Title Survival of foodborne viruses in soil amendments and subsequent interaction with fresh produce

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Abstract

Fresh produce has been increasingly associated with outbreaks of foodborne illness caused by human enteric viruses. Viruses are shed in human feces and can contaminate leafy greens through soil amendments and irrigation water. The usage of manure and biosolids on farmland increases the potential dissemination of human pathogen into agricultural environments. Among enteric viruses, human norovirus and hepatitis A virus (HAV) are leading foodborne pathogens causing non-bacterial gastroenteritis, and adenovirus is an important human pathogen also responsible for enteric illness. All three viruses are transmitted through the fecal-oral route and are prevalent in water and soil throughout the environment. The survival of three viruses in different types of biosolids and animal manure was evaluated. Murine norovirus-1 (MNV, a widely used surrogate of human norovirus), HAV and adenovirus type 41 (Ad41) were able to maintain viability in solid manure and biosolids after 60 days at both 20 and 4°C. However, all three viruses were inactivated quickly in liquid dairy manure and almost lost infectivity immediately in alkaline treated biosolids. Virus survival in manure and biosolids is virus and manure type dependent, e.g. aluminum sulfate added to poultry litter during the processing could inactivate HAV to some extent, but had no effect on MNV. Overall, enteric viruses were able to survive in manure and biosolids and thus application of soil amendments on farmlands may pose a risk to crop contamination.

Contaminated water used in irrigation or washing of fresh produce is a means of contamination, and was studied here on leafy greens. Pure MNV or MNV in manure/biosolids were suspended in buffer and used to wash Romaine lettuce. Virus was detected on lettuce after 5 min of washing and MNV in animal manure had similar attachment as pure virus; however, a significantly higher viral quantity was found on lettuce washed with buffer containing MNV in biosolids. Confocal microscopy showed that MNV was on lettuce surface, inside cut leaves and occasionally within the stomata. In general, water contaminated with run-off from biosolids could increase the viral contamination of produce during the washing process and virus inside cut leaves or stomata may be protected from sanitization and thus increase the risk of foodborne illness. Environmental contamination was assessed as if there was a

flooding event with a high amount of contamination or if a lower amount of contamination in water was applied consistently over a period of time. Romaine lettuce was grown hydroponically or in soil and challenged with MNV via contaminated irrigation water. Again virus was detected in lettuce leaves under both conditions, with both high and low inoculums. Although only a few viral particles were still infectious, contamination through root uptake could still occur.

Good agricultural practices are important to protect the plant and crops in the field as well as well managed and maintained soil and water systems. Soil amendments must be well treated and composted as suggested by the USEPA to reduce microbial pathogens of fecal origin. Both MNV and Aichi virus, a Picornavirus closely related to hepatitis A virus, were inactivated in composted dairy manure achieving temperatures of more than 55°C for 3 days. Ad41 was more heat resistant compared to the other two viruses, but still lost infectivity during the composting processing. The treatment of manure and biosolids is critical to prevent spread of pathogens and composting is an efficient method for viral inactivation and reduction.