# Microbial source tracking and spatial analysis of *E. coli* contaminated private well waters in southeastern Ontario

Julia Krolik, Gerald Evans, Paul Belanger, Allison Maier, Geoffrey Hall, Alan Joyce, Stephanie Guimont, Amanda Pelot and Anna Majury

## ABSTRACT

Private water supplies, which are the primary source of drinking water for rural communities in developed countries, are at risk of becoming fecally contaminated. It is important to identify the source of contamination in order to better understand and address this human health risk. Microbial source tracking methods using human, bovine and general *Bacteroidales* markers were performed on 716 well water samples from southeastern Ontario, which had previously tested positive for *Escherichia coli*. The results were then geospatially analyzed in order to elucidate contamination patterns. Markers for human feces were found in nearly half (49%) of all samples tested, and a statistically significant spatial cluster was observed. A quarter of the samples tested positive for only general *Bacteroidales* markers (25.7%) and relatively few bovine specific marker positives (12.6%) were found. These findings are fundamental to the understanding of pathogen dynamics and risk in the context of drinking well water and will inform future research regarding host-specific pathogens in private well water samples.

**Key words** | cluster, fecal contamination, private drinking water, public health, source tracking

**Julia Krolik**
**Allison Maier**
**Alan Joyce**
**Stephanie Guimont**
**Amanda Pelot**
**Anna Majury** (corresponding author)
Public Health Ontario Laboratories,
181 Barrie Street, Kingston, Ontario,
Canada K7L 4V6
E-mail: *anna.majury@oahpp.ca*

**Julia Krolik**
**Gerald Evans**
**Anna Majury**
Department of Pathology and Molecular Medicine,
Queen's University, Richardson Laboratory,
88 Stuart Street, Kingston, Ontario,
Canada K7L 3N6

**Gerald Evans**
Division of Infectious Diseases,
Department of Medicine,
Queen's University & Kingston General Hospital,
76 Stuart Street, Kingston, Ontario,
Canada K7L 2V7

**Gerald Evans**
**Anna Majury**
Department of Biomedical and Molecular Sciences, Queen's University,
Botterell Hall – Room 915, Stuart Street,
Kingston, Ontario,
Canada K7L 3N6

**Paul Belanger**
Department of Geography,
Queen's University, Mackintosh-Corry Hall,
Room D201, Kingston, Ontario,
Canada K7L 3N6
and
Kingston, Frontenac and Lennox & Addington Health Unit, 221 Portsmouth Avenue,
Kingston, Ontario,
Canada K7M 1V5

**Paul Belanger**
**Anna Majury**
Department of Public Health Sciences,
Queen's University, Carruthers Hall,
Kingston, Ontario,
Canada K7L 3N6

**Geoffrey Hall**
Department of Civil Engineering,
Queen's University,
Ellis Hall, 58 University Ave,
Kingston, Ontario,
Canada K7L 3N6

doi: 10.2166/wh.2013.192

## LIST OF ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| BCIG | 5-bromo-4-chloro-3-indolyl-D-glucuronide |
| CFUs | colony forming units |
| EO | Eastern Ontario |
| *E. coli* | *Escherichia coli* |
| GI | gastrointestinal |
| GIS | geographic information system |
| HPEC | Hastings Prince Edward County |
| KFL&A | Kingston, Frontenac and Lennox and Addington |
| LGL | Leeds Grenville and Lanark |
| MST | microbial source tracking |
| PHO | Public Health Ontario |
| PHU | public health unit |
| qPCR | quantitative real-time polymerase chain reaction |

## INTRODUCTION

Waterborne pathogens remain a leading cause of morbidity and mortality globally. While urban populations in the developed world generally have access to regulated public drinking water sources, rural populations in these countries often remain dependent on private supplies leaving them at an increased risk for gastrointestinal (GI) illness. Microbiological contamination of private drinking water wells has been demonstrated in many countries including Ireland, England and Wales, Norway, the United States, and Canada (Raina *et al.* 1998; Smith *et al.* 2006; Hanne *et al.* 2010; Hynds *et al.* 2012; Allevi *et al.* 2013). Pathogens typically found in water supplies are those that are transmitted through the fecal-oral route (Simpson 2004).

*Escherichia coli* (*E. coli*) has been used as fecal indicator bacteria since the early 1900s to identify contaminated water sources, as they are a normal inhabitant of the GI tract and found in most mammals, including humans, livestock and wildlife (Klein & Houston 1898). Although the presence of *E. coli* is considered to be an effective indicator of fecal contamination in drinking water, it does not reveal any information about the fecal source (Okabe *et al.* 2007). Microbial source tracking (MST) methods have been recently developed, and successfully applied to address this limitation. MST methods fall into two general categories: library dependent and independent, the latter of which relies on source-specific targets (Hagedorn *et al.* 2011). In order for MST to be effective, it is crucial that the targeted gene sequences are only present in the feces of hosts under investigation. The 16S rRNA gene is a frequently selected bacterial target for library independent MST as it is highly conserved and therefore host specific and detectable in diluted quantities (Shanks *et al.* 2008). The order *Bacteroidales* is the most widely used microbial source marker, because it is easily detected in environmental waters due to its great fecal abundance. Furthermore, high host-specificity, strict anaerobic physiology, regional stability and direct analytic capability with polymerase chain reaction (PCR) make this marker a prime candidate for MST studies (Kirs *et al.* 2011). Recently, Lee *et al.* (2010) developed a quantitative TaqMan real-time PCR assay using the 16S rRNA genetic markers of *Bacteroidales*; the assay successfully detected fecal pollution sources from human and bovine hosts. *Bacteroidales* MST methods have been utilized in freshwaters such as river watersheds, creek watersheds and alpine karst spring catchments (Hagedorn *et al.* 2011).

Furthermore, MST methods can aid with identifying the origin of contaminant(s), especially when combined with geographic information system (GIS) tools. The use of molecular techniques, alongside GIS, has previously allowed researchers to characterize water pollution sources. Peed *et al.* (2011) traced point sources of human fecal pollution to septic systems following increases in seasonal precipitation. Patterns and, in turn, possible contributing factors, can be identified through spatial cluster analysis; for example, a study by Odoi *et al.* (2004) used spatial scan statistics to investigate possible clusters of giardiasis in southern Ontario.

This study investigated a region that includes four public health units (PHUs) in southeastern Ontario, where bacteriological analysis of private well drinking water is available to all well owners through Public Health Ontario (PHO). This region has a 47% rural population (in comparison to 14.4% for the province of Ontario) (Statistics Canada 2013). Properties with private wells, which lack access to municipal services (drinking water provision and wastewater treatment), were targeted in this study.

The overall goal of this study was to characterize the source of fecal contamination in private drinking water wells in southeastern Ontario in order to assess the risk of host-specific pathogens, and to establish a knowledge base

for identifying potential origins of contamination. The two major objectives were to: (1) determine the host-specificity of fecal contamination through the application of MST; and (2) investigate possible geospatial patterns of host-specific contamination.

## METHODS

### Sample collection and DNA extraction

This study was comprised of private well water samples that were submitted for bacteriological analysis in 2012 to a single PHO laboratory. The well water was collected by well owners in 200 mL bottles containing sodium thiosulfate, which quenches possible chlorine inhibition. One-hundred milliliters of the sample was filtered using a 0.45 μm pore sized mixed cellulose esters filter (Millipore Billerica, MA) and partial vacuum. Each filter was placed on a differential coliform medium with 5-bromo-4-chloro-3-indolyl-D-glucuronide (BCIG), incubated for 24 h ± 2 h at 35 °C, and the number of *E. coli* colony forming units (CFUs) were counted. When released into the medium via glucuronidase activity, BCIG is insoluble, accumulating within the cell, and colouring presumptive *E. coli* colonies blue (Ogden & Watt 1991). Only presumptive *E. coli* positive submissions were included in this study. Each presumptive *E. coli* positive sample underwent a second identical filtration step, using the remaining 100 mL of well water in the sample bottle. The filter was then rolled, placed in a cryovial and suspended in 2 mL of NucliSENS® easyMAG® Lysis buffer for 1 hour at 37 °C. The DNA was extracted using an automated NucliSENS® easyMAG® DNA extractor to a final volume of 60 μL and extracts were stored at −20 °C. Filtration controls were obtained by filtering 100 mL of buffered water and applying the extraction procedure above.

### Real-time PCR assays

Primer and probe sequences, designed for detecting host-specific *Bacteroidales* 16S rRNA genetic markers in environmental samples (Lee *et al.* 2010) and shown in Table 1, were used in this study. Three quantitative real-time PCR (qPCR) assays, described as BacHuman, BacBovine 1 and BacGeneral by Lee *et al.* (2010), were employed to determine the quantity of host-specific genetic markers present in well water samples using the ViiA™ 7 Real-Time PCR System. Specifically, each qPCR reaction contained 10 μL of extracted DNA, primers and probes (final concentrations of 500 and 176 nm, respectively) and PCR TaqMan® Environmental Mastermix 2.0 (Applied Biosystems, Carlsbad, CA) to a final volume of 25 μL. qPCR conditions were as follows: 2 min at 50 °C and 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C for each cycle. Two types of negative controls were tested with each PCR run – a negative filtration control (described above) as well as a no template control, where TE buffer was added instead of DNA to the PCR mastermix.

**Table 1** | Sequences of primers and probes used for the detection of host-specific *Bacteroidales* 16s rRNA genetic markers in this study. Developed by Lee *et al.* (2010)

| Assay | Target | Oligo name | Oligo sequence (5′ → 3′) | Tm (°C) |
|---|---|---|---|---|
| BacGeneral[a] | Order *Bacteroidales* | BacGen-F[c] | CTGAGAGGAAGGTCCCCCAC | 60 |
| | | BacGen-TP[d] | AGCAGTGAGGAATATT | 70 |
| | | BacGen-R[e] | CACGCTACTTGGCTGGTTCAG | 60 |
| BacBovine 1[b] | Bovine-specific *Bacteroidales* | BacBov-F1 | AAGGATGAAGGTTCTATGGATTGTAAA | 59 |
| | | BacBov-TP1 | ATACGGGAATAAAACC | 68 |
| | | BacBov-R1 | GAGTTAGCCGATGCTTATTCATACG | 59 |
| BacHuman[b] | Human-specific *Bacteroidales* | BacHum-F | CGCGGTAATACGGAGGATCC | 59 |
| | | BacHum-TP | AAGTTTGCGGCTCAAC | 70 |
| | | BacHum-R | CGCTACACCACGAATTCCG | 59 |

[a]VIC™.
[b]6-FAM.
[c]Forward primer.
[d]TaqMan probe.
[e]Reverse primer.

Standard quantification plasmids for each assay were created using cloning methodology previously described by Lee *et al*. (2010) with one modification – a TOPO® TA Cloning® Kit with a PCR™ 2.1-TOPO® vector was used according to manufacturer instructions (Life Technologies, 2012, Carslbad, CA). The plasmids were screened for target marker presence using colony PCR, and a one-to-one vector-to-insert ratio was verified using vector/insert primers and end-point PCR. Products were verified using agarose gel electrophoresis. Seven plasmid standards were prepared for each assay as a ten-fold serial dilution (ranging from 6.5 to $6.5 \times 10^6$ gene copies per reaction) and tested in duplicate to confirm PCR efficiency and sensitivity for each assay using standard curves. A set of four standards (1, 3, 5 and 7 in duplicate) was included with each PCR assay quantification. To identify a natural gene copy cut-off for the qPCR assay, at the level of the test, the numbers for each assay were visualized using histograms.

### Data preparation and analysis

The entire data set for the study was comprised of the 716 presumptive *E. coli* positive samples that were tested using the qPCR assay. The following *Bacteroidales* statuses were assigned to each record in the data set based on established gene copy thresholds: negative for all *Bacteroidales,* positive for general *Bacteroidales* only, positive for human *Bacteroidales*, positive for bovine *Bacteroidales,* and positive for both human and bovine *Bacteroidales*. Addresses for these wells were provided at the time of water submission; geographical coordinates (geocodes) were obtained for these addresses using Google Earth™ version 5.1 (Google Inc., Mountain View, CA), ArcGIS version 10.0 (ESRI Inc., Redlands, CA) and LIOcoder (a service provided by the Ontario Ministry of Natural Resources) (Ontario Ministry of Natural Resources, Peterborough, ON). Geocodes were not available for all addresses due to insufficient information provided by the submitter or the address not existing in any of the geocode databases used. Geocodes were obtained for 584 samples (82% geocoding rate).

In some instances, multiple samples were submitted (and tested) for a single well location; there were 208 samples that represented 78 distinct locations (defined by identical geocoordinates); in these cases a positive/negative status was manually determined for each of human, bovine and general *Bacteroidales* using the previously established gene copy threshold. Where both positive and negative results existed for a single location, one status was manually assigned taking into account the number of positives and negatives along with the gene copy range. An additional analysis was performed to ensure no bias was introduced during the manual process, where all locations with multiple submitted samples were removed. In total, there were 460 distinct geocoded locations. Of these, 456 were located within the established study region of Hastings & Prince Edward Counties (HPEC), Kingston, Frontenac and Lennox & Addington (KFL&A), Leeds Grenville & Lanark (LGL), and Eastern Ontario (EO); that is, four PHUs regions in southeastern Ontario. These 456 comprised the geocoded data set.

Spatial analysis was performed on the geocoded data set using SaTScan™ software version 9.1.1 (M. Kuldorff and Information Management Service, Inc., Boston, MA). Potential clusters of human and bovine specific contamination and general fecal contamination were investigated. Human and bovine cases also included instances where the sample tested positive for both human and bovine assays. The spatial scan statistic investigates the occurrence of clusters by using a circular window of variable radius that systematically moves across the map. The radius of the window varies from zero to a user-defined maximum limit, in this case 50%. Clusters are determined by comparing observed cases within the radius of the window to the number of expected cases, provided they are randomly distributed. The location and statistical significance ($p$-values) were determined by carrying out 999 Monte Carlo replications, using the Bernoulli distribution (Kuldorff 1997). A $p$-value greater than 0.05 was used for primary clusters for the rejection of the null hypothesis of no clusters.

Ethics approval for the study was obtained from the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board in Kingston, Ontario, Canada.

## RESULTS

The PCR reaction efficiencies were 97.1, 96.8 and 94.8% for the BacHuman, BacBovine 1 and BacGeneral assays,

respectively, with corresponding correlations ($R^2 > 0.99$ for all assays) as shown in Figure 1. The detection sensitivity of each assay was 6.5 gene copies per reaction, which equates to 1–2 cells since *Bacteroidales* species possess an average of 5.5 copies of the 16S rRNA gene per cell (Lee *et al.* 2009). The entire data set was used to generate histograms for the BacHuman, BacBovine 1 and BacGeneral assays (BacBovine 1 not shown) from which a natural cut-off value of 10 gene copies (per reaction) was selected for each marker (Figure 2). The range of gene copies per 100 mL for each of the three markers was: 60 to 19,000,000 for BacGeneral, 60 to 2,300,000 for BacHuman and 60 to 180,000 for BacBovine 1.

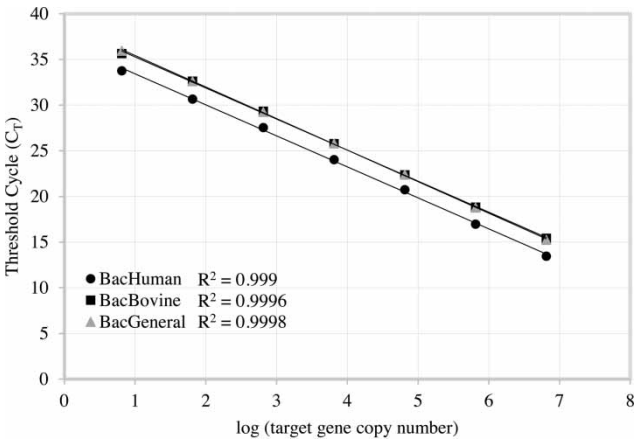


**Figure 1** | Standard curves for the quantification of general and host-specific *Bacteroidales* markers.

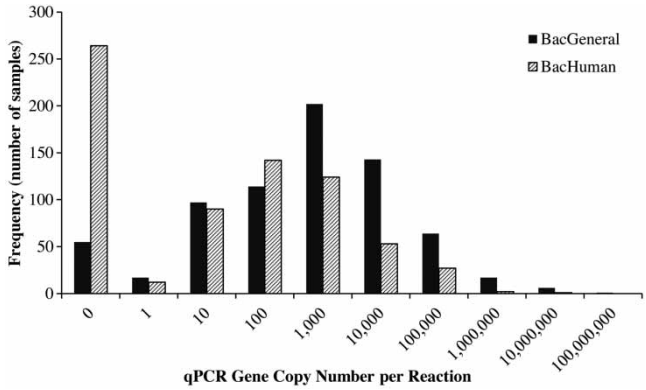


**Figure 2** | General and human *Bacteroidales* PCR assay gene copy number histograms (for the determination of positive cut-off values).

Of the presumptive *E. coli* positive well water samples submitted and microbially source tracked (the entire data set), 38.1% were designated positive for only human *Bacteroidales*, 25.7% for general *Bacteroidales* only, 11.0% for both human and bovine *Bacteroidales*, and 1.5% for bovine *Bacteroidales* only. 23.6% of presumptive *E. coli* positive samples tested negative for all *Bacteroidales* assays. In all, 49.2% of all samples tested positive for human *Bacteroidales* and 12.6% for bovine *Bacteroidales*, as seen in Figure 3. All samples that tested positive for human and/or bovine also tested positive for general, and thus other non-tested host species may also be present. Additionally, the figure displays these distributions stratified geographically by the four PHUs within the study region (the geocoded data set). HPEC had the highest percentage of human positives (53.6%) and the lowest percentage of all *Bacteroidales* assay negatives.

The geographic distribution of geocoded samples can be found in Figure 4. Spatial cluster analysis revealed a significant cluster ($p = 0.012$) of human-sourced contamination located within the southern regions of the HPEC and KFL&A PHUs (latitude 44.043285 N, longitude 77.092273 W, radius 38.08 km, log likelihood ratio 10.19) (also displayed in Figure 4). Fecal contamination in wells within this cluster was 1.52 times more likely to originate from human hosts than for wells outside of the cluster. No differences in cluster location, radius or statistical significance were observed during the analysis performed to ensure no bias. There were no significant clusters of bovine or general contamination identified from the spatial clustering analysis.

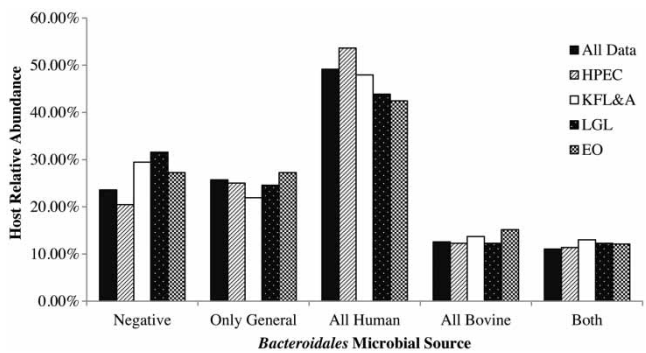


**Figure 3** | Distribution of microbial sources, overall and geographically stratified by PHU.

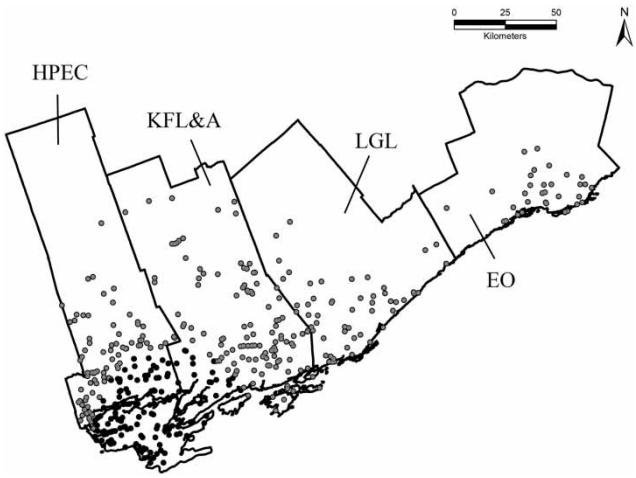


**Figure 4** | The study region (as defined by PHUs), the geographic distribution of tested samples and the statistically significant human *Bacteroidales* positives cluster. (Black wells within cluster; grey wells out of cluster.)

## DISCUSSION

This study is among the first worldwide to apply quantitative real-time PCR assays to microbially sources to track the fecal contamination present in private well water submissions, and the only study of its kind for Canada. A few factors were considered during qPCR assay selection. First, geographic stability of independent MST methods is critical in widespread point source investigations as genetic markers need to be highly conserved across the study region. The target sequences used here have demonstrated such stability across North America, as previously published by Lee *et al.* (2010). Second, the assays selected have previously demonstrated high target specificity and sensitivity. The use of gene copy numbers as reportable units has been previously documented in watershed studies (Lee *et al.* 2012; Drozd *et al.* 2013). It is essential to establish a gene copy threshold, which forms the basis for positive sample identification. Few qPCR MST studies describe this specific methodology in the context of the reportable range of the assay. For this investigation, natural cut-off values identified using histograms of gene copy numbers were used to define positives for each of the assays. The identified cut-offs (10 gene copies per reaction) were slightly higher than the PCR sensitivity for the three assays (6.5 gene copies per reaction); hence, the *Bacteroidales* positive definition was a conservative adjustment on the sensitivity of the test. The three ranges of gene copy values per 100 mL for *Bacteroidales* markers cannot be cross-compared with the literature as standardized ranges have not yet been established.

A human source was identified in the majority (49%) of contaminated private well water samples. This is in contrast to the only previous MST well water study (which was limited to 26 samples), where no samples tested positive for human-specific markers and only a single sample tested positive for general *Bacteroidales* markers (Allevi *et al.* 2013). Additionally, this study identified a significant spatial cluster of human-sourced fecal contamination in the southern portions of the HPEC and KFL&A PHUs. Human feces can contain pathogenic bacteria (e.g., *Shigella* and *Salmonella*), viruses (e.g., *Hepatitis A* and *Norovirus*) and protozoa (e.g., *Giardia lamblia* and *Cryptosporidium* spp.) (Heymann 2008). Furthermore, research indicates private well water may be a source of transmission for many of these pathogens (Simpson 2004), resulting in a human health risk. For example, *Norovirus* has been implicated as the most significant infectious pathogen in Norway's groundwater (Hanne *et al.* 2010), and can maintain its infectivity for a minimum of 61 days and detectability for 3 years (Seitz *et al.* 2011). Rates of GI infectious outbreaks have been found to be 35 times higher in recipients of private water supplies in England and Wales (compared to public water supplies) (Smith *et al.* 2006) and enteric disease rates 5.2 times higher for individuals living on land parcels serviced by private wells in British Columbia, Canada (compared to municipal groundwater systems) (Uhlmann *et al.* 2009).

For the region studied, humans are the primary source of fecal contamination in compromised private wells. To address this human health risk, prevention strategies should be implemented at the origin of contamination (which first needs to be identified). Likely origins for human contamination may be private wastewater systems (i.e. septic systems) and/or nearby land application of municipal biosolids.

Previous research has attributed private well water contamination to several septic system characteristics, including the distance between well and septic system. Arnade (1999) demonstrated a directly proportional relationship between septic tank distance and fecal coliform concentration. A study by Hynds *et al.* (2012) found a significant association

between septic tank location and thermotolerant coliform presence. Conversely, Raina *et al.* (1998) described an inverse relationship between septic tank distance and GI illness (associated with *E. coli* positive private wells), which may have occurred as a result of repeated exposure to nearby contamination enabling acquired immunity. Septic system density has also been implicated as a factor in private well water contamination. A study by Bremer & Harter (2012) demonstrated that the likelihood of pumping septic leachate is increased with septic system spatial density. High spatial concentrations of septic systems have also been linked to infectious diarrhea in children (Borchardt *et al.* 2003); however, holding tanks comprised a large portion of the septic systems in his analysis. The impact of density may explain the cluster of human-sourced contamination identified by our study. Additionally, septic system leakage has been shown to be a contamination source for private wells. Borchardt *et al.* (2011) conducted a case study that tracked *Norovirus*-containing septic waste from a code compliant septic system to a restaurant groundwater well. Given that our study area is rural, septic systems are prevalent and may be the contributing source of human fecal contamination, though further research is required to confirm this link.

In Ontario, land application of biosolids (a potential source of human fecal contamination), with *E. coli* levels below two million CFU per gram of total solids dry weight, is acceptable practice (Ontario Ministry of Agriculture and Food 2012). Groundwater contamination associated with biosolids land application has been studied; notably, fractured bedrock and karstic regions have been implicated in providing the least protection from biosolids pathogens (Eisenberg *et al.* 2008; Viau *et al.* 2011). This has relevance to the results of this investigation as the cluster region identified here is located in an area comprised mainly of bedrock geology (Ontario Geological Survey 2010). Furthermore, land application of biosolids has been practiced in this region (City of Quinte West, personal communication, September 24th, 2013).

Testing for bovine fecal contamination is of particular significance given that cattle act as a reservoir for numerous pathogens including Shiga toxin-producing *E. coli*. In 2000, cattle-based agricultural runoff containing this bacterium contaminated a municipal groundwater well in Walkerton, Ontario, and resulted in the deaths of seven individuals and over 2000 infections (Bruce-Grey-Owen Sound Health Unit 2000). In our study, only 12.6% of the samples tested positive for bovine fecal contamination and no significant cluster was found. This low prevalence may be explained by the low cattle density within the study area (Statistics Canada 2011).

Approximately one-quarter (24%) of samples tested negative for all *Bacteroidales* assays. Given that all samples had tested presumptively positive for *E. coli,* non-fecal origins need to be considered. Fecally deposited *E. coli* has been shown to survive in the environment in matrices including soil and water (Ishii & Sadowsky 2008). This naturalized *E. coli* exhibits genetic differences from its animal GI origins (Byappanahalli *et al.* 2006; Ishii *et al.* 2006). Furthermore, a review by Ishii & Sadowsky (2008) affirmed that environmental *E. coli* can survive extensive freezing, and multiply within a wide range of temperatures (7.5–49°C) (Ishii & Sadowsky 2008). Additionally, a study by VanderZaag *et al.* (2010) documented the presence of naturalized *E. coli* populations in shallow groundwater in Canada. Therefore, naturalized *E. coli* populations may be contributing to private well water contamination.

In addition, biofilms can exist within drinking well systems and may undergo bacterial separation, which can act as a source of contamination (Environmental Protection Agency 2002). The presence of infectious organisms originating from biofilms in drinking water systems has been documented and can include human and zoonotic pathogens (Environmental Protection Agency 2002). Given that a quarter of presumptive *E. coli* positives tested negative for *Bacteroidales* and multiple possible sources have been identified in the literature, further research is required to determine the source of these presumptive *E. coli* in private wells.

The BacGeneral assay targets a conserved region for 10 host species: feline, bovine, deer, canine, anserine, gull, equine, human, swine, and raccoon (Lee *et al.* 2010). A significant portion (25.7%) of samples tested positive exclusively for this assay, which implicates hosts other than human and bovine as possible contributors to the fecal contamination present in the well water samples. It is likely that no cluster was observed for exclusive general

marker positives because of the varied sources of contamination. MST qPCR assays have been developed targeting many of these host markers and can be applied to determine exact sources (Mieszkin *et al.* 2009; Hagedorn *et al.* 2011). Furthermore, it is important to note that the exclusive BacGeneral positives may actually be false negatives for the BacHuman and BacBovine 1 MST assays as a recent study by Arumugam *et al.* (2011) discovered that individuals fall into three main enterotypes (*Bacteroides*, *Prevotella* and *Ruminococcus*) based on their GI flora composition, with the latter two possessing reduced concentrations of species belonging to the order *Bacteroidales*. These findings were supported in a study by Drozd *et al.* (2013), where a human fecal sample tested negative for *Bacteroidales* markers. Similarly, the BacBovine 1 assay may also elicit false negative results, as rumen microbiota composition varies with diet and its genome sequencing is relatively new (Morgavi *et al.* 2013). Thus, the actual number of positives for these host-specific assays may be underrepresented.

The data presented here were collected for a 1-year period; however, the distribution of positives among the MST assays is consistent with unpublished data from 2013 (collected for an alternate investigation, but which included the same study area). Furthermore, the spatial aspect of the significant cluster of human contamination is supported by a 2003–2004 *E. coli* cluster result from a study by Perkins *et al.* (2009), which tested rural tap water and implicated the same region.

## CONCLUSIONS

This was the first study to combine MST methods with geospatial analysis techniques to investigate private well water quality. We demonstrated that human feces were the primary contributor to private well water contamination in southeastern Ontario, with a statistically significant cluster of human contamination existing within the region. These findings will enable further research into the detection of human-specific pathogens, which are of public health concern. Furthermore, establishing a human cluster region allows for future investigation into the origins of contamination and the targeting of public health interventions based on source and elevated risk.

## ACKNOWLEDGEMENTS

The authors wish to thank the employees of the Public Health Ontario Laboratory in Kingston for their kind support, especially Cindy Froats for her contribution to this project. A special thank you also to Dai Young Lee and Susan Weir who shared their molecular expertise, to David Ryding and Eric Wagar for their early work on the assay development and the analyses, and to Susan Greaves, Kayla Poirier, Lydia Cheng and Connor Korsten. The funding for this study was provided by Physicians' Services Incorporated Foundation.

## REFERENCES

Allevi, R. P., Krometis, L. H., Hagedorn, C., Benham, B., Lawrence, A. H., Ling, E. J. & Ziegler, P. E. 2013 Quantitative analysis of microbial contamination in private drinking water supply systems. *J. Water Health* **11**, 244–255.

Arnade, L. J. 1999 Seasonal correlation of well contamination and septic tank distance. *Ground Water* **37**, 920–923.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., Kurokawa, K., Leclerc, M., Levenez, F., Manichanh, C., Nielsen, H. B., Nielsen, T., Pons, N., Poulain, J., Qin, J., Sicheritz-Ponten, T., Tims, S., Torrents, D., Ugarte, E., Zoetendal, E. G., Wang, J., Guarner, F., Pederson, O., de Vos, W. M., Brunak, S., Doré, J., MetaHIT Consortium, Weissenbach, J., Ehrlich, S. D. & Bork, P. 2011 Enterotypes of the human gut microbiome. *Nature* **473**, 174–180.

Borchardt, M. A., Chyou, P. H., DeVries, E. O. & Belongia, E. A. 2003 Septic system density and infectious diarrhea in a defined population of children. *Environ. Health Perspect.* **111**, 742–748.

Borchardt, M. A., Bradbury, K. R., Alexander Jr, E. C., Kolberg, R. J., Alexander, S. C., Archer, J. R., Braatz, L. A., Forest, B. M., Green, J. A. & Spencer, S. A. 2011 Norovirus outbreak caused by a new septic system in a dolomite aquifer. *Groundwater* **49**, 85–97.

Bremer, J. E. & Harter, T. 2012 Domestic wells have high probability of pumping septic tank leachate. *Hydrol. Earth Syst. Sci.* **16**, 2453–2467.

Bruce-Grey-Owen Sound Health Unit 2000 Waterborne outbreak of gastroenteritis associated with a contaminated municipal water supply, Walkerton, Ontario, May-June 2000. *Can. Commun. Dis. Rep.* **26**, 170–173.

Byappanahalli, M. N., Whitman, R. L., Shively, D. A., Sadowsky, M. J. & Ishii, S. 2006 Population structure, persistence, and seasonality of autochthonous *Escherichia coli* in temperate, coastal forest soil from a Great Lake watershed. *Environ. Microbiol.* **8**, 504–513.

Drozd, M., Merrick, N. N., Sanad, Y. M., Dick, L. K., Dick, W. A. & Rajashekara, G. 2013 Evaluating the occurrence of host-specific bacteroidales, general fecal indicators, and bacterial pathogens in a mixed-use watershed. *J. Environ. Qual.* **42**, 713–725.

Eisenberg, J. N., Moore, K., Soller, J. A., Eisenberg, D. & Colfrod, J. M. J. 2008 Microbial risk assessment framework for exposure to amended sludge projects. *Environ. Health Perspect.* **116**, 727–733.

Environmental Protection Agency 2002 *Health Risks from Microbial Growth and Biofilms in Drinking Water Distribution Systems*. EPA, Washington, DC.

Hagedorn, C., Blanch, A. R. & Harwood, V. J. 2011 *Microbial Source Tracking: Methods, Applications and Case Studies*. Springer Science + Business Media, New York.

Hanne, M. L., Kvitsand, H. M. L. & Fiksdal, L. 2010 Waterborne disease in Norway: emphasizing outbreaks in groundwater systems. *Water Sci. Technol.* **61** (3), 563–571.

Heymann, D. L. 2008 *Control of Communicable Diseases Manual* (19th edition). American Public Health Association, Washington, DC.

Hynds, P. D., Misstear, B. D. & Gill, L. W. 2012 Development of a microbial contamination susceptibility model for private domestic groundwater sources. *Water Resour. Res.* **48**, 1–13.

Ishii, S. & Sadowsky, M. J. 2008 *Escherichia coli* in the environment: implications for water quality and human health. *Microbes Environ.* **23**, 101–108.

Ishii, S., Ksoll, W. B., Hicks, R. E. & Sadowsky, M. L. 2006 Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. *Appl. Environ. Microbiol.* **72**, 612–621.

Kirs, M., Harwood, V. J., Fidler, A. E., Gillespie, P. A., Fyfe, W. R., Blackwood, A. D. & Cornelisen, C. D. 2011 Source tracking faecal contamination in an urbanized and a rural waterway in the Nelson-Tasman region, New Zealand. *New Zeal. J. Mar. Fresh.* **45**, 43–58.

Klein, E. & Houston, A. C. 1898 Report on bacteriological evidence of presumable recent, and therefore dangerous, sewage pollution of elsewise potable waters. 27th Report Annual Report of the Local Government Board 1897–98, Supplement Report of Local Officer 4, 318–325.

Kuldorff, M. 1997 A spatial scan statistic. *Commun. Stat. Theory Methods* **26**, 1481–1496.

Lee, Z. M. P., Bussema III, C. & Schmidt, T. M. 2009 rrnDB: documenting the number of rRNA and tRNA genes in bacteria and archaea. *Nucl. Acids Res.* **37**, D489–D493.

Lee, D., Weir, S. C., Lee, H. & Trevors, J. T. 2010 Quantitative identification of fecal water pollution sources by TaqMan real-time PCR assays using *Bacteroidales* 16S rRNA genetic markers. *Appl. Microbiol. Biotechnol.* **8**, 1373–1383.

Lee, C., Agidi, S., Marion, J. W. & Lee, J. 2012 Arcobacter in Lake Erie Beach waters: an emerging gastrointestinal pathogen linked with human-associated fecal contamination. *Appl. Environ. Microbiol.* **78**, 5511–5519.

Mieszkin, S., Furet, J. P., Corthier, G. & Gourmelon, M. 2009 Estimation of pig fecal contamination in a river catchment by real-time PCR using two pig-specific *Bacteroidales* 16S rRNA genetic markers. *Appl. Environ. Microbiol.* **75**, 3045–3054.

Morgavi, D. P., Kelly, W. J., Janssen, P. H. & Attwood, G. T. 2013 Rumen Microbial (meta)genomics and its application to ruminant production. *Animal* **7**, 184–201.

Odoi, A., Marin, W. S., Michel, P., Middleton, D., Holt, J. & Wilson, J. 2004 Investigation of clusters of giardiasis using GIS and a spatial scan statistic. *Int. J. Health Geogr.* **3**, 1–11.

Ogden, I. D. & Watt, A. J. 1991 An evaluation of fluorogenic and chromogenic assays for the direct enumeration of Escherichia-coli. *Lett. Appl. Microbiol.* **13**, 212–215.

Okabe, S., Okayama, N., Savichtcheva, O. & Ito, T. 2007 Quantification of host-specific *Bacteroides-Prevotella* 16S rRNA genetic markers for assessment of fecal pollution in freshwater. *Environ. Biotechnol.* **74**, 890–901.

Ontario Geological Survey 2010 Surficial geology of Southern Ontario; Ontario Geological Survey, Miscellaneous Release–Data 128-REV.

Ontario Ministry of Agriculture and Food 2012 *Information on Analysis Requirements for Non-Agricultural Source Materials*. Queen's Printer for Ontario, Toronto, ON.

Peed, L. A., Nietch, C. T., Kelty, C. A., Meckes, M., Mooney, T., Sivaganesan, M. & Shanks, O. C. 2011 Combining land use information and small stream sampling with PCR-Based methods for better characterization of diffuse sources of human fecal pollution. *Environ. Sci. Technol.* **45**, 5652–5659.

Perkins, N. R., Kelton, D. F., Hand, K. J., MacNaughton, G., Berke, O. & Leslie, K. E. 2009 An analysis of the relationship between bulk tank milk quality and wash water quality on dairy farms in Ontario, Canada. *J. Dairy Sci.* **92**, 3714–3722.

Raina, P. S., Pollari, F. L., Teare, G. F., Goss, M. J., Barry, D. A. & Wilson, J. B. 1998 The relationship between *E. coli* indicator bacteria in well-water and gastrointestinal illnesses in rural families. *Can. J. Public Health* **90**, 172–175.

Seitz, S. R., Leon, J. S., Schwab, K. J., Lyon, G. M., Dowd, M., McDaniels, M., Abdulhafid, G., Ferandez, M. L., Lindesmith, L. C., Baric, R. S. & Moe, C. L. 2011 Norovirus infectivity in humans and persistence in water. *Appl. Environ. Microbiol.* **77**, 6884–6888.

Shanks, O. C., Atikovic, E., Blackwood, D. A., Lu, J., Noble, R. T., Domingo, J. S., Seifring, S., Sivaganesan, M. & Haugland, R. A. 2008 Quantitative PCR for detection and enumeration

of genetic markers of bovine fecal pollution. *Appl. Environ. Microbiol.* **74**, 745–752.

Simpson, H. 2004 Promoting the management and protection of private water wells. *J. Toxicol Env. Health A* **67**, 1679–1704.

Smith, A., Reacher, M., Smerdon, W., Adak, G. K., Nichols, G. & Chalmers, R. M. 2006 Outbreaks of infectious intestinal disease in England and Wales, 1992–2003. *Epidemiol. Infect.* **134**, 1141–1149.

Statistics Canada 2011 *Census of Agriculture, Farm and Farm Operator Data*, Catalogue No. 95-640-XWE, Ottawa, Canada.

Statistics Canada 2013 *Health Profile*. Catalogue No. 82-228-XWE, Ottawa, Canada.

Uhlmann, S., Galanis, E., Takaro, T., Mak, S., Gustafson, L., Embree, G., Bellack, N., Corbett, K. & Isaac-Renton, J. 2009 Where's the pump? Associating sporadic enteric disease with drinking water using a geographic information system, in British Columbia, Canada, 1996–2005. *J. Water Health* **7**, 692–698.

VanderZaag, A. C., Campbell, K. J., Jamieson, R. C., Sinclair, A. C. & Hynes, L. G. 2010 Survival of *Escherichia coli* in agricultural soil and presence in tile drainage and shallow groundwater. *Can. J. Soil Sci.* **90**, 495–505.

Viau, E., Bibby, K., Paez-Rubio, T. & Peccia, J. 2011 Toward a consensus view on the infectious risks associated with land application of sewage sludge. *Environ. Sci. Technol.* **45**, 5459–5469.

First received 4 October 2013; accepted in revised form 24 November 2013. Available online 24 December 2013