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Title: The effect of well depth and septic tank distance on *Escherichia coli* and total coliform presence in private wells at Vilas County rental properties

Running Title: Effect of well factors on coliform contamination

**Abstract**

Because *Escherichia coli* and other coliform bacteria pose a risk to public health through waterborne illnesses, it is necessary to investigate the impact of well depth and septic tank proximity on bacterial contamination in rural private wells. I hypothesized that coliform contamination in wells correlates to shallow well depth and proximity to a household septic tank. To test this hypothesis, I sampled 182 wells at rental properties in Vilas County, a rural county in north central WI. My results showed that well depth and septic tank proximity had no significant influence on total coliform bacteria or *E. coli* contamination. Additional studies with larger sample sizes are required to determine the effect of well depth, septic tank distance, and other well construction factors on coliform contamination in groundwater wells.

**Keywords:** bacterial contamination, environmental health, groundwater, water quality, waterborne diseases, well construction

Waterborne diseases continue to be a leading cause of illness in the United States (Benedict et al. 2017). Benedict and colleagues reported that 42 waterborne disease outbreaks occurred in the United States in 2013 and 2014; these were responsible for at least 1,006 reported illnesses and 13 deaths. Of the 42 outbreaks, 28 were associated with drinking water systems with groundwater sources. Furthermore, their report found that disease-causing bacteria were responsible for the majority of outbreaks and that bacteria were responsible for all reported deaths. In many cases, *Escherichia coli* O157:H7, a bacterial species that inhabits all mammalian intestinal tracts, was the cause of drinking water contamination. *Escherichia coli* and other coliform bacteria contamination can cause diarrhea and dehydration, which may lead to serious illness or death (Anon. 2018). Therefore, *E. coli* and coliform bacteria can pose an important health risk to the public. Given the danger of these bacteria, it is necessary to examine factors that cause bacterial contamination in wells to help promote public health and ensure safe drinking water quality.

Drinking water systems are susceptible to bacterial and other microbial contamination, which may cause outbreaks of severe gastrointestinal illnesses (Chhetri et al. 2017, Kuusi et al. 2004, Strauss et al. 2001). Recent studies have identified numerous environmental factors that increase the risk of water system contamination and waterborne illness. For example, Chhetri et al. (2017) found that the risk of waterborne illness increases after extreme precipitation events. Surprisingly, they found that the risk became greater after a long drought. Because climate change is expected to lead to more extremes in rainfall and drought, they concluded that drinking water contamination will likely increase with climate change.  Furthermore, over 50% of the United States population, including 99% of rural residents, depends on groundwater as a drinking water supply, so drinking water contamination threatens communities that rely on well water. Moreover, 37% of all agricultural water use relies on groundwater, so drinking water contamination may impact crop output and potentially cause food scarcity, which illustrates its far-reaching consequences (Anon. 2019a). Thus, it is important to understand how precipitation and other environmental factors affect drinking water quality to prepare an adequate strategy to limit drinking water contamination.

Previous studies have observed an increased prevalence of bacterial contamination in private wells in rural areas. For example, Strauss et al. (2001) sampled over 200 rural wells and discovered that 20% had levels of total coliform or *E. coli* greater than the national standard for safe drinking water. While that study found no serious gastrointestinal illnesses associated with bacterial contamination, the researchers suggested that additional data was needed on the prevalence of coliform bacteria in rural wells to protect the well user's health and ensure safe drinking water.

It is also important to understand temporal variations in bacterial contamination of private wells to prevent future waterborne illness outbreaks. For example, recent studies have investigated seasonal coliform bacteria patterns to determine whether seasonal environmental changes affect bacterial contamination (Arnade 1999). Atherholt et al. (2017) found that seasonal changes in coliform bacteria levels were caused by changes in the amount of groundwater extracted, precipitation levels, and seasonal temperature changes. Alternatively, bacterial contamination may be caused by the development of biofilms in wells (Oliphant et al. 2002). Oliphant and colleagues discovered that coliform colonies found in contaminated wells originated from outside the well and quickly reappeared after well chlorination. Since the presence of coliform colonies in the soil varies seasonally, a well's susceptibility to biofilm formation may be affected by seasonality (Arnade 1999). In addition to temporal variation and other environmental factors, well construction factors may also cause bacterial contamination.

Well construction and well maintenance have been identified as a potential source of bacterial contamination. For example, Zimmerman et al. (2001) found that unsanitary wells (e.g., wells with a loose-fitting well cap) were more susceptible to contamination than sanitary wells (i.e., wells with a sealed well cap). That study also found that wells drawing water from an aquifer in a carbonate bedrock had the highest amount of total coliform and *E. coli* contamination. Zimmerman et al. (2001) concluded that a combination of well construction and aquifer contamination may cause bacterial contamination. On the other hand, Won et al. (2013) found no discernible correlation between well structure and coliform contamination for groundwater wells in a predominantly limestone and sandstone bedrock aquifer. In addition, Swistock and Sharpe (2008) concluded that good well construction practices help prevent bacterial contamination, but significant contamination can still occur, even in properly constructed wells. Thus, because the causes of bacterial contamination in private wells are complex, it is necessary to conduct a study to determine whether well depth and septic tank distance affect bacterial contamination in private wells.

Unfortunately, very few studies have investigated the impact of well depth and septic tank distance on bacterial contamination in private wells. Shallow well depth may leave the water source susceptible to contamination from coliform bacteria near the surface. Therefore, wells with a shallow well depth may test positive for total coliform bacteria and *E. coli* at a higher frequency than deeper wells. While shallow well depth has caused bacterial contamination in 30 private wells in rural Colorado, the effect of shallow well depth on a greater sample size of private wells in rural Wisconsin is unknown (Gonzales 2008). Furthermore, septic tanks that are close to the wellhead may lead to contamination because nearby fecal bacteria from the septic tank may leak into the adjacent well. Therefore, wells located close to a septic tank may test positive for total coliform bacteria and *E. coli* at a higher frequency than wells with an increased septic tank distance. While a statistically significant relationship between coliform bacteria concentrations and decreased septic tank distances has been found in suburban Florida, it is unclear how septic tank distance affects private wells in rural northern Wisconsin (Arnade 1999).

To examine the importance of that question, I conducted a survey of private wells of rental properties in Vilas County. Vilas County has more than 1300 lakes, which permits a thriving tourist industry that serves as the county's main economic source. Therefore, hundreds of tourist rooming houses (i.e., resorts and rental cabins) are found throughout the county (Anon. 2019b). As a part of the Vilas County Public Health Department's annual inspection program, wells at tourist rooming houses were sampled to ensure safe drinking water for tourists using the property. To investigate effects of well construction on bacterial contamination in private wells, I sampled private wells at Vilas County rental properties to determine whether well depth and septic tank distance influenced *E. coli* and total coliform presence in these wells. I predict (1) greater bacterial contamination in wells with shallow depth and (2) in wells located close to a septic system.

**Methods**

*Study Area and Well Sampling*

To investigate whether well depth and the distance of a septic tank to a well affects total coliform and *E. coli* presence, I sampled 182 private wells at rental properties in Vilas County, Wisconsin from 15 May 2018 to 22 August 2018 (Fig. 1). My study does not include the property address or other personal information of the well owner to ensure each property owner's confidentiality.

I collected all water samples from properties using standard sterile techniques for well water sampling. When sampling, the kitchen tap or bathroom tap served as the sample tap because they are most often used for water consumption. When I gathered a water sample, I removed the faucet's aerator and I heated its rim with a butane torch for 10 s to disinfect the faucet head of any residual bacteria. Next, I ran the faucet for 3-5 min with cold water to circulate the well water prior to taking the sample. If I could not remove the faucet's aerator, I applied a 70% ethanol solution to the faucet head to disinfect it and I ran the water for 5-7 min. After I ran the faucet, I used a sterile, IDEXX 120 mL-vessel to collect the sample and I filled the vessel past the 100 mL line. The IDEXX vessel is used for Colilert® and Colilert-18® testing for coliform bacteria and *E. coli*. The vessels are shrink banded to protect the entire perimeter from possible contamination (Anon. 2017a). After sample collection, I placed the water sample in a cooler and I transported it back to the Vilas County Public Health Department's Environmental Health Laboratory for bacterial testing.

*Water Testing*

Analysis for presence or absence of total coliform bacteria and *E. coli* used the Colilert® detection method. This method is recommended to determine the presence of total coliform bacteria and *E. coli* in drinking water samples by the U.S. Environmental Protection Agency (EPA) and American Public Health Association (Clesceri et al. 1998).

I followed the Colilert® test procedure when testing samples. First, I added the contents of one Colilert® packet to a 100 mL water sample in a sterile IDEXX vessel. Next, I capped and thoroughly shook the vessel and placed it in an incubator at 35˚ C for 24 hrs. After incubation, I read the results using a positive comparator sample. I used a 6 watt, 365 nm UV light within 12.7 cm (5 in) of the sample in a dark environment to determine whether the water sample was fluorescent and positive for *E. coli.* If the incubated sample remained clear or was less yellow than the positive comparator, the water sample tested negative for both total coliform bacteria and *E. coli*. A sample tested positive for total coliform bacteria, but not *E. coli* if it was equal to or more yellow than the positive comparator and did not fluoresce. The water sample tested positive for total coliform bacteria and *E. coli* if it was equal to or more yellow than the positive comparator and was fluorescent (Anon. 2017a). After the sample was assessed, I recorded its result and discarded the sample in a sanitary bag.

Water samples from properties that tested positive for presence of total coliform bacteria and/or *E. coli* were resampled using the same sampling procedure. If the resampled well again tested positive, I prompted the owner to chlorinate their well to eliminate coliform bacteria and/or *E. coli*. After chlorination, I conducted another resample to confirm that well chlorination was successful. My study considered a well to be contaminated when both the original sample and the resample tested positive for coliform bacteria and/or *E. coli* and well chlorination was required.

*Well Depth and Septic Tank Distance Analysis*

I used the Wisconsin Department of Natural Resources (WDNR) Drinking Water System to determine well depth and septic tank proximity at each property. The WDNR Drinking Water System is an online database that contains well construction reports for private homeowners. By entering a property's address or other pertinent information, the database provides a well construction report, which features many well construction features, such as well depth, wellhead protection, septic tank distance, and geology (Anon. 2017b). I used this database to find well construction reports for each sampled rental property and obtain well depth and septic tank distance for each property.

I analyzed the effect of well depth and septic tank distance on well contamination using multiple logistic regression. I ran separate multiple logistic regression models to determine the effect of septic tank distance and well depth on *E. coli* presence and total coliform presence. The significance level for each test was set at P = 0.05. I performed this statistical analysis on John C. Pezzulo's Logistic Regression Calculator, which was provided by the Handbook of Biological Statistics (Pezzullo 2015, McDonald 2014).

**Results**

Of the 182 wells, 174 tested negative for coliform bacteria, while eight tested positive for coliform bacteria and were considered contaminated (Fig. 1). The eight contaminated wells occurred in northwestern Vilas County, while the remainder of the county had no contaminated wells. The contaminated wells were chlorinated and each contaminated well tested negative for coliform bacteria after chlorination. All 182 wells sampled tested negative for *E. coli* presence.

The logistic regression model for determining the impact of well depth and septic tank distance on coliform presence was not significant ($X^2 = 2687.05$, *d.f.* = 2, *P* = 0.25). Furthermore, as measured by the odds ratio (OR), there was no significant influence of well depth (*OR* = 1.03, P = 0.48) or septic tank distance (*OR* = 0.88, *P* = 0.18) on coliform contamination of wells. Because no wells tested positive for *E. coli*, the logistic regression model was unable to assess the effect of well depth and septic tank distance on *E. coli* well contamination.

**Discussion**

The multiple logistic regression model of my data did not support my hypothesis that shallow well depth caused greater bacterial contamination. Surprisingly, the model found that a 1 m increase in well depth actually increased probability of a coliform-positive sample by approximately 3%. While this result was not statistically significant, it illustrates the lack of influence that well depth had on coliform contamination in this study. However, this result is not consistent with a previous study by Gonzales (2008), which found that decreased well depth caused increased coliform contamination in wells in rural Colorado. Unfortunately, that study had a very small sample size (n = 30 wells) and used a Chi-square test as its statistical model rather than multiple logistic regression. On the other hand, Won et al. (2013) used multiple logistic regression to analyze 180 private wells in northeastern Ohio and found no discernible association between well depth and total coliform well contamination. This study is consistent with my results and features the same statistical model and nearly an identical sample size as my study. While Won et al. (2013) had markedly more positive samples than my study, their study failed to discover any correlation between well depth and well contamination. Given the disparity in results among these studies, future studies with large sample sizes are required to clarify the relationship between well depth and coliform contamination.

The multiple logistic regression model of my data also did not support my second hypothesis that decreased septic tank disease caused coliform well contamination. While the model found that a 1 m increase in septic tank distance decreased probability of well contamination by ~12%, this finding was not statistically significant, likely because of my study's small number of positive samples. This result is not consistent with Arnade (1999), who used ANOVA analysis to examine data from 60 wells in suburban Florida and found a statistically significant correlation between coliform contamination and septic tank distance. Furthermore, Arnade's study tested for coliform and *E. coli* concentrations by using a membrane filtration technique that measured colony growth on agar plates. Their methodology provided an accurate determination of bacterial concentrations in water samples. Thus, I suggest that future studies use these methods.

The small sample size and low number of coliform-positive results in my study may have led to these unexpected results as multiple logistic regression requires a sample size of approximately 2000 for studies with a low prevalence rate of positive results (Hsieh et al. 1998). Moreover, use of a presence or absence procedure may have limited data interpretation as samples could only be classified as coliform and/or *E. coli* positive or negative. However, if specific coliform concentration was analyzed in each water sample, this may have provided a more accurate representation of the impact of well depth and septic tank distance on coliform contamination. In fact, previous studies using colony count and membrane filtration techniques have often discovered more significant correlations between well construction factors and well contamination than studies with presence/absence procedures (Arnade 1999, Zimmerman et al. 2001). Therefore, future studies should have sample sizes greater than 2000 and use colony count and membrane filtration methods to best determine the influence of well construction factors on well contamination.

The impact of well depth and septic tank distance on *E. coli* contamination was not analyzed due to the absence of *E. coli* positive samples in my study. While the lack of positive recordings of *E. coli* is a great indicator for public health, it is difficult to study its effect without a larger sample size. Thus, I propose a controlled study to determine whether there is a correlation between well depth and *E. coli* contamination. In that study, wells of varying depths will be drilled in the same control area where each well is circulated daily to simulate the conditions of an operating well. Other than well circulation, wells should be left untreated and sampled for *E. coli* concentration annually for 10-15 years. This controlled well experiment will illustrate the influence of well depth on *E. coli* contamination without harming public health. This study could be repeated to investigate the effect of septic tank distance or other well construction factors on *E. coli* contamination.

Seasonality is also an important factor. However, my study was conducted during summer months, so seasonal variations in contamination levels could not be assessed. Seasonality has been shown to affect coliform presence as temperature and groundwater extraction rates vary seasonally (Atherholt et al. 2017). Also, coliform concentration is influenced by seasonal precipitation levels as increased precipitation levels caused greater coliform well contamination (Arnade 1999). Given the effect of season and precipitation on well contamination, future studies should perform seasonal sampling and record precipitation levels to determine the impact of seasonality and precipitation on coliform contamination in Vilas County wells. In addition, Vilas County has a complex aquifer comprised of sand, gravel, and crystalline bedrock systems, which may influence bacterial well contamination in unknown ways (Kassulke and Chern 2016). However, carbonate bedrock aquifers are most susceptible to total coliform and *E. coli* contamination (Zimmerman et al. 2001). Given the unknown effects of bedrock in the aquifer of Vilas County on coliform well contamination, future studies should record the bedrock of sample sites to analyze the effect of bedrock type on bacterial well contamination in the county.

My study illustrates the complexity of determining sources of bacterial contamination in groundwater wells. In particular, small sample size and a lower number of positive samples limited detailed interpretation of the results. Moreover, research on private water wells is often limited by logistical problems of reluctant well owners and travel costs associated with well sampling. However, analyzing the impact of well construction factors on well contamination is vital to protecting public health and ensuring clean water quality. Thus, future research that investigates potential sources of well contamination is necessary to improve well construction practices and protect the health and well-being of private well users.

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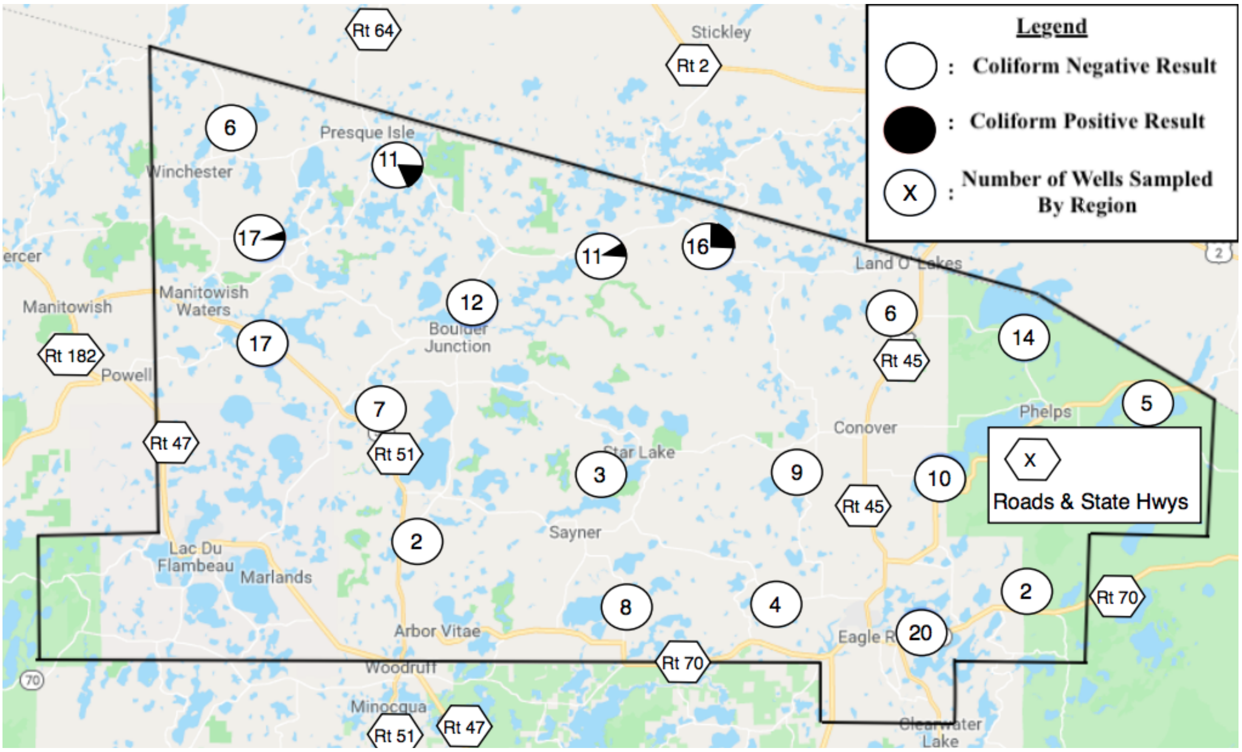


Figure 1. Coliform bacteria results from wells at rental properties in Vilas County. Wells were sampled from 15 May 2018 to 22 August 2018 as a part of the Vilas County Public Health Department's annual tourist rooming house inspection program. Sampled wells are organized in clusters that correspond with a region of Vilas County.