Survival of infectious human norovirus in water and on leafy greens

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- Dr. Christine Moe (Eugene J. Gangarosa Chair in Safe Water and Sanitation, Emory University)
- Dr. Lilly Pang (Professor, Department of Laboratory Medicine and Pathology, University of Alberta, Canada)

Summary

Human norovirus (HuNoV) is the leading cause of foodborne outbreaks in the United States. The virus has been recalcitrant to cultivate in a cell culture system since its discovery in the 1970s. The lack of a cell culture system makes it difficult to determine whether a sample has infectious viruses or not. A new breakthrough in organoid cell biology allowed the use of human stem cells to grow human mini-guts in a petri dish. In 2016 this novel human intes tinal enteroids (HIE) cell culture system was adapted for the determination of the infectivity of HuNoV. The overall goal of this project is to adapt the use of HIE cell culture for answering basic questions regarding the recovery and persistence of infectious HuNoV from lettuce and water.

Objectives

1. Determine the survival of infectious HuNoV in water and in relation to generic *E. coli*.
2. Determine the pre-harvest survival of infectious HuNoV on leafy greens.

Methods

The enteroids were differentiated into cell monolayers to be susceptible for norovirus replication. Known fecal samples positive for HuNoV or recovered samples from HuNoV-spiked lettuce or water were incubated on HIE for 1 and 72 hours. The HIE were then subjected to RNA extraction followed by reverse transcription real-time PCR targeting HuNoV. If a sample shows higher HuNoV RNA titer at 72 h as compared to the 1 h time point, the sample is deemed to contain infectious virus.

Lettuce and pond water samples were spiked with fecal samples containing infectious HuNoV. Recovery of infectious HuNoV at time zero was achieved by an optimized protocol based on centrifugation, followed by treatment with antimicrobial cocktail to eliminate bacterial communities from the water. The samples were tested on HIE as described above.

Results to Date

The novel human intestinal enteroids (HIE) cell culture system for human norovirus (HuNoV) was established and successfully implemented in our lab. Intestinal stem cells were grown in 3D culture for about one month to form mini-human guts with morphology similar to the human intestine (**Figure 1**).

The HIE system was validated using fecal samples positive for HuNoV that were obtained from our collaborators at the CDC (**Figure 2**).

The HIE system was used to recover infectious human norovirus from small pieces of HuNoV-spiked lettuce samples (**Figure 3A**). In addition, the HIE system was used to optimize a protocol to recover infectious HuNoV from small volumes of HuNoV-spiked pond water containing natural microflora (**Figure 3B**). Investigating the survival of infectious human norovirus in water and on lettuce is ongoing.

Benefits to the Industry

The produce industry will benefit from knowledge of die-off rates of infectious norovirus in water, on pre-harvest lettuce, and in relation to indicator organisms, such as *E. coli*, to better guide the industry in terms of agricultural water safety and the design of effective treatments.

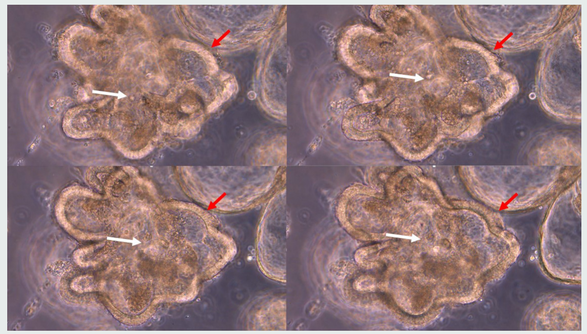

Figure 1. One month old human mini-gut grown in vitro from intestinal stem cells, shown at different depths. Red arrow indicates intestinal wall, and white arrow points to intestinal lumen.

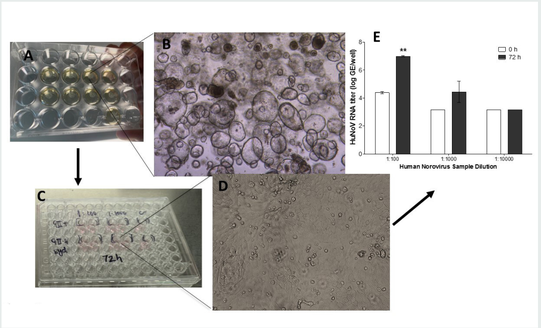

Figure 2. (A) Human intestinal enteroids (HIE) inside wells of a 24-well plate shown on 3D matrigel droplets. **(B)** HIE after one month of passage in cell culture, as seen under light microscopy showing the 3D structure of the mini-guts. **(C)** Enteroids dispersed into monolayers in 96-well plates grown on collagen. **(D)** Differentiated cell monolayer after 4 days in cell culture, as seen under light microscopy. **(E)** Successful replication of human norovirus from fecal suspension, as shown by significant increase in log copies of viral RNA after 72 h of incubation on differentiated HIE monolayers.

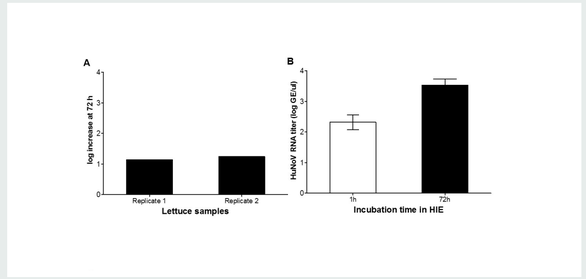

Figure 3. (A) Example of infectious human norovirus (HuNoV) recovery from lettuce at time zero (following a drying period), as shown by log increase in RNA titer at 72 h. **(B)** Example of infectious HuNoV recovery from one replicate of spiked pond water, as shown by increase in viral titer at 72 h when tested on HIE system.