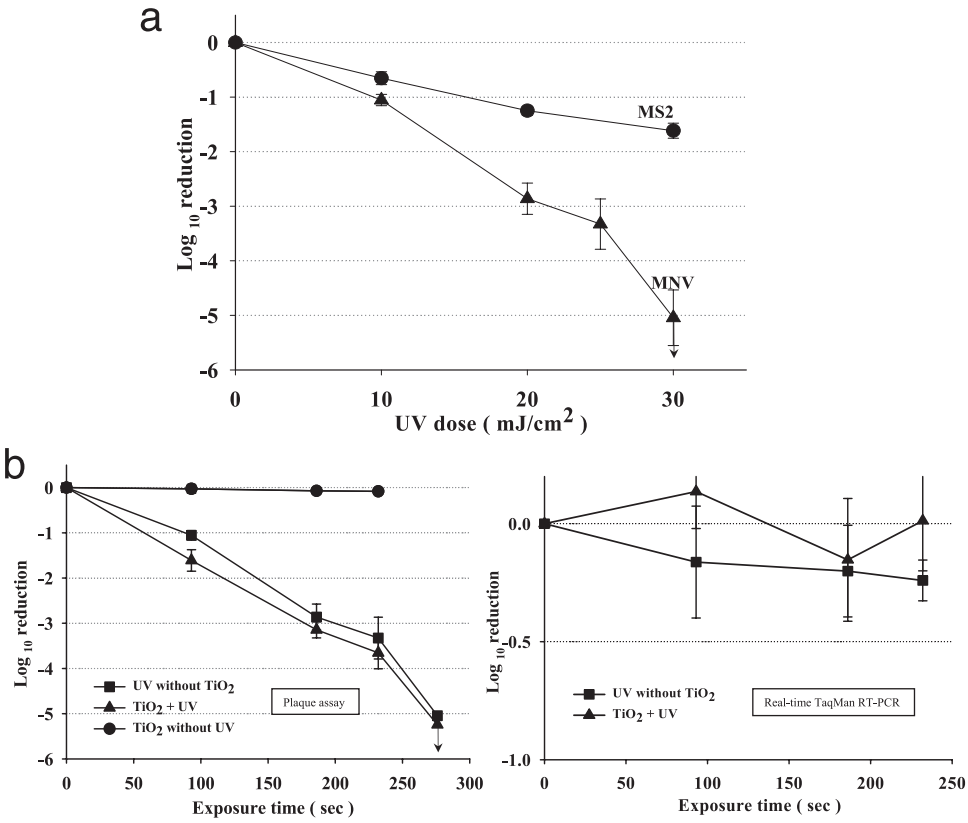

FIG. 5. (a) Inactivation of MS2 (●) and MNV (▲) with 254-nm UV. The arrows indicate detection limits ($n = 5$). (b) Inactivation of MNV by UV with TiO_2. The arrows indicate detection limits ($n = 3$).

likely because of the efficient removal of PCR inhibitors during the nucleic acid extraction procedure.

Inactivation of the MNV was characterized as a function of salt concentration; a high salt concentration facilitated inactivation of the MNV. These results suggest that inactivation of NoVs is likely more rapid in seawater than in freshwater because seawater contains 3% NaCl (~0.5 M NaCl). Greater inactivation with a higher salt concentration was previously reported for another virus as well (7). However, the fact that there was only a 2-$\log_{10}$ reduction in the presence of 0.5 M NaCl after 3 days suggests that there can be prolonged survival in seawater conditions as well. These results provide important insights for investigating NoV contamination in seafood, such as shellfish. NoV contamination in shellfish is frequent and causes outbreaks due to improper control of wastewater and the concentrating effects of the feeding of the shellfish themselves (32). None of these studies that investigated NoV contamination using RT-PCR assays could determine the infectivity of viruses. Further research is needed to investigate the survival of MNVs in shellfish.

Our study confirmed that the detection of viral RNA by the RT-PCR assay did not confirm the presence of infectious viruses (14, 33, 36). Simultaneous assays for detecting infectious viruses and viral RNA were performed by using the plaque and real-time TaqMan RT-PCR methods, respectively. Compared to the plaque assays measuring the infectivity of MNVs, the real-time TaqMan RT-PCR assays revealed only minor reductions in the levels of viral RNA. Regardless of the temperature conditions, only a <1-$\log_{10}$ reduction in the level of viral RNA was observed on the surfaces of both the gauze and the diaper material even after 40 days. A greater reduction in the amount of viral RNA was observed at −20°C than at the other temperatures used (4, 18, and 30°C). These data suggest that viral RNA is more sensitive to freezing and thawing than to the actual temperature. Our results demonstrate that the number of infectious MNVs is not correlated with the amount of viral RNA if the viral contamination is not recent. Although we cannot completely rule out the possibility that the viral RNA of inactivated MNVs is infectious, it is generally accepted that molecular assays for detecting viral RNA overestimate the number of infectious NoVs under most environmental conditions. Our results indicate that the difference between the amount of viral RNA and the number of infectious viral particles could be more than 5 $\log_{10}$ PCR units. The use of RT-PCR assays of NoVs for evaluating human health risk should be evaluated cautiously, particularly for environmental samples.

To our knowledge, this is the first report describing disinfection of NoVs by 254-nm UV and UV with TiO_2. The UV resistance of the MNV was greater than that of other calicivirus surrogates, such as FCV. UV doses of 10, 20, and 25 mJ/cm^2 caused 1.0-, 2.8-, and 3.3-$\log_{10}$ reductions in the amount of MNV, respectively. Previous studies reported a 4-$\log_{10}$ reduction in the amount of FCV after treatment with 19.4 mJ/cm^2 (39) and a 3-$\log_{10}$ reduction in the amount of FCV after treatment with 12 mJ/cm^2 (9). Another study reported that a 3-$\log_{10}$ reduction in the amount of FCV was obtained with 26 mJ/cm^2 of 254-nm UV (38). However, MNVs are much more susceptible to UV than other enteric viruses, such as adenovirus (24, 38). Our study suggests that human NoVs are likely to be more resistant to UV than previously reported and that UV disinfection could be an effective means of inactivating NoVs in water or on surfaces.

We also investigated whether addition of TiO_2 facilitates the inactivation of MNVs. Prior to the investigation of UV disinfection of MNVs with TiO_2, the optimal TiO_2 concentration was determined using the MS2 bacteriophage. Addition of low concentrations of TiO_2 particles (1 to 10 mg/liter) increased the rate of inactivation of MS2. Hydroxyl radicals generated from a photocatalytic reaction can synergistically increase the inactivation of MS2 by 254-nm UV. However, higher concentrations of TiO_2 particles (100 to 1,000 mg/liter) reduce the efficiency of MS2 disinfection, possibly because TiO_2 particles block the penetration of 254-nm UV and reduce the exposure of viruses to UV light. The rate of inactivation of the MNV by UV with TiO_2 was slightly higher than rate of inactivation by UV alone; however, the difference was not significant. Therefore, addition of TiO_2 appears to be of little help in disinfecting NoVs in water.

In summary, our findings demonstrate that MNVs can persist in the environment for a prolonged period of time, especially at low temperatures and in the presence of low salt concentrations. In addition, UV with and without TiO_2 can be an effective tool for disinfection of NoVs on surfaces and in liquid suspensions. Future research should evaluate field applications of tested disinfection methods for controlling NoV-contaminated surfaces or water.

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