chemistry). We note that AdV belonging to the genus Mastadenovirus that have yet to be isolated and sequenced could share one or both of the 3'DNP around which our assay derives its specificity. This uncertainty is not unique to the present study; the design of PCR primers and probes (and their resulting specificity) is always limited by the availability of sequence data upon which primers are based. That said, the primer designed here (1) are based on two sets of 3' dual nucleotide mismatches, when one 3'DNM alone would likely provide sufficient discrimination, and (2) are not predicted to amplify any known non-target AdV (based on an evaluation of our oligonucleotides with the BLAST algorithm of the NCBI database).

While poor recovery of AdV discouraged further evaluation of NanoCeram® filters, the HFUF unit is ready to be deployed by the WSLH for simultaneous concentration of multiple pathogens and indicators (including AdV) of interest for fecal source tracking. 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 enhanced, allowing for continuing evaluation/validation of the specificity of the primers/probes designed here. In addition, considering the improved HFUF configuration, which allows for sample concentration without user supervision, a logical next step is the modification/evaluation of this HFUF system for the collection of very large (500- to 1000-L) water samples. Specifically, modifications of the current system facilitating (1) continuous filtration from a water source, (2) injection of the NaPP dispersing agent during filtration (as opposed to one-step addition at when commencing to acquire 50- to 100-L samples), and (3) real-time sample preservation (e.g., installation of a cooling jacket around the sample concentrate bottle) would significantly expand an investigator’s ability to efficiently concentrate viral (and other) pathogens from potentially contaminated groundwater.

REFERENCES


