

## Evaluation of the Persistence of Infectious Human Noroviruses on Food Surfaces by Using Real-Time Nucleic Acid Sequence-Based Amplification<sup>∇</sup>

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**Noroviruses (NoV) are the major cause of nonbacterial gastroenteritis. However, there is no published study to ascertain their survival on foodstuffs which are directly related to human health risk. In the present study, we developed a rapid, simple, and sensitive real-time nucleic acid sequence-based amplification (NASBA) combined with an enzymatic treatment for distinguishing infectious from noninfectious human NoV. The developed method was validated using spiked ready-to-eat food samples. When feline calicivirus (FCV) was used as a NoV surrogate in the preliminary assays, it appeared more sensitive to heat inactivation and enzymatic pretreatment than the human NoV. This suggests that FCV may not be an ideal model for studying NoV. Our results reveal clearly that the developed enzymatic pretreatment/real-time NASBA combination successfully distinguished the infectious from heat-inactivated NoV. Moreover, we demonstrate that NoV survived for at least 10 days on refrigerated ready-to-eat foods, such as lettuce and turkey. However, the survival rate was higher on turkey than on lettuce, probably because of their different surface natures. The approach developed in this study may be suitable for more in-depth studies of the persistence and inactivation of human NoV and may be applied to other nonculturable RNA viruses. Moreover, the evaluation of infectious NoV survival provided valuable information concerning its persistence on ready-to-eat food.**

Noroviruses (NoV), members of the *Caliciviridae* family, are the major cause of viral gastroenteritis in humans worldwide (6, 29). Epidemiological investigation of outbreaks has shown that more than 67% of all food-borne disease outbreaks due to known agents are caused by NoV (29). These viruses are transmitted mainly by person-to-person contact or by consumption of food or water contaminated directly by fecal matter or vomitus or indirectly by exposure to contaminated surfaces (13, 27). Enteric viruses such as NoV are able to survive outside the host and to remain relatively stable under food processing and storage conditions (17). In fact, any type of food product is a potential vehicle for NoV transmission and can cause infection with only a few infectious NoV particles (7). However, the consumption of fresh produce and ready-to-eat food contaminated by ill or asymptomatic food handlers has been identified as the most common source of NoV outbreaks (9, 10, 37).

Numerous efforts have been made to develop cell culture systems (gastric cells, duodenal cells, and small intestinal enterocyte-like cells) that mimic the intestinal environment for the in vitro propagation of NoV. Unfortunately, none of these monolayer cell culture combinations has been successful (12). Straub et al. (39) recently developed an infectivity assay for human NoV using a three-dimensional INT-407 small intestinal epithelium model. These three-dimensional cell cultures may be used to study host-pathogen interactions, such as attachment and intracellular replication, in addition to genomic and proteomic profiling (30). However, this is a long, costly,

and laborious procedure (maintaining the cell culture for approximately 35 generations in a rotating wall vessel with daily monitoring and replenishing the medium every 24 to 72 h). Moreover, this procedure has never been used to study persistence and inactivation of human NoV.

Due to the lack of animal models or suitable tissue culture, several studies have evaluated the persistence of human NoV using surrogates such as feline calicivirus (FCV) (11, 40), MS2 phage (10), and more recently murine NoV (7) in various foodstuffs and environmental conditions. FCV is a respiratory pathogen that it is unstable under acidic and environmental conditions (7, 15). Murine NoV 1, the first NoV found in mice, induces lethal systemic diseases (encephalitis, vasculitis, meningitis, hepatitis, and pneumonia) in immunodeficient mice and does not produce clinical symptoms in wild-type mice (24). Although it can be detected in intestines and feces and appears to be more stable than FCV at low pHs (7), it still differs from human NoV and may not reflect all of the characteristics of the latter.

Consequently, molecular methods are currently the most widely used technologies for the detection of NoV in food and clinical samples (1, 14, 33, 41). Although highly sensitive and specific, none of these techniques discriminates between infectious viruses and those that have been inactivated and thus rendered noninfectious and no threat to health. Since they target specific nucleic acid sequences and thus detect only the presence of the viral genome, these molecular assays do not provide any information about viral infectivity (14, 35, 38).

To discriminate between infectious viruses and those that have been inactivated using molecular methods, Nuanalsuwan and Cliver (31) have proposed an enzymatic pretreatment. The suggested enzymatic treatment uses simultaneously proteinase K and RNase for noninfectious virus particle digestion.

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TABLE 1. Primers and probes used to detect NoV and FCV by NASBA

Assay <sup>a</sup>	Oligonucleotide	Sequence <sup>b</sup> (5'–3')	Polarity or description	Reference
Conv. NASBA for NoV	NVP1	aattctaatacagactcactataggagaTCTCATCATCACCATA	–	20
	NVPa	AATTCCATCGCCCACTGGCT	+	20
	NVG2a	Biotin-GTCCCTGACATCATAACAGGCT	+	20
	NVG2b	Biotin-ACAGGACTAGGCCCGACAT	+	20
	NVG2c	Biotin-TCAGGTCTCTACCAGAT	+	20
Conv. NASBA for FCV	FCVf	GATAGCCCCAGCGTCAAG	+	14
	FCVr	aattctaatacagactcactataggagaTCGGAAAAGTAACGAAGG ATGT	–	14
	FCVr	Biotin-TCGACCCAATCGCCTCGTGTC	+	14
Real-time NASBA for NoV	JJV2F	CAAGAGTCAATGTTTAGGTGGATGAG	+	21
	COG2R	aattctaatacagactcactataggagaTCGACGCCATCTTCATTACA	–	22
	Saf1-MBP	FAM- <u>CCAAGCGGAGGGCGATCGCAATCTGGGCTTGG</u> - DabcyI	Molecular beacon probe	This study

<sup>a</sup> Conv., conventional.

<sup>b</sup> Lowercase letters correspond to the T7 RNA promoter sequence. Underlined nucleotides represent the stem structure of the molecular beacon. FAM, 6-carboxyfluorescein as the reporter dye; DabcyI, 4-(4'-dimethylaminophenylazo)benzoic acid as the quencher dye.

However, earlier reports have described the use of proteinase K for RNase inactivation during the isolation of viral RNA (32, 42). Thus, this enzymatic pretreatment must be reevaluated and readapted for infectious NoV detection.

In addition, insufficient data have been published to allow reliable estimation of the persistence of infectious human NoV on foodstuffs commonly associated with the risk of infection. The present study was therefore undertaken with two objectives: (i) to develop a new approach to the rapid, sensitive, and specific detection of putatively infectious NoV GII (Pi-NoV), using enzymatic treatment followed by real-time nucleic acid sequence-based amplification (NASBA) targeting the highly conserved ORF1-ORF2 domain, and (ii) to evaluate for the first time the persistence of Pi-NoV GII in common refrigerated foods.

#### MATERIALS AND METHODS

**Propagation of FCV-F9.** Seed cultures of Crandell's feline kidney cells and FCV-F9 virus were kindly provided by Sabah Bidawid, Health Canada, Ottawa, Ontario. Crandell's feline kidney cells were cultured as described previously (5) in Dulbecco's modified Eagle's medium (Aldrich Sigma Canada Ltd., Oakville, Ontario, Canada) supplemented with 10% (vol/vol) newborn calf serum, 1% (vol/vol) glutamine, 1% (vol/vol) nonessential amino acids, and 1% (vol/vol) penicillin-streptomycin (Invitrogen Canada Inc., Burlington, Ontario). The cells were maintained in a similar medium but with only 2% serum.

Norovirus strain GII was provided by a provincial public health laboratory in the form of clinical stool samples from patients with confirmed cases of GII gastroenteritis.

**Thermal inactivation.** Thermal inactivation data for FCV-f9 and NoV were obtained using a procedure described by Nuanualsuwan et al. (31) with some modifications. FCV stock suspension was diluted to a final concentration of approximately  $6 \times 10^5$  PFU/ml in 500  $\mu$ l of preheated phosphate-buffered saline (pH 7.0) held at 72°C. The viral suspensions were incubated for a selected time at 72°C to establish the heat inactivation curve and to set up the minimal treatment required to eliminate all infectivity. After the inactivation time, the viral suspensions were diluted 10-fold in Dulbecco's modified Eagle's medium and infectious particles were titrated immediately (5). One hundred microliters of GII NoV was heated in phosphate-buffered saline at 72°C for 45, 60, or 75 min at  $10^6$  NASBA particle units (NPU) per ml.

**Extraction of viral RNA.** The extraction of nucleic acid from FCV and NoV was performed with the Nuclisens isolation kit (BioMérieux, Boxtel, The Netherlands) according to the manufacturer's instructions. Briefly, 100  $\mu$ l of FCV suspension or 20% (vol/vol) of a stool sample was added to 900  $\mu$ l of lysis buffer,

and 50  $\mu$ l of activated silica was added to allow binding of RNA. After centrifugation, the bound nucleic acids were washed once with 1 ml wash buffer, twice with 1 ml 70% ethanol, and once with 1 ml acetone. The silica pellet was then dried at 56°C for 15 min. After drying, the RNA was eluted in 50  $\mu$ l of elution buffer and stored at –80°C until NASBA quantification.

**Conventional NASBA, gel electrophoresis, and dot blot hybridization.** The NASBA reaction was performed on extracted RNA in 25- $\mu$ l volumes as previously described (18, 20). A reaction mixture with 5  $\mu$ l of RNase-free water instead of extracted RNA served as a negative control. Analysis by agarose gel electrophoresis was done immediately after amplification. The amplified RNA was stored at –80°C for dot blot hybridization, which was performed as described previously (19).

**Real-time NASBA.** The real-time NASBA reaction was carried out in a Nuclisens EasyQ reader using a Nuclisens amplification basic kit according to the manufacturer's instructions (BioMérieux, Boxtel, The Netherlands) with some modifications. Briefly, reagent spheres were reconstituted in the diluents and KCl was added to a final concentration of 80 mM, followed by primers and a molecular beacon probe (Table 1) at final concentrations of 0.16 and 0.08  $\mu$ M, respectively. The reaction started upon mixing of 2.5  $\mu$ l of extracted RNA and 5  $\mu$ l of primer-beacon-reagent solution. A virus-free fecal sample (2.5  $\mu$ l) served as a negative control. Reaction mixtures were incubated at 65°C for 2 min, followed by further incubation at 41°C for 2 min and then addition of 2.5  $\mu$ l of reconstituted enzyme mixture and incubation at 41°C for 90 min.

**Estimation of human NoV concentration.** The human NoV concentration, expressed in NPU, was based on a standard curve obtained by molecular beacon-based NASBA using 10-fold serial dilutions of a NoV GII extract of known RNA concentration. The measured variable was the time at which the fluorescence exceeded the baseline response of the negative control by 20%, called the  $C_T$  time.

**Recovery of added Pi-NoV from foodstuffs.** Lettuce and cooked turkey slices purchased from local supermarkets were cut into 1.5- by 1.5-cm squares, which were then rinsed in sterile water for 2 min, allowed to dry in a laminar flow hood for 30 min, exposed on both sides to UV light for 1 min, and then placed in sterile six-well microplates. A 50- $\mu$ l droplet of suspension containing approximately  $5 \times 10^5$  NPU of NoV GII was deposited on the center of each square and left to dry in a laminar flow hood for 30 min. The microplates were then stored at 7°C and sampled in triplicate on days 0, 2, 4, 6, 8, and 10 for lettuce and 0, 2, 3, 5, 8, and 10 for turkey. Virus was eluted from each sampled square by repeated pipetting of the contaminated area using 200  $\mu$ l of 10% glycine-tryptone phosphate broth (pH 9.0) and analyzed.

**Enzymatic treatment.** The enzymatic digestion was carried out as described by Nuanualsuwan and Cliver (31) with major modifications. Briefly, 100  $\mu$ l of NoV or 500  $\mu$ l of FCV (heated and nonheated as a control) were incubated at 37°C for 1 h with 22 U of proteinase K (Aldrich Sigma Canada Ltd., Oakville, Ontario, Canada) freshly dissolved in phosphate-buffered saline. The proteinase K reaction was stopped by adding 2  $\mu$ l of 200  $\mu$ M phenylmethane sulfonyl fluoride (Aldrich Sigma Canada Ltd., Oakville, Ontario, Canada), and the suspensions

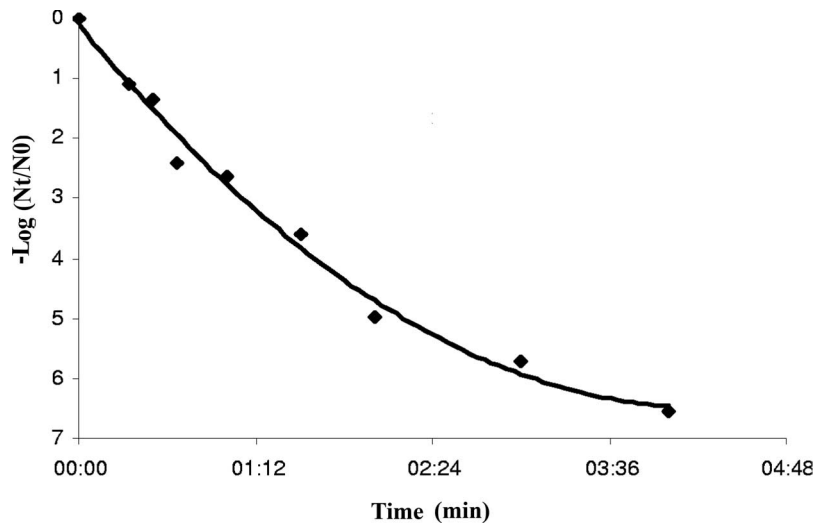


FIG. 1. Inactivation curve of FCV at 72°C. Nt/N0, ratio of the concentration of FCV at time *t* to the concentration of FCV at time zero.

were left at room temperature for 30 min. RNase (Roche Diagnostics, Indianapolis, IN) was added (100 ng), and the mixtures were incubated at 37°C for 1 h, after which 80 U of RNase inhibitor solution (Promega US, Madison, WI) was added. Viral RNA was then immediately extracted as described above.

**Statistical analyses.** Statistical analyses were performed with STATGRAPHICS Plus 4.1 (Manugistics Inc., Rockville, MD). Significant differences among mean values of each tested parameter over the 10 days of storage at 7°C of the lettuce and turkey squares were tested by analysis of variance. Treatment comparisons were performed using Fisher's least-significant-difference test, with a *P* value of ≤0.05 considered significant.

**RESULTS**

**Thermal inactivation.** Both FCV and NoV inactivation experiments were conducted at 72°C. Figure 1 shows the thermal inactivation curve of FCV-f9. After only 4 min, the initial infectivity of 6 × 10<sup>5</sup> PFU/ml of FCV-f9 was no longer detectable (~6.5-log reduction). For NoV inactivation, reduction in the levels of RNA was monitored by conventional NASBA detection. The NoV genome still cannot be completely degraded despite exposure for 45 and 60 min (Fig. 2B, lanes 8 and 10, respectively). However, the reduction in the inactivated NoV RNA signal was significantly greater after 60 min than after 45 min. Our results show that NoV appears to be much more resistant to heat inactivation than FCV and indicate that inactivated particles still can be detected by molecular methods.

**Effect of enzymatic digestion on detection of heat-inactivated FCV and NoV by conventional NASBA.** Detection of infectious and heat-inactivated FCV and NoV after enzymatic pretreatment was carried out at least in duplicate. The effect of the combination of 4.4 U of proteinase K and 20 ng of RNase per 100 μl on 6 × 10<sup>5</sup> PFU/ml of infectious and heat-inactivated FCV is shown in Fig. 2A. Conventional NASBA showed positive results for heat-inactivated FCV and infectious FCV with or without enzymatic digestion (lanes 1, 2, and 4). On the contrary, the digested heat-inactivated FCV gave no false-positive signal (lane 3). To achieve better results with Pi-NoV, higher concentrations of proteinase K (22 U/100 μl) and RNase (100 ng/100 μl) were used (Fig. 2B). Indeed, both agarose gel electrophoresis and dot blot hybridization showed positive results for untreated Pi-NoV (lane 6), Pi-NoV subjected to enzymatic treatment (lane 7), and heat-inactivated NoV not subjected to enzymatic treatment (lanes 8 and 10). However, heat-inactivated NoV subjected to enzymatic treatment (lanes 9 and 11) gave no detectable response.

**Effect of enzymatic digestion on the detection of heat-inactivated NoV by real-time NASBA.** Heating NoV for 45 to 75 min at 72°C produced a significant reduction in the fluorescence intensity obtained by real-time NASBA (Fig. 3A), although NoV was still detected at all heating times. However, when enzymatic digestion was combined with even the shortest

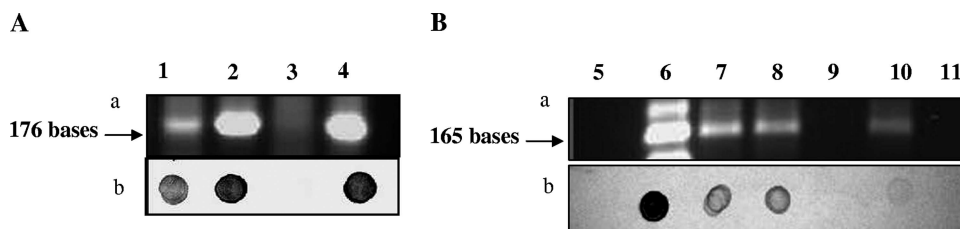


FIG. 2. Detection of FCV (A) or NoV (B) NASBA products by gel agarose electrophoresis (a) or dot blot hybridization (b). Lane 1, heat-inactivated FCV; lane 2, infectious FCV positive control; lane 3, enzyme-treated heat-inactivated FCV; lane 4, enzyme-treated infectious FCV; lane 5, blank; lane 6, infectious NoV positive control; lane 7, enzyme-treated infectious NoV; lane 8, NoV held at 72°C for 45 min; lane 9, NoV held at 72°C for 45 min followed by enzyme treatment; lane 10, NoV held at 72°C for 60 min; lane 11, NoV held at 72°C for 60 min followed by enzyme treatment.

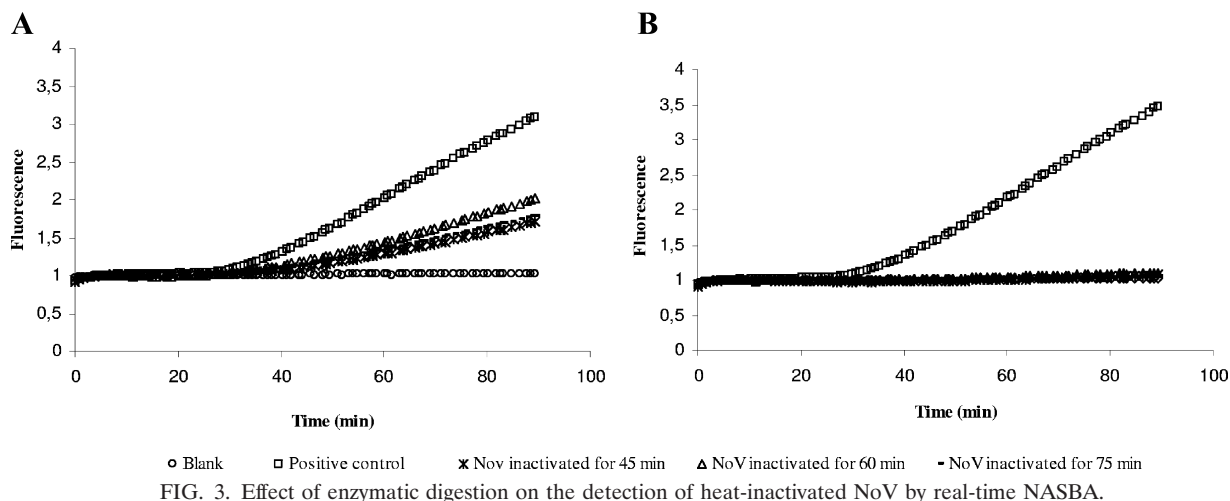


FIG. 3. Effect of enzymatic digestion on the detection of heat-inactivated NoV by real-time NASBA.

heat treatment, no signal and hence no false-positive result was obtained in any case, unlike the case for inactivated NoV not subjected to enzymatic digestion (Fig. 3B).

#### Persistence of Pi-NoV on refrigerated lettuce and turkey.

The standard curve and the equation used to establish the titer of NoV are shown in Fig. 4. For the persistence of Pi-NoV on lettuce and on turkey, data are expressed as means ( $\pm$  standard deviations) of the NoV titer for three experimental repetitions. Figure 5A shows NoV detected on lettuce at 7°C over a period of 10 days. The amount of NoV recovered after the 30-min drying at room temperature was approximately 40% of the initial viral titer of  $5 \times 10^5$  NPU. Although the titer thereafter dropped by more than one log cycle, our results demonstrate that NoV persisted for at least 10 days on refrigerated lettuce, that the decline was linear ( $P < 0.05$ ) whether enzyme-treated or untreated eluate was measured (1.37 and 1.79 log reductions, respectively), and that there was no significant difference ( $P > 0.05$ ) between the two assay procedures. Figure 5B shows the profile of NoV detected on turkey slices at 7°C over 10 days. Only 1% of the virus was recovered from the

meat surface after the 30-min drying at room temperature. However, there was no significant decline in viral titer throughout the 10-day period whether enzyme-treated or untreated turkey eluates were measured ( $P > 0.05$ ) and no significant difference between the two assays.

## DISCUSSION

According to several epidemiological studies, NoV are recognized as the most common cause of nonbacterial gastroenteritis worldwide (6, 29, 37). Molecular methods are currently the most promising technology for NoV detection. However, these assays do not provide any information about infectious risk (14). Moreover, epidemiological investigations during NoV outbreaks to identify the cause and source of illness are limited by the lack of a specific detection method for infectious NoV (9). The purpose of the present study was to develop a novel beacon-based NASBA assay using an enzymatic treatment to distinguish noninfectious from infectious human NoV. This method was then used to evaluate the survival of Pi-NoV on ready-to-eat food.

Our initial results demonstrate that FCV is rapidly inactivated at 72°C, as detected by plaque formation and by conventional NASBA. As shown previously (14), these findings indicate that moderate heating may alter the viral protein capsid and cause a loss of infectivity, although the viral genome may still be protected from RNase and other environmental factors. Breakdown of viral RNA therefore may not occur until it is released from the degraded capsid. Consequently, positive results obtained by molecular assays do not distinguish infectious from noninfectious viruses. Earlier studies have reported the same phenomenon using other inactivation methods. Simonet and Gantzer (38) have studied degradation of poliovirus 1 by chlorine dioxide and demonstrated that the detection of genome fragments by real-time reverse transcription (RT)-PCR must be interpreted with caution because positive results are insufficient to confirm the infectious risk for the human population. They also demonstrated that the stability of the smallest genome fragments is superior to that of the longest fragments, thus increasing the likelihood that detection of short genome fragments by RT-PCR overestimates the real infec-

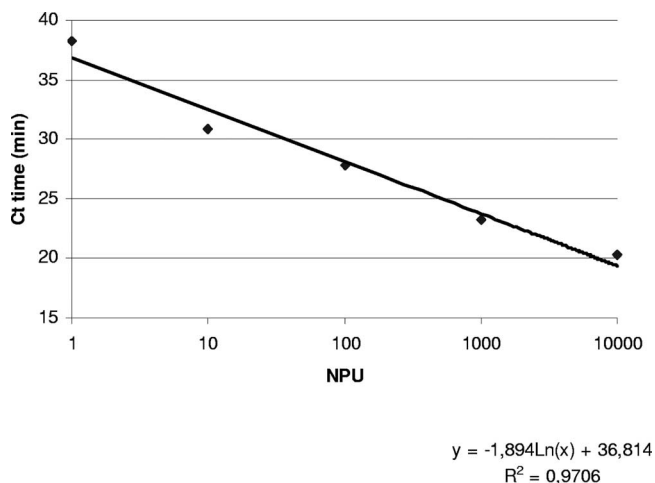


FIG. 4. Standard curve used to calculate the NoV count.  $C_T$  time is the time required for fluorescence to exceed the negative control level by 20%.

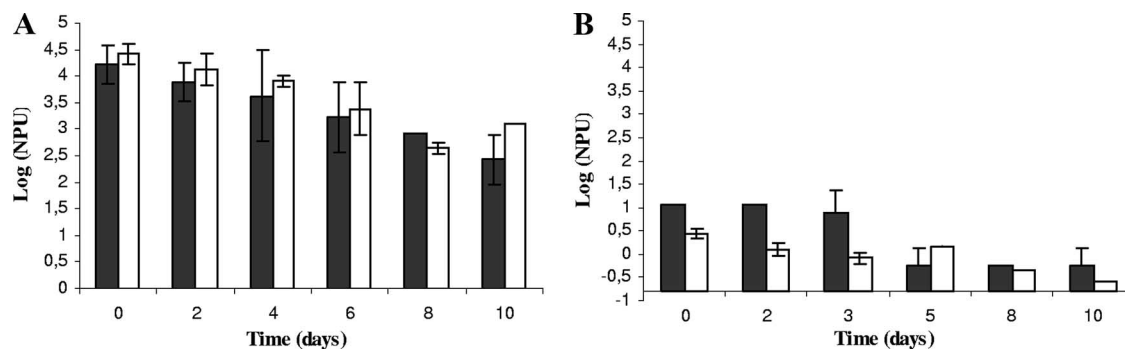


FIG. 5. Real-time NASBA detection of NoV GII eluted from refrigerated lettuce (A) or turkey slices (B). Black, untreated eluate; white, enzyme-treated eluate.

tious risk. It was also reported that from 68 samples of river water, 60 were positive for enterovirus RNA by RT-PCR but only 2 samples were positive for infectious enterovirus by cell culture (16).

In using enzymatic digestion combined with molecular beacon-based NASBA to distinguish infectious NoV from non-Pi-NoV and overcome the false-positivity problem, there is no practical way to test the infectivity of human NoV virions and prove that infectious particles are refractory to the enzymatic steps. It is assumed that noninfectious virions, regardless of how they become so, are digested by the treatment and that positive results are due to infectious particles. Preliminary assays using conventional NASBA showed that only infectious FCV and Pi-NoV were detected once the enzyme dosage was correctly adjusted (data not shown). Since our results indicate that human NoV was five times more resistant to the enzymes, there appear to be major limitations on what may be anticipated for NoV based on the response of FCV. Hewitt and Greening (15) have reported that compared to NoV, FCV is sensitive to environmental factors in general and therefore may not remain viable under acidic or alkaline processing conditions.

The choice of specific primers and probes is an important factor for the detection of genetically diverse NoV strains. Several recent studies have explored the ORF1-ORF2 junction as a target because it is the most conserved part of the NoV genome (23, 25, 36). In an effort to improve NoV GII primer specificity, Jothikumar et al. (21) designed the broadly reactive forward NoV GII primer JJV2F, which targets the junction domain and contains eight bases at the 3' end that are 100% identical among the majority of NoV GII sequences. To increase sensitivity and to detect a large variety of circulating NoV strains, the molecular beacon-based NASBA designed in this study was performed using a specific molecular beacon probe targeting the most conserved ORF1-ORF2 domain and using the sensitive forward primer (JJV2F). Using endpoint dilution, the developed real-time NASBA appeared to be 100-fold more sensitive than the conventional RT-PCR (data not shown). When the method is combined with the enzymatic pretreatment, our results clearly demonstrate that molecular beacon-based NASBA successfully distinguishes Pi-NoV from heat-inactivated NoV. This finding suggests that enzymatic pretreatment is essential to specifically detect infectious viruses.

The use of enzymatic treatment to avoid false-positive RT-PCR results with inactivated viruses has been previously described by Nuanualsuwan and Cliver (31), who optimized conditions for hepatitis A virus, vaccine poliovirus 1, and FCV, which differ in terms of stability and resistance to environmental conditions. They reported that simultaneous treatment with proteinase K and RNase for 30 min at 37°C resulted in a negative signal by conventional RT-PCR. We found no reports of this type of treatment combined with NASBA. It is well documented that proteinase K is very useful in the isolation of undamaged bacterial or viral RNA in high yield (8, 32), since ribonucleases are digested by this enzyme, particularly in the presence of sodium dodecyl sulfate or guanidinium chloride at low concentrations (32, 42). The simultaneous use of proteinase K and RNase proposed by Nuanualsuwan and Cliver (31) to eliminate RNA from inactivated virus suspensions could yield inconsistent results for this reason.

As reported in previous studies, the human enteric viruses are present in low concentrations in foods (17, 37), since no virus is able to replicate outside live cells. Moreover, enteric viruses may be inactivated during food storage or processing (35). However, our results indicate that NoV persists in what may be an active form for at least 10 days on refrigerated lettuce and turkey. Since the minimal infectious dose of this virus is very low (7), if a method for the detection of infectious NoV in food is to be useful, it must be very sensitive.

To the best of our knowledge, there is no study of the stability of Pi-NoV in foods or on surfaces, but it is well documented that a low temperature is favorable to most of the enteric viruses (2, 37). We therefore compare our results with caution to others obtained using NoV surrogates under similar experimental conditions. In general, the results of our study are consistent with reports of the persistence of human NoV under refrigerated storage conditions. Dawson et al. (10), using MS2 phage as a surrogate to predict the persistence of NoV on fresh produce at refrigeration temperatures, showed that only one log reduction occurred during the first 39 days on tomato, cabbage, and lettuce and concluded that virus survival exceeded the shelf life of the produce. More recently, Mattison et al. (28), using FCV as a surrogate to predict the survival of NoV on different food samples and surfaces, demonstrated that infectious virus was able to survive over 7 days at both room and refrigeration temperatures in lettuce and ham and on stainless-steel surfaces. Hewitt and Greening (15), compar-

ing the persistence of NoV RNA and survival of FCV in marinated mussels, did not observe any reduction in NoV RT-PCR units after 4 weeks at 4°C. However, a pronounced decline in the FCV titer was obtained under these experimental conditions. Our finding in the present study that NoV survival on turkey was slightly greater than that on lettuce suggests that the food surface may affect the persistence of NoV infectiousness or recovery of the virus by elution. This protective effect of food matrices has already been reported for other studies (4, 28, 34). As found by Mattison et al. (28), the smallest reduction in the FCV titer over 7 days at refrigeration temperature was observed for ham, compared to lettuce, strawberries, and stainless-steel surfaces. These authors suggested that smooth surfaces, such as that of lettuce, might provide less protection to the virus than coarse surfaces like that of ham. These results were in agreement with those reported by Kurdziel et al. (26). Other studies suggest that on plant surfaces, viruses are exposed to potentially toxic compounds, such as phenolics, ethanol, and acetaldehyde, which could accelerate the inactivation processes (3, 10). However, the great stability in turkey may be due to the protective effect of protein and fat (37). The evaluation of enteric virus survival using virus models or surrogates should therefore be interpreted with caution despite the similar physical and chemical properties of these viruses. In addition to virus type, factors such as the nature of the food and experimental conditions must be considered.

To the best of our knowledge, this is the first study to report the survival of infectious GII NoV on ready-to-eat foods. The results of this study reveal that enzymatic treatment combined with molecular beacon-based NASBA is a useful approach for the detection of infectious GII NoV in food samples. This approach may be applied to other nonculturable RNA viruses and may also be suitable for more in-depth studies of the persistence and inactivation of NoV.

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