Abstract of the article: Watershed managers and utilities responsible for source water protection are looking for ways to minimize risk from both human and nonhuman pathogen reservoirs. The ability to accurately identify microbial input source(s) would allow water managers to develop and apply effective, cost-efficient corrective measures. In this study, the potential role of F-specific coliphages with a special focus on the F+DNA coliphages as delineators of sources of surface water microbial pollution was investigated. A variety of source samples from across the United States were analyzed including wastewater and septic system samples, animal slurry lagoon samples, and freshly voided animal fecal samples. Samples were analyzed for fecal coliforms, F-specific coliphages, and somatic coliphages. F-specific phages were isolated and typed to determine whether they were F+RNA or F+DNA coliphages, and were subsequently serotyped and genotyped. F-specific coliphages were detected more often in wastewater samples and slurry lagoon samples when compared to individual fecal samples, where they were rarely detected. F+DNA coliphages were detected in all wastewater samples, in two cow manure samples, and were absent from avian fecal samples. Serotyping and genotyping analysis of F+DNA coliphages did not exclusively associate one subtype with human or animal wastes. A significant proportion of F+DNA isolates demonstrated inconclusive serotyping results, although a majority of the solely M13 serotyped isolates were from domestic wastewater sources. In wastewater samples, 77% were F+DNA while 23% were F+RNA coliphages. Consistent with previous studies, F+RNA coliphone serotyping analysis demonstrated a predominance of group II and III F+RNA coliphages in the wastewater samples, whereas group I and group IV predominated in animal fecal samples and animal slurry lagoon samples. Overall, the results from this study suggests that the presence of F+DNA coliphages, and especially M13 type F+DNA coliphages, could be a potential indicator of human-related wastes.
Abstract of the article: In recent years, there has been increased interest in the use of male-specific or F+ coliphages as indicators of microbial inputs to source waters. Sero- or genotyping of these coliphages can also be used for microbial source tracking (MST). Among the male-specific coliphages, the F+ RNA (FRNA) viruses are well studied, while little is known about the F+ DNA (FDNA) viruses. We have developed a reverse line blot hybridization (RLB) assay which allows for the simultaneous detection and genotyping of both FRNA as well as FDNA coliphages. These assays included a novel generic duplex reverse transcription-PCR (RT-PCR) assay for FRNA viruses as well as a generic PCR for FDNA viruses. The RT-PCR assays were validated by using 190 field and prototype strains. Subsequent DNA sequencing and phylogenetic analyses of RT-PCR products revealed the classification of six different FRNA clusters, including the well-established subgroups I through IV, and three different FDNA clusters, including one (CH) not previously described. Within the leviviruses, a potentially new subgroup (called JS) including strains having more than 40% nucleotide sequence diversity with the known levivirus subgroups (MS2 and GA) was identified. We designed subgroup-specific oligonucleotides that were able to genotype all nine (six FRNA, three FDNA) different clusters. Application of the method to a panel of 351 enriched phage samples from animal feces and wastewater, including known prototype strains (MS2, GA, Qß, M11, FI, and SP for FRNA and M13, f1, and fd for FDNA), resulted in successful genotyping of 348 (99%) of the samples. In summary, we developed a novel method for standardized genotyping of F+ coliphages as a useful tool for large-scale MST studies.