HHS Public Access

Author manuscript

J Appl Microbiol. Author manuscript; available in PMC 2024 June 14.

Published in final edited form as:

J Appl Microbiol. 2024 February 01; 135(2): . doi:10.1093/jambio/lxae033.

Heat inactivation of aqueous viable norovirus and MS2 bacteriophage

Marlee Shaffer[1], **Kimberly Huynh**[2], **Verónica Costantini**[2], **Jan Vinjé**[2], **Kyle Bibby**[1,*]

[1]Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, IN 46556, United States

[2]National Calicivirus Laboratory, Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States

Abstract

Aims: This study aimed to compare the heat inactivation kinetics of viable human norovirus with the surrogate, MS2 bacteriophage as well as assess the decay of the RNA signal.

Methods and results: Human intestinal enteroids were used to analyze the heat inactivation kinetics of viable human norovirus compared to the surrogate MS2 bacteriophage, which was cultured using a plaque assay. Norovirus decay rates were 0.22 min^{-1}, 0.68 min^{-1}, and 1.11 min^{-1} for 50°C, 60°C, and 70°C, respectively, and MS2 bacteriophage decay rates were 0.0065 min^{-1}, 0.045 min^{-1}, and 0.16 min^{-1} for 50°C, 60°C, and 70°C, respectively. Norovirus had significantly higher decay rates than MS2 bacteriophage at all tested temperatures ($P = .002–.007$). No decrease of RNA titers as measured by reverse transcription-PCR for both human norovirus and MS2 bacteriophage over time was observed, indicating molecular methods do not accurately depict viable human norovirus after heat inactivation and treatment efficiency is underestimated.

Conclusions: Overall, our data demonstrate that MS2 bacteriophage is a conservative surrogate to measure heat inactivation and potentially overestimates the infectious risk of norovirus. Furthermore, this study corroborates that measuring viral RNA titers, as evaluated by PCR methods, does not correlate with the persistence of viable norovirus under heat inactivation.

Keywords

human norovirus; surrogates; heat inactivation; persistence; viability

*Corresponding author. Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, IN 46556, United States. kbibby@nd.edu.

Author contributions

Marlee Shaffer (Conceptualization, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing), Kimberly Huynh (Investigation, Writing – review & editing), Verónica Costantini (Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – review & editing), Jan Vinjé (Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing), and Kyle Bibby (Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing)

Supplementary data

Supplementary data is available at *JAMBIO Journal* online.

Conflict of interest: No conflict of interest declared.

Introduction

Human noroviruses are icosahedral, non-enveloped, single-stranded RNA viruses of the family *Caliciviridae* (Bae and Schwab 2008, Ahmed et al. 2014). Norovirus is transmitted through the fecal-oral route via contaminated water and food and person-to-person contact. Recent estimates demonstrate that noroviruses are the most common cause of acute gastroenteritis among all age groups worldwide, resulting in 685 million cases annually. Noroviruses cause 58% of foodborne illnesses in the United States each year with an estimated cost of $2 billion annually (Lopman et al. 2012, Ahmed et al. 2014). Norovirus outbreaks linked to contaminated recreational, agricultural, and drinking waters have been reported throughout the past decade (Zhu et al. 2020). Symptomatic norovirus infections are typically relatively mild for healthy individuals; however, clinical symptoms can be more severe and require hospitalization for the elderly, young children, and immunocompromised persons (Lopman et al. 2012).

Due to the lack of a robust cell culture system for human norovirus, the effect of inactivation experiments has typically been measured by molecular methods, by cultivable surrogate viruses, or human challenge studies (Costantini et al. 2018, Zhu et al. 2020). Molecular methods include polymerase chain reaction (PCR), which is unable to discern between viable and non-viable virions, leading to overestimation of viable viruses after treatments (Hirneisen and Kniel 2013, Knight et al. 2016). Surrogate viruses, such as murine norovirus, feline calicivirus, Tulane virus, and MS2 bacteriophage, are morphologically similar to human norovirus (Hirneisen and Kniel 2013, Knight et al. 2016). However, there is an ongoing debate on the relevance of surrogate viruses and viable human norovirus (Hirneisen and Kniel 2013, Knight et al. 2016). Direct comparisons of the behavior of surrogate viruses to human norovirus in terms of infectivity have yet to be made (Hirneisen and Kniel 2013, Knight et al. 2016). Human intestinal enteroids (HIEs) have successfully shown to support replication of human norovirus indicating the presence of viable and infectious norovirus (Costantini et al. 2018, Shaffer et al. 2022). HIEs recapitulate the complex environment of the gastrointestinal tract (Blutt et al. 2018, Costantini et al. 2018, Green et al. 2020, Hagbom et al. 2021).

Heat treatments are widely employed in food and wastewater processing since they are a cost-effective means of inactivating enteric viruses and bacteria (Lau et al. 2020, Zhu et al. 2020). Historically used in the food industry, these treatments involve relatively higher temperatures and shorter contact times to prevent product alterations, like pasteurization ranging from 55°C to 75°C for 15 s to 30 min (Peng et al. 2017). Additionally, anaerobic digestion, occurring for days at temperatures between 25°C and 45°C, highlights the varied temperature and time requirements across different heat treatment processes (Wu et al. 2006). In this study, we compared the effect of heat, at relevant time and temperature combinations for current pasteurization and anaerobic digestion techniques, on viable norovirus assessed by HIEs and inactivation of cultivable surrogate MS2 bacteriophage. Viral RNA titers were also determined for both viruses.

Materials and methods

MS2 bacteriophage heat inactivation

All experiments using MS2 bacteriophage were conducted at the University of Notre Dame. *Escherichia coli* bacteriophage MS2 (ATCC® 15597-B1™) was cultured and recovered from *E. coli* C-3000 (ATCC® 15597™), as described previously (Cormier and Janes 2014). After filtration, the phage was stored at 4°C until used for the heat inactivation experiments within 48 h of production. The double layer agar method was used to determine the titer of the MS2 bacteriophage stock (Baird and Bridgewater 2017).

Each 2 ml sample tube had 900 µl of ultrapure molecular water and 100 µl of MS2 bacteriophage stock, which had an initial concentration of 6×10^9 plaque-forming units (PFU)/ml. A heat block was used to achieve the desired temperatures (50°C, 60°C, or 70°C), and the temperature of the heat block was continually monitored throughout each experiment to ensure it remained constant. Post-experiment testing showed that all target temperatures were reached for an aliquot within 2 min. At each time point, as shown in Table S1, a tube was removed from the heat block and immediately added to an ice bath, and subsequently cultured using the double agar technique as previously described (Baird and Bridge-water 2017). A volume of 200 µl from each sample tube was aliquoted for downstream extractions and quantification and frozen at −80°C until used. All experiments were performed in duplicate with three technical replicates, used for the serial dilution of the plaque assay, for each time point. All experiments used ultrapure molecular water as a negative control.

Nucleic acids of MS2 bacteriophage samples and controls were extracted from each 200 µl aliquot using the AllPrep PowerViral DNA/RNA Kit (Qiagen) using Glass PowerBead Tubes included with the kit. Solution PM1 was heated to 55°C, and 600 µl was added to each PowerBead Tube with 6 µl of ß-mecaptoethanol (MP Biomedicals). Each tube was vortexed and homogenized on a FastPrep 24 Bead Beating Instrument for 20 s at 4.5 M/s for four rounds. PowerBead Tubes were centrifuged at 13 000 *g* for 1 min, and 700 µl of supernatant was transferred to a clean 2-ml microcentrifuge tube. The remaining steps followed the Qiagen protocol, and 100 µl of RNase-free water was used to elute the nucleic acids. Negative controls of ultrapure water were also extracted to ensure no contamination occurred during the extractions.

Extracted MS2 Bacteriophage RNA was quantified using reverse transcription droplet digital polymerase chain reaction (RT-ddPCR) performed on the BioRad QX2000 Droplet Digital PCR System with thermal cycling on the C1000 Touch Thermal Cycler (BioRad). RNA reverse transcription and PCR amplification were performed in a single reaction using the One-Step RT-ddPCR Advanced Kit for Probes (BioRad) per the manufacturer's instructions. Oligonucleotide primer and probe sequences and thermocycling conditions are summarized in Tables S2 and S9, and Table S10 shows the dMIQE and MIQE guidelines, respectively. Each RT-ddPCR plate included a negative control from the experiments, a MS2 gblock positive control, and a no-template control of molecular water. QuantaSoft Version 1.7.4 (BioRad) was used to manually threshold the RNA copy number for each reaction.

Norovirus heat inactivation

CDC's internal program for research determination deemed that this study is categorized as public health non-research and that human subject regulations did not apply. All experiments utilizing the HIE assay were conducted at the CDC.

A fresh 10% stool suspension was prepared by adding 0.5 g of GII.4 Sydney[P31] positive stool that was previously collected and stored at −70°C as aliquots to 4.5 ml of PBS. The stool suspension was vortexed for 30 s, kept at room temperature for 5 min, and vortexed again. The sample was sonicated for 10 s, and solids were removed by centrifugation for 10 min at 10 000 × *g*. The supernatant was sequentially filtered through 5, 1, 0.45, and 0.22 μm filters to remove aggregates of virus and other stool components, and the final 0.22 μm filter removed bacteria, which could immediately affect the HIE cell culture (Costantini et al. 2018). The resulting 10% stool filtrate was aliquoted and stored at −70°C until tested.

Adult secretor-positive jejunal HIE cultures (J2 cell line) were grown at 37°C and 5% CO_2 as undifferentiated 3D cultures as described previously with minor modifications (Ettayebi et al. 2016, Costantini et al. 2018). Briefly, HIEs were recovered from liquid nitrogen, suspended in 20 μl of Matrigel™ (Corning), plated in 24 well plates, and grown as 3D cultures in 500 μl IntestiCult™ (INT) organoid growth medium (stem cell technologies) supplemented with 10 μM Y-27632 (Sigma-Aldrich).

Duplicate 96 well plate monolayers were prepared as previously described (Costantini et al. 2018). Briefly, HIE cultures were dissociated into a single-cell suspension in 100 μl of INT medium supplemented with 10 μM Y-27632 (Sigma-Aldrich) and plated as undifferentiated monolayers in collagen IV (Sigma-Aldrich) pre-coated 96 well plates. After 24 h, we replaced the INT medium with a differentiation medium to induce cell differentiation. Differentiation media was prepared by mixing equal volumes of IntestiCult™ (INT) organoid basal medium (stem cell technologies) and complete media without growth factors (CMGF-; advanced DMEM/F12 medium supplemented with 1x Glutamax, 10 mM HEPES, and 100 U/ml penicillin–streptomycin). Monolayers differentiated for 4 days at 37°C and 5% CO_2, and we refreshed differentiation media every other day, ensuring 100% confluence before use.

Inactivation kinetics for viable human norovirus were determined at 50°C, 60°C, and 70°C. Two experiments were conducted per temperature, with three technical replicates for each experiment. Two experimental replicates were conducted due to cost and resource limitations involved with using the HIE assay, which is in agreement with previous infection experiments using the HIE assay (Shaffer et al. 2022). In each sample tube, 800 μl of infection media [CMGF- supplemented with 500 μM glycochenodeoxycholic acid (GCDCA) and 50 μM ceramide (C2)] was added to diluted 10% stool suspension. Samples were placed on a digital dry heat block at the desired temperature, and at each time point, a sample tube was removed for HIE infections and placed in an ice bath. Due to the complex media, the temperature of the sample was not directly measured. Negative controls (no virus) and treatment (no heat) controls were included.

Spiked aliquots consisted of infection media spiked with norovirus suspensions (Figs S1 and S2) and non-spiked aliquots were solely infection media. Duplicate 96-well plates were inoculated with 100 µl of each sample. All HIE infections were performed in triplicate wells of 100% confluent 4-day-old differentiated HIE monolayers. After a 1-h incubation at 37°C and 5% CO_2, HIE monolayers were washed twice with CMGF- and 100 µl differentiation medium containing 500 µM GCDCA and 50 µM C2 was added. For each set of experimental plates for the HIE assay, one plate was frozen immediately at −70°C, and a duplicate plate was incubated at 37°C, 5% CO_2, for 72 h and then frozen at −70°C.

Viral RNA from spiked and non-spiked aliquots, cells, and media at 1 and 72 h after infection was extracted using the MagMax-96 Viral RNA Isolation Kit (Applied Biosystems, Foster City, CA, USA) and an extraction control (MS2 bacteriophage) according to the manufacturer's instructions. Norovirus genomic copies were quantified by real-time reverse-transcription quantitative PCR as described previously (Cannon et al. 2017). The primer and probe sequences are shown in Table S2 (Cannon et al. 2017). Each reaction was prepared as 22 µl volume consisting of 12.50 µl 2X RT-PCR Buffer, 1.15 µl of primer and probe mixture, 1.00 µl of 25X RT-PCR Mix, 7.35 µl of molecular grade water, and 3 µl of nucleic acid extract. A standard curve using 10-fold serial dilutions of quantified GII.4 Sydney RNA transcripts was generated, and norovirus genomic copies from each sample were extrapolated from the curve. The RT-qPCR limit of detection was 28.6 RNA copies per 1 µl (or 2.86×10^3 RNA copies/well for infection). Measurements below the limit of detection were assigned to half of the limit of detection (i.e. 14.3 RNA copies per 1 µl of RNA or 1.43×10^3 RNA copies/well for infection).

Statistical analysis

All statistical analyses were completed R Studio 2022.07 (RStudio Team, n.d.). The plots below show all experimental runs and technical replicates' mean and standard error. Compiled raw data showing the mean and standard deviation for the pooled replicates can be found in the SI ($n = 6$). Significant differences in genomic copies and PFU were analyzed using a Student's t-test with multiple comparisons. Viable human norovirus and MS2 bacteriophage decay were analyzed using a monophasic decay model, which assumes first-order decay (Equation 1).

$$C_t = Ce^{-kt}, \tag{1}$$

where C_t is the genome copies or PFU at time t, C_0 is the genome copies or PFU at time 0, k is the decay rate, and t is the time in minutes. Half-life and T_{90} values, the time for a 90% reduction in starting concentration, were also evaluated.

Results

Heat inactivation of MS2 bacteriophage

Monophasic decay of MS2 bacteriophage was calculated for each point using Equation (1) (Fig. 1a). The decay rate increased with temperature for viable MS2 bacteriophage;

however, the molecular data showed no significant change in RNA signal with temperature ($P = .12–.93$). The calculated decay rates for MS2 bacteriophage were 0.0065 min^{-1}, 0.045 min^{-1}, and 0.16 min^{-1} for 50°C, 60°C, and 70°C, respectively. Summaries of the decay rates, half-life, and T_{90} are shown in Table 1, and all viable decay rates were statistically different ($P = .01 –< .0001$). The decay rates found for each temperature were plotted against temperature (Fig. 2); for every degree increase in temperature (°C), the decay rate increased by 0.008 min^{-1}.

Heat inactivation of norovirus

The monophasic decay of norovirus is shown in Fig. 1b. The decay rate of viable human norovirus, shown by the slope of the line of best fit, increased as the temperature increased. The molecular data showed no significant change in the RNA signal with temperature. The decay rates for human norovirus were 0.22 min^{-1}, 0.68 min^{-1}, and 1.11 min^{-1} for 50°C, 60°C, and 70°C, respectively. Summaries of the monophasic decay rates, half-life, and T_{90} are shown in Table 1, and human norovirus decay rates were plotted against temperature, as shown in Fig. 2; for each degree increase in temperature, the decay rate increased by 0.04 min^{-1}.

Discussion

Heat is an effective treatment for the inactivation of enteric viruses in various matrices, including food (e.g. pasteurization) and wastewater (e.g. anaerobic digestion). Heat treatments are an effective and economical method for inactivating pathogens and have historically been used in the food industry, typically with low temperatures and long contact times to avoid product alterations (Lau et al. 2020, Zhu et al. 2020). For example, pasteurization of for ready-to-eat foods typically range between 55°C and 75°C for 15 s to 30 min for non-spore forming bacteria (Peng et al. 2017). Conversely, anaerobic digestion occurs on the magnitude of days and at temperatures between 25°C and 45°C for mesophilic and above 45°C for thermophilic digestion, showing the diversity of temperature and time ranges for different processes (Wu et al. 2006). This study showed heat is a highly effective treatment method for the inactivation of human norovirus, especially at relevant temperatures and times used, and shorter exposure times are necessary at higher temperatures to achieve the same level of norovirus inactivation.

MS2 bacteriophage is a surrogate of interest for human norovirus as it has the same capsid shape (icosahedral) and genome makeup (ssRNA), similar seasonality to human norovirus with respect to illness, and high concentrations of phage particles (10^{10} PFU/ml), which are necessary for inactivation studies, are relatively easy to achieve (Rzeżutka and Cook 2004, Dawson et al. 2005, Pecson et al. 2009). Furthermore, MS2 bacteriophage has no requirement for mammalian cell culture facilities and the ease of culturing allows for relatively simple infectivity analyses compared to human norovirus (Rzeżutka and Cook 2004, Pecson et al. 2009). MS2 bacteriophage had significantly higher decay rates at each temperature than human norovirus, indicating less heat susceptibility for MS2 bacteriophage, as shown in Fig. 2. MS2 bacteriophage is a conservative surrogate virus for human norovirus as inactivation occurs much faster with viable norovirus.

Previous studies using RT-qPCR also determined MS2 bacteriophage to be a conservative surrogate virus; however, this study is the first to analyze viable norovirus instead of solely using the molecular signal for comparisons (Bae and Schwab 2008). Due to the cultivation methodologies, there were different matrices used for human norovirus and MS2 bacteriophage in this study, and the impact of these matrices is not known and should be assessed in future work. There is also the potential for heat shock to have impacted the samples after exposure to heat when they were transferred to an ice bath. As culturing protocols started immediately after the heat inactivation experiments, the samples were not frozen, and the ice bath was used to stop heat exposure immediately.

Experiments using other enteric viruses and human norovirus surrogates were conducted by Bozkurt et al., who analyzed the thermal inactivation of murine norovirus, feline calicivirus, and hepatitis A virus (Bozkurt et al. 2013, 2014). In cell culture media, the T_{90} ranged from 19.95 to 20.23 min at 50°C and 0.56–0.94 min for 60°C for feline calicivirus, 34.49–36.28 min at 50°C and 0.57–1.09 min for 60°C for murine norovirus, and 56.22 min at 50°C and 2.67 min at 60°C for hepatitis A virus. Compared to the results of this study, MS2 bacteriophage has higher T_{90} than other human norovirus surrogates, indicating these surrogates using mammalian cells may be more indicative of the behavior of viable human norovirus.

Molecular methods are commonly used to determine the presence of viruses in samples; however, these methods cannot discern between viable and non-viable viruses. The results from these experiments show that there was no significant difference in the starting RNA signal and the RNA signal at the end of the experiment for both norovirus and MS2 bacteriophage, whereas there were significant differences in viable virus concentrations (Fig. 2). A study conducted by Pecson et al. found an 8.6 log loss of MS2 bacteriophage at 72°C for 3 min; however, the qPCR signal loss was 1.6 log, further showing the discrepancy between loss of infectivity and loss of molecular signal (Pecson et al. 2009). Overall, molecular methods largely underestimate treatment efficiency, especially when used to determine the efficacy of heat treatments (Bertrand et al. 2012).

The limitations of this study include using a single stool sample and using a costly and labor-intensive cell culture. With using a single stool sample, the variability between different genotypes of norovirus is not assessed and could be done in future work. Due to the HIE assay being costly and labor-intensive, only two replicate experiments were conducted, with three technical replicates used for each experiment. Each temperature and time combination therefore had six total replicates. As this is the first study to assess the heat inactivation of norovirus, the data are novel and can inform future decisions on time and temperature combinations.

Viability pre-treatments, such as intercalating dyes and RNases, could eliminate free RNA and RNA from structurally compromised virions to further demonstrate the disconnect between molecular detection methods and viability. Further research is needed to determine optimal methods for these assays, as inhibition still results in the molecular signal not representing the viability of inactivated viruses (Knight et al. 2016).

This study shows heat is an effective treatment to inactivate human norovirus in an aqueous solution. The data provided can be used to inform treatment time and temperatures for heat inactivation and details the effectiveness of current anaerobic digestion and pasteurization techniques. Our data also show that human norovirus decay is significantly faster than MS2 bacteriophage decay, showing that MS2 bacteriophage is a conservative surrogate for human norovirus under heat inactivation. Finally, our data demonstrate that molecular measurements do not correlate with viable human norovirus under heat inactivation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding

Funding for this work was provided by National Science Foundation grant [2006326]. The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Data availability

The data supporting the findings of this study are found in the SI in Tables S4–S8.

References

Ahmed SM, Hall AJ, Robinson AE et al. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. Lancet Infect Dis 2014;14:725–30. 10.1016/S1473-3099(14)70767-4 [PubMed: 24981041]

Bae J, Schwab KJ. Evaluation of murine norovirus, feline calicivirus, poliovirus, and MS2 as surrogates for human norovirus in a model of viral persistence in surface water and groundwater. Appl Environ Microbiol 2008;74:477–84. 10.1128/AEM.02095-06 [PubMed: 18065626]

Baird R, Bridgewater L. 9224 B. Somatic Coliphage Assay. In: Lipps WC, Baxter TE, Braun-Howland E (eds.), Standard Methods for the Examination of Water and Wastewater. Washington, D.C.: American Public Health Association, 2017.

Bertrand I, Schijven JF, Sánchez G et al. The impact of temperature on the inactivation of enteric viruses in food and water: a review: virus inactivation. J Appl Microbiol 2012;112:1059–74. 10.1111/j.1365-2672.2012.05267.x [PubMed: 22380614]

Blutt SE, Crawford SE, Ramani S et al. Engineered human gastrointestinal cultures to study the microbiome and infectious diseases. Cell Mol Gastroenterol Hepatol 2018;5:241–51. 10.1016/j.jcmgh.2017.12.001 [PubMed: 29675450]

Bozkurt H, D'souza DH, Davidson PM. Determination of the thermal inactivation kinetics of the human norovirus surrogates, murine norovirus and feline calicivirus. J Food Prot 2013;76:79–84. 10.4315/0362-028X.JFP-12-327 [PubMed: 23317860]

Bozkurt H, D'Souza DH, Davidson PM. A comparison of the thermal inactivation kinetics of human norovirus surrogates and hepatitis a virus in buffered cell culture medium. Food Microbiol 2014;42:212–7. 10.1016/j.fm.2014.04.002 [PubMed: 24929739]

Cannon JL, Barclay L, Collins NR et al. Genetic and epidemiologic trends of norovirus outbreaks in the United States from 2013 to 2016 demonstrated emergence of novel GII.4 recombinant viruses. J Clin Microbiol 2017;55:2208–21. 10.1128/JCM.00455-17 [PubMed: 28490488]

Cormier J, Janes M. A double layer plaque assay using spread plate technique for enumeration of bacteriophage MS2. J Virol Methods 2014;196:86–92. 10.1016/j.jviromet.2013.10.034 [PubMed: 24211298]

Costantini V, Morantz EK, Browne H et al. Human norovirus replication in human intestinal enteroids as model to evaluate virus inactivation. Emerg Infect Dis 2018;24:1453–64. 10.3201/eid2408.180126 [PubMed: 30014841]

Dawson DJ, Paish A, Staffell LM et al. Survival of viruses on fresh produce, using MS2 as a surrogate for norovirus. J Appl Microbiol 2005;98:203–9. 10.1111/j.1365-2672.2004.02439.x [PubMed: 15610433]

Ettayebi K, Crawford SE, Murakami K et al. Replication of human noroviruses in stem cell-derived human enteroids. Science 2016;353:1387–93. 10.1126/science.aaf5211 [PubMed: 27562956]

Green KY, Kaufman SS, Nagata BM et al. Human norovirus targets enteroendocrine epithelial cells in the small intestine. Nat Commun 2020;11:2759. 10.1038/s41467-020-16491-3 [PubMed: 32488028]

Hagbom M, Lin J, Falkeborn T et al. Replication in human intestinal enteroids of infectious norovirus from vomit samples. Emerg Infect Dis 2021;27:2212–4. 10.3201/eid2708.210011 [PubMed: 34287131]

Hirneisen KA, Kniel KE. Comparing human norovirus surrogates: murine norovirus and tulane virus. J Food Prot 2013;76:139–43. 10.4315/0362-028X.JFP-12-216 [PubMed: 23317870]

Knight A, Haines J, Stals A et al. A systematic review of human norovirus survival reveals a greater persistence of human norovirus RT-qPCR signals compared to those of cultivable surrogate viruses. Int J Food Microbiol 2016;216:40–49. 10.1016/j.ijfoodmicro.2015.08.015 [PubMed: 26398283]

Lau M, Monis P, Ryan G et al. Selection of surrogate pathogens and process indicator organisms for pasteurisation of municipal wastewater—a survey of literature data on heat inactivation of pathogens. Process Saf Environ Prot 2020;133:301–14. 10.1016/j.psep.2019.11.011

Lopman B, Gastañaduy P, Park GW et al. Environmental transmission of norovirus gastroenteritis. Curr Opin Virol 2012;2:96–102. 10.1016/j.coviro.2011.11.005 [PubMed: 22440972]

Pecson BM, Martin LV, Kohn T. Quantitative PCR for determining the infectivity of bacteriophage MS2 upon inactivation by heat, UV-B radiation, and singlet oxygen: advantages and limitations of an enzymatic treatment to reduce false-positive results. Appl Environ Microbiol 2009;75:5544–54. 10.1128/AEM.00425-09 [PubMed: 19592538]

Peng J, Tang J, Barrett DM et al. Thermal pasteurization of ready-to-eat foods and vegetables: critical factors for process design and effects on quality. Crit Rev Food Sci Nutr 2017;57:2970–95. 10.1080/10408398.2015.1082126 [PubMed: 26529500]

RStudio Team. n.d. RStudio: integrated development for R. http://www.rstudio.com/ (5 January 2024, date last accessed).

Rzeżutka A, Cook N. Survival of human enteric viruses in the environment and food. FEMS Microbiol Rev 2004;28:441–53. 10.1016/j.femsre.2004.02.001 [PubMed: 15374660]

Shaffer M, Huynh K, Costantini V et al. Viable norovirus persistence in water microcosms. Environ Sci Technol Lett 2022;9:851–55. 10.1021/acs.estlett.2c00553 [PubMed: 37179819]

Wu M, Sun K, Zhang Y. Influence of temperature fluctuation on thermophilic anaerobic digestion of municipal organic solid waste. J Zhejiang Univ Sci B 2006;7:180–5. 10.1631/jzus.2006.B0180 [PubMed: 16502503]

Zhu S, Barnes C, Bhar S et al. Survival of human norovirus surrogates in water upon exposure to thermal and non-thermal antiviral treatments. Viruses 2020;12:461. 10.3390/v12040461 [PubMed: 32325896]

Impact Statement

Norovirus is the leading cause of epidemic and endemic acute gastroenteritis worldwide. Treatments to inactivate norovirus are critical to reducing the risk associated with contaminated food and water. The recent developments to replicate human norovirus in human intestinal enteroids enable the evaluation of heat inactivation kinetics of viable norovirus. Historically, cultivable surrogate viruses have been used to approximate the environmental fate of human norovirus. Our findings indicate that compared to human norovirus, MS2 bacteriophage is a conservative surrogate to measure the effect of heat inactivation.

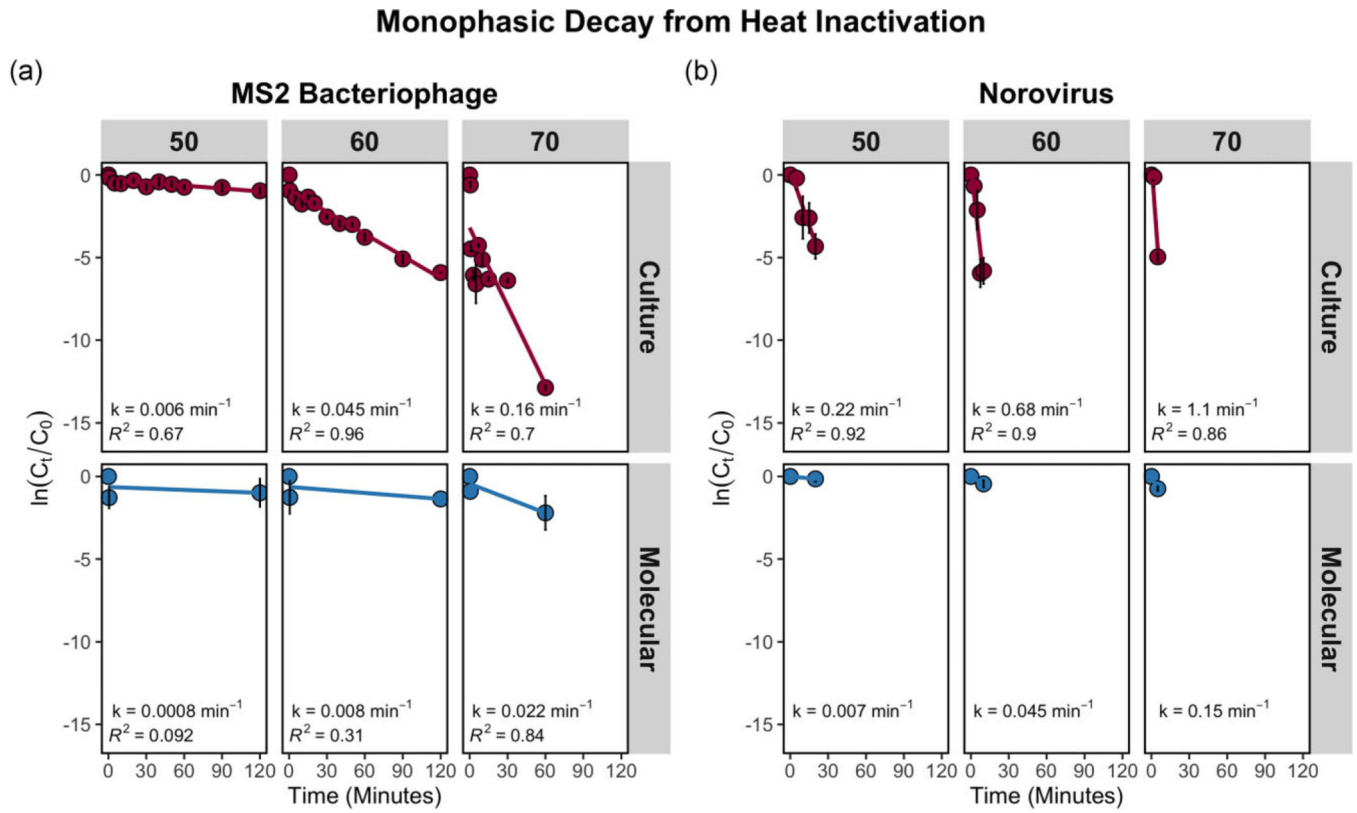

Figure 1.

Monophasic decay from heat inactivation. Decay rates and R^2 are shown on the plots. (a) Monophasic decay of MS2 bacteriophage with each column of panels representing a different experimental temperature. The top panels are from the culture assay, and the bottom panels are from RT-ddPCR. (b) Monophasic decay of norovirus with each column of panels representing a different temperature and the rows showing the results from the HIE assay and the molecular detection of the input virus.

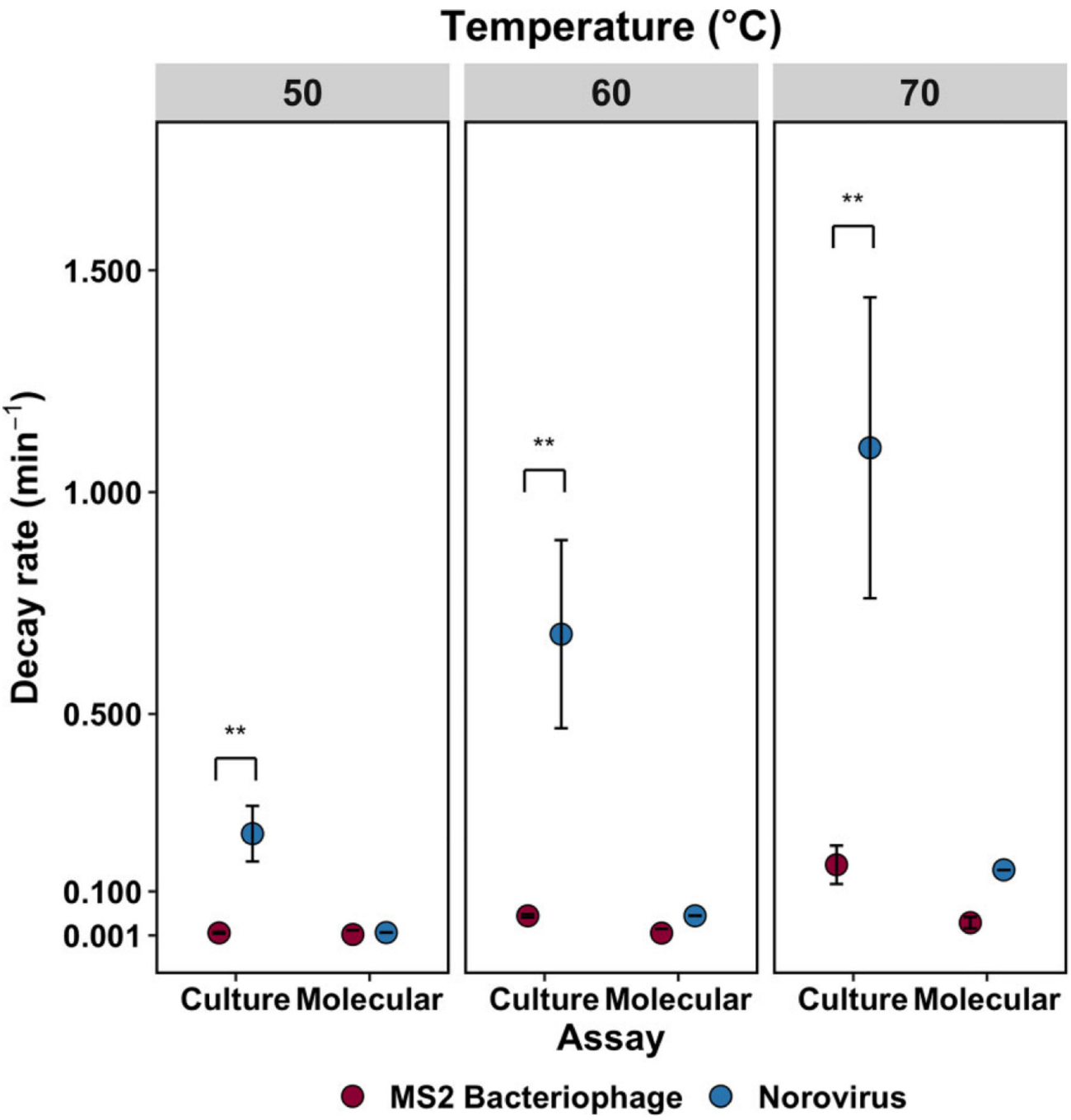

Figure 2.
Decay rate, *k*, versus temperature. The decay rates for each temperature for both viruses were plotted against temperature.

Table 1.

Summary of decay equations for viable MS2 bacteriophage and norovirus.

	MS2 bacteriophage (50°C)	MS2 bacteriophage (60°C)	MS2 bacteriophage (70°C)	Norovirus (50°C)	Norovirus (60°C)	Norovirus (70°C)
Decay rate (min^{-1})	0.0065 (0.003–0.010)	0.045 (0.038–0.052)	0.16 (0.073–0.24)	0.22 (0.098–0.34)	0.68 (0.26–1.09)	1.11 (0.44–1.77)
Half-life (min)	130.8 (67.9–256.7)	15.6 (13.5–18.3)	4.3 (2.9–9.5)	3.1 (2.02–7.1)	1.03 (0.6–2.7)	0.62 (0.4–1.6)
T_{90} (min)	354.2 (225.7–852.8)	51.6 (44.7–60.9)	14.4 (9.5–31.4)	10.4 (6.7–23.5)	3.4 (2.1–8.9)	2.1 (1.3–5.2)

The values shown are the mean and the 95% CI.