Investigating the Potential of Tulane Virus, a Human Norovirus Surrogate, to Develop Enhanced Recalcitrance After Serial Subfatal Heat Treatments

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ABSTRACT

Human noroviruses are the leading cause of foodborne illness and fourth leading cause of foodborne death in the United States.[1,2] Along with the substantial threat posed on public heath, foodborne noroviruses are estimated to cause $2.7-3.9 billion of economic loss to the U.S. economy annually.[3] In efforts to try to reduce the spread of human noroviruses, disinfection measures, both physical and chemical, has been attempted to inactivate viruses in food industry settings. However, in an FDA report on the occurrence of foodborne illness shows that sanitation non-conformances are the number one reason for infractions in food service.[4] The potential for subfatal disinfection of noroviruses exist in food preparation and service. Norovirus' prevalence and rapid evolution suggests the potential for evolution of variants with resistance to commonly used inactivation agents. To study this, serial subfatal inactivation of norovirus surrogate Tulane using heat as an inactivation measure was used to see if heat would act as a selective pressure causing the populations of viruses to resist inactivation in later passages. A series of temperatures and times were tested to cause subfatal disinfection of Tulane virus with one $\log_{10}$ of virus remaining after treatment. When exposing Tulane to 61.1°C for four minutes one $\log_{10}$ of virus remained and was used to grow the next generation of virus. This process will be continued for each generation of Tulane and each pool of virus will be evaluated for molecular and genetic changes.

INTRODUCTION

Human noroviruses are small, 38 nm in diameter, nonenveloped viruses that have (+)ssRNA enveloped in an icosahedral protein capsid.

Human Noroviruses are extremely diverse with over 30 genotypes reported.

- GII.4 is the most prevalent pandemic causing genotype, in which new strains emerge and circulate globally every few years.[6]

Noroviruses have multiple properties that make them hard to control:

- Low infection dose (18-100 virus particles)
- Ability to persist on environmental surfaces for weeks to months
- High mutation rate and ability to escape long term immunity
- High diversity
- General resistance to most common disinfectants

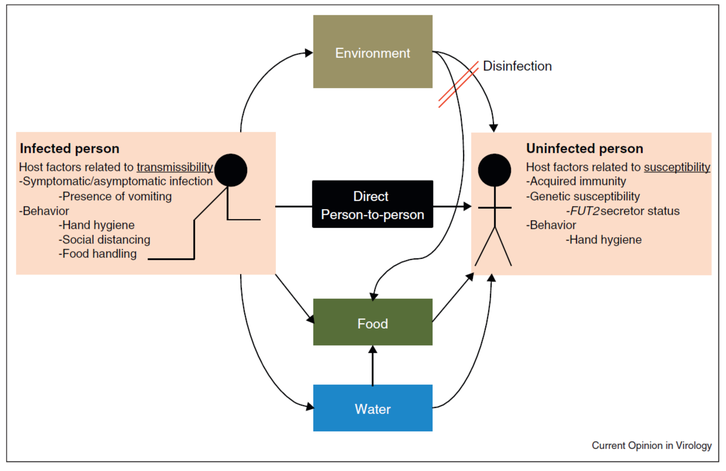
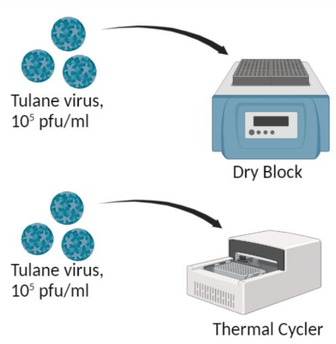


Figure 1. Routes of human norovirus transmission. (Lopman et al.)

Person-person contact is the main cause of norovirus transmission, however, there is a significant amount of foodborne caused infections.

- Food service accounts for 81% of food preparation reported norovirus outbreaks in the United States.[5]

Methods



Tulane virus titered at 10^5 pfu/ml was used for heat inactivation: Tulane virus was treated by exposure to various temperatures for 4-5 minutes via the dry block or thermal cycler.

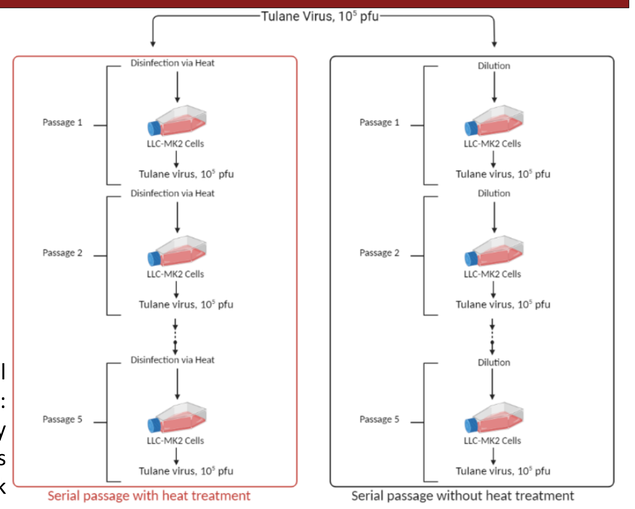


Figure 2. Schematic diagram of serial passages as performed in experimental adaptation of Tulane Virus. When initial infection occurs in flask 1, the virus is collected and used to infect cells in flask 2. This will be repeated for 5 series.

RESULTS

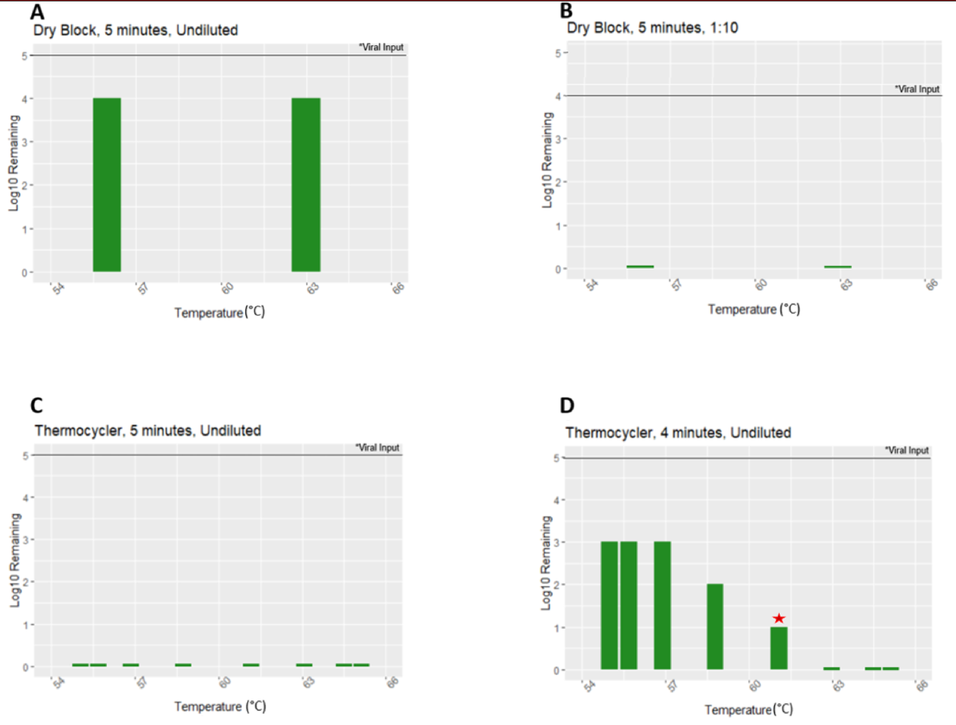


Figure 3. Viral $\log_{10}$ reduction during heat treatment using dry block and thermocycler. **A.** Undiluted Tulane Virus heated to 56°C and 63°C for 5 minutes via dry block; Viral input started at 10^5 pfu/ml. **B.** Tulane virus diluted 1:10 heated to 56 °C and 63 °C for 5 minutes via dry block; Viral input started at 10^4 pfu/ml. **C.** Undiluted Tulane virus heated to 55, 55.7, 56.9, 58.8, 61.1, 63, 64.3, and 65 °C for 5 minutes via thermal cycler; Viral input started at 10^5 pfu/ml. **D.** Undiluted Tulane virus heated to 55, 55.7, 56.9, 58.8, 61.1, 63, 64.3, and 65 °C for 4 minutes via thermal cycler; Viral input started at 10^5 pfu/ml. Star indicates what pool of virus was saved reestablishing next generation of virus. *No statistical difference between trials documented.

CONCLUSIONS

- The thermal cycler produced less variable results compared to a dry heat block.
- When Tulane virus, 10^5 pfu/ml, is exposed to 61.1 °C for 4 minutes, a 4 $\log_{10}$ reduction of the virus occurs leaving 1 $\log_{10}$ of virus.
- The pool of Tulane virus exposed to 61.1 °C for 4 minutes was used for the first serial passage.

FUTURE DIRECTIONS

Serial passage of Tulane virus will be continued until a total of 5 generations have been collected.

For each pool of virus collected at each serial passage, evaluation of infectivity and susceptibility to heat treatment will be evaluated.

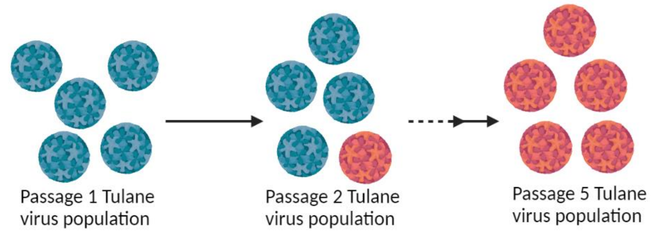


Figure 4. Schematic diagram of viral pools in passages exposed to heat treatment. Blue virus particles represent Tulane virus susceptible to heat inactivation. Red virus particles represent Tulane virus resistant to heat inactivation.

Isolation and plaque purification of Tulane from pools of interest will be identified, evaluated for susceptibility to inactivation, and sequenced.

Bioinformatic analysis of each passage comparing pools of virus exposed to heat treatment and virus that has not will be completed. The potential genetic changes that arose will be evaluated for contribution to susceptibility/ resistance to heat inactivation.

Acknowledgements

Thank you to the funders and supporters of our work: UMass Amherst, United States Department of Agriculture, National Institute of Food and Agriculture, the Moore Lab, Erica Light, and William Miller!

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