Exploring links between water quality and *E. coli* O157:H7 survival potential in well waters from a rural area of southern Changchun City, China

Meiyue Ding, Jiahang Li, Xiaodan Liu, Huiru Li, Rui Zhang and Jincai Ma

**ABSTRACT**

Waterborne infectious disease outbreak associated with well water contamination is a worldwide public health issue, especially for rural areas in developing countries. In the current study, we characterized 20 well water samples collected from a rural area of southern Changchun city, China, and investigated the survival potential of *Escherichia coli* O157:H7 in those water samples. The results showed that nitrate and ammonia concentrations in some well water samples exceed the corresponding China drinking water standards, indicating potential contamination by local agricultural farms. Our results also revealed that the average survival time ($t_{td}$) of *E. coli* O157:H7 in all well water samples was 30.09 days, with shortest and longest $t_{td}$ being 17.95 and 58.10 days, respectively. The $t_{td}$s were significantly correlated with pH and the ratio of total nitrogen to total phosphorus. In addition, it was found that the shape parameter ($\rho$) and first decimal reduction parameter ($\delta$) were negatively ($P < 0.05$) and positively ($P < 0.05$) correlated to $t_{td}$, respectively. Our study showed that *E. coli* O157:H7 could survive up to two months in well water, suggesting that this pathogen could constitute a great public health risk.

**Key words** | *Escherichia coli* O157:H7, survival, well water

**INTRODUCTION**

*Escherichia coli* O157:H7 is a Gram-negative, rod-shaped, facultative anaerobic, and shiga toxin-producing pathogen. It was identified during an investigation into a food-borne disease outbreak in the United States (Riley et al. 1983). Later evidence has shown that *E. coli* O157:H7 is one of the most commonly isolated bacterial pathogens (Mead et al. 1999). In the USA, *E. coli* O157:H7 alone was estimated to result in a total of 73,480 cases of disease per year, including more than 1,800 hospitalizations and 52 deaths (Mead et al. 1999). In addition, many large outbreaks of *E. coli* O157:H7 infections have been reported in more than 50 other countries and areas, making *E. coli* O157:H7 an increasing worldwide public health concern. In China, five *E. coli* O157:H7 strains have been isolated from nearly 500 stool samples during several *E. coli* O157:H7 outbreaks that occurred in Jiangsu Province, and those strains were believed to be the cause of the outbreaks (Xu et al. 1990). The typical clinical symptoms of *E. coli* O157:H7 infections are watery diarrhea and hemorrhagic colitis (Riley et al. 1983), which might ultimately develop into life-threatening hemolytic uremic syndrome. Therefore, it is essential to investigate the fate of *E. coli* O157:H7 in the environment to prevent its potential health risks.

Research has shown that cattle are a major reservoir of *E. coli* O157:H7, with other potential carriers including a large variety of wild and domestic animals (e.g., goose, dog, and deer). Among cattle, there are some supershedders, and the concentrations of *E. coli* O157:H7 in their feces may be as high as $10^7$ cfu/g feces (Chase-Topping et al. 2007). Once *E. coli* O157:H7 is in the environment, this organism...
can be transferred to other sites by rainwater, wind, and spreading of manure. It is a great threat to environmental safety and public health because of its low infective dosage (as few as 10 cells can result in diseases) (Girill et al. 1994). During those processes, inter- and intra-animals and humans, transmission is possible. Outbreaks may occur when drinking water and food become contaminated by E. coli O157:H7. Contaminated drinking water is responsible for 15% of reported E. coli O157:H7 according to the Centers for Disease Control and Prevention (CDC) of the USA (Rangel et al. 2003). It was reported that during an outbreak in Canada, people became infected via drinking water contaminated by cattle feces (Krewski et al. 2002). Similarly, contaminated well water has led to several major outbreaks in Washington County (CDC 1999), and the ground water was later confirmed to be polluted by wildlife or nearby cattle feces (Barwick et al. 2000). Previous survival studies have shown that E. coli O157:H7 can persist for weeks and even months in groundwater, tap water, and bottled drinking water (Rice et al. 1992; Artz & Killham 2002). Thus, the persistence of E. coli O157 in drinking water deserves further investigation, since longer survival may increase the chance of potential waterborne infections by this pathogen.

Groundwater is one of the major drinking water resources for people to survive on this planet. Well water supplies approximately half of the US population with drinking water, yet only 55% of ground water systems receive any sort of disinfection (Fujikawa & Yoneyama 2001). According to statistics, about 80% of the total population use shallow well water and lake or river water as their drinking water, while most of such drinking water resources are contaminated to some extent (Leclerc et al. 2002). Compared to deeper groundwater, shallow well water deserves more attention due to its vulnerability to fecal contamination, which ultimately may lead to major outbreaks, as reported previously (Barwick et al. 2000).

Changchun city is the capital of Jilin Province. The population in the urban area has a reliable drinking water supply; however, the inhabitants living in the rural area only about 20 kilometers south of Changchun city do not have access to a municipal water supply. All of the people there use well water as their sole drinking water source. The wells there are shallow (typically <40 m in depth), and most wells are surrounded by small family-owned cattle farms and, in many cases, they are close to a port-a-potty outside toilet, therefore well water might have a significant chance of being contaminated by fecal bacteria, among which there might be some potential human pathogens, such as E. coli O157:H7 and Salmonella enterica spp. (Reano et al. 2015).

In the current study, we collected 20 well water samples from 18 villages located in a rural area of southern Changchun city, and investigated the survival potential of E. coli O157:H7 under normal household storage conditions in those samples. The objectives were to: (1) study the survival behavior of E. coli O157:H7 in well water; (2) investigate the relationships between E. coli O157:H7 survival time and water physicochemical properties; and (3) provide insights into the potential health risks associated with E. coli O157:H7 in well water used as drinking water.

MATERIALS AND METHODS

Study site

The well water samples were collected from a rural area, which was about 20 km south of Changchun city, China. The local people there use well water as their sole drinking water source due to the unavailability of tap water. Wells shared by a maximum number of people within a village were selected for sampling. Twenty wells from 18 small villages were sampled, and location, well depth, water temperature, as well as the surrounding environmental conditions (e.g., proximity to family animal farms and port-a-potty outside toilet locations) were also recorded. The map of the sampling area is illustrated in Figure 1.

Well water sampling and characterization

Among 20 wells sampled, two of them were covered open wells, the rest were tube wells. For open wells, grab sampling technique was applied; for tube wells, water samples were collected after the existing water in tubing was completely replaced by fresh well water. All samples were collected in the morning when maximum water usage was reached for the local community. Duplicate
samples per well were taken, and a larger volume (>2.0 liter) of well water samples was collected to increase their representation. Samples were saved in sterile polypropylene containers and transported to the laboratory on ice, and their characterization was done within 6 h of collection.

Total organic carbon (TOC) and total nitrogen (TN) in water samples were determined by TOC (TOC-L, Shimazu, Japan), NH₄⁺-N was determined by Nessler’s reagent spectrophotometry method, NO₃⁻-N was assayed by double wavelength ultraviolet spectroscopy method, total phosphorus (TP) was quantified by potassium persulfate oxidation coupled with ammonium phosphomolybdate colorimetry, and total iron was examined by ascorbic acid reduction-phenanthroline spectrophotometric analysis method. Total plate count (TPC, is total coliform count here) was conducted according to China National Food Safety Standard, GB4789.2-2010. Well water samples were subjected to serial dilution, and inoculated into nutrient agar (peptone 10 g/L, beef extract 3 g/L, NaCl 5 g/L, agar 15 g/L) by thoroughly mixing 0.1 ml diluted or raw well water sample with about 20 mL molten agar (46°C). The plates were incubated at 37°C for 24 h. The colonies that formed inside the agar and on the agar surface were counted as TPC. Triplicate plates were prepared to increase the accuracy of the results. The results of well water characterization are listed in Table 1.

**E. coli O157:H7 strain**

*E. coli* O157:H7 EDL931 (ATCC 35150) was used in the well water survival assays. EDL951 was initially isolated from human feces, and it confers *stx₁*, *stx₂*, and *eas* genes (Beery *et al.* 1984). To facilitate the enumeration of EDL951 on sorbitol MacConkey agar (Lab M, UK), the EDL951 wild type was tagged with nalidixic acid and rifampicin resistance, and its growth in LB (Luria–Bertani) broth and survival in rich media was found to be identical to that of the non-tagged wild-type strain, which was consistent with the other *E. coli* O157 strain we used previously (Ma *et al.* 2011).

**Growth and numeration of E. coli O157:H7**

Cells from stock cultures were streaked on LB agar (without antibiotics), and incubated at 37°C overnight. Single
colonies were picked and restreaked on to LB agar with rifampicin and nalidixic acid. Single colonies were streaked onto SMAC (sorbitol MacConkey) agar (Lab M, Lancashire, UK). The isolated colonies were inoculated into 100 mL LB broth with rifampicin and nalidixic acid, and incubated at 37°C for 16 h. Stationary phase cells were used because in the natural environment, the majority of bacteria exist in this condition (Berry et al. 2015). The overnight cultures were harvested by centrifugation at 4°C, washed three times with 0.1% NaCl, resuspended in sterile deionized water, and inoculated into water samples. The NaCl wash step was essential to remove the nutrient, typically organic carbon from the LB broth, since E. coli O157 is able to grow at low carbon concentrations in freshwater (Vital et al. 2010). Cells were added into well water samples to a final density of about 1×10⁴ CFU/mL. Rifampicin and nalidixic acid were added into the media at 100 μg mL⁻¹ and 20 μg mL⁻¹, respectively. The water samples were put in 5 mL glass tubes at room temperature (21 ± 1°C) in the dark and mixed twice per day to simulate regular drinking water storage conditions in the sampling area. All samples were prepared in triplicate.

Survival data modeling

Survival of E. coli O157:H7 was analyzed by fitting the experimental data to the Weibull survival model using GIna-FiT Excel Add-In version 1.5 (Geeraerd et al. 2005). The Weibull survival model was constructed based on the hypothesis that the deactivation kinetics of the bacteria population follows a Weibull distribution. The size of the surviving population can be calculated using the following

### Table 1 | Properties of well waters

<table>
<thead>
<tr>
<th>Well water #</th>
<th>Village</th>
<th>Depth m</th>
<th>Temp °C</th>
<th>pH</th>
<th>EC S·m⁻¹</th>
<th>TOC mg·L⁻¹</th>
<th>TN</th>
<th>NH₄-N</th>
<th>NO₃-N</th>
<th>TP</th>
<th>Fe</th>
<th>TPC CFU·mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
<td>Xinlicheng</td>
<td>15</td>
<td>7.2</td>
<td>6.97</td>
<td>22.4</td>
<td>3.61</td>
<td>20.2</td>
<td>0.300</td>
<td>6.59</td>
<td>0.137</td>
<td>0.015</td>
<td>25</td>
</tr>
<tr>
<td>W2</td>
<td>Jiaguan</td>
<td>13</td>
<td>7.5</td>
<td>6.12</td>
<td>82.9</td>
<td>2.50</td>
<td>47.5</td>
<td>0.401</td>
<td>34.4</td>
<td>0.005</td>
<td>0.019</td>
<td>15</td>
</tr>
<tr>
<td>W3</td>
<td>Changshan</td>
<td>50</td>
<td>7.8</td>
<td>7.36</td>
<td>45.5</td>
<td>1.35</td>
<td>25.4</td>
<td>0.357</td>
<td>4.16</td>
<td>0.123</td>
<td>0.025</td>
<td>45</td>
</tr>
<tr>
<td>W4</td>
<td>Xinnong</td>
<td>20</td>
<td>7.5</td>
<td>7.20</td>
<td>125</td>
<td>2.60</td>
<td>62.8</td>
<td>0.150</td>
<td>54.5</td>
<td>0.042</td>
<td>0.022</td>
<td>5</td>
</tr>
<tr>
<td>W5</td>
<td>Liushu</td>
<td>40</td>
<td>7.5</td>
<td>7.32</td>
<td>20.6</td>
<td>1.10</td>
<td>21.0</td>
<td>0.101</td>
<td>1.05</td>
<td>0.023</td>
<td>0.258</td>
<td>65</td>
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<tr>
<td>W6</td>
<td>Liushu</td>
<td>20</td>
<td>7.5</td>
<td>7.13</td>
<td>141</td>
<td>3.76</td>
<td>125</td>
<td>0.344</td>
<td>75.2</td>
<td>0.046</td>
<td>0.067</td>
<td>45</td>
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<tr>
<td>W7</td>
<td>Yanjia</td>
<td>40</td>
<td>8.4</td>
<td>7.28</td>
<td>28.6</td>
<td>0.681</td>
<td>23.8</td>
<td>0.212</td>
<td>8.37</td>
<td>0.049</td>
<td>0.021</td>
<td>0</td>
</tr>
<tr>
<td>W8</td>
<td>Xinxingxiang</td>
<td>40</td>
<td>8.9</td>
<td>6.38</td>
<td>29.3</td>
<td>0.120</td>
<td>17.0</td>
<td>0.329</td>
<td>0.483</td>
<td>0.012</td>
<td>0.089</td>
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<tr>
<td>W9</td>
<td>Yushu</td>
<td>34</td>
<td>7.9</td>
<td>6.60</td>
<td>20.1</td>
<td>0.715</td>
<td>17.2</td>
<td>0.254</td>
<td>2.52</td>
<td>0.056</td>
<td>1.575</td>
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<tr>
<td>W10</td>
<td>Xintun</td>
<td>20</td>
<td>8.1</td>
<td>6.82</td>
<td>50.7</td>
<td>1.80</td>
<td>7.29</td>
<td>0.158</td>
<td>0.800</td>
<td>0.002</td>
<td>0.027</td>
<td>0</td>
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<tr>
<td>W11</td>
<td>Changsheng</td>
<td>12</td>
<td>9.3</td>
<td>6.20</td>
<td>173</td>
<td>4.05</td>
<td>68.8</td>
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<td>0.130</td>
<td>0.005</td>
<td>0.049</td>
<td>0</td>
</tr>
<tr>
<td>W12</td>
<td>Yueshan</td>
<td>15</td>
<td>7.9</td>
<td>7.38</td>
<td>82.2</td>
<td>2.69</td>
<td>49.1</td>
<td>0.696</td>
<td>19.8</td>
<td>0.028</td>
<td>0.017</td>
<td>5</td>
</tr>
<tr>
<td>W13</td>
<td>Lvhua</td>
<td>5</td>
<td>5.5</td>
<td>7.40</td>
<td>52.0</td>
<td>1.38</td>
<td>29.4</td>
<td>0.367</td>
<td>27.9</td>
<td>0.026</td>
<td>0.016</td>
<td>5</td>
</tr>
<tr>
<td>W14</td>
<td>Nonglin</td>
<td>7</td>
<td>5.4</td>
<td>6.53</td>
<td>69.8</td>
<td>0.363</td>
<td>21.1</td>
<td>0.290</td>
<td>19.5</td>
<td>0.007</td>
<td>0.038</td>
<td>95</td>
</tr>
<tr>
<td>W15</td>
<td>Yongjiu</td>
<td>10</td>
<td>7.2</td>
<td>7.12</td>
<td>123</td>
<td>2.34</td>
<td>62.2</td>
<td>0.228</td>
<td>59.2</td>
<td>0.023</td>
<td>0.022</td>
<td>40</td>
</tr>
<tr>
<td>W16</td>
<td>Changlingzi</td>
<td>60</td>
<td>7.8</td>
<td>7.23</td>
<td>92.0</td>
<td>2.19</td>
<td>34.4</td>
<td>0.422</td>
<td>27.2</td>
<td>0.004</td>
<td>0.000</td>
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<tr>
<td>W17</td>
<td>Changlingzi</td>
<td>20</td>
<td>7.7</td>
<td>7.39</td>
<td>194</td>
<td>3.90</td>
<td>118</td>
<td>0.758</td>
<td>97.7</td>
<td>0.011</td>
<td>0.004</td>
<td>35</td>
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<tr>
<td>W18</td>
<td>Pingan</td>
<td>60</td>
<td>7.7</td>
<td>6.95</td>
<td>19.6</td>
<td>0.382</td>
<td>12.2</td>
<td>0.282</td>
<td>10.6</td>
<td>0.011</td>
<td>0.003</td>
<td>225</td>
</tr>
<tr>
<td>W19</td>
<td>Liujia</td>
<td>8</td>
<td>6.8</td>
<td>6.79</td>
<td>100</td>
<td>1.17</td>
<td>58.8</td>
<td>0.566</td>
<td>30.6</td>
<td>0.024</td>
<td>0.032</td>
<td>55</td>
</tr>
<tr>
<td>W20</td>
<td>Yihe</td>
<td>50</td>
<td>7.4</td>
<td>6.82</td>
<td>18.3</td>
<td>0.001</td>
<td>20.2</td>
<td>0.212</td>
<td>9.24</td>
<td>0.009</td>
<td>0.066</td>
<td>0</td>
</tr>
</tbody>
</table>

EC, electrical conductivity; TOC, total organic carbon; TN, total soluble nitrogen; TP, total soluble phosphorus; Fe, total soluble iron; TPC, total plate (coliform) count; CFU, colony forming unit.

Numbers in bold indicate the value exceeds the China Food Safety Standard, GB4789.2-2010.
equation:

$$\log(N_t) = \log(N_0) - \left(\frac{t}{\delta}\right)^p$$

where $N$ is the number of survivors, $N_0$ is the inoculum size; $t$ is the time; $p$ is the shape parameter; when $p > 1$ a convex curve is observed; when $p < 1$ a concave curve is observed, when $p = 1$ a linear curve is observed. The scale parameter, $\delta$, represents the time needed for the first decimal reduction. A very important and useful parameter, $ttD$ (time needed to reach detection limit, 100 CFU mL$^{-1}$) can also be calculated when using GInaFiT to model the experimental survival data.

**Statistical analysis**

Detrended correspondence analysis (DCA) of well water samples based on their physicochemical and biological characteristics was performed by using PC-ORD v5.0 (MjM Software, Gleneden Beach, OR). Cluster analysis of well water samples based on the survival parameters obtained in the survival experiments was also conducted with PC-ORD v5.0. Principal component analysis (PCA) of water characterization, including pH, electrical conductivity (EC) (dS m$^{-1}$), TOC (mg/L), TP (μg/L), TN (mg/L), NH$_4^+$-N (mg/L), NO$_3^-$-N (mg/L), Fe (mg/L), and $ttD$ (day), were conducted by using SPSS (Statistical Product and Service Solutions) 19 (IBM Analytics, Armonk, New York). The log$_{10}$ ($X + 1$) transformed variables were used in both DCA and PCA. Stepwise multiple regression analysis was done to establish the correlation between survival time ($ttD$) and the soil properties by applying SPSS statistic software version 19.0.

**RESULTS**

**Well water characterization**

The well sample characterizations are shown in Table 1. According to National Food Safety Standard of China, various fractions of water samples were not suitable for drinking due to higher concentrations of NH$_4^+$-N (4/20, 20%), NO$_3^-$-N (11/20, 55%), and total plate counts (1/20, 5%). DCA was used to cluster the well water samples according to their physicochemical properties, and the results showed that 10 water samples (W2, W4, W6, W11, W12, W13, W15, W16, W17, W19) seemed to share more similarities compared to the rest of the well water samples (Figure 2).

**Survival profiles of EDL931 in well waters**

The survival profiles of EDL931 in each well water sample are shown in Figure 3. When inoculated into the water samples (W3, W5, W6, W7, W19), EDL931 could die-off to a level below detection within 20 days, while in W2, W10, W11, W16, this pathogen could survive for more than 40 days. The average survival time was 30.09 days. In addition, most of the survival profiles showed a convex curve, since $p$ values were less than 1 in the Weibull distribution model. Only six samples (W2, W9–11, W13, and W17) showed a concave survival curve. On average, the first decimal time ($\delta$) was 14 days, with the largest $\delta$ value being 30 days (W16) and smallest $\delta$ value being 9.2 days (W17).

**Cluster analysis of survival time ($ttD$)**

Cluster analysis based on survival time ($ttD$) yielded three clusters: cluster 1 contained five water samples, cluster 2 included seven samples, and the rest of the samples belonged to cluster 3 (Figure 4(a)). Further analysis revealed...
that $ttd$ of cluster 1 samples was significantly longer than those of cluster 2 and cluster 3 samples. The shape parameter, $p$, of cluster 3 samples, was greater compared to both cluster 1 and cluster 2 samples, while for the first decimal parameter, $\delta$, no significant different was found among all three clusters (Figure 4(b)–4(d)).

**PCA of well water physicochemical parameters**

PCA was conducted to reconstruct the correlation between survival time ($ttd$) and well water physicochemical parameters (Figure 5). The results showed that the first two PCs (principal components) accounted for 64% of the total variance with PC1 accounting for 42.1%. According to PC1, Fe, NH$_4$–N, NO$_3$–N, and EC exhibited positive scores, indicating that these factors may have positive effects on EDL931 survival, whereas P, TOC, TN, and pH had negative scores, indicating these factors may negatively affect the survival of EDL931 in well waters.

**Multiple step-wise regression analysis**

In order to identify the water physicochemical parameters significantly affecting the survival of EDL931, multiple step-wise regression analysis was carried out and the results (Table 2) revealed that the survival time of EDL931 could be best predicted by pH and the ratio of TN to TP ($P < 0.05$).

**Correlation between survival time ($ttd$) and the first decimal reduction time ($\delta$) and the shape parameter ($p$)**

Linear regression analysis was done to probe the relationship between $ttd$ and $\delta$ and $p$. The results showed that $ttd$
Figure 4 | Cluster analysis of well water samples based on the survival parameters ($ttd$, $p$, and $\delta$) (a), and the survival parameters, $ttd$, $p$, and $\delta$ for each cluster of well water samples (b), (c), and (d), respectively.

Figure 5 | PCA of survival time ($ttd$) and water physicochemical properties. EC, electrical conductivity; TOC, total organic carbon; TN, total soluble nitrogen; TP, total soluble phosphorus; Fe, total soluble iron.
was positively \( P < 0.05 \) and negatively \( P < 0.05 \) correlated with \( \delta \) and \( p \), respectively (Figure 6(a) and 6(b)).

**DISCUSSION**

Inactivation of *E. coli* O157:H7 was significantly influenced by groundwater quality (e.g., physicochemical and biological properties) (John & Rose 2005). Expanding populations and intensification of agricultural activities may lead to an increase in coliform counts and nutrients loading in groundwater, which may amplify the public health risks associated with human pathogens (Williams et al. 2012). Analysis of the well water samples indicated that the overall quality of the well water samples in the current study might be subjected to fecal contamination as evidenced by the high levels \( (>0.5 \text{ mg/L}) \) of \( \text{NH}_4^+ - \text{N} \) (Table 1). Generally, the appearance of ammonia indicates fecal contamination (Cabral 2010), and a high level of \( \text{NO}_3^- - \text{N} \) may favor an extended survival of *E. coli* O157:H7 (Lim & Flint 1989).

In addition, the higher concentrations of \( \text{NO}_3^- - \text{N} \) \( (>10 \text{ mg/L}) \) in the well water samples indicated there might be a significant non-point agricultural pollution around the sampling areas. No significant correlation was found between \( \text{NO}_3^- - \text{N} \) concentration and the survival time of *E. coli* O157:H7, which could be explained by the fact that nitrogen was not a limiting nutrient factor compared to phosphorus (see below). Generally, indigenous bacteria, typically total coliform counts, negatively affect the persistence of *E. coli* O157:H7 inoculated in water samples, since *E. coli* O157 generally survived longer in sterile or filtered water samples (Williams et al. 2012). However, no significant correlation was found between total coliform counts and survival times; this phenomenon might be explained by the larger inoculation size of *E. coli* O157:H7 \( (10^6 \text{ cfu/mL}) \). Therefore, the inculated *E. coli* O157:H7 might outnumber the indigenous heterotrophic bacteria and hold an advantageous position in competing nutrients. In short, the well water quality might have been deteriorated potentially by both animal feces and agricultural fertilizers, which might influence the survival of *E. coli* O157:H7.

Our survival study revealed that *E. coli* O157:H7 displayed various survival profiles in each well water sample under normal storage conditions. In the current study, we found the longest survival time was nearly two months, while the shortest survival time was only about 18 days.

### Table 2 | Stepwise multi-regression analysis of water properties and survival time (\( ttd \)) of *E. coli* O157:H7 EDL933 in well water samples

<table>
<thead>
<tr>
<th>Regression equation</th>
<th>( R^2 )</th>
<th>( F ) value</th>
<th>( T ) value and partial correlation coefficients (( r ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>( ttd = 38.134 - 10.498 \times \text{pH} + 8.214 \times \log_{10}(\text{TN/TP}) )</td>
<td>0.522</td>
<td>9.299**</td>
<td>( \text{pH} ) 2.114* ( \log_{10}(\text{TN/TP}) ) 3.015**</td>
</tr>
</tbody>
</table>

\( ttd \), time to reach detection limit; \( \text{TN} \), total soluble nitrogen; \( \text{TP} \), total soluble phosphorus.

* and ** denotes statistical significance at the 0.05, and 0.01 level, respectively.

![Figure 6](https://example.com/f6.png)

**Figure 6** | Linear regression analysis of survival time (\( ttd \)) and first decimal reduction time (\( \delta \)) (a) and shape parameter (\( p \)) (b), respectively.

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On average, *E. coli* O157:H7 could survive about 30 days in all well waters tested. Overall, the survival time found in the current study is largely comparable with previous reports (Rice *et al.* 1992). Under 20°C, *E. coli* O157:H7 strain 932, C4195, and RI survived about 23, 33, and 25 days, respectively, in ground water collected from a well used as drinking water source in Cabool, MO, USA. This drinking water was believed to be associated with a waterborne disease outbreak of hemorrhagic colitis (Rice *et al.* 1992). Similarly, *E. coli* O157:H7 could persist for about 2 weeks when inoculated into unfiltered well water samples, while this pathogen survived significantly longer when inoculated into filtered well water samples (filtration through 3 and 0.2 µm to remove protozoa). In addition to groundwater, *E. coli* O157:H7 could also survive a long period of time in pond water, and it was still viable after two months post-inoculation (Jenkins *et al.* 2011). On the other hand, the survival patterns as revealed by cluster analysis (Figure 4(b)–4(d)) could be largely explained by the location of the wells when comparing Figure 1 to Figure 4. Most of the samples from the west side of the sampling area (Figure 1) were grouped together, similarly, the survival patterns of *E. coli* O157:H7 in those samples were also within the same big cluster (clusters 1 and 2), suggesting that survival of *E. coli* O157:H7 was more likely to be influenced by the similar physicochemical properties shared by those well waters.

We observed that pH was negatively correlated with the persistence of *E. coli* O157 in well water samples (Table 2). Water pH may indirectly affect the *E. coli* O157:H7 community by changing water physicochemical properties, including nutrient availability, cationic metal solubility (e.g., trace elements), organic carbon characteristics (e.g., low-molecular-weight organic acids), and EC, which might exert a more direct influence on bacterial community structure (Leclerc *et al.* 2002). Water pH might also directly stress and select for different aqueous bacteria taxa. Water microbes that are more sensitive to pH change might die off faster than those more tolerant of pH changes (Cabral 2010). Extreme pH values may impose a significant stress to certain taxa while others may have higher tolerance, as described in related works (Ma *et al.* 2013). Results from this study showed that pH had a significantly positive effect on the *ttd* values. *E. coli* O157:H7 strains can grow at a variety of pH values (Nauta & Dufrêne 1999), and the effect of pH on bacterial inactivation in aquifers is lowest at pH levels between 6 and 8 (Foppen & Schijven 2006) and greatest in acid conditions (Inglis & Sagripanti 2006). Overall, our conclusion that pH may affect *E. coli* O157:H7 is largely in agreement with survival results (Ma *et al.* 2012, 2014). However, it should be noted that the pH range in our current study was not large enough to cover extreme cases, and such results should be interpreted with caution.

It is interesting that the ratio of TN to TP is significantly correlated with the survival time (*ttd*), as shown in Table 2. In the well water samples, the phosphorus concentration was really low compared to the higher concentration of TN. Thus, compared with nitrogen source, phosphorus source might be limited to support the survival of *E. coli* O157:H7 cells inoculated into the water samples, which might be a reasonable explanation for the close correlation between *ttd* and the ratio of TN to TP. In the current study, TOC was limited compared with TN, and organic carbon should be one of the limiting factors influencing the survival of *E. coli* O157:H7; however, no pronounced correlation between total organic carbon and survival time was found. This could be explained by the fact that the lower molecular weight organic carbon fraction, assimilable organic carbon (AOC), which could be readily utilized by *E. coli* O157:H7, in the water samples, might vary from sample to sample, thus no strong correlation was expected to be established. In fact, a correlation between apparent AOC concentration and the final cell concentration of *E. coli* O157:H7 was established in some water samples (Vital *et al.* 2008). Obviously, further study should focus on the dynamic changes of AOC in well water and study its effect on the persistence of *E. coli* O157:H7.

In the current study, it was found that the first log reduction time (δ) was significantly correlated with *ttd*. This was in line with our previous report that showed δ was positively related with *ttd* of *E. coli* O157:H7 in agricultural soils (Ma *et al.* 2012). While in the current study, the *ttd* was found to be negatively correlated with the shape parameter, *p*, suggesting that a concave (*P < 1*) survival curve may correspond to a greater *ttd*, and a convex (*P > 1*) survival curve may correspond to a smaller *ttd*. This phenomenon is in contrast with our previous findings that
ttd is positively correlated with $p$ when investigating \textit{E. coli} O157 and non-O157 strain in agricultural soils (Ma \textit{et al.} 2012, 2014). One of the potential explanations was the differences in the environmental matrix: one is soil, which is really complex, and the other one is groundwater, whose matrix is not that complex. In soil, the bioavailable nutrient is not evenly distributed and each cell may not be able to access the nutrients, typically AOC at the same rate, while in aqueous media, each cell could take advantage of available nutrients at the same speed due to the more homogeneous property of water samples compared to soil samples. When the cells were inoculated into the water, the initial nutrient level might be high enough for all the cells to persist for a while, as a result, the number of survivors did not change a great deal at the beginning of the experiments (Vital \textit{et al.} 2008). The cells in the more persistent phase might deplete the nutrients and lead to a sharp decrease in the nutrient level later, thus the survival curve may display a convex-like curve, which differs from that observed in soils.

It should be noted that the cells counted in the current study only represented culturable ones, while our counting methods were unable to count viable but non-culturable cells. For those cells, different protocols, including quantitative RT-PCR (reverse transcription-polymerase chain reaction), flow cytometry, could be applied. In addition, the persistence of \textit{E. coli} O157:H7 might also be affected by other factors, such as the composition and structure of the indigenous microbial community. Potential mechanisms have been shown to include predation, substrate competition, and antagonism. An aqueous ecosystem with a reduced diversity (e.g., sterilization) index might favor the survival of \textit{E. coli} O157:H7 spiked into the samples (John \& Rose 2005). Since the water samples used in the current study were not filtered through 0.45 or 0.22 $\mu$m filters, the bacteria existing in the water samples may at least compete for nutrients (e.g., carbon, nitrogen, and trace element) with the \textit{E. coli} O157:H7 introduced into the samples (Artz \& Killham 2002). The overall survival of \textit{E. coli} O157:H7 in water might be a function of a combination of water samples physical, chemical, and biological factors as observed in soil samples (Ma \textit{et al.} 2013). Obviously, further investigation is required to elucidate the correlation between \textit{E. coli} O157: H7 survival behavior and the composition and structure of indigenous bacterial communities in well waters. Additionally, a survey of the total population, total number of cattle, and identification of the supershedders among cattle in the sampling area would be of great value to explain the overall well water quality and the survival behavior of \textit{E. coli} O157:H7 in well water samples. Last, the well water quality might be subjected to seasonal change in the sampling area, which may in turn influence the persistence of pathogens introduced into those well waters.

In summary, the current study offers additional information regarding the survival of \textit{E. coli} O157:H7 in well waters, and highlights the longer survival of this pathogen in groundwater used for drinking water. This study showed that \textit{E. coli} can persist in well water for up to two months, which might considerably increase the opportunity for their transmission into the human food chain.

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