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Effect of land application wastes on virus fate

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Abstract Text:

Noroviruses cause over 90% of nonbacterial epidemic gastroenteritis worldwide and epidemiological projects in 1999 indicate that noroviruses may account for over 60% of all foodborne disease in the US. Norovirus and other enteric viruses are shed in the feces of infected humans and can contaminate fresh produce by contamination of the irrigation waters. The objective of the study is to understand factors controlling the fate and transport of viruses in agricultural systems. Noroviruses that infect humans can not be cultured routinely under standard laboratory conditions; therefore, the closely related Murine Norovirus Virus 1 (MNV-1) was used. MNV-1 (~ 2*10⁵ PFU/ml) was added into two different types of biosolids, pellet treated (PT) and Alum treated (AT). After virus was extracted with 3% beef extract or Phosphate buffer (15% Na₂PO₄, pH 9.5), no MNV-1 was detected either by RT-PCR or plaque assay. Alternatively 10 ml Phosphate buffer was added to 1 g biosolid and viral RNA was directly extracted using various methods. RNA extracted with Qiashredder and a Qiagen viral RNA extraction kit was detected by RT-PCR, but failure to detect RNA occurred with the Trizol Reagent. A standard curve for real-time PCR was generated by serial dilution of biosolid solution and Qiashredder extracted RNA. MNV-1 contaminated biosolids (PT and AT) were incubated at 20 or 4 °C for 10, 20 and 30 days. After 10 days, there was ~1 log reduction of MNV-1 at both temperatures in PT, but no reduction in AT as determined by real-time PCR. Using plaque assays there were ~10⁴ PFU/ml MNV-1 still infectious at both temperatures in PT and AT.

Using real-time PCR after 30 days, there was ~2 log reduction of MNV-1 in PT at 20 °C and ~1 log at 4 °C; and ~1 log reduction at 20 °C and no reduction at 4 °C for MNV-1 in AT. Plaque assay showed that ~3 logs MNV-1 in PT and ~4 logs in AT biosolids were still infectious.

Impact Statement:

We will be able to better assess the fate of viruses in land applied wastes and how they may enter the water supply by addressing the survival of various pathogenic viruses that may be present in these waste samples.