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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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ARTICLE INFO

Article history:
Received 3 October 2012
Received in revised form
27 March 2013
Accepted 31 March 2013
Available online 5 July 2013

Keywords:
Microbial source tracking

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

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0043-1354/$ – see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.watres.2013.03.061

Fecal pollution
Ruminants
Cattle

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-associated CowM2 qPCR method combined with either the BacR or Rum2Bac ruminant-associated methods are most suitable for implementation.

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1. Introduction

The presence of fecal contamination in recreational waters from ruminant animals, in particular cattle can pose a threat to public health (Soller et al., 2010). For example, cattle feces are commonly associated with the spread of *Salmonella*, *Escherichia coli* O157:H7, *Giardia*, and *Cryptosporidium*. Human populations may be exposed to cattle-derived fecal pathogens via a number of routes (Fayer and Lewis, 1999; MacKenzie et al., 1994) including swimming or bathing in recreational waters (Cabelli et al., 1982; Keene et al., 1994). Waterborne disease outbreaks due to suspected cattle fecal contamination are documented worldwide (ie. *Cryptosporidium* in Milwaukee, Wisconsin, USA in 1993). Currently, health authorities rely on the enumeration of fecal indicators (ie. enterococci or *E. coli*) to identify the presence of fecal contamination. However, a variety of warm-blooded, and even some cold-blooded (Harwood et al., 1999; McLain et al., 2009), animals contain these same fecal indicators making these approaches insufficient for the determination of cattle fecal pollution.

As a result, many methods have been developed to detect and/or quantify ruminant fecal pollution sources (Bernhard and Field, 2000; Kildare et al., 2007; Mieszkin et al., 2010; Reischer et al., 2006; Shanks et al., 2008). A recent study designed to assess the performance of several of these methods with a collection of cattle fecal samples collected from different geographic locations across the United States found that the shedding of ruminant-associated fecal indicators dramatically changed based on local animal feeding practices (Shanks et al., 2010). The notion that performance can vary from one geographic location to another due to local animal diets or other uncharacterized factors suggests that these methods must be tested before implementation in a particular region.

In California, it is estimated that there are over six million ruminant animals including cattle (5.35 million), sheep (570,000), goat (3500), deer (445,000), as well as alpaca and llama (1800) (USDA, 2012). Because of the prevalence of ruminant animals in this geographic region, cattle- and ruminant-host associated fecal identification approaches were included in a large multiple laboratory fecal source identification method evaluation study to identify top performing technologies for the State of California (Boehm et al., 2013). The overall report of this study provides an excellent overview of the findings submitted by 27 different laboratories using a total of 41 different fecal source identification technologies designed to identify fecal animal sources ranging from cattle to pigeons. However, the overall report leaves several important factors that may influence the performance of ruminant/cattle-associated methods unaddressed warranting further study in the present work.

In this study we describe the performance of two end-point PCR assays (CF128 and CF193) and five qPCR assays (Rum2Bac, BacR, BacCow, CowM2, and CowM3) previously reported to be associated with either ruminant and/or cattle feces (Bernhard and Field, 2000; Kildare et al., 2007; Mieszkin et al., 2010; Reischer et al., 2006; Shanks et al., 2008) using reference fecal samples collected from the state of California. Issues such as lack of standardization of protocols, use of extremely high concentrations of fecal material, influence of selected performance benchmark definition (unit of measure and test concentration), and the high degree of similarity in primer design between most ruminant methods are explored.

2. Materials and methods

2.1. Sample collection and preparation

Fecal material was collected from more than 100 individual animals representing 10 different species (human, horse, cow, deer, pig, goose, chicken, pigeon, gull, and dog), nine primary effluent wastewater samples, and six septage samples collected from Northern, Central and Southern California (Ervin et al., 2013). Fecal slurries were prepared for each pollution source by mixing equal wet weight masses or volumes of respective individual samples to generate composites. Blinded, composite samples, of both single sources and mixed sources (two pollution types), were prepared for each slurry at two concentrations (undiluted and 1:10) using 47 mm diameter, 0.4 μm polycarbonate membranes and distributed to participating laboratories in duplicate sets ($n = 38$ filters/laboratory). More detailed information about fecal sample collection and creation of blinded reference samples is reported elsewhere (Boehm et al., 2013).

2.2. Participating laboratories and method selection

Eleven laboratories from the United States ($n = 7$) and the European Union ($n = 4$) contributed data from seven host-associated methods (Table 1). Methods originally reported to