Microbial quality and bacteria pathogens in private wells used for drinking water in northeastern Ohio
Gayeon Won, Amy Gill and Jeffery T. LeJeune

ABSTRACT
In agricultural intensive areas, drinking contaminated water from private wells is considered an important cause of acute gastroenteric illnesses (AGI), particularly among high-risk populations. In the summer of 2009, the microbial water quality of 180 randomly selected private wells in two northeastern Ohio counties, a region with a high concentration of dairy farms, was assessed. Forty-five percent (82/180) of water samples were contaminated with total coliforms. Generic Escherichia coli were present in 9% (16/180) of samples. Using real-time polymerase chain reaction, E. coli O157:H7 was identified in 4% (7/180) of specimens. Campylobacter spp. DNA could not be amplified from 70 of the samples tested for this organism. The frequency of generic E. coli contamination varied among townships ($P < 0.001$). Well structure (i.e. age and depth) or other common measures of pollution potential (depth of water, hydrology, topography, net recharge soil media) was not correlated with coliforms and E. coli contamination. Importantly, the presence of the pathogen E. coli O157:H7 was not associated with the presence of fecal indicators in the water samples: Only one of the seven E. coli O157-positive samples was also positive for generic E. coli. Appropriate risk management and communication processes are needed to reduce the potential waterborne disease outbreaks in agricultural intensive areas.

Key words | coliforms, E. coli O157, generic E. coli, private well drinking water

INTRODUCTION
In agricultural intensive areas, water quality deterioration is a growing concern for public health. Surface waters are often used for recreational, irrigation and drinking purposes. Surface water sources such as ponds, streams and reservoirs are easily contaminated by surface runoffs originating from non-point sources such as animal and human wastes (Johnson et al. 2003; Ahmed et al. 2009), resulting in water-borne disease outbreaks in the USA (Yoder et al. 2008; Craun et al. 2010; Hlavsa et al. 2011). Even though ground water from private wells is generally perceived safe for drinking by consumers (Jones et al. 2005), during 2007 and 2008, contaminated ground water was responsible for 36% of all waterborne disease outbreaks in the USA (Brunkard et al. 2011). Two recent case–control studies in the USA identified the drinking of well water as a risk factor for children’s enteric infections (Denno et al. 2009; Gorelick et al. 2011).

Approximately 15% (15.8 million) of the population in the USA obtains drinking water from domestic private wells (Hlavsa et al. 2011). Most private well owners reside in rural areas. Although the US Environmental Protection Agency (US EPA) recommends that fecal indicators should be completely absent in drinking water from private wells (US EPA 2011), these wells have the potential to be contaminated with fecal organisms from nearby agricultural and other anthropogenic activities. Krapac et al. (2002) reported that ground water near swine manure storage pits was adversely affected (Krapac et al. 2002). Septic tank density (Borchardt et al. 2003) and human and animal feces in the vicinity of wells (Licence et al. 2001) also affects microbial quality of private well water. Arnade (1999) indicated significant impact of precipitation on microbial quality of well water located near septic tanks. Water may also be

contaminated during conveyance in distribution systems (Payment et al. 1997; Trevett et al. 2005).

Individuals living on farms, or living in regions with high density of livestock, are at higher risk for infections for Campylobacter and Escherichia coli O157 (Stanley & Jones 2005; Oliver et al. 2005) and may have increased hospitalization rate (Febriani et al. 2009) for bacterial gastroenteritis (Febriani et al. 2009). However, the direct or indirect routes of exposures to these pathogens among rural residents are not clearly defined (Friesema et al. 2011). Hence, we hypothesized that wells in close proximity to bovine operations might be a source of these pathogens. The purpose of this study was to determine the frequency and magnitude of private well water contamination with microbial indicators (coliforms, generic E. coli) and two specific pathogens, E. coli O157: H7 and Campylobacter jejuni in an agriculture intensive region of Ohio. In addition, the influence of factors anticipated to affect microbial contamination in this agriculturally intensive region were assessed.

MATERIALS AND METHODS

Study area and sample collection

Sampling was conducted in six townships of Wayne and Holmes Counties, Ohio during summer (May–August 2009). This region is characterized by the presence of a large number of dairy farms (Clark et al. 2008). Three townships were selected from each of these two counties to represent areas of variable dairy farm densities and differing ground water pollution potential as indicated by the Ohio Department of Natural Resources Division (GWMTS 2010). This pollution index map was developed based on the major hydrological factors (i.e. depth of water, net recharge, aquifer media, soil media, topography, impact of the vadose zone media hydraulic conductivity of the aquifer) (Figure 1). The ground water pollution potential index was compared among selected townships and the level of potential risk from ground water pollution index was determined (Table 1). Dairy farm densities were determined by the number of grade A licensed dairy farms located in each township. Selected townships were categorized into high risk (>50 dairy farms; Saltcreek, Paint and Milton), medium risk (<20 dairy farms; Wayne, Franklin) and low risk (<5 dairy farms; Knox) townships for dairy farm density (Thorn et al. 2011). Thirty households in each six townships (i.e. total 180 households) were randomly selected based on addresses present in a well log recorded by the Ohio Department of Natural Resources (GWMTS). During summer 2009 (June–August), we visited the randomly selected 180 households to collect water samples from their private well. Each sampling location (i.e. households) and the location of dairy farms were plotted on a regional map using Arc GIS program (version 9.2, Esri, CA, USA) (Figure 1). Approximately 4 L of water was collected in sterile containers from indoor residential cold water faucets or outside faucets connected to the private well serving the household. Other details of selected wells such as age and depth were also recorded from Ohio well logs. Samples were kept chilled and analyzed within 12 hours of sampling.

Bacterial analysis

Total coliform and generic E. coli were enumerated in 100 mL aliquots of the water sample using a commercial MPN tests following the manufacturer’s instructions (Quanti-Tray2000 Idexx Laboratories, Westbrook, ME, USA). In addition, two 2-L aliquots of each water sample were filtered through separate 0.22 μm pore membrane filters (Millipore Corporation, Billerica, MA, USA) using a vacuum pump. One filter membrane was transferred into 10 mL of buffered peptone water (BPW) and shaken for 3 min on an orbital shaker (250 rpm) to dislodge entrapped bacteria. Subsequently, the rinseate (i.e. 10 mL of BPW after shaking) was incubated at 37 °C overnight (i.e. enriched samples with BPW). This overnight broth culture was frozen at −70 °C, with 50% buffered glycerol. The other filter membrane was frozen at −70 °C and the DNA from attached organisms was extracted at a later time using a commercial kit according to the manufacturer’s instructions (Rapid Water TM DNA Isolation Kit, MOBIO Laboratories, Inc.). These later specimens were called the non-enriched samples.

Statistical analysis

Multivariate logistic regression models (STATA ver.10, STATA Corporation, College Station, TX, USA) were used
to assess the association between well attributes (well age, depth, and township, as a surrogate for pollution index/cattle density) and the probability ($pi$) that the fecal indicators were found in the sample (Hosmer & Lemeshow 2000).

**Detection of pathogens by real-time polymerase chain reaction (PCR)**

Commercial real-time PCR kits were used to amplify and detect targets specifics for *E. coli* O157 and
Campylobacter jejuni (MicroSEQ® E. coli O157:H7 detection kit, TaqMan® Campylobacter jejuni detection kit, Applied Biosystems, Foster City, USA) following the manufacturer’s directions. Targeted sequences and primer sets are proprietary. From enriched samples, template DNA was extracted from the frozen stocks stored at −70°C using a simple boiling method: 1 mL of enriched samples was centrifuged at 13,000 rpm during 3 min and cell pellets were resuspended in 300 μL sterile water, boiled for 10 min, and then cooled on ice for 20 min. Cellular debris was pellets by centrifugation (13,000 rpm, 3 min) and the supernatant was used as PCR template. Fluorescent probes included reporter dye (VIC, proprietary), quencher dye (FAM, 6-carboxyfluorescein) and internal positive control (NED) that targeted a synthetic piece of DNA in the sample for E. coli O157 detection. The fluorescence intensities of amplification reactions was measured and analyzed using an automated 7500 Real time RT-PCR system (Applied Bio Systems). Standard curves were generated with 10-fold serial dilutions ranged between 10^6 and 10^8 CFU per mL (spiked CFU/mL) from E. coli O157:H7 strain (American Type Culture Collection strain ATCC 43888). In order to quantify the initial concentration of E. coli O157 in the samples, the PCR analysis was re-performed with non-enriched samples that tested positive from enrichments under the same conditions.

The extracted DNA non-enriched filters from the first 76 collected water samples were also tested for Campylobacter jejuni following the manufacturer’s recommended amplification conditions (TaqMan® Campylobacter jejuni detection kit, Applied Biosystems).

### RESULTS

#### Fecal indicator detection

Total coliforms were found in 45.5% (82/180) of total sampled well water and 9% (16/180) of the samples were positive for generic E. coli. There were differences in the frequencies of fecal indicators present among townships (Table 1). For example, total coliforms and generic E. coli were detected in 16 (16/30, 53.3%) and seven of water samples (7/30, 23%) collected in Saltcreek township, respectively, while in Franklin township, both total coliforms (8/30, 26.7%) and E. coli (1/30, 3.3%) were detected at the lowest frequency. Total coliforms and generic E. coli were moderately correlated (r = 0.463, P < 0.001). However, there was no association between the presences of fecal indicators and E. coli O157:H7 found in water samples (Table 2).

#### Bacterial pathogen detection

From our standard curve, as few as 10^-6 genomic targets were detectable after 38 amplification cycles in this PCR assay (for VIC, y = −3.94312x + 15.742, R^2 = 0.9527; for FAM, y = −4.38946x + 14.71, R^2 = 0.9807).

E. coli O157:H7 was detected in seven enrichment samples (Table 2). All positive samples showed fluorescent signal from FAM in 24–37 cycles, VIC in 17–38 cycles of VIC signal, and IPC (NED) in 28–38 cycles at the 0.2 of cutoff Ct value. Out of seven positive samples, there were two samples that tested positive for E. coli O157:H7 from the direct (non-enriched) specimens. These specimens showed fluorescent signals from FAM in 34 and 37 cycles,
VIC in 39 and 37 cycles and IPC at 34 cycles. The quantity of template of *E. coli* O157:H7 DNA in the non-enriched was calculated by using the standard curve and was estimated to be between equivalent to $10^{-5} - 10^{-6}$ CFU/mL in the two non-enriched positive samples. Based on these calculations, if the organism was evenly distributed in the water source, there would be one CFU of *E. coli* O157 in 2 L of the well water. The amount of amplicon in the five other samples was below detection threshold without enrichment.

For *Campylobacter jejuni*, although all positive control samples provided expected results, no fluorescent signals were detected from any of the direct (non-enriched) specimens tested.

**Logistic regression model**

The binomial probability of detecting total coliform in the sample was not associated with well depth, age or well location ($P > 0.2$). Well age also did not have any relation with the presence of generic *E. coli*. Even though well location (i.e. township) significantly affected the probability of presence of *E. coli* (coefficient $= 0.416$, $P = 0.038$) in the water sample, these results did not show any significant associations among dairy farm density, ground water pollution potential index and microbial water quality.

**DISCUSSION**

Rural wells located in the region were frequently contaminated with bacteria indicative of fecal contamination. Even though the probability of detecting generic *E. coli* in the sample was significantly different among townships, the association with dairy farm density and the pollution index was not evident. *E. coli* O157:H7 was also detected in seven water samples (3.9%, 7/180). Among the *E. coli* O157-positive samples, one sample was positive for both coliforms and generic *E. coli*; and two samples were positive for coliforms alone, one at high levels. The four remaining samples that tested positive for *E. coli* O157 were negative for both total coliforms and generic *E. coli*. Schets *et al.* (2008) described similar results that *E. coli* O157:H7 was isolated in 2.7% of well water samples which had no fecal indicators (i.e. intestinal enterococci, total coliforms and generic *E. coli*) in the Netherlands.

The detection of *E. coli* O157 in drinking water is of serious concern. An association between the potential factors (i.e. dairy farms, ground water pollution potential) and occurrences of *E. coli* O157 and fecal indicators was not detected (Tables 1 and 2). This indicates that other unmeasured risk factors (e.g. presence of grazing animals, other livestock farms including hog and poultry, septic tanks and other activities near sampling locations) may affect the water quality (Arnaud 1999; Licence *et al.* 2001; Krapac *et al.* 2002; Borchardt *et al.* 2003). Another explanation for this observation is also the possibility of false-positive results on the PCR. However, given the reported specificity of the detection kit used (Sen *et al.* 2011), and the consistent negative results on our negative control assays, we considered this unlikely. Sen *et al.* indicated that the *E. coli* O157:H7 detection kit can correctly identify 37 different *E. coli* O157 strains and does not react with a large number of

<table>
<thead>
<tr>
<th>Table 2</th>
<th>The number of fecal indicators in water samples that was positive for <em>E. coli</em> O157:H7 detected using the PCR analysis (Northeast Ohio 2009)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample No.</td>
<td>Sampling location (township)</td>
</tr>
<tr>
<td>8</td>
<td>Saltcreek</td>
</tr>
<tr>
<td>15</td>
<td>Saltcreek</td>
</tr>
<tr>
<td>17</td>
<td>Paint</td>
</tr>
<tr>
<td>29</td>
<td>Milton</td>
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<tr>
<td>55</td>
<td>Paint</td>
</tr>
<tr>
<td>57</td>
<td>Paint</td>
</tr>
<tr>
<td>128</td>
<td>Wayne</td>
</tr>
</tbody>
</table>

*This water sample tested positive by PCR for *E. coli* O157:H7 from both enriched and non-enriched specimens.*
other pathogenic E. coli or three other common waterborne bacteria (Sen et al. 2011). In that same study (Sen et al. 2011), regarding the sensitivity of the kit, they detected low concentration of E. coli O157:H7 in the presence of high concentration of other E. coli species (i.e. 10^8 cells).

Importantly, in the context of waterborne pathogen detection, viable but non-culturable (VBNC) complicates the estimation of targeted pathogens in water by PCR (Rozsak & Colwell 1987). Since PCR is based on the detection of nucleic acids, not viable cells, nucleic acids from dead cells can result in positive signals in PCR analyses (Josephson et al. 1993; Fode-Vaughan et al. 2003). Considering the rate of nucleic acid degradation (i.e. 3 weeks) even if the DNA entered in this study was from dead cells (Josephson et al. 1993), residents drinking well water positive for E. coli O157 may have been exposed to risks of E. coli O157 infection during the period prior to the sampling point. PCR-positive samples would still be important as an indication of recent past contamination with this organism.

The total coliform group consists of closely related bacteria which can be inherently found in soil, water and feces (WHO 2011). All coliforms are not necessarily of fecal origin. The presence of total coliform in municipal water systems has implicated the deficiency of distribution system or treatment due to the potential of environmental contamination of non-fecal origin as described in the Total Coliform Rule (TCR) (WHO 2011) regulated by the US EPA (Bennear et al. 2009). Generic E. coli, a subset of a total coliform group, is more likely to indicate fecal contamination in water (Edberg et al. 2000). However, the capacity of generic E. coli to predict the occurrence of pathogens with a fecal origin has not been determined.

Lack of association between the presence of generic E. coli and E. coli O157:H7 found in the water samples may be attributed to several factors. First, E. coli O157:H7 is distinguished from generic E. coli by the enzymatic activity of β-glucuronidase (GUD) (Hayes et al. 1995). Generic E. coli has the capacity to hydrolyse 4-methylumbelliferyl β-D-glucuronide (MUG) in the media to release 4-methylumbelliferone (MU) but E. coli O157 is consistently MUG negative. Thus, the indicator commonly used in E. coli detection tests, including the tests employed herein, would not be cleaved to a fluorescent by-product and the test would yield a negative result in the presence of E. coli O157. Notwithstanding, contamination of water with E. coli O157, in the absence of other generic E. coli, is unlikely. E. coli O157 does not typically persist longer in water than generic E. coli counterparts. A second, and more likely explanation, for the apparent discrepancy in PCR and MPN results for E. coli O157 and generic E. coli respectively, is the fact that the numbers E. coli O157 estimated in the water were very low: they were detectable only when 2 L of water was tested, whereas generic E. coli counts were determined in 100 mL aliquots. Had larger volumes of water been tested for generic E. coli, low levels of contamination may have been detected.

One limitation of this study is that only fecal indicators and bacterial pathogens were used for microbial water quality parameters. If waterborne parasites and viruses of fecal origin were used in this monitoring, these organisms may have been detected in the water samples.

The Foodborne Disease Active Surveillance Network reported that 15 per 10,000 infants (i.e. younger than one year of age) are annually diagnosed with campylobacteriosis in the USA (Fullerton et al. 2007). Despite the low survival rate of Campylobacter spp. in the environment due to their microaerophilic characteristics (Stintzi 2005), the low infectious dose and conversion to a VBNC state in adverse environmental conditions may affect the high prevalence of human campylobacteriosis attributed to water despite the low detection rate. Notwithstanding, we used a Taq-Man®Campylobacter jejuni Detection Kit (Life Technologies) with high sensitivity for Campylobacter jejuni detection (Toplak et al. 2012), yet identified no positive samples.

In the USA, multiple outbreaks of E. coli O157:H7 associated with drinking water sources have occurred, some of which were quite large (>100 illnesses) (Olsen et al. 2002; Bopp et al. 2003). Even though those outbreaks were attributed to untreated well or surface water supplied from municipal water system, they may also reflect a potential role of untreated private drinking water as an important exposure route of this pathogen. Moreover, reported cases of waterborne disease from drinking well sources occur more frequently among rural visitors rather than permanent residents, even when they were exposed to the same contaminated water sources (Licence et al. 2001; Olsen et al. 2002). Belongia et al. (2003) found that 59% of farm
residents have C. jejuni antibodies and 14% have anti-E. coli O157 LPS. Multidrug resistance E. coli was also observed in private well drinking water in Canada (Mataseje et al. 2009). These reports indicate that rural residents have frequent exposure to numerous pathogens, some of which may have been via water. Even though past exposure may enhance immunity (Frost et al. 2005), precautionary measures to prevent such exposure, especially among the young or otherwise immune-compromised, are warranted to prevent disease. Well maintenance practices, including regular water testing and well head protection, are also important to reduce well contamination (Macler & Merkle 2000).

CONCLUSIONS

The residents using private well water located in the test area were unknowingly exposed to the risk of E. coli O157:H7 infection. These studies show that AGI disease observed in agricultural intensive regions might be attributed to human exposure to pathogens via drinking water. The physical factors typically used to identify the characteristics of the wells at high risk for contamination (pollution potential index based on hydrologic parameters, depth, etc.), were not predictive of likelihood of contamination with indicators or pathogens. Instead, contamination potential may be associated with other factors for which owners have greater control such as well maintenance. Efforts should be made to prevent contamination of rural wells, as microbiological testing alone is not a reliable assurance of safety.

REFERENCES


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