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Comparison of PCR and quantitative real-time PCR methods for the characterization of ruminant and cattle fecal pollution sources

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ABSTRACT

The State of California has mandated the preparation of a guidance document on the application of fecal source identification methods for recreational water quality management. California contains the fifth highest population of cattle in the United States, making the inclusion of cow-associated methods a logical choice. Because the performance of these methods has been shown to change based on geography and/or local animal feeding practices, laboratory comparisons are needed to determine which assays are best suited for implementation. We describe the performance characterization of two end-point PCR assays (CF128 and CF193) and five real-time quantitative PCR (qPCR) assays (Rum2Bac, BacR, BacCow, CowM2,and CowM3)reported to be associated with either ruminant or cattle feces. Each assay was tested against a blinded set of 38 reference challenge filters (19 duplicate samples) containing fecal pollution from 12 different sources suspected to impact water quality. The abundance of each host-associated genetic marker was measured for qPCR-based assays in both target and non-target animals and compared to quantities of total DNA mass, wet mass of fecal material, as well as Bacteroidales, and enterococci determined by 16S rRNA qPCR and culture-based approaches (enterococci only).Ruminant- and cow-associated genetic markers were detected in all filters containing a cattle fecal source. However, some assays cross reacted with non-target pollution sources. A large amount of variability was evident across laboratories when protocols were not fixed suggesting that protocol standardization will be necessary for widespread implementation. Finally, performance metrics indicate that the cattle-associatedCowM2qPCR method combined with either the BacR orRum2Bacruminantassociated methods are most suitable for implementation.