RESEARCH COMPLETED

Enteric Viruses as New Indicators of Human and Cattle Fecal Contamination of Irrigation Water

SUMMARY

The standards used by the produce industry to detect fecal contamination in irrigation waters are based on tests developed for drinking waters and include risk threshold levels that may not be appropriate for determining if there is a "risk relevant" level of contamination for crops irrigated with waters tested in this manner. To improve these standards, novel viral targets that have shown to better correlate with the presence of fecal contamination were assessed for their potential use as more sensitive and specific fecal indicators for evaluating the safety of irrigation waters.

OBJECTIVES

Objective 1 - Develop protocol for using a negatively charged membrane method that optimizes volume, cost, and time requirements for surveillance of viruses in irrigation waters.

Objective 2 - Identify the following novel viral indicator species within irrigation water samples from four regions and relate them to bacterial indicators/ pathogens:

Human	Cattle
Aichivirus (high #s in human feces) Pepper Mild Mottle Virus (high #s in feces) Human adenoviruses (human pathogen) Enteroviruses (human pathogen)	Bovine polyomavirus (high #s in cattle feces) Bovine adenovirus (high #s in cattle feces)

Objective 3 - Estimate the amount of fecal contamination present based on the known levels of viral shedding from cow and human sources (using quantitative PCR).

Objective 4 - Perform a quantitative microbial risk assessment (QMRA) to determine what levels of fecal contamination from irrigation water pose a public health risk.

METHODS

Irrigation water samples (4 L each) collected in four regions:

Yuma, Arizona (n = 190) **California (n = 60)**

Maricopa, Arizona (n = 60) **Georgia (n = 20)**

- 1 L examined for the presence of *Salmonella/Escherichia coli (*STEC) by cultural methods (confirmed by API biochemical strips and/or PCR [*stx1, stx2, eae* genes])
- 3 L examined for the presence of enteric/fecal viruses:
- Concentration from 3L --> 10 ml --> 0.65ml using series of filtration steps
- Viruses detected via qPCR
- Evaluated potential correlations between the presence of indicators/bacterial pathogens and proposed virus indicators.
- The number of virus genomes present quantified and used to estimate the amount of fecal material based on known amounts in feces.
- QMRA performed to determine what levels of fecal contamination pose a public health risk (Table 1).

RESULTS TO DATE

- The method developed for this study is feasible for use by growers for the detection of viruses in irrigation waters.
- Physical/chemical/microbial parameters can vary significantly between regions.
- Although *E. coli* isolates were found in nearly all samples, no STEC strains were identified.
- *Salmonella* isolates were found in approximately 15% to 40% of samples from different regions, but likely were low levels (no outbreaks).
- Pathogenic and indicator viruses were identified in a number of samples (Table 2).
- No correlations were observed between viruses and fecal indicators/bacterial pathogens, which could be due to the low occurrence of viruses or due to the limited size of the sample set.
- Pepper mild mottle virus was readily detected in irrigation waters (34% to 63% of samples) and was used to estimate the amount of fecal contamination present (Table 3).
- QMRA was performed for the pathogens, adenoviruses and enteroviruses using measured values (Table 4). QMRA was also performed for four foodborne pathogens using estimated contamination levels (Table 3). Both risk assessments used the following assumptions:
  - Single irrigation event with the contaminated water as the only input of pathogens to produce surface
  - Does not account for any microbial removal/reduction at the point of harvest or at any point post-harvest
  - Observed viral genome copies detected by qPCR are infectious
  - A one-time (1 day) consumption of contaminated lettuce, not annual risk
- **Conservative estimate of risk.** Likely an order of magnitude (at least) lower.

BENEFITS TO THE INDUSTRY

The development of a more accurate and quantitative method for the detection of fecal contamination in irrigation water will provide the industry with necessary tools to evaluate water quality through a scientifically based approach by targeting novel indicators that better fit the definition of effective indicator organisms. With this information, growers and government agencies are provided a statistically driven approach to setting limits of fecal contamination that is acceptable for irrigation purposes.

By obtaining quantitative data regarding the level of fecal contamination present in irrigation water through these new viral targets (namely pepper mild mottle virus), there can be improved exposure assessments for various irrigation waters. With this information, we will be able to fill a major data gap regarding exposure for more sophisticated risk analyses to be conducted. The assessment of risk could be further improved by including harvest/post-harvest outcomes for pathogens on produce, such as through the removal of the outer leaves of lettuce during harvest, removal via washing/sanitization, or microbial die-off during processing and transport, prior to the produce reaching the consumer.

Table 1. Final quantitative microbial risk model and the values used for variables.

$$P(response) = 1 - \exp(-k_{pathogen} \times M(p * c_{iw})V_{prod}e^{(-k_{viral\ decay}t)})$$

Variable	Variable Description	Value	Reference
M	Lettuce consumed daily per capita (g/person per day)	12.1	Stine et al. 2005a
c_{iw}	Concentration of organism in irrigation water (copies/ml)	Observed / estimated for this study	
V_{prod}	Volume of irrigation water retained on produce surfaces (ml/g)	0.108	Hamilton et al. 2006
p	Pathogen transfer from the water to the surface of the lettuce (%)	1.5 (viruses) 0.007 (bacteria)	Stine et al. 2005a
$K_{pathogen}$	Kinetic decay constant (% per day)	69 (viruses) 35 (bacteria)	Hamilton et al. 2006 Stine et al. 2005a
t	Time between last irrigation and harvest (days)	4	Recommended for water of poor quality

Table 2. Occurrence of viruses in irrigation waters from four different regions in the United States.

Agricultural Region	Bovine Polyomavirus	Bovine Adenovirus	Aichivirus	Pepper Mild Mottle Virus	Adenoviruses	Enteroviruses
Yuma, Arizona	0/190 (0%)	0/190 (0%)	3/190 (2%)	65/190 (34%)	3/190 (2%)	7/190 (4%)
Maricopa, Arizona	0/60 (0%)	0/60 (0%)	0/60 (0%)	38/60 (63%)	0/60 (0%)	0/60 (0%)
California	0/60 (0%)	0/60 (0%)	0/60 (0%)	28/60 (47%)	2/60 (3%)	0/60 (0%)
Georgia	0/20 (0%)	0/20 (0%)	0/20 (0%)	12/20 (60%)	0/20 (0%)	20/20 (100%)

Table 3. Estimated pathogen levels in irrigation water samples and the associated risk of illness following one exposure event.

Pathogen of interest	Estimated concentration*	Risk of Illness (per # of exposures)
Norovirus	$3.2 \times 10^{-4} \pm 3.9 \times 10^{-3}$ PDU/ml	1.2 cases per 10^{7}
Rotavirus	$1.6 \times 10^{-8} \pm 1.9 \times 10^{-7}$ FFU/ml	1.2 cases per 10^{10}
E. coli O157	$9.9 \times 10^{-7} \pm 1.4 \times 10^{-5}$ CFU/ml	5.6 cases per 10^{14}
Shigella	$1.6 \times 10^{-3} \pm 2.0 \times 10^{-2}$ CFU/ml	8.3 cases per 10^{10}

* PDU = PCR-detectable units; FFU = fluorescent focus forming units; CFU = colony forming units

Table 4. Risk associated with the measured values of viral pathogens following one exposure event.

Pathogen	Risk of Illness (per # of exposures)
Human adenoviruses	3.1 per 100,000
Enteroviruses	1.2 per 100,000

CONTACT

Kelly Bright
The University of Arizona
bright@email.arizona.edu
520.626.8094,

AUTHORS

Kelly Bright, Marc Verhougstraete, Kelly Reynolds, Lisa Casanova, Brandon Iker

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Jonathan Sexton and Jennifer Pearce-Walker for their efforts in the collection and processing of irrigation water samples and Jennifer Pearce-Walker for her work on the QMRA. They would also like to thank the students in the collaborating labs for their various contributions.

LENGTH OF FUNDING

January 1, 2015 – February 15, 2017

CENTER *for* PRODUCE SAFETY