

Survival of infectious human norovirus in water and on leafy greens



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Methods

Lettuce will be grown in the greenhouse to maturity (8 weeks) (**Figure 1**). The plants will be spiked with infectious human norovirus known to replicate in human intestinal enteroids (HIE). The virus will be incubated for 2 weeks under pre-harvest conditions. The infectivity and RNA titers of the virus will be determined using HIE and real-time qPCR. Water will be sampled from multiple produce growing regions and used to set microcosms in which the virus will be spiked with or without generic *E. coli*. The microcosms will be incubated under pre-harvest conditions and sampled weekly for 2 months. The infectivity and RNA titers of the virus and *E. coli* counts will be determined using HIE, real-time qPCR and standard microbiological enumeration methods, respectively.

Results to Date

A material transfer agreement (MTA) was signed with Baylor College of Medicine to obtain the new human norovirus cell culture system based on human intestinal enteroids. These enteroids are based on stem cells taken from human jejunum biopsies. These cells are being propagated in a three-dimensional cell culture system (**Figure 2**). The cells are currently maintained undifferentiated and ready to be differentiated into monolayers, which are then susceptible to infection by human norovirus. Another MTA was signed with our collaborators at the CDC and Emory University to obtain human norovirus GII.4 and GI.1 human norovirus-positive fecal samples.

Summary

Lettuce is a popular salad vegetable consumed fresh with minimal processing. However, lettuce and other leafy greens are often implicated in human norovirus foodborne outbreaks. In the US, under the Produce Safety Rule, agricultural water applied to produce is required to be tested for indicator organism *Escherichia coli* but not for viruses. The genetic materials of human norovirus often can be detected in surface water used for irrigation; however, it is unknown how long the virus remains infectious in water or on irrigated leafy greens. A breakthrough in human norovirus research was the discovery of replication of the virus in human intestinal enteroids. This cell culture system can potentially be used to answer questions regarding the infectivity of the virus on leafy greens and in agricultural water.

Benefits to the Industry

Advances in molecular biology allow the detection of genetic materials specific to human norovirus in produce and water; however, historically no known cell culture system was available to assess whether the virus is infectious or not. Molecular methods such as RT-qPCR tend to overestimate the quantity of norovirus due to detection of non-infectious damaged viral particles. The produce industry would benefit from knowledge of die-off rates of infectious norovirus in water, on pre-harvest lettuce, and in relation to *E. coli* to better guide the industry in terms of agricultural water safety and the design of effective treatments. The overall knowledge generated from this project will allow better assessment of the microbiological risks associated with exposure to norovirus from leafy greens contaminated via irrigation water.

Objectives

The overall goal of this project is to determine the survival of infectious human norovirus (HuNoV) in agricultural water and in relation to indicator organism *Escherichia coli* as well as its survival on leafy greens under preharvest conditions. Specifically:

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

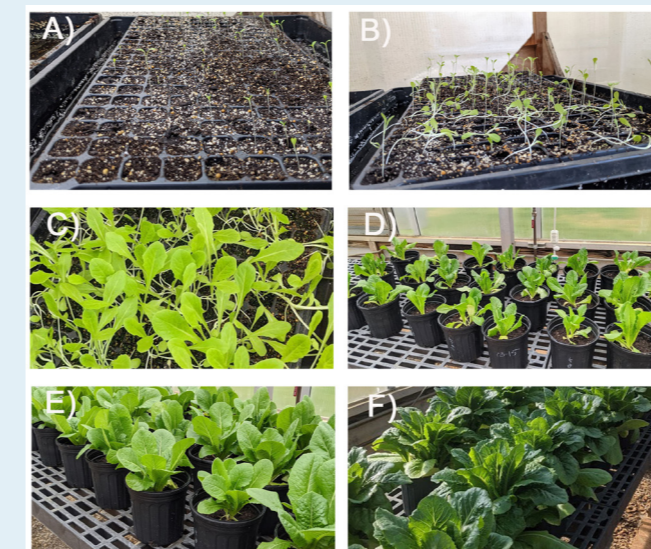


Figure 1. Lettuce grown under greenhouse conditions is shown at different ages: A) germination, B) week 2, C) week 4, D) week 4 after transfer to larger pots, E) week 6, and F) week 8 (mature).

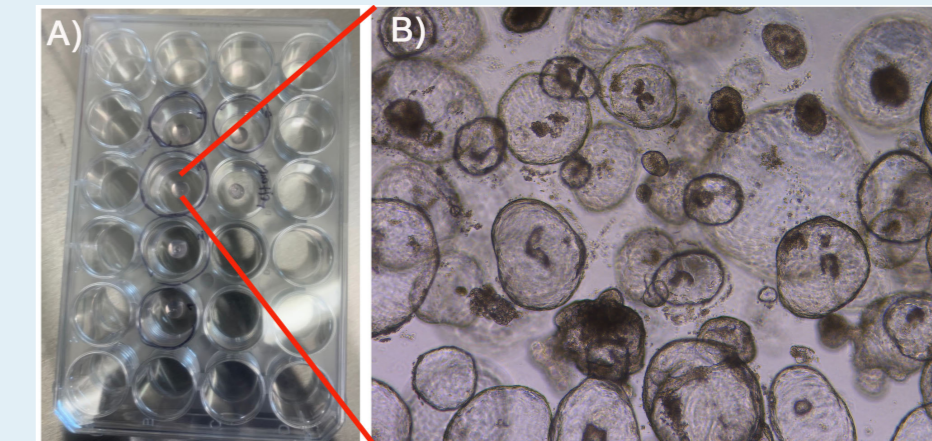


Figure 2. A) Human intestinal enteroids inside wells of a 24-well plate shown on 3D matrigel droplets (before addition of growth media); B) Human intestinal enteroids as seen under light microscopy showing the 3D structure of the mini-guts.