Title: **Use of Human and Bovine Adenovirus for Fecal Source Tracking**

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Background/Need: Confirming the presence and determining the source(s) of fecal contamination to water is critical to protect human health and environmental quality. This is especially true in large portions of Wisconsin, where fractured and/or karst bedrock is located within a few feet of the ground surface, facilitating rapid transport of fecal pathogens to aquifers. Detection of commonly targeted fecal indicators (e.g., coliform bacteria, caffeine) suggests that water quality has been compromised. However, these indicators do not adequately track or confirm the presence of several enteric pathogens of concern, including viruses. In addition, current indicators do not discriminate between important sources of fecal contamination without labor-intensive and time-consuming investigation. Therefore, the causes of elevated indicator concentrations may be misjudged or remain unidentified.

Adenoviruses (AdV) have been advocated as fecal indicators with potential to both distinguish contamination sources and track environmental transport of enteric viruses. Despite these promising features, the following challenges have prevented efficient and confident use of AdV as source-specific fecal indicators: (1) current polymerase chain reaction (PCR) methods for AdV detection cannot simply or definitively discriminate between AdV of human and livestock origin; (2) filters commonly used to collect/concentrate viruses in 10- to 100-L water samples often exhibit poor virus recovery and are costly; and (3) compounds present in concentrated samples often interfere with DNA amplification by PCR.

Objectives: The objectives of this study were (1) to develop a quantitative PCR (qPCR) method capable of simply and confidently distinguishing between human and bovine AdV; and (2) to optimize virus recovery from environmental samples by two newly-advocated, competitively priced filters while minimizing concomitant concentration or introduction of PCR-inhibiting compounds.

Methods: Oligonucleotides for the detection of HAdV and of BAdV were designed based on alignments of all available non-redundant sequences of the hexon gene (or complete AdV genome) present in the NCBI GenBank database. Primers were evaluated first by conventional PCR, agarose gel electrophoresis, and quantitative PCR. Cationic Nanoceram® filters were evaluated for the retention and recovery of two bacteriophages (MS2 and PRD1) and two AdV (bovine 1 and human 41) by virus adsorption-elution. A variety of eluents were investigated based on their previously demonstrated or hypothesized ability to disrupt virus-filter interactions. Fresenius hollow-fiber hemodialysis ultrafilters were evaluated for the recovery of bacteria (*Escherichia coli* and *Enterococcus faecalis*), bacteriophages (MS2 and PRD1), BAdV 1 and HAdV 41. AdV were assayed by qPCR, while bacteriophage were enumerated via plaque-forming-unit (pfu) assay on host bacteria cultures. *E. coli* and *E. faecalis* were enumerated using the quantitray application of the Colilert and Enterolert assays (IDEXX, Inc.).

Results and Discussion: Published PCR methods were unavailable (BAdV) or deemed inadequate (HAdV) for purposes proposed here. While designing a PCR assay capable of distinguishing between (i.e., amplifying specifically) human vs. group I (GI) bovine AdV, both belonging to the genus *Mastadenovirus*, represented a significant challenge, we successfully designed and evaluated a set of primers capable of specific HAdV vs.

group I BAdV amplification. These primers take advantageous of three sets of previously undescribed consecutive mismatches between HAdV vs. BAdV hexon gene sequences that are conserved within the sequences of each of these groups. The specificity of the technique is derived from the placement of consecutive dual nucleotide mismatches (DNM) at the 3' end of both the forward and reverse primers: our data show that extension (duplication) of DNA from the 3' end of a primer/template complex by Taq Polymerase, the enzyme responsible for PCR, is completely inhibited when otherwise homologous primers demonstrate these consecutive 3' mismatches with the target sequence. To our knowledge, ours represents the first promulgated qPCR assay for BAdV.

NanoCeram® filters demonstrated quantitative removal and 0-90% recovery of phages from feed water, depending on eluent composition and bacteriophage identity. Optimized eluents showed no PCR inhibition but were surprisingly ineffective at eluting AdV (HAdV 41 and BAdV 1) from the filters. We observed >99% AdV retention on NanoCeram® filters, but <5% AdV recovery with eluents optimized for bacteriophage. Favorable microorganism recoveries (≥58%), including for HAdV 41 (72%), were observed for the Fresenius hollow fiber ultrafilter. The hollow-fiber ultrafiltration system (HFUF) designed here is therefore preferred for future virus and pathogen collection and is ready for deployment by Wisconsin State Laboratory of Hygiene (WSLH) personnel.

Conclusions/Implications/Recommendations: The novel PCR assay developed represents a significant advance toward implementing AdV-based fecal source tracking. The primers designed are based on two sets of 3' dual nucleotide mismatches, and are not predicted to amplify any known non-target AdV. The HFUF unit is ready to be deployed by WSLH for simultaneous concentration of multiple pathogens and indicators (including AdV) of interest for fecal source tracking. Poor AdV recovery discouraged further evaluation of NanoCeram® filters.

Future research into the spatial and seasonal distribution of livestock and wildlife AdV is recommended, as the information acquired during such surveys will make AdV-based fecal source tracking assays more robust. Any work completed in this regard should be accompanied by the acquisition of genetic data through cloning/sequencing of AdV-positive PCR products. In this way, the database of available animal AdV sequences will be enlarged, allowing continual evaluation/validation of primer/probe specificity. In addition, considering the improved HFUF configuration, which allows sample concentration without user supervision, a logical next step is the modification/evaluation of the HFUF system for collection of very large (500- to 1000-L) water samples.

Related Publications:

Sibley, S.; McMahon, K.D.; Pedersen, J.A. Development and evaluation of a novel fecal source tracking TaqMan PCR assay for discriminating bovine and human adenoviruses based on specific polymerase extension from 3' dual nucleotide mismatches. (*in prep*.)

Sibley, S.; McMahon, K.D.; Pedersen, J.A. Capture and recovery of bacteriophage MS2 and PRD1, bovine adenovirus Type 1 and human adenovirus type 41 from novel nanoaluminum oxide fiber filters. (*in prep*.)

Key Words: Adenovirus, bovine, bacteriophage, nanoaluminum, NanoCeram©, hollow fiber ultrafiltration, fecal source tracking, indicator.

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Final Report: A final report containing more detailed information on this project is available for loan from Wisconsin's Water Library, University of Wisconsin, 1975 Willow Drive, Madison, Wisconsin 53706 (608) 262-3069.