Method Optimization and Evaluation of Modified Moore Swab for Recovery of Enteric Viruses in Agricultural Water

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Abstract

Human enteric viruses are the major cause of epidemic gastroenteritis caused by contaminated food and water. Agricultural water plays an important role in contamination of produce during irrigation, growing, and harvest of produce, leading fresh produce to be a major vehicle of foodborne viral illnesses. To develop pre-harvest preventive controls, surveillance of viruses in agricultural water is crucial. The objective of this project was to evaluate the feasibility of a Modified Moore swab (MMS) method for the recovery and extraction of enteric viruses from agricultural water for molecular detection. MMS was used to collect agricultural water samples at domestic farms in the Ohio and Georgia regions. In seeded samples with murine norovirus (MNV) as a process control, the recovery rate of virus ranged from 0.1% to 9%. The limit of detection (LOD50) was estimated to be at 2.1E+03 PFU/swab. The results indicate that MMS is an effective method for the detection of viruses in water samples, and the method can be used for enteric virus environmental surveillance.

Introduction

Human enteric viruses, especially norovirus and hepatitis A virus, are the major cause of epidemic gastroenteritis. Wastewater, containing virus particles discharged from infected patients, can become a source of contamination to agricultural irrigation systems that transfer enteric viruses to produce, which can ultimately lead to outbreaks. Numerous studies have found norovirus in the environmental water. Detection of enteric viruses in the agricultural water system continues to be a major challenge to public health.

Several methods have been developed and proven to be effective to recover enteric viruses from environmental water samples. Modified Moore swab (MMS) captures microorganisms within the packed gauze as water flows through the cartridge. This is a capture-filtration sampling method, successfully used for in large-volume sample collections for waterborne pathogen detection. Moore swabs are inexpensive, simple to use, and do not require collecting and transporting large volumes of water.

The objective of this study was to optimize and evaluate the effectiveness of an MMS method for the recovery and isolation of enteric viruses from agricultural water for molecular detection.

Materials and Methods

A Modified Moore swab (MMS) consists of a polyvinyl chloride (PVC) cartridge filled with gauze (Fig. 1). Irrigation water from various sources (streams, ponds, wells, or drip tapes) was collected from domestic farms. One to ten liters of water per sample were pumped through an MMS. After TGBE (Tris-Glycine-Beef Extract) elution, murine norovirus (MNV), ranging from 7.9E+05 to 7.9E+02 PFU, was spiked as a process control into selected swab eluates to estimate the efficacy of virus extraction and the presence of RT-PCR inhibitors. Viruses were further concentrated by ultracentrifugation. RNA was extracted with guanidinium thiocyanate (GITC) and a QIAGEN RNeasy kit. The recovery efficiency of the process control virus was evaluated by RT-qPCR. The 50% end-point limit of detection (LOD50) was determined using the Reed–Muench method.

Modified Moore Swab Cassette | Field Collection | Lab Eluates

Figure 1

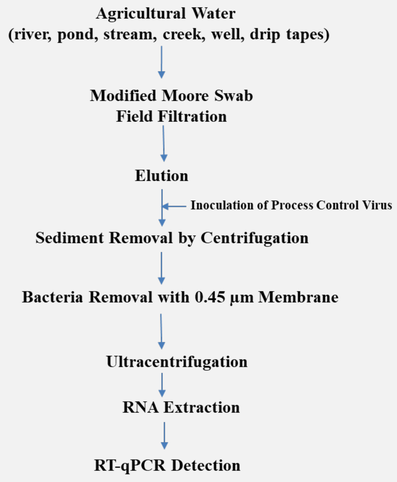

Figure 2. Flow chart of MMS method

Results and Discussion

A Modified Moore Swab method for the isolation of viruses from water samples is being evaluated and optimized (Fig. 2). We observed that the elution, concentration, and extraction of viruses were greatly affected by the turbidity and sediments of the eluates from the various water sources (Fig.1). Metagenomics analysis showed the crude eluates predominantly contain bacteria and that a 0.45 µm membrane filtration step could efficiently remove bacteria (Fig. 3). Thus, high-speed centrifugation and membrane filtration steps were added prior to the ultracentrifugation of viruses.

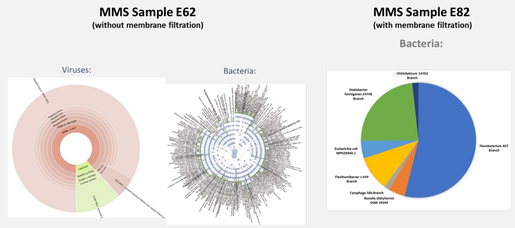

Figure 3. Metagenomics of Water Samples

RT-qPCR was used to determine the recovery efficiency of the inoculated process control (MNV) in the virus particle concentration and RNA extraction procedures (Table 1). Using the optimized method (Fig.2), we found that the recovery rate of MNV ranged from 0.1% to 9% (data not shown). The co-extracted PCR inhibitors presumably prevented the detection of virus when the inoculant was at the lower levels. Results are presented as positive or negative (below the RT-qPCR detection limit) as the variance of PCR inhibitors among samples made quantitative analysis inaccurate. The 50% end-point limit of detection (LOD50) determined by the Reed–Muench method was estimated to be at 2.1E+03 PFU/swab.

Sample Type	Number of MNV-Spiked Samples	Inoculum Dose (PFU)	MNV RT-qPCR Positive Rate	Ct Value (Mean ± SD)
MMS	1	7.9E+05	1/1	32.3 ± 0.6
	5	7.9E+04	5/5	36.3 ± 0.6
	13	7.9E+03	8/13	36.5 ± 1.8
	12	7.9E+02	5/12	37.8 ± 2.0

Table 1. Recovery of Process Control Virus from Water Samples

Challenges in the Detection of Viruses from Agricultural Water

Sampling:
- Low contamination level in agricultural environment
- Requires large sample volumes

Elution:
- Water composition: solid sediments (sand, silt, clay), organic matter, inorganic salts, bacteria, fungi, algae,etc.
- Enteric viruses bind to environmental matrices (type and strain dependence):
  * Non-specific: electrostatic and hydrophobic interactions
  * Specific receptor-ligand interactions: HBGA/HBGA-like moieties on bacteria

Concentration and Detection:
- Isolation efficiency
- Enzymatic Inhibitors
- Detection sensitivity

Conclusion

We developed and optimized the Modified Moore Swab method for the concentration and isolation of foodborne viruses in agricultural water.

1. Foodborne viruses captured by MMS can be eluted and extracted for molecular detection.
2. MMS is simple, inexpensive, and effective at sampling large volumes of water.
3. The recovery rate of viruses varied from sample to sample, and virus detection may be affected by the turbidity and particulate matter in eluates.

Mission Relevance:

The method development effort toward multi-pathogen recovery and identification in agricultural water will provide the scientific basis and practical tools for the risk assessment, prevention, and investigation of foodborne outbreaks.