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Characterization of sources and loadings of fecal pollutants using microbial source tracking assays in urban and rural areas of the Grand River Watershed, Southwestern Ontario

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ABSTRACT

Sources of fecal water pollution were assessed in the Grand River and two of its tributaries (Ontario, Canada) using total and host-specific (human and bovine) *Bacteroidales* genetic markers in conjunction with reference information, such as land use and weather. In-stream levels of the markers and culturable *Escherichia coli* were also monitored during multiple rain events to gain information on fecal loadings to catchment from diffuse sources. Elevated human-specific marker levels were accurately identified in river water impacted by a municipal wastewater treatment plant (WWTP) effluent and at a downstream site in the Grand River. In contrast, the bovine-specific marker showed high levels of cattle fecal pollution in two tributaries, both of which are characterized as intensely farmed areas. The bovine-specific *Bacteroidales* marker increased with rainfall in the agricultural tributaries, indicating enhanced loading of cattle-derived fecal pollutants to river from non-point sources following rain events. However, rain-triggered fecal loading was not substantiated in urban settings, indicating continuous inputs of human-originated fecal pollutants from point sources, such as WWTP effluent. This study demonstrated that the *Bacteroidales* source tracking assays, in combination with land use information and hydrological data, may provide additional insight into the spatial and temporal distribution of source-specific fecal contamination in streams impacted by varying land uses. Using the approach described in this study may help to characterize impacted water sources and to design targeted land use management plans in other watersheds in the future.

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1. Introduction

PCR-based fecal source tracking tools targeting *Bacteroidales* genetic markers have become increasingly recognized as an identification tool for various fecal sources, including humans (Bernhard and Field, 2000), cows (Bernhard and Field, 2000), dogs (Dick et al., 2005a), pigs (Dick et al., 2005b), horses (Dick et al., 2005b), gulls (Jeter et al., 2009), and geese (Fremaux et al., 2010). However, quantification of *Bacteroidales* markers alone may not provide a complete set of information to accurately identify fecal source(s) as well as to characterize fecal loadings from diffuse sources in the environment. As an approach to improve source-tracking capability, the outcomes of marker-based tools can be interpreted in combination with environmental reference information including catchment hydrology and/or watershed land use (Peed et al., 2011; Reischer et al., 2008). This approach is expected to allow more accurate interpretation of microbiological fecal source tracking data (Reischer et al., 2011).

Rainfall is one of the most important hydrological factors that affect transport of microorganisms in the environment (Jamieson et al., 2004). Microbial loadings to surface water typically increase during rainfall via overland surface transport, in-stream routing, and sediment resuspension (Dorner et al., 2006). The effects of rain have been extensively studied for fecal indicator bacterial loading in an urban inland watershed (Gentry-Shields et al., 2012), coastal storm water outfalls (Converse et al., 2011), freshwater streams affected by faulty septic systems (Peed et al., 2011), tidal creeks (Stumpf et al., 2010), coastal river watersheds (Surbeck et al., 2006), and tributaries of a drinking water reservoir (Kistemann et al., 2002).

The effects of rainfall on loading of fecal *Bacteroidales* markers to surface water have been reported in a few cases (Converse et al., 2011; Sauer et al., 2011; Gentry-Shields et al., 2012). Most of these reports focused on transport of human-specific *Bacteroidales* markers in urban settings during intense storm events. More recent studies have applied genetic markers of agricultural origin for fecal source identification and to interpret its outcome in relation to environmental and land use parameters (Jent et al., 2013; Marti et al., 2013), however, these studies remain limited. In addition to human sources, farm animals, in particular cattle, may be responsible for fecal pollution of many watersheds. Cattle livestock in Canada, the US, and the EU produce approximately 100 million, 860 million, and 1.1 billion metric tons of manure annually, which account for 78%, 82%, and 79%, respectively, of the total manure produced by all forms of farm livestock (Foged et al., 2011; Hofmann and Kemp, 2001; US Department of Agriculture, 2000). Therefore, it is important to assess fecal loadings from these two primary sources (i.e., human and cattle) for proper management and remediation of fecal water pollution in many North American watersheds.

In this study, the sources and loadings of fecal water pollutants were characterized in the Grand River Watershed including both urban and rural-agricultural areas. Specific aims included (a) identification of fecal pollution source(s) at each sampling site using *Bacteroidales* 16S rRNA gene-based quantitative PCR (qPCR) assays and land use information; and (b) analysis of fecal loadings to streams from both human and cattle origins during rain events.

2. Materials and methods

2.1. Study sites

The present study was conducted in the Grand River and two of its tributaries (Conestogo River and Canagagigue Creek) in Ontario, Canada between July 18th, 2012 and May 7th, 2013. The five sampling sites included four river sites and one point source of fecal contamination. These sites were selected to represent (a) the most upstream site on the Grand River with relatively limited human or agricultural impacts (GRU), (b, c) two agricultural sites in the Conestogo River (COR) and Canagagigue Creek (CAC), both of which are characterized by intensely farmed watershed, (d) a point source of human fecal pollution [municipal wastewater treatment plant (WWTP) effluent] in the Grand River (WTE), and (e) an urban location at the most downstream on the Grand River (GRD) (Table 1, Fig. 1). Land-use and hydrological summaries for each watershed included in this study are shown in Table 1.

2.2. Sample collection

On 25 separate occasions, a total of 125 water samples (25 occasions × 5 sites) were collected. Samples were taken a minimum of twice per month at each site to capture a range of hydrological conditions, including base-flow and rain events. On fifteen occasions, there was no substantial rainfall and stream levels were maintained at or near base flow; and on the other ten occasions of rain event, accumulated precipitation exceeded 15 mm within 12 h from the onset of rain and stream levels surpassed the base flow level by at least two times as measured by gauges. Precipitation data were obtained from the National Climate Data and Information Archive (Environment Canada, 2012).

During a sampling occasion, a single water sample (about 250 mL) was collected in a sterile polyethylene bottle (300 mL size) at each study site. As some sites were downstream of wastewater treatment facilities that use chemical disinfectants, the water sampling bottle contained sodium thiosulfate (about 50 mg/L final concentration when dissolved) to quench the activity of any chlorine residues in the sample. All water samples were stored on ice during transport to the laboratory and processed within 24 h from the sample collection.

2.3. Nucleic acid extraction and Bacteroidales qPCR assays

All water samples were divided into two 100-mL aliquots; one for nucleic acid extraction followed by *Bacteroidales* qPCR assays and the other for *Escherichia coli* enumeration. *Bacteroidales* cells were collected by passing 100 mL of water samples through a 0.45-μm-pore-size mixed cellulose esters membrane filter (Pall Canada Ltd., St. Laurent, QC, Canada) under partial vacuum. The filter was placed in a sterile disposable tube containing 1.5 mL of 5 M GITC lysis buffer (5 M guanidine