Development of an Infectivity Assay for Noroviruses in Cells
[Project #3027]

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OBJECTIVES:
This project sought to develop the first in vitro cell culture infectivity assay for human noroviruses, a U.S. Environmental Protection Agency Contaminant Candidate List (CCL-2) pathogen, isolated from source and drinking water supplies.

BACKGROUND:
Noroviruses (NoV) are the leading cause of non-bacterial, self-limiting, gastrointestinal illness worldwide. The most important routes of norovirus transmission are via contaminated water and food, and secondary person-to-person spread is significant. Due to the importance of this family of viruses, Caliciviridae are listed on USEPA’s Contaminant Candidate List (CCL-2). The USEPA uses this list of unregulated contaminants to prioritize research and data collection efforts to help them determine whether they should regulate a specific contaminant.

Research on NoV occurrence and risks in municipal drinking water supplies has relied on reverse transcription real-time PCR. However, real-time RT-PCR methods may not provide an accurate assessment of the infectivity potential for these viruses. While this method can provide pathogen occurrence data to the USEPA and the water industry, viral infectivity assays provide the best evidence of the public health risks associated with pathogen detection in water supplies. For NoV research, the problem has always been the lack of a suitable viral infectivity assay either in cell culture or animal models.

HIGHLIGHTS:
An in vitro 3-dimensional model of human small intestinal epithelial cells, generated by growing the cells on porous collagen-I coated microspheres in rotating wall vessel bioreactors under conditions of physiological fluid shear, provided a physiologically relevant tissue system that was permissive to human NoV infection.

Evidence of NoV infection was demonstrated through microscopic examination of the tissues, fluorescent in situ hybridization of the viral RNA inside cells, and conventional presence/absence PCR. Optimization of quantitative reverse transcription real-time PCR in conjunction with infectivity demonstrated reproducible increases in viral titer for a diverse group of NoV strains.

APPROACH:
Optimization of conditions for generating human physiological meaningful 3-D models of the small intestinal epithelium had been previously demonstrated for studies on Salmonella pathogenesis of the small intestine. Using this cell culture model, the research team tested a number of documented NoV outbreaks from cruise ships, nursing homes, and human challenge trials. Both Genogroup I and II strains were tested in this system to demonstrate robustness of the infectivity assay. Infectivity was assessed by light microscopy, transmission electron microscopy, hybridization of viral RNA inside cells, and conventional and real-time PCR. In addition, assays were conducted to determine if the viruses could be passed through cell culture.
RESULTS/FINDINGS:
1. Success in developing an infectivity assay for NoV in cells was demonstrated by visible cytopathic effect of the 3-D tissue aggregates using simple inverted light microscopy.
2. Sub-cellular pathology was demonstrated by conventional light microscopy and viral particle accumulation was demonstrated through transmission electron microscopy.
3. Harvesting viruses from an initial infection, the researchers demonstrated that these new viral particles will infect and replicate in unchallenged cells. This was demonstrated for up to five passages in cell culture. Evidence for this success was provided by light microscopy, transmission electron microscopy, fluorescence in situ hybridization, and end-point dilution PCR.
4. Quantitative reverse transcription real-time PCR demonstrated that for higher dose infection, between 10 and 1,000 fold replication occurs within the first 24 hours of infection. For low dose (<100 viral particles), significant increases in viral titer were observed within 72–96 hours post infection.
5. Additional evidence of viral infection was provided from proteomic and metabolomic studies of the infected hosts versus their corresponding uninfected host cells. In addition, this host cell response was demonstrated at the fifth passage of the viruses through cell culture.

IMPACT:
This particular project required basic fundamental research to develop this assay. Nonetheless, the water industry will benefit from this research in two important ways. First, by using an integrated cell culture reverse transcription real-time PCR approach, utilities can begin to determine if NoV isolated from their water supplies is infectious. Second, with careful experimental design, assays to determine efficacy of current water disinfection and treatment practices to remove NoV from water supplies can now be performed.

RESEARCH PARTNER:
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