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Utility of Microbial Source-Tracking Markers for Assessing Fecal Contamination in the Portage River Watershed, Northwestern Ohio, 2008

Scientific Investigations Report 2010–5036

U.S. Department of the Interior
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Conversion Factors

Multiply	By	To obtain
	Volume	
milliliter (mL)	0.06102	cubic inch (in^3)
milliliter (mL)	0.03381	fluid ounce (oz)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8×°C)+32$$

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Abstract

An influx of concentrated animal feeding operations in northwest Ohio has prompted local agencies to examine the effects of these industrial farms on water quality in the upper Portage River watershed. The utility of microbial source-tracking (MST) tools as a means of characterizing sources of fecal contamination in the watershed was evaluated. From 2007 to 2008, scientists with the U.S. Geological Survey, Bowling Green State University, and the Wood County Health Department collected and analyzed 17 environmental samples and 13 fecal source samples for *Bacteroides*-based host-associated DNA markers. At many of the environmental sites tested, MST marker results corroborated the presumptive fecal contamination sources. Results from this demonstration study support the utility of using MST with host-specific molecular markers to characterize the sources of fecal contamination in the Portage River watershed.

Introduction

The upper Portage River watershed in northwestern Ohio is an agricultural area whose water quality has historically been influenced by runoff from row crops and small-scale livestock operations and discharges from septic systems and small wastewater-treatment plants. Recently, however, the area has been targeted for construction and operation of large dairy farms. As of July 2007, two large-scale dairies already were operating, and three more were proposed. Local officials and the public are concerned that discharges from these animal feeding operations (AFOs) will result in the degradation of water quality. Little information is available, however, on the influence of existing fecal sources on water quality (including the two large-scale dairies) within the watershed.

In order to establish current (2008) or baseline conditions before any watershed protection steps are undertaken, the relative contributions of contaminants from AFOs, septic systems, and treated wastewater within the Portage River watershed need to be understood. Contaminants include pathogens of fecal origin, wastewater compounds (including hormones and antibiotics), nutrients, and suspended sediment. Research indicates that the best approach for characterizing the sources of fecal contamination in a watershed is to establish multiple lines of evidence (Boehm and others, 2003; Francy and others, 2006). This is done by identifying the spatial distribution of bacterial and chemical indicators, understanding the hydrologic factors that affect their distribution, and applying microbial source-tracking (MST) techniques. The use of culture-independent host-specific molecular markers is gaining acceptance among researchers as the preferred MST tool (Santo Domingo and others, 2007). Molecular markers for MST are common in research; however, their utility for forensic assessment of fecal sources in a specific location must be demonstrated before being applied.

The U.S. Geological Survey (USGS), in cooperation with Bowling Green State University (BGSU) and the Wood County Health Department (WCHD), have taken the first step towards understanding the relative contributions of fecal contaminants by identifying the source-tracking tools that can best be used in the Portage River watershed. Fecal source and environmental water samples were analyzed for *Bacteroides* DNA markers of general fecal contamination, as well as markers that have been associated with human or bovine feces. The *Bacteroides* are the most numerous members of the normal flora of the human intestinal system (Finegold and others, 1983) and are a group of enteric bacteria thought to have co-evolved with their hosts (Dick and others, 2005). For these reasons, their host specificity has been investigated for use in microbial source tracking (Bernhard and Field, 2000). In this study, samples were analyzed by means of quantitative polymerase chain reaction (qPCR), which results in a relative quantity of each DNA marker. The results from this study may be used as preliminary information for a larger, long-term source-tracking study to identify and assess relative source contributions of fecal contamination in the changing Portage River watershed.

This report documents the demonstration study to identify MST tools that can be used to understand the relative contributions of fecal contaminant sources in the Portage River

watershed. A total of 17 environmental water samples and 13 known-source fecal samples (treated wastewater, septage, and bovine slurry) were tested for the presence and relative quantity of MST markers by qPCR. Four *Bacteroides*-based markers were evaluated: two human-associated MST markers, one bovine-associated MST marker, and one marker of general fecal contamination.

Methods

Fecal source sampling.—Sample collection of potential fecal sources (treated wastewater, septage, and cattle slurry) was done on November 6, 2007, by USGS, BGSU, and WCHD to ensure that the selected DNA markers were found in potential sources. These samples consisted of

- two wastewater samples (primary-treated influent and final effluent) collected from two treatment plants in or near the watershed (designated as WWTP-1 and WWTP-2),
- septic-tank samples collected from six households in the watershed,
- two replicate cattle-slurry samples collected from the primary settling lagoon at a local dairy farm, and
- a composite goose fecal sample collected from a golf course in the watershed to confirm that human or bovine-associated markers would not be detected.

Wastewater, septage, and bovine slurry samples were collected in sterile polypropylene bottles by using a grab-sampling technique described in Myers and others (2007). A total of 20 individual goose fecal droppings were composited into a sterile 50-mL centrifuge tube using sterile toothpicks. All source samples were preserved on ice and were transported to the USGS for further analysis.

Water sampling.—Water samples were collected on June 26, 2008, and September 15, 2008, at the sites described in table 1 and illustrated in figure 1. The June samples, which were collected by USGS and BGSU, were collected after a recent rain event. The September samples were collected by BGSU. Samples were collected in sterile polypropylene bottles by using a grab-sampling technique described in Myers and others (2007). All water samples were preserved on ice and were transported to BGSU and USGS for further analysis.

Sample analyses.—Fecal source samples were analyzed by the USGS within 24 hours of collection by membrane filtration on modified mTEC agar (U.S. Environmental Protection Agency, 2006). Water samples were analyzed for *E. coli* by BGSU within 24 hours of collection by means of a most-probable number (MPN) technique using Colilert in Quanti-Tray/2000 wells (Idexx, Westbrook, Maine). At USGS, all samples were filtered and stored at −70°C for subsequent DNA marker analysis. All samples were analyzed for three *Bacteroides* DNA markers; fecal source samples were also analyzed for a fourth marker (Btheta):

- General fecal marker, AllBac (Layton and others, 2006)
- Human fecal marker, qHF183 (Seurnick and others, 2005)
- Bovine fecal marker, BoBac (Layton and others, 2006)
- Human fecal marker, Btheta (Carson and others, 2005)

Table 1. Sampling site locations and descriptions.

[WWTP, wastewater-treatment plant; BGSU, Bowling Green State University; WCHD, Wood County Health Department]

Site name	Latitude	Longitude	Site description[a]
Poe Ditch 1	41.38513	−83.61185	Upstream from WWTP
Poe Ditch 2	41.38511	−83.61086	Downstream from WWTP
Huffman Ditch	41.33953	−83.59162	Thought to have septic inputs
Unnamed Tile 1	41.24818	−83.77061	Field with no manure application
Unnamed Tile 2	41.24817	−83.77083	Field with possible manure application
Unnamed Tile 3	41.25075	−83.76598	Field with no manure application
Bays Ditch 1	41.26963	−83.73242	Thought to drain manure-applied field
Bays Ditch 2	41.26461	−83.72824	Thought to drain manure-applied field and have septic inputs
Bays Tile 1	41.26963	−83.73100	Thought to drain manure-applied field
Bays Tile 2	41.26962	−83.72825	Thought to have septic inputs
Ostego Pike Ditch	41.24782	−83.78533	Mixed sources
Rangeline Ditch	41.28486	−83.76642	Not expected to have high levels of fecal contamination

[a]Descriptions are based on presumptions, input from local agencies, and historical data collected by BGSU and WCHD. Discussions of possible sources are based only on observations, not on scientific evidence.

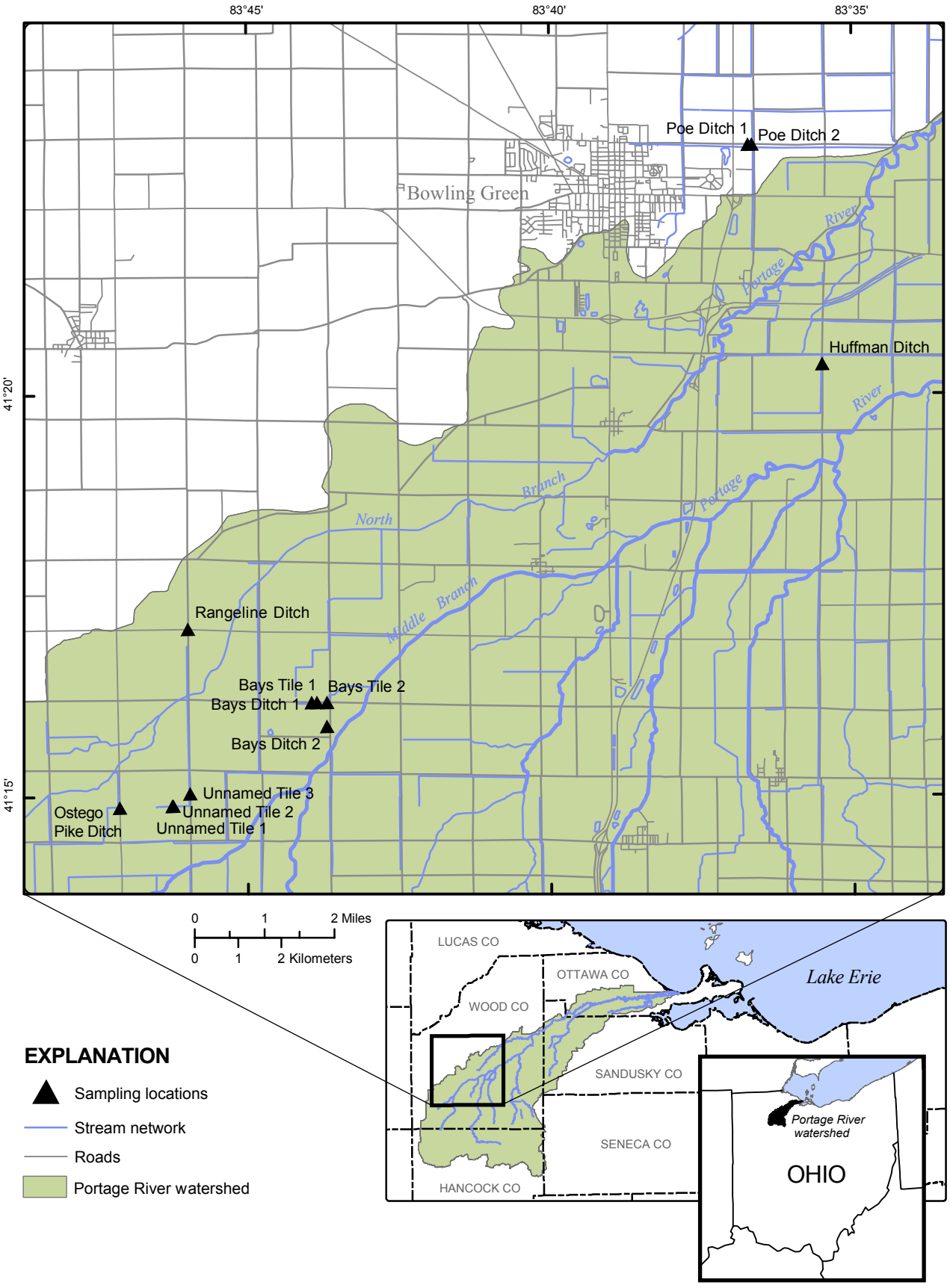

Figure 1. Location of sampling sites within the Portage River watershed, northwestern Ohio.

Quantitative PCR.—Quantification was done by use of standard curves calculated from threshold cycles observed for decimal dilutions of plasmid-borne DNA target. Calculation of standard concentrations was based on the total concentration of DNA in the plasmid solution and the known size of the target-containing plasmid. Characteristics of the standard curves are presented in table 2.

Each qPCR run included a standard curve, an extraction blank (negative control at the extraction step), and a no-template control (negative control at the qPCR step). The upper and lower limits of quantification (ULQ and LLQ, represented as the dynamic range in table 2) were based on the range of standards that contributed to the linear part of the standard curve. In all cases, the standard curve remained linear at both the highest and lowest standards.

No-template-control and extraction-blank data were used to measure a limit of detection (LOD). The no-template controls and extraction blanks sometimes showed nonspecific fluorescent signal during late cycles. In these cases, the mean and the 99-percent confidence interval among cycle thresholds were calculated. To guard against false-positive results, the target concentration that corresponded with detection at the lower 99-percent confidence interval of multiple detections was used as the LOD. A cycle threshold higher than the LOD was not considered credible evidence that the sample contained detectable quantities of the marker.

The LLQ and the LOD were used to qualify low-concentration data. In cases where the LLQ was greater than the LOD, results higher than the LLQ were not qualified. Results between the LLQ and the LOD were qualified "detected, not quantified" (DNQ). Results below the LOD were considered nondetects. Conversely, when the LOD was greater than the LLQ, results higher than the LOD were not qualified. Results below the LOD were considered nondetects.

Exogenous internal standard.—DNA encoding red-fluorescent protein dsRed2 (Matz and others, 1999) was used as an internal standard that is not expected to be detected from natural sources in the freshwater environment. Approximately 2.5×10^6 cells of *E. coli* containing a dsRed2 plasmid were added to each filtered sample immediately before extraction. Recovery of dsRed2 marker was measured by qPCR and used as a measure of matrix inhibition. Cycle threshold values of all samples were within 2 cycles of the expected *C*t value for the positive control and thus were not considered to be matrix inhibited.

Utility of Microbial Source-Tracking Markers for Assessing Fecal Contamination

A total of 13 fecal source samples and 17 water samples were analyzed for DNA markers. Tables 3 and 4 list the results for the source and water samples, respectively. These tables show the quantities of the markers, in copies per 100 milliliters, which were based on multiple runs of composite standard curves of known concentrations of each marker.

Table 2. Standard-curve characteristics for AllBac, BoBac, Btheta, and qHF183 microbial source-tracking (MST) markers.

[R^2, Pearson correlation coefficient; ND, not detected]

MST marker	Number of compiled curves	Dynamic range (copies per 100 milliliters)	Range of amplification efficiency (percent)	Range of R^2 values	Limit of detection (copies per 100 milliliters)
AllBac	11	1.2×10^1–1.2×10^7	82–102	0.985–0.999	290
BoBac	11	6.1×10^1 – 6.1×10^7	82–101	0.992–0.999	ND[a]
Btheta	4	5.7×10^1 – 5.7×10^7	84–91	0.992–0.999	ND[a]
qHF183	23	2.3×10^1 – 2.3×10^7	84–108	0.991–1.000	ND[b]

[a]The marker was never detected in a blank.

[b]The marker was detected in one of 10 blanks; however, it was considered an outlier and was removed from the dataset because it was atypical and the replicate result was not detected.

Table 3. DNA marker results for fecal source samples collected on November 6, 2007, Portage River watershed, northwestern Ohio.

[WWTP, wastewater-treatment plant; ND, not detected; DNQ, detected but not quantified; --, not analyzed for]

Sample name[a]	*Escherichia coli* (colony-forming units per 100 milliliters)	Marker concentration (copies per 100 milliliters)			
		AllBac general marker	BoBac bovine marker	Btheta human marker	qHF183 human marker
WWTP-1 influent	7.6×10^{5}	1.3×10^{8}	1.4×10^{4}	2.7×10^{4}	3.7×10^{6}
WWTP-1 effluent	3.0×10^{2}	4.0×10^{3}	ND	DNQ	4.5×10^{1}
WWTP-2 influent	9.8×10^{5}	2.3×10^{8}	9.9×10^{3}	7.2×10^{4}	2.9×10^{6}
WWTP-2 effluent	1.0×10^{3}	3.5×10^{3}	ND	DNQ	4.8×10^{2}
Septic 1	9.8×10^{5}	7.9×10^{8}	ND	1.7×10^{3}	8.4×10^{5}
Septic 2	$<1.0\times10^{3}$	3.4×10^{8}	ND	DNQ	6.0×10^{5}
Septic 3	2.1×10^{5}	2.6×10^{8}	ND	1.1×10^{3}	2.4×10^{2}
Septic 4	1.0×10^{3}	3.8×10^{8}	ND	DNQ	DNQ
Septic 5	2.9×10^{4}	4.6×10^{8}	ND	1.9×10^{3}	1.1×10^{5}
Septic 6	4.6×10^{4}	1.5×10^{8}	ND	6.5×10^{3}	5.8×10^{5}
Cattle 1	9.1×10^{6}	3.7×10^{9}	1.1×10^{8}	DNQ	DNQ
Cattle 2	--	2.5×10^{9}	5.5×10^{7}	DNQ	ND
Goose	--	1.1×10^{7}	ND	DNQ	ND

[a] Sample names defined in methods section.

Table 4. DNA marker results for water samples, Portage River watershed, northwestern Ohio.

[ND, not detected; DNQ, detected but not quantified]

Date	Sample name	*Escherichia coli* (most-probable number per 100 milliliters)	Marker concentration (copies per 100 milliliters)		
			AllBac general marker	BoBac bovine marker	qHF183 human marker
6/26/2008	Poe Ditch 1	7.7×10^{3}	5.7×10^{6}	1.5×10^{3}	1.0×10^{3}
9/15/2008	Poe Ditch 1	2.1×10^{3}	5.0×10^{6}	6.2×10^{2}	8.4×10^{3}
6/26/2008	Poe Ditch 2	4.0×10^{2}	1.7×10^{7}	9.8×10^{3}	4.7×10^{4}
9/15/2008	Poe Ditch 2	2.8×10^{3}	8.8×10^{6}	3.0×10^{3}	5.6×10^{4}
6/26/2008	Huffman Ditch	1.7×10^{4}	1.5×10^{8}	3.0×10^{4}	1.8×10^{5}
9/15/2008	Huffman Ditch	3.3×10^{2}	2.9×10^{6}	DNQ	1.4×10^{2}
6/26/2008	Unnamed Tile 1	3.8×10^{2}	1.7×10^{6}	DNQ	DNQ
6/26/2008	Unnamed Tile 2	3.7×10^{3}	5.2×10^{6}	2.8×10^{3}	DNQ
9/15/2008	Unnamed Tile 2	2.3×10^{3}	1.0×10^{6}	DNQ	DNQ
9/15/2008	Unnamed Tile 3	2.8×10^{2}	1.1×10^{6}	ND	DNQ
6/26/2008	Bays Ditch 1	2.9×10^{3}	2.2×10^{6}	2.3×10^{2}	9.6×10^{2}
9/15/2008	Bays Ditch 1	3.8×10^{3}	1.7×10^{6}	ND	DNQ
6/26/2008	Bays Ditch 2	6.9×10^{3}	2.6×10^{6}	DNQ	2.2×10^{3}
9/15/2008	Bays Tile 1	2.1×10^{2}	6.5×10^{5}	ND	DNQ
9/15/2008	Bays Tile 2	1.6×10^{4}	1.3×10^{7}	9.7×10^{2}	1.1×10^{4}
6/26/2008	Ostego Pike Ditch	6.1×10^{3}	1.5×10^{6}	DNQ	1.1×10^{2}
6/26/2008	Rangeline Ditch	6.2×10^{2}	5.4×10^{5}	ND	DNQ

Fecal source samples.—The AllBac general fecal marker was detected in all 13 fecal source samples at concentrations ranging from 3.5×10^3 to 3.7×10^9 copies per 100 milliliters. The BoBac bovine-associated marker was detected at high concentrations, as expected, in the two cattle samples; however, it also was detected at considerably lower levels in both wastewater-influent samples. The BoBac marker was not detected in any of the six septic-source samples. The Btheta human-associated marker was detected at quantifiable levels in only 6 of the 10 human-source samples; it was detected at levels below the LLQ in 4 human-source samples and all of the non-human samples (2 cattle and 1 goose). Because the Btheta marker was detected in nonhuman sources, it does not appear to be a useful human-associated marker for use in this study; consequently, stream samples were not analyzed for this marker. The qHF183 human marker was detected at quantifiable levels in 9 of the 10 human-source samples and was detected in the remaining human-source sample, but at a nonquantifiable level. This marker also was detected in one of the cattle samples but at a level below the LLQ.

Water samples.[1]—In the Poe Ditch 1 samples, collected upstream from a WWTP outfall, concentrations of AllBac, BoBac, and qHF183 were lower on both collection dates than in Poe Ditch 2 samples, collected downstream from the WWTP outfall. At Poe Ditch 2, the concentrations of BoBac and qHF183 markers were among the highest observed in the water samples collected during this study. High levels of the qHF183 were expected at Poe Ditch 2 because qPCR analysis detects genetic targets regardless of cell viability. According to personnel at this WWTP, possible sources of bovine-origin fecal contamination at these Poe Ditch sites include drainage that goes to the WWTP from nearby county fairground lands and combined-sewer overflow (CSO) events flowing into Poe Ditch upstream from Poe Ditch 2 after heavy rain. (Heavy rainfall before the June sample may have led to contribution of fairground drainage and/or a CSO event to Poe Ditch at this site).

The Huffman Ditch site was expected to receive inputs from upstream septic sources. This expectation was corroborated by its having the highest concentration of qHF183 observed in this study (in the June sample). This sample, which was collected after a significant rain event, also had the highest concentrations of *E. coli*, AllBac, and BoBac. The high BoBac concentration was unexpected because adjacent fields were not known to receive manure application. This finding demonstrates that other sources of fecal contamination may be influencing this site, especially after rainfall.

The Unnamed Tile 1 and 3 sites were known to drain fields that do not receive cattle-manure application; results show concentrations of BoBac and qHF183 that were either below the LLQ or not detected. Unnamed Tile 2 was thought to drain a cattle-manure-treated field; results show a detectable concentration of BoBac in the June sample. The fact that the September sample collected at Unnamed Tile 2 had a BoBac concentration below the LLQ was not unexpected because flow from the tile was very low, and at least 2 months had elapsed since the last cattle manure application.

Before sampling, there was less confidence in the presumptions about the inputs of contamination to the Bays Ditch and Bays Tile sites than at the other sites. Concentrations of the BoBac and qHF183 markers generally were low, if detected, with the exception of high levels of qHF183 in the Bays Tile 2 site, which was thought to have septic inputs.

The Ostego Pike Ditch sample was thought to be affected by both human and animal sources of contamination. The BoBac marker was detected below the LLQ, and the qHF183 marker was detected at a low concentration.

The Rangeline Ditch site was included to serve as a control site and was not expected to have high levels of contamination. The concentration of the AllBac marker was the lowest measured in the stream samples for this study. The BoBac marker was not detected in this sample, and the qHF183 marker was detected at levels below the LLQ.

The results described above suggest that microbial source tracking by means of host-associated molecular markers is a viable option for characterizing fecal contamination sources in the upper Portage River watershed. With the exception of the Btheta marker, the markers tested exhibited good host specificity, based on analysis of known-source fecal samples. In addition, marker results for most ditch and tile sites were reflective of expected fecal sources. This study provides a foundation from which future investigations could be conducted concerning fecal contamination in the Portage River watershed. In addition to demonstrating usefulness of these MST methods, this study provides background information on putative sources and sites that may be helpful in future investigations in the watershed.

Summary and Conclusions

Water-quality impacts to the Portage River watershed originate from a variety of sources, including various agricultural operations, septic-system discharges, and wastewater-treatment-plant (WWTP) effluent. Recent and planned expansion of industrial livestock operations, such as large-scale dairy farms, in and surrounding the watershed has prompted concern about the possible effects on water quality. Characterization of the sources of fecal contamination was undertaken in 2008 to assist water-resource managers in determination of the most effective watershed protection steps to take. Along with the Wood County Health Department and Bowling Green State University, the U.S. Geological Survey investigated the usefulness of microbial source tracking (MST) with host-associated molecular markers to characterize cattle and human-origin fecal contamination in the Portage River watershed.

Of the three host-associated MST markers evaluated, two markers—BoBac (bovine-associated) and qHF183

[1] Names of ditches in this report are unofficial and follow the local convention of reference to the adjoining road name.

(human-associated)—performed well enough during analysis of relevant fecal samples to be considered for evaluation of water samples. For many of the water-sampling locations, observations and input from local agencies were provided about what fecal inputs could be affecting water quality. At many of these sites, BoBac and qHF183 marker results supported the presumptive fecal inputs. At Poe Ditch, samples collected downstream from WWTP effluent had increased levels of qHF183 compared to samples collected upstream from the effluent. At Huffman Ditch, the presumption of septic-dominant inputs was corroborated by elevated levels of qHF183 but was complicated by concurrently elevated levels of BoBac. At the Unnamed Tile sites, all drainage samples from fields not expected to receive manure application had levels of BoBac that were undetected or below the lower limit of quantitation (LLQ). Conversely, the BoBac marker was detected at quantifiable levels from the drainage tile of a field known to receive manure applications. At a site expected to receive minimal fecal contamination, Rangeline Ditch, marker results were either undetected or below the LLQ.

Results from this study demonstrate the utility of using microbial source tracking with host-associated markers to help characterize fecal contamination sources in the Portage River watershed. Future studies to investigate contamination sources could include use of the BoBac and qHF183 markers, along with other new or refined MST markers that have been validated for use in the watershed. If expansion of large-scale or industrial livestock facilities continues in this watershed, then host-associated MST marker analysis can potentially be a useful tool in elucidating fecal contamination sources and pathways, and could provide water-resource managers and public health officials with data needed for effective watershed protection.

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