

## Microbial quality and bacteria pathogens in private wells used for drinking water in northeastern Ohio

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### 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

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### 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 2003; 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 ( $\geq 50$  dairy farms; Saltcreek, Paint and Milton), medium risk ( $\leq 20$  dairy farms; Wayne, Franklin)

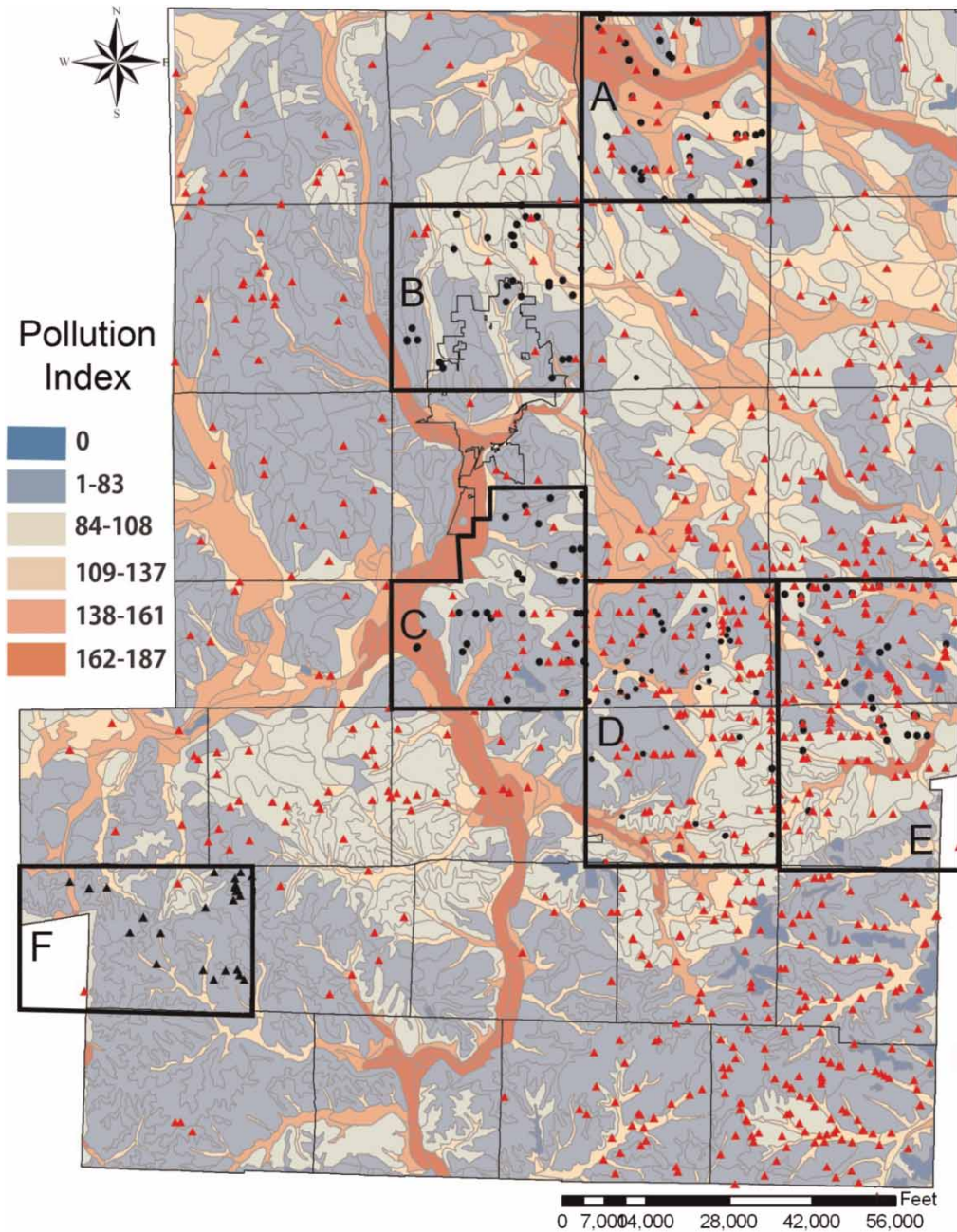
and low risk ( $\leq 5$  dairy farms; Knox) townships for dairy farm density (Thorn *et al.* 2011). Thirty households in each six townships (i.e. total 180 households) were randomly selected based on addresses present in a well log recorded by the Ohio Department of Natural Resources (GWMTS). During 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  $\mu\text{m}$  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 rinsate (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 30% 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



**Figure 1** | Locations of sampling (i.e. households, black points) and dairy farms (red triangles) projected on the ground water pollution indexed map (A: Milton Township, B: Wayne Township, C: Franklin Township, D: Saltcreek Township, E: Paint Township, F: Knox Township).

to assess the association between well attributes (well age, depth, and township, as a surrogate for pollution index/cattle density) and the probability ( $p_i$ ) 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 specific for *E. coli* O157 and

**Table 1** | Frequency of fecal indicators and *E. coli* O157:H7 detected in well drinking water sample (northeast Ohio 2009)

Township	Total coliform	Generic <i>E. coli</i>	<i>E. coli</i> O157:H7	Pollution potential	Dairy farm density
Milton (A)	43.3	3.3	3.3	High	High
Wayne (B)	40.0	3.3	3.3	Medium	Medium
Franklin (C)	26.7	3.3	0	High	Medium
Saltcreek (D)	53.3	23.0	6.7	Medium	High
Paint (E)	46.7	6.7	9.9	Medium	High
Knox (F)	60.0	13.2	0	Low	Low
Total	45.6	8.9	3.9		

*Campylobacter jejuni* (MicroSEQ<sup>®</sup> *E. coli* O157:H7 detection kit, TaqMan<sup>®</sup> *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  $-70^{\circ}\text{C}$  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  $\mu\text{L}$  sterile water, boiled for 10 min, and then cooled on ice for 20 min. Cellular debris was pelleted by centrifugation (13,000 rpm, 3 min) and the supernatant was used as PCR template. Fluorogenic 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  $10^0$  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<sup>®</sup> *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,

**Table 2** | The number of fecal indicators in water samples that was positive for *E. coli* O157:H7 detected using the PCR analysis (Northeast Ohio 2009)

Sample No.	Sampling location (township)	Sampling date	<i>E. coli</i> O157:H7 (P/A)	Generic <i>E. coli</i> (MPN/100 mL)	Total coliforms (MPN/100 mL)
8	Saltcreek	6/4/2009	P	0	2
15	Saltcreek	6/5/2009	P	1	5
17	Paint	6/8/2009	P	0	0
29	Milton	6/12/2009	P	0	0
55	Paint	6/22/2009	P	0	2,419.2
57	Paint	6/22/2009	P <sup>a</sup>	0	0
128	Wayne	7/16/2009	P <sup>a</sup>	0	0

<sup>a</sup>This water sample tested positive by PCR for *E. coli* O157:H7 from both enriched and non-enriched specimens.

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.* (2005) 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 (Arnade 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

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 (Roszak & 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 (Benear *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  $\beta$ -glucuronidase (GUD) (Hayes *et al.* 1995). Generic *E. coli* has the capacity to hydrolyze 4-methylumbelliferyl  $\beta$ -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 13 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 2003), 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<sup>®</sup> *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

resident children 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.

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