Temporal Assessment of the Impact of Exposure to Cow Feces in Two Watersheds by Multiple Host-Specific PCR Assays

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Exposure to feces in two watersheds with different management histories was assessed by tracking cattle feces bacterial populations using multiple host-specific PCR assays. In addition, environmental factors affecting the occurrence of these markers were identified. Each assay was performed using DNA extracts from water and sediment samples collected from a watershed directly impacted by cattle fecal pollution (WS1) and from a watershed impacted only through runoff (WS2). In WS1, the ruminant-specific *Bacteroidales* 16S rRNA gene marker CF128F was detected in 65% of the water samples, while the non-16S rRNA gene markers Bac1, Bac2, and Bac5 were found in 32 to 37% of the water samples. In contrast, all source-specific markers were detected in less than 6% of the water samples from WS2. Binary logistic regressions (BLRs) revealed that the occurrence of Bac32F and CF128F was significantly correlated with season as a temporal factor and watershed as a site factor. BLRs also indicated that the dynamics of fecal-source-tracking markers correlated with the density of a traditional fecal indicator ($P < 0.001$). Overall, our results suggest that a combination of 16S rRNA gene and non-16S rRNA gene markers provides a higher level of confidence for tracking unknown sources of fecal pollution in environmental samples. This study also provided practical insights for implementation of microbial source-tracking practices to determine sources of fecal pollution and the influence of environmental variables on the occurrence of source-specific markers.