Food Research Institute
UNIVERSITY OF WISCONSIN–MADISON

# FRESH Seminar: "News and Views from an Under-Appreciated Foodborne Virus: Astrovirus"

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MADISON, Wis. (FRI) – The astrovirus is a non-enveloped, positive-sense, single-stranded RNA virus that causes gastroenteritis, particularly in children. It's not commonly tested for, the illnesses it triggers are not notifiable, and it gets less publicity than norovirus, despite the fact it is the leading cause of childhood diarrhea, with more than 90% of the U.S. population showing evidence of exposure to it. At a recent FRESH seminar, Stacey Schultz-Cherry, PhD (St. Jude Children's Research Hospital) described her lab's work establishing novel models and analytical methods to study this emerging public health concern. In addition to providing detailed insight into astrovirus's unique mechanism of enterotoxicity, her work has shown the virus is more prevalent in the population (and in commercially sourced food such as oysters) than previously thought.

Astroviruses can infect virtually any species, and although individual astrovirus strains were previously believed to be species-specific, more recent work has demonstrated its zoonotic potential. Schultz-Cherry's group has evidence that astroviruses may be transferrable between turkeys and humans, while South American investigators have shown that children who play with piglets become infected with astroviruses that share genetic information with pig astroviruses. In Hong Kong, humans have been shown to harbor antibodies to rodent astrovirus strains, while conversely, nonhuman primates in Southeast Asia (who may be exposed to the human virus from the water supply) have been shown to shed human astrovirus.

The mechanism by which astroviruses cause gastroenteritis has been a puzzle. The virus neither elicits cell death nor stimulates an overt inflammatory response in the gut. Schultz-Cherry's group hypothesized that astrovirus instead wreaks havoc in the gut by disrupting the intestinal epithelial cell barrier. To test and refine this hypothesis, *in vivo* and *in vitro* model systems were needed.

Astroviruses are a major problem in the poultry industry, as early infection with astrovirus results in lifelong growth suppression in turkeys, and the virus can be found throughout the meat. This problem, however, has been turned into one positive: it has led to the development of a baby turkey astrovirus model, which is particularly important since no *in vivo* human astrovirus model currently is available.

To study astrovirus effects *in vitro*, Schultz-Cherry's lab grew a human intestinal carcinoma cell monolayer on a support and measured transepithelial resistance (TER) across the membrane in

response to infection of the cells with astrovirus. Human astrovirus decreased TER, supporting the hypothesis that membrane permeability changes cause the virus's pathogenic effects. The increased permeability occurred independent of viral replication, as virus-like particles generated from the recombinant capsid protein were sufficient to cause TER decreases. Surprisingly, astrovirus was able to disrupt non-intestinal cell barriers as well, including lung epithelial barriers and blood-brain barriers, even though astrovirus doesn't grow in these cells.

This *in vitro* work provided clues that the mechanism by which astrovirus triggers gastroenteritis may be related to tight junctions, which are the areas between cells that form a barrier between them and also maintain polarity of cells. Astrovirus appears to disrupt tight junctions through an indirect interaction with occludin, a key protein found in tight junctions, and through a dismantling of the actin cytoskeleton. Cytokine and chemokine release may be involved; astrovirus capsid stimulates *in vitro* epithelial cell release of type 1 interferon mRNA and protein in a time frame consistent with *in vivo* pathogenic effects, and capsid-mediated effects on epithelial membrane permeability can be blocked when type I interferon is added exogenously in the *in vitro* system.

Surprisingly (as it was not previously known to affect epithelial cells), vascular endothelial growth factor (VEGF) also is involved in the ability of astrovirus to impact epithelial permeability, as inhibition of VEGF results in much less permeability. A more complete understanding of VEGF's role may help explain why astrovirus has an effect on the blood/brain barrier.

A tentative model has emerged in which the virus initially binds the cell via integrin, which triggers the involvement of secondary messengers such as cAMP and nitric oxide and activates toll-like receptors in the process. These changes alter interferon and VEGF levels, resulting in actin disruption, which then increases epithelial permeability.

The *in vitro* model predicts that the capsid protein alone should be sufficient to trigger diarrhea *in vivo*. To test this idea, the turkey poult model was put to use. Administration of the recombinant capsid to the birds resulted in time- and dose-dependent diarrhea associated with increased barrier permeability *in vivo*. The crystal structure of the human and turkey capsid proteins has been solved, and a spike-like projection on the protein surface was hypothesized and subsequently shown to be sufficient for causing barrier permeability.

Significantly, this is the first time a viral structural protein has been shown to function as an enterotoxin.

In addition to her elegant basic science work, Schultz-Cherry's research has expanded into the clinical and food safety areas.

Working at St. Jude Children's Research Hospital provided the opportunity to investigate whether the nearly universal gastroenteritis afflicting pediatric cancer patients might be due not just to chemotherapeutic drugs, but also to astroviral infections. Current clinical tests for astrovirus only look

for the canonical viral sequence, but the rapid mutation of the virus may result in the test missing true infections. Schultz-Cherry developed a multiplex real-time PCR test which recognizes multiple genotypes of the virus. Using this test, a 20% prevalence of astrovirus was observed among children with gastroenteritis at both St. Jude (oncology patients) and Vanderbilt Medical Center (non-oncology patients), much higher than the 3–5% prevalence expected based on previous results from conventional tests. This is a higher prevalence than the more notorious norovirus, suggesting that astrovirus infections are a more significant problem than has been realized previously.

The multiplex PCR test has also been adapted for food applications, and results obtained from oysters purchased from a commercial market were presented. Three of 42 tested oysters were positive for astrovirus, with 1 positive for sapovirus and none positive for norovirus. These results again suggest that astrovirus may be a more significant emerging health problem than has been appreciated.

The unique ability of astrovirus capsid to target the gut is also being explored for drug delivery.

*Summary by Wendy Bedale, Science Writer, Food Research Institute*

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