ABSTRACT

Title of Thesis: DETERMINING THE IMPACT OF WELL MAINTENANCE, CONDITION, TYPE, AND LOCATION FACTORS ON *E. COLI* AND TOTAL COLIFORMS IN MARYLAND FARM PRIVATE DRINKING WATER WELLS

Cameron Nicole Smith, Master of Science, 2023

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Even with the establishment of the Safe Drinking Water Act in 1974, private wells are still not regulated or monitored for drinking water quality or the presence of contaminants such as total coliform bacteria and *Escherichia coli*. The presence of microbiological contaminants in private wells poses a public health risk. With Agricultural Agents from the University of Maryland Extension, we collected 67 water samples from Maryland farms with private wells located in seven regions and 19 counties of Maryland. We evaluated water samples for total coliforms and *E. coli* to understand the risk of contamination for Maryland private well owners. We also analyzed the impact of well factors, location, and climate on the presence of total coliforms and *E. coli* in well water by analyzing participant survey responses and climate data. Our results found that 39% (26/67) of the well water samples were positive for total coliforms and 10% (7/67) were positive for *E. coli*. Region ($p<0.01$), county ($p=0.03$), previously testing for pH ($p<0.01$), and ambient temperature ($p=0.05$) were significant factors impacting total coliform concentration. Region ($p<0.01$) and precipitation in the last 24 hours of collection ($p<0.01$) were the only significant factors impacting *E. coli* concentration. These findings emphasize the importance of well water testing for private well owners.

DETERMINING THE IMPACT OF WELL MAINTENANCE, CONDITION, TYPE, AND LOCATION FACTORS ON E. COLI AND TOTAL COLIFORMS IN MARYLAND FARM PRIVATE DRINKING WATER WELLS

by

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List of Abbreviations

SDWA	Safe Drinking Water Act
E. coli	*Escherichia coli*
EPA	US Environmental Protection Agency
CDC	Centers for Disease Control
USDA	United States Department of Agriculture
WHO	World Health Organization
HUS	Hemolytic Uremic Syndrome
MC	MacConkey Agar
TSA	Tryptic Soy Agar
STEC	Shiga Toxin-Producing *E. coli*
LS	Lower Shore (Maryland region)
US	Upper Shore (Maryland region)
SO	Southern (Maryland region)
CA	Capital (Maryland region)
CE	Central (Maryland region)
WE	Western (Maryland region)
NO	Northern (Maryland region)

Chapter 1: Introduction

1.1 Preamble

To improve public drinking water quality, the Safe Drinking Water Act (SDWA) was passed in 1974 by the United States Congress to set a limit for acceptable contaminant levels determined by the Environmental Protection Agency (EPA) [1]. The EPA provides regulations or guidelines for approximately 90 microbial and chemical contaminants in drinking water [2]. However, private drinking water wells are not monitored by the EPA [3]. According to the USGS, over 43 million people in the United States currently utilize private well drinking water [41]. The potential for contaminants being present poses a public health risk for individuals that rely on private wells for drinking water.

The goal of this study was to better understand the risk of total coliform bacteria and *Escherichia coli* (*E. coli*) being present in private drinking water wells from Maryland farms. Sixty-one participants were selected from 19 counties in seven regions of Maryland and were provided a 32-question online survey focusing on current well factors (maintenance, type, age, depth, etc.). The 61 selected participants provided water samples to be tested for the presence of total coliforms and *E. coli* which were filtered and processed with the U.S. EPA Standard Method 1604 [4]. The participant survey questions were analyzed to determine if there were any differences between the presence or absence of the microbial contaminants and well factors such as maintenance, conditions, type, and age of well. Comparisons were also made between the seven regions of Maryland for differences in microbial contaminant presence to understand what role location plays in the presence of total coliforms and *E. coli*. Climate data was collected to look for differences in microbial contamination depending on ambient temperature and precipitation. Through analyzing the presence of total coliforms and *E. coli* by region, well factors and survey results, and climate data, we were able to gain a better understanding of which factors could impact the presence of *E. coli* and total coliforms in Maryland farm private drinking water wells.

1.2 Identification of the problem

My project focuses on the contamination of Maryland farm private drinking water wells with total coliforms and *E. coli* and the factors that could lead to the presence of microbial contaminants. This is to better understand the risk of contamination and the risk of negative health implications for the approximately 350,000 Maryland homes with private well drinking water [7]. More specifically this thesis is looking to understand the risk of total coliforms and *E. coli* contamination in Maryland private drinking water wells on farms. To our knowledge, this is the first study to evaluate the risk of total coliform and *E. coli* contamination in Maryland farm private drinking water wells and analyze factors (well factors, location, and climate) that impact well water quality.

A study conducted in Maryland, Murray et al., 2018, highlighted the risk of microbial and chemical contaminants being present in Maryland private drinking water wells and if the water quality was impacted by the presence of nearby livestock and animal operations and facilities [5]. This thesis is different from the study by Murray et al., 2018 because we are focusing on farms with private drinking water wells and on seven regions rather than four [5], this offers differences in geological factors. Therefore, there is a difference in risk of contamination from Murray et al., 2018 and this thesis. A recently published study in Canada, White et al., 2021, identified the importance of certain well factors (well depth, year that the well was built, location, and type of bedrock) and climate and seasonal factors for the presence of *E. coli* [12]. This thesis is different from the study by White et al., 2021 because we are focusing on 14 different well factors through participant surveys and are collecting samples from seven regions during the summer months. These studies provide a preliminary understanding about the risk of microbial contamination in private drinking water wells and the importance of conducting additional research on this topic. However, more research concerning the factors that could contribute to presence of *E. coli* and total coliforms needs to be conducted. More information about these two previously published studies can be found in *Section 1.4 Literature Review*. In *Section 1.5 Identification of Knowledge Gaps*, I describe what questions remain after these studies were conducted. This thesis project contributes to the limited data available by investigating the impact of several well factors, climate factors, and location by region to see how these play a role in the presence of total coliform and *E. coli.* Overall, we are looking to investigate what external factors play a role in microbial contamination in private well water to better understand what could be done to protect human health.

1.3 Importance of the research

This research is important because it connects the water quality results for the Maryland farm private drinking water wells to the participant survey results and climate data. This is to identify the sources or factors that play a role in the presence of *E. coli* and total coliforms. This provides a better understanding of the factors that lead to a higher risk of contamination of total coliforms and *E. coli* in Maryland farm private drinking water wells. We are working to determine the factors that play a role in the presence of total coliforms and *E. coli* which can be utilized by farmers and private well owners in Maryland. This research is also important because it will research the water quality and factors in seven different regions of Maryland. Therefore, comparisons can be made between the results in each region to understand which location is more at risk of contamination in private drinking water wells. Current data that compares and analyzes well drinking water quality, well factors, and region is limited therefore this project will add to what we do know about the risk of contamination in private drinking water wells on Maryland farms.

This research is important because of the approximately 350,000 Maryland homes with private drinking water wells that are not protected under the SDWA [7]. While the number of farms that use private well water is currently unknown, it can be assumed that a large percentage of the 12,400 farms operating in the state of Maryland [42] utilize private drinking water wells due to the rural location of many Maryland farms. Even without this exact number, it is apparent that the presence of total coliforms and *E. coli* is a public health risk for the Maryland farming population. With no guidelines and regulations for private well water, these individuals need to understand how to protect their drinking water and be educated on the resources available to test, treat, and maintain their well. This project is important because it is researching various factors to try to understand what conditions impact water quality. Depending on the results of this thesis, Maryland farmers could utilize the results to understand their individual risk and understand what they can do to prevent or minimize the presence of total coliforms and *E. coli*.

If farmers know that they are in a region at higher risk of total coliforms and *E. coli* contamination than they can make decisions to prevent it from being present in the well water. Similar to many areas of public health, prevention should be a focus and by researching various factors of contamination this brings us closer to finding the conditions that lead to higher presence of total coliforms and *E. coli* contamination and then make decisions to prevent it. The data from this small group of farmers in Maryland can be used to better understand the potential risk of total coliforms and *E. coli* present in the Maryland farming population among the seven regions of Maryland. The results from this thesis could be used to educate farmers on the risk of total coliforms and *E. coli* presence in their region and/or county. This thesis is also important as it depicts the importance of testing for microbial contaminants in private wells for farms in Maryland. Total coliforms and *E. coli* can potentially lead to negative human health complications and can also indicate pathogens and contaminants present in drinking water [10] thus understanding your risk and consistently testing is crucial.

1.4 Literature review

A study in New Jersey, Procopio et al., 2017, studied the relationship of precipitation and total coliform presence in private wells with the use of a statistical model [34]. Data was collected from 78,207 total private wells from October 2002 to September 2012 from four provinces in New Jersey (Piedmont, Highlands, Valley, Ridge) which all offer various geologic characteristics [34]. With the use of logistic regression to predict potential impact of precipitation on total coliform concentration the study reported that during a 10-day period there would be an increase in total coliforms when there were over 1.4 inches of rain and there would be an increase in *E. coli* when there were over 2.1 inches of precipitation [34]. This model also predicted that there was greater risk of total coliform presence in a bedrock well in the Coastal Plain which is similar to the results of the study by White et al., 2021 found from data collected from Ontario, Canada [34, 12].

A recently published Canadian study, White et al., 2021, focuses on identifying the correlation of well factors and climate factors for the presence of *E. coli* in private wells [12]. Data was collected from an Ontario groundwater dataset for 253,136 wells and 795,023 well samples that were collected from 2010 to 2017 [12]. The well water where data was collected from was used for various purposes such as agriculture, domestic, commercial, livestock, and public [12]. There was significance in climate and seasonal factors when broken down by month [12]. Analysis depicts that consolidated or wells made from bedrock and sedimentary or igneous material had higher risk of *E. coli* presence compared to unconsolidated well units and metamorphic material [12]. When looking at well characteristics (depth and the year that the well was built) shallow wells (up to 60m depth) had higher rates of *E. coli* present but deep wells do not necessarily prevent *E. coli* contamination [12, 32.] Therefore, even if a private well owner has a deeper well, they are still at risk of *E. coli* being introduced to their water source [12]. The temperature and precipitation changes in the White et al., 2021 study were mostly referring to changes between the seasons whereas this thesis is identifying differences in temperature and precipitation and the impact on water quality in the Summer.

An article by MacDonald Gibson & Pieper, 2017 focused on ways that private well drinking water quality could be improved in North Carolina and the four primary challenges that private well owners face [6]. These challenges are important and can be used to understand challenges that private well owners throughout the United States face. The first challenge is that there is not a database that lists the population in North Carolina that utilizes private wells which makes it difficult to offer check-in services for private well owners [6]. A similar concern is present in Maryland, where there appears to be lack of data portraying a published number of farms in Maryland that rely on private wells. The second challenge is racial disparities [6]. In North Carolina specifically, individuals in peri-urban communities have larger population sizes which increases risk of water contamination [6]. The third challenge is that many private well owners lack the knowledge of the importance of testing their well water or do not have access to the resources to test and maintain their well water [6]. North Carolina data suggests that a significantly low percentage of well owners regularly test their well water and that many individuals do not have the ability to install treatment devices [6]. This article by MacDonald Gibson & Pieper, 2017 recommends educating the community about the importance of testing, providing resources to well owners and designing a database of areas that use well water and areas at higher risk of contamination [6]. The final challenge is that there is a lack of programs offered for private well owners to get these resources and educational opportunities [6]. Overall, this article provides a much-needed look at the challenges that private well owners face.

A study by Murray et al., 2018 collected 118 private well water samples from four Maryland counties (Cecil, Kent, Montgomery, and Queen Anne's) and was evaluating potential environmental sources that could contribute to water contamination [5]. Murray et al., 2018 found that 43% of the studied wells had contamination concentrations that exceeded the EPA standards for at least one of the studied microbial and chemical contaminants [5]. Total coliforms were found in 25% of the water samples and *E. coli* were found in 3% of the water samples [5]. In addition to these contaminants, many of the private wells were also positive for chemical contaminants and fecal bacteria [5]. In terms of environmental factors that could be a source of the contamination, the study found that there was no connection between broiler, cattle, turkey, dairy and aquaculture presence, and the presence of fecal bacteria [5]. Murray et al., 2018 concluded that more research is needed for the factors causing the microbial and chemical contamination in Maryland private wells [5].

1.5 Identification of knowledge gaps

Currently, there appears to be a knowledge gap in the understanding of well factors, region, and climate (precipitation and ambient temperature) with water quality in Maryland. In the state of Maryland, there is currently only one published study that focuses on private drinking well water quality and contamination of total coliforms and *E. coli* [5]. Therefore, there is a lack of studies that focus on the connection between private well factors and well water quality and the connection between climate factors and well water quality in Maryland. Although the Murray et al. study points to contamination of total coliforms and *E. coli* in Maryland private wells [5], there is still a need for more information about the well factors, conditions and region factors that impact the presence of *E. coli* and total coliforms. While Murray et al., 2018 provided an idea on the risk of microbial and chemical contaminants being present in private well water, samples were collected from only four counties of Maryland [5]. There is currently a lack of understanding of how well water quality differs between regions in Maryland.

The recently published Canadian study by White et al., 2021, provided an understanding of the significance of climate and well factors however the well factors were mainly limited to four specific factors [12]. There are still questions pertaining to other well factors such as well type, type of wellhead cover, recent well repairs, age of home plumbing, type of home plumbing, and if well water has been tested previously, among other factors. Location was only related to latitude and longitude data rather than specific regions of Canada [12]. It is also important to note that this study was using datasets collected over eight-years from a database from previously collected samples, therefore as mentioned by the authors the results could be limited by the recorded *E. coli* counts [12]. It is also important to realize that this study was conducted in Canada therefore the geography and environmental conditions may differ than what is typically seen in Maryland.

Overall, more information is needed on how the age of the well, current condition, maintenance, and testing practices, among other well factors can impact the presence or absence of total coliforms and *E. coli* in Maryland farm private drinking water wells. More information is needed on whether there is a difference in the presence of *E. coli* for shallow and deep wells in Maryland [32, 12]. More information is needed on how the various precipitation values and ambient temperatures for each sampling location can impact the presence or absence of total coliforms and *E. coli*. There is evidence that shows that precipitation levels could provide more contamination in a water source [31, 11, 34], but research focusing on Maryland wells needs to be investigated further. Lastly, more information is needed on how water quality differs between the seven regions in Maryland.

To date, a comparison between well factors, climate data and water quality and making comparisons between various regions has not been completed for Maryland farm private drinking water wells prior to this study.

Chapter 2: Background

2.1 E. coli and total coliform characteristics

Total coliforms are microorganisms found in the environment and in human and animal digestive systems [13, 53, 54]. According to the EPA, total coliforms consist of different bacteria [10] and *E. coli* is an example of a type of total coliform (Figure 1). Total coliforms act as an indicator of harmful pathogens and contaminants that could be present in drinking water, help determine the drinking water quality and help determine if treatment of the water source is needed [10]. Exposure to total coliforms may not always lead to illness, however testing for total coliforms indicates the risk of pathogens that could potentially lead to illness [13, 53, 54]. Identifying the specific pathogen present in water may be more difficult to complete through water testing procedures, therefore total coliform testing is more commonly used. Testing for total coliforms indicates if the water source has become contaminated [13, 53, 54] and if there is a concern with equipment or treatment protocol [33].

The Centers for Disease Control and Prevention (CDC) define *E. coli* as being present in our intestines however when the human population is exposed to *E. coli* the various strains can lead to different levels of risk [8, 53, 54]. *E. coli* symptoms include fever, vomiting, diarrhea, nausea, and abdominal cramps [9, 53]. *E. coli* falls into the category of fecal coliform and total coliform (Figure 1). *E. coli* is an indicator of fecal contamination in the water [33]. According to the CDC, a fecal coliform is a total coliform that originates from human or animal feces [13, 53, 54]. When *E. coli* is discovered in drinking water, it may not always cause illness, however it does signify that the drinking water source has been contaminated, thus it could still pose a potential health risk to the individuals relying on that water source [8, 53, 54].

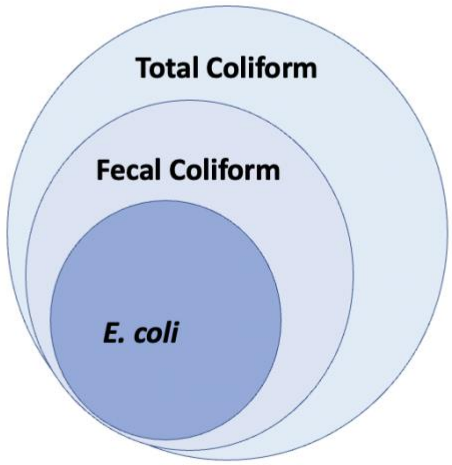

Figure 1. E. coli is a type of total coliform and fecal coliform.

An *E. coli* strain that could cause serious symptoms and illness if present in an individual's drinking water source is *E. coli* O157:H7 [14]. This is a Shiga-toxin producing *E. coli* (STEC) that according to the World Health Organization (WHO) humans can be exposed through contaminated water and food sources including raw milk and ground meat [14]. Previous research has shown that this strain of *E. coli* can be present in various water sources such as drinking water, well water, and recreational sources and for those that are exposed it can lead to illness, needing to be hospitalized, and death [23, 24, 25, 26, 27, 28]. Symptoms of *E. coli* from the STEC strain include diarrhea, bloody diarrhea, abdominal cramps, fever, and vomiting and can sometimes lead to the development of hemolytic uremic syndrome (HUS) which includes hemolytic anemia and thrombocytopenia and acute renal failure [14]. This project is not looking to identify if *E. coli* O157:H7 is present in Maryland private drinking water wells. However, it is important to understand the severity of this *E. coli* strain when discussing the public health impact of *E. coli* presence.

By studying both total coliforms and *E. coli* we will be able to gain a better understanding as to whether the private drinking water wells are at risk of microbial contamination and thus could lead to a negative human health concern. The presence of *E. coli* is a public health concern because it would identify the presence of fecal contamination [33] and the presence of total coliforms would identify the potential presence of harmful pathogens [10]. The presence of total coliforms and *E. coli* could also potentially signify that the well water may not be thoroughly treated or maintained or that treatment devices are not working properly [10]. Overall, water that has total coliforms and *E. coli* present may not be adequate water quality or safe for drinking water. Thus, determining the cause of the contamination is vital so that individuals with private well water can make decisions to limit their risk. The more that is understood about the factors that impact water quality and public health, the more that prevention measures could be put in place and better protect private well water owners.

2.2 Well and climate factors and the presence of *E. coli* and total coliforms

Private well water sources are not regulated by the EPA for the presence of chemical and microbial contaminants such as total coliforms and *E. coli* which is a public health concern [3]. Private well water owners are at risk of continuously being exposed to potentially harmful contaminants and may not have the understanding, resources, or experience to receive proper testing or treatment devices. The EPA only provides owners of private wells with recommended resources on testing, treatment, and prevention but does not provide any strict requirements [3]. Therefore, responsibility is solely on the owner of the private well to ensure that the water is safe for drinking purposes.

Based on the recommendations of the EPA, private wells should conduct annual testing for nitrate, pH, total coliform, and total dissolved solids (TDS)[15]. It is also recommended that private well owners who live in an area that already has groundwater quality concerns, experience recent environment changes, experience water quality issues or have completed a well repair should test their private well water [15]. Testing for total coliforms specifically is recommended if individuals using the private well water source are experiencing ongoing issues of gastrointestinal disorders [15]. The maximum contaminant level goal for total coliforms, fecal coliforms, and *E. coli* for drinking water is 0 mg/L [16].

Private drinking water wells that have a higher concentration of total coliforms and *E. coli* than the recommended contaminant level can use a variety of treatment processes including distillation, filtration, and disinfection [15]. Disinfection technique recommendations consist of the use of ozone, chlorine, electronic radiation, and ultra-violet light [15]. Current use of treatment devices could help prevent the presence of total coliforms and *E. coli,* however if contamination still occurs with the use of treatment devices it could signify that the technique needs to be re-evaluated or changed.

When the environmental conditions are dry and the temperatures are hot, the ground can potentially experience cracking which can introduce microbial contaminants into groundwater sources, which has been previously discovered in

Canada [12, 29]. Precipitation events can help *E. coli* to travel further and contaminate water bodies [31, 11]. Previous research has discovered that precipitation allows for easier travel of the contaminant from the surface to groundwater [35, 36, 37, 38]. Research and the use of statistical models for private wells in New Jersey displayed that there is a higher risk of total coliform presence with over 1.4 inches of precipitation and higher risk of *E. coli* presence when precipitation was greater than 2.1 inches over a 10 day time period [34]. The EPA recommends that the well is not located in the direction where rainwater will directly flow [40].

Well factors that could play a role in the presence of *E. coli* include the depth of the well [12, 32]. A shallow well could introduce more microbial contaminants into the well drinking water [12, 32]. Bedrock wells have also been found to have higher rates of total coliform in certain locations such as in Canada and in New Jersey [12, 34]. Past studies have presented that factors that play a role in the survival and presence of microbial contaminants specifically in aquifers includes the weather, hydrogeology, and well factors mostly concerning where the well is located, how it was built, and well maintenance [12, 30]. Based on information provided by the CDC, introduction of contaminants could also be determined by the placement of the well and by what it is adjacent to the well [39, 53, 54]. This includes the overall distance that the well is from potential contamination sources such as septic system, livestock, fertilizer, and manure storage sites [39, 53, 54]. Maintaining a proper distance could prevent the introduction of microbial contaminants and chemical contaminants.

Chapter 3: The Impact of Well Factors and Region on Presence of *E. coli* and Total Coliforms

3.1 Objectives and hypothesis

This project consists of four objectives and hypotheses which together focus on analyzing water quality data from farms with private drinking water wells throughout Maryland and comparing these results to various well and climate factors. The aim of this study is to understand the risk of total coliforms and *E. coli* present in Maryland farms with private drinking water wells and the potential association of water quality to well factors, region, and climate. The study variables consist of presence or absence of total coliforms, presence, or absence of *E. coli*, well factors, climate factors, and region (Table 1).

3.1.1 Objective 1

Objective: Evaluate well water quality for the presence of total coliforms and *E. coli* from private drinking water wells for farms throughout Maryland.

Hypothesis: The results for whether total coliforms and *E. coli* is present or absent will differ by the region. The presence or absence could also be dependent on well factors which will be studied more closely in objective 3.

Approach: Filter and process water samples with the U.S. EPA Standard Method 1604 [4] to determine if total coliforms and *E. coli* are present. Conduct descriptive statistical analyses with Microsoft Excel to determine the percentage of water samples with *E. coli* and total coliforms present.

3.1.2 Objective 2

Objective: Evaluate survey data from water testing participants to understand current well practices and conditions (e.g., well type, maintenance, and testing).

Hypothesis: Total coliform and *E. coli* presence will be higher in participants that responded that they have not previously tested their well water, have not installed treatment devices, and have a shallow well depth.

Approach: Evaluate the participant survey responses on well condition, maintenance, location, and type with a descriptive analysis for participants who provided well water samples. Evaluate demographic data through descriptive analysis. This will be conducted with a one-way frequency table and Fisher's Exact Test in SAS to determine the frequency and percentage of each response for the survey questions.

3.1.3 Objective 3

Objective: Determine the significance of the well factors and the correlation between the well water quality data (presence or absence of total coliforms and *E. coli*) and participant survey data (well factors and region) for the participating Maryland farms.

Hypothesis: Well region and current well factors such as well maintenance, well water testing, well depth and well type will be significant factors for concentration of total coliforms and *E. coli*.

Approach: Conduct a Fisher's Exact test in SAS to compare the water quality results to the survey factors to determine what factors are statistically significant.

3.1.4 Objective 4

Objective: Determine the significance of the collected climate data and the correlation between the well water quality data (presence of absence of total coliforms and *E. coli*) and the climate data (precipitation and ambient temperature) for the participating Maryland farms.

Hypothesis: The presence or absence of *E. coli* and total coliforms will be associated with higher levels of precipitation and temperatures that are hot similar to what was found in White et al., 2021 [12]. Similar to the Procopio et al., 2017 study, cumulative precipitation levels of 1.4 inches or greater and 2.1 inches or greater will have increased risk of total coliform and *E. coli* presence respectively [34].

Approach: Conduct a Fisher's Exact test in SAS to compare the water quality results to the climate data to determine if there is significance for the collected precipitation values and ambient temperature values.

Study Variables	**Description**
Total coliform	Presence or absence in each sampling location.
E. coli	Presence or absence in each sampling location.
Well factors	Collected from participant survey data focusing on well maintenance, type, condition, depth, etc.
Climate factors	Precipitation in the past 24 hours, 7 days, and 14 days of collection and ambient temperature when the sample was collected.
Region	Collected from participant survey data to compare the presence of total coliforms and *E. coli* in the seven regions of Maryland.

Table 1. The variables studied in this thesis to look at water quality, survey data, and climate data to complete all objectives.

3.2 Approach and methods

3.2.1 Subject recruitment

Participants from seven regions of Maryland (Lower Shore, Upper Shore, Sothern, Capital, Central, Western, Northern) were selected based on a convenience sample. Information about the project and an invitation to complete a 32-question online survey was sent to approximately 2,000 farmers through the Agricultural Agents from the University of Maryland Extension and their mailing lists, Agronomy News newsletter, and the Agriculture Law Newsletter. One hundred fifty-seven farmers filled out the 32-question online survey through Qualtrics expressing their interest to have their water tested for total coliforms and *E. coli* (Appendix E). Sixty-one of the 157 farmers that completed the survey confirmed their interest with the Agricultural Agents for their region and were then selected to have their well water tested. Selected participants also had their farms private drinking water wells tested for chemical contaminants such as heavy metals and pesticides; however, these results were not analyzed for the current thesis. The remaining 96 potential participants did not have their well water tested for total coliforms and *E. coli* and their survey responses were not included in the analysis of this thesis.

3.2.2 Survey instrument

The 32- question online survey consisted of a variety of questions focusing on the farm’s location (i.e., zip code, county, and region), participant demographics (i.e., race, ethnicity, education level, age, and sex), and their interest in attending educational events related to well protection, sources of contamination, and water testing among others (Appendix E). The survey also consisted of questions related to well factors such as maintenance, age, depth, current condition, and current water testing practices among others (Appendix E). Participants completed the online survey through Qualtrics, an online tool to create and distribute surveys, and the responses were evaluated and organized to complete objective 2 and objective 3. Surveys for participants who provided more than one water sample were included in the survey analysis for the number of times they provided a sample because some participants had variations in the survey responses depending on if they collected from two different well sources, or locations. The survey was reviewed and approved by the University of Maryland Institutional Review Board.

3.2.3 Sample collection

Sixty-seven water samples were collected from 61 participants from May 2, 2022, to August 29, 2022. Prior to collecting water samples, a kickoff meeting was held where Dr. Rachel Rosenberg Goldstein recorded and demonstrated how to collect a water sample. The Agricultural Agent from University of Maryland Extension also provided each participant with written instructions for water sample collection (Appendix D). The water samples were either collected by the trained participants or the Agricultural Agent. Agents and participants were instructed to collect water samples from point of use since the project is focusing on drinking water (i.e., kitchen sink). Participants were also instructed to write the date and time that the sample was collected to complete the climate data.

In addition to the written instructions for sample collection, each participant was provided with a sterile 1L Nalgene bottle, sterile 500 mL Nalgene bottle, a lab marker, and a pair of gloves in a large Ziploc bag. After collecting the water samples all samples were either shipped on ice overnight or delivered on ice within 24 hours of sampling by the Agricultural Agent. Water samples were taken to the Water Quality, Outreach and Wellness Laboratory (WOW) at the University of Maryland School of Public Health to test for the presence of total coliforms and *E. coli* for objective 1.

3.2.4 Climate data collection

For each sample, climate information was collected and recorded (Appendix B). This includes the ambient temperature at the time of sampling in Fahrenheit (F) by using National Oceanic and Atmospheric Administration National Weather Service [17]. This was completed by inserting the location and recording the ambient temperature listed under the "3-day history" tab [17]. The ambient temperature at the time of sampling was also collected with Weather Underground [18]. This was completed by using the historical weather page and inserting the location, date and finding the ambient temperature by time [18]. A series of precipitation data in inches was collected with Weather Underground [18]. This includes the collection of precipitation in the last 24 hours, precipitation in the last 7 days, and precipitation in the last 14 days. This was completed by using the historical weather page and inserting the location and date(s) and week(s). For both the ambient temperature and precipitation data, the County Seat [19] for the County that the sample was collected from was used as the location. This was to complete objective 4, to analyze for significance of climate data and water quality data.

3.2.5 Objective 1. Analyze water samples

Water Sample Analysis Procedure: The water samples were processed with U.S. EPA Standard Method 1604 which is detailed below in this section [4]. Culture-based testing was conducted to test for the presence of total coliforms and *E. coli.* Ten, 100, and 500 mL of each water sample were filtered and rinsed with 10 mL of PBS. The filtered water sample was plated on MI agar plates at 35ºC to incubate for 24 hours (Figure 2). After 24 hours, a UV light was used to identify total coliforms on the MI agar plate by counting all blue/white fluorescent colonies for each sample (Figure 2). This number was recorded as number of total coliforms present. The number of *E. coli* was identified by counting all blue colonies under ambient light on the MI agar plates. The number of *E. Coli* and total coliforms present was recorded for each sample and for each volume filtered (10, 100, 500 mL). Water samples presumptively positive for *E. coli* went through two purification steps. The first purification step included plating colonies from the MI agar plates to MacConkey (MC) agar plates to incubate overnight at 35ºC. The colonies then went through the second purification step by plating on Tryptic Soy Agar (TSA) plates to incubate overnight at 35ºC. Purified isolates were taken from the TSA plates and archived in 1 mL LB Broth with 15% glycerol and stored in the -80 freezer for further testing.

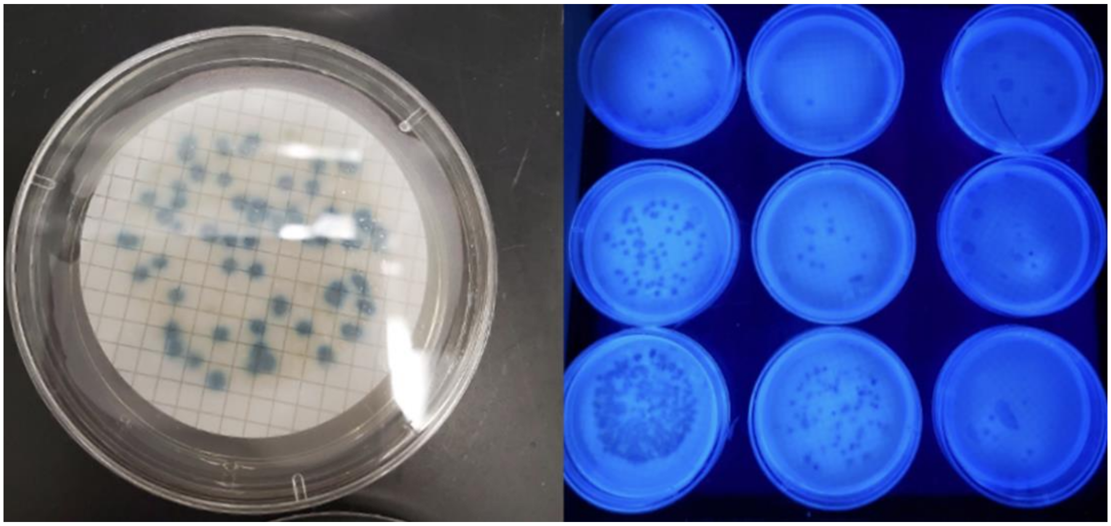

Figure 2. Well water samples plated on MI plates to identify total coliforms and E. coli present.

For each sampling period we filtered ten mL of phosphate buffered saline (PBS) for a negative *E. coli* control and 500 mL of DI Water and *E. coli* ATCC 8739 for a positive *E. coli* control. Total Dissolved Solids (TDS) were also measured with a TDS meter and was recorded for each sample (Appendix A).

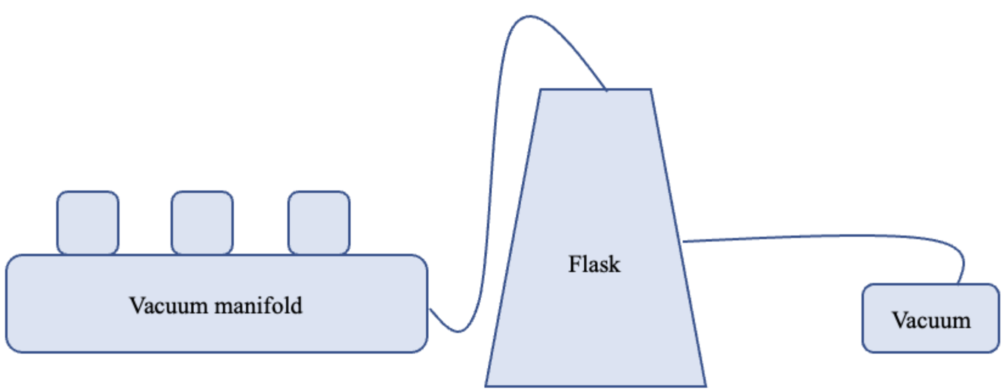

Figure 3. Diagram of water filtration to process water samples to test for the presence of E. coli and total coliforms.

PCR Confirmation Procedure: All archived isolates will go through the process of DNA extraction following the Heat Shock DNA Extraction from Pure Culture protocol. A colony of *E. coli* taken from a TSA plate and submerged into an Eppendorf tube of 200 ul of molecular grade water and vortexed thoroughly. The Eppendorf tube with the *E. coli* colony was put on a heat block for 5 minutes at 100 C and then put in ice for 5 minutes. The heating and cooling step was repeated. Lastly, samples were vortexed, centrifuged at 10,000 RCF for 1 minute and then supernatant was transferred to a clean Eppendorf tube.

E. coli PCR confirmation was conducted following the *E. coli* PCR Confirmation protocol based on Solaiman et al., 2022 to confirm presence of *E. coli* in water samples from the individual isolates of each water sample [43]. With 10x buffer, 25mM MGCl2, 10mM dNTPs, 10um 16SF, 10um 16SR, 10um F, 10um R, Taq, and PCR grade water Master Mix were created [43]. A gel was run to confirm that the samples that were initially recorded as positive for *E. coli* are positive for *E. coli* [43]. Table 2 lists the sequence of primers based on the findings from Solaiman et al. 2022 [43]. The reagents and primers were used to create a master mix that went through the thermocycler for "an initial single cycle at 95C for 30s, followed by 30 cycles of denaturation at 95C for 30s, annealing at 55C for 30s, elongation at 72C for 30s and a final single cycle at 72C for 5 min" (Table 3) [43].

Gene	Size (bp)	Primer	Sequences (5'-3')	References

uidA	192	Forward	CAGTCTGGATCGCGAAAA	20
		Reverse	ACCAGACGTTGCCCACATA	
16S rRNA	357	Forward	AGAGTTTGATCCTGGCTCAG	21
		Reverse	TGACGGGCGGTGTGTACAAG	22

Table 2. Primers used for E. coli PCR confirmation (Source: Solaiman et al. 2022 [43]).

Step	Temperature (Celsius)	Time (seconds)	Notes
Single cycle	95 C	30 s	
Denaturation	95 C	30 s	
Annealing	55 C	30 s	
Elongation	72 C	30 s	Go to step 2 x 29
Final cycle	72 C	300 s	
	4 C	Forever	

Table 3. Settings for thermocycler for E. coli PCR confirmation (Source: Solaiman et al. 2022 [43]).

Statistical Analysis Procedure: A descriptive analysis of the well water quality results was conducted with Microsoft Excel to determine the frequency and percentage of water samples with total coliforms and *E. coli* present and absent for all the water samples collected in Maryland. The water quality results were also analyzed by region to look for differences in the presence of total coliforms and *E. coli* throughout Maryland.

3.2.6 Objective 2. Analyze survey results.

To analyze the survey results for objective 2 and objective 3, the survey responses were narrowed down to focus on the multiple choice and multiple selection questions that focused on well factors, region and county, and demographic questions.

Short answer responses and questions focusing on participants interest in educational events were excluded from the study.

The selected questions were organized on an Excel sheet and codes were created for the response options for each question to prepare for the statistical analysis for objective 2 and objective 3. To complete the Fisher’s Exact test and to test for significance of well factors, certain participant response options were grouped together for the questions. Appendix C shows the selected questions and the combined responses and codes. For question 14, each of the options that participants could have tested their private well water for previously were analyzed separately (Appendix C). For question 15, the responses were analyzed as observed issues with water quality vs no issues (Appendix C). For question 16, the responses were analyzed as no water treatment devices vs installing water treatment devices (Appendix C).

Table 8 includes the 14 survey questions selected from the 32-question online survey that focus specifically on well factors and current well practices. The categories in Table 8 consist of the combined categories that were created to complete the Fisher’s Exact Test. For the original questions and answer options see the full survey in Appendix E.

Statistical Analysis Procedure: A descriptive analysis of the participant survey results was conducted in SAS with the use of a one-way frequency table and a Fisher’s Exact test. This provided data for the frequency and percentage of the participant responses for 32 questions regarding various factors such as region, demographics, well maintenance, well care, well type, and well testing (n=67).

Frequency of participant responses was recorded for each of the selected questions for all the collected samples (n=67). Frequency was recorded for each of the selected questions for the samples positive for *E. coli* (n=7) and samples positive for total coliforms (n=26).

3.2.7 Objective 3. Statistical analysis of the significance between well water quality and survey data

Statistical Analysis Procedure: Fisher's Exact test in SAS was used to determine the most statistically significant survey questions/factors for the selected questions related to the water quality results. A p value <0.05 from the two-sided $Pr \leq P$ was considered significant.

3.2.8 Objective 4. Statistical analysis of the significance between well water quality and climate data

Statistical Analysis Procedure: The climate data for each sample was collected and focused on ambient temperature and precipitation data (Appendix B). Categories for the ambient temperature were created for temperatures 55 F to 94 F and categorized by 55-64 F, 65- 74 F, 75-84 F, 85-94 F to compare between cool to hot temperatures (Table 9). An analysis of the original values was analyzed for the precipitation data for the last 24 hours, the last 7 days, and the last 14 days from sample collection to see if there is significance in specific precipitation values (Table 10). Categories for the precipitation values for the last 24 hours, the last 7 days, and the last 14 days to collapse the precipitation values collected for analysis (Table 11). Categories for analysis of total coliforms and precipitation included 0-0.07 inches, 0.18-0.26 inches, and 0.56-0.62 inches for precipitation in the last 24 hours, <1.4 inches and >1.4 inches for precipitation in the last 7 days precipitation in the last 14 days. The values of <1.4 inches and >1.4 inches were determined based on Procopio et al., 2017 which found precipitation values greater than 1.4 inches to have higher risk of total coliforms [34]. The categories for analysis of *E. coli* and precipitation included 0-0.07 inches, 0.18-0.26 inches, 0.56-0.62 inches for precipitation in the last 24 hours, < 2.1 inches and > 2.1 inches for precipitation in the last 7 days and precipitation in the last 14 days. The values of <2.1 inches and >2.1 inches were determined based on Procopio et al., 2017 which found precipitation values greater than 2.1 inches to have higher risk of total coliforms [34]. The collected precipitation and ambient temperature values were then put into each of these categories for the 67 water samples (Appendix B).

Fisher's Exact test in SAS was used to determine the most statistically significant climate factors (precipitation amount and ambient temperature) to see if any of the factors played a role in the presence of total coliforms and *E. coli* and. A *p* value <0.05 from the two-sided Pr≤P was considered significant.

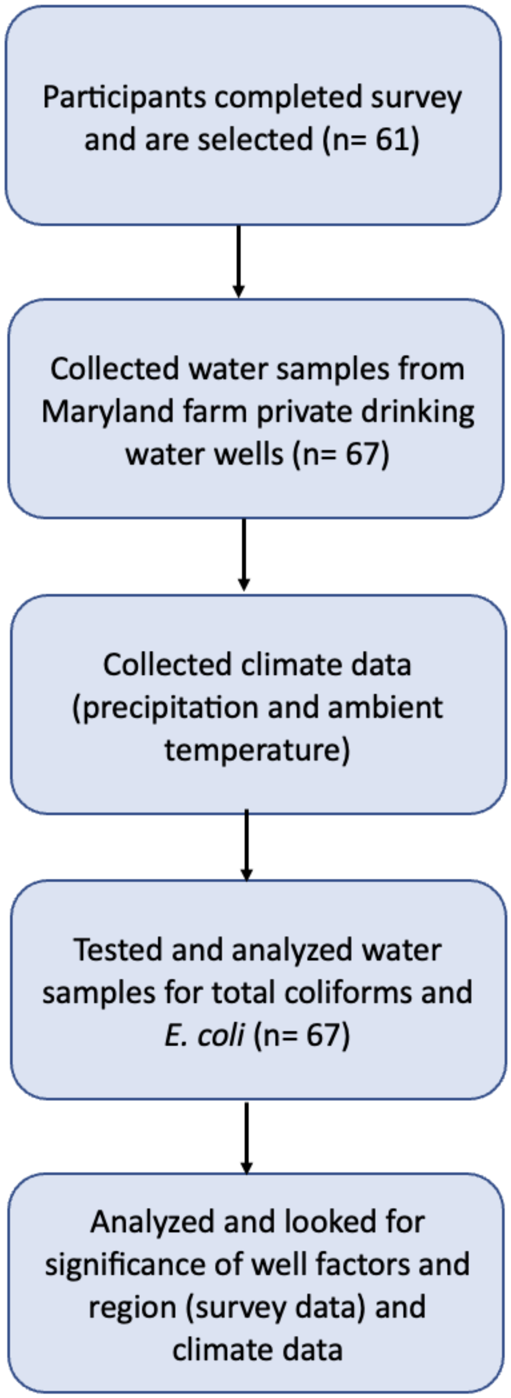

Figure 4. Thesis methods outline.

3.3 Results

3.3.1 Participant demographic characteristics

Out of the 61 participants, 67 water samples were collected from 19 counties in seven regions of Maryland. Water samples were collected from one to four different counties in each region (Table 4). The number of samples collected from each county and region varied. The largest percentage of samples came from the Lower Shore (n=13; 19%) (Table 4). The smallest percentage of samples came from the Northern region (n=6; 9%) (Table 4).

Lower Shore (n, %)	**Upper Shore (n, %)**	**Southern (n, %)**	**Capital (n, %)**	**Central (n, %)**	**Western (n, %)**	**Northern (n, %)**
Somerset (5, 38%)	Talbot (3, 38%)	St. Mary's (11, 100%)	Charles (8, 67%)	Frederick (4, 50%)	Washington (7, 78%)	Carroll (2, 33%)
Worcester (3, 23%)	Cecil (1, 13%)		Anne Arundel (2, 17%)	Montgomery (4, 50%)	Allegany (2, 22%)	Harford (3, 50%)
Wicomico (4, 31%)	Queen Annes (3, 38%)		Prince Georges (2, 17%)			Baltimore (1, 17%)
Dorchester (1, 8%)	Kent (1, 13%)					
Total n=13	**Total n=8**	**Total n=11**	**Total n=12**	**Total n=8**	**Total n=9**	**Total n=6**

Table 4. The number (n) of water samples collected from each of the seven regions and 19 counties of Maryland.

Most of the participants that provided water samples and completed the survey were White (n= 57; 93%) and nearly half were in the 50-69 age group (n=32;

51%) (Table 5). A large percentage of participants were male (n=43; 69%) (Table 5). Most participants were educated with a bachelor's degree (n=23; 35%) or a graduate or professional degree (n=22; 34%) (Table 5).

Characteristic	**Category**	**Number (%) (n=67)**
Race	White	57 (93%)
	Asian	1 (2%)
	Black or African American	1 (2%)
	Other	2 (3%)
	Total	61 (100%)
Age	18-29	1 (2%)
	30-49	17 (27%)
	50-69	32 (51%)
	70-89	13 (21%)
	Total	63 (100%)
Sex	Female	19 (31%)
	Male	43 (69%)
	Total	62 (100%)
Highest Level of Education	Less Than High School	3 (5%)
	High School/GED	9 (14%)
	Bachelor's degree	23 (35%)
	Graduate or Professional Degree	22 (34%)
	Some College or Associate's Degree/ Certificate	8 (12%)
	Total	65 (100%)

Table 5. Participant demographic data for race, age, sex, and highest level of education.

3.3.2 Sample collection sources

Out of the 61 participants, 56 participants included one well water sample and five participants included two to three well water samples. Sample 22 and sample 15 were from the same individual but were from two different house and well locations and different days (Table 6). Sample 60, sample 52, and sample 51 were from the same individual but collected on different days. Sample 60 represents filtered water source from the well house, sample 52 and sample 51 were collected from the same location from an unfiltered water source (Table 6). Due to a power outage on July 12 that impacted the lab at the University of Maryland, sample 52 was not finished with the analysis of chemical contaminants for the second portion of this project. Therefore sample 51 was collected later in July and retested for microbial contaminants as well so the participants had a total of 3 water samples (Table 6). Sample 27 and sample 24 were collected from the same individual on the same day but from an untreated water source and a treated water source from the location (Table 6).

Sample 39 and sample 46 were collected on the same day but from different sources at the farm. Sample 39 was collected from the house kitchen sink and sample 46 was collected from the barns old hose on a hydrant (Table 6). Sample 42 and Sample 43 were collected from the same individual and same date but from different sampling locations and residences (Table 6). All 11 of these samples were still included in the analysis of water quality, region, well factors, and climate data since none of the samples were collected on the same day at the same sampling location so comparisons of well factors, conditions, and climate can still be tested. Table 6 lists all the samples where more than one water sample was collected. Each time that a participant provided a water sample, their survey response was added to the analysis since some survey responses had varying responses if the well location was different or if the location was operated by another individual.

Sample Number (From Same Participant)	**Date Collected**	**Water Source Notes**
22	5/2/22	First house/well source
15	8/29/22	Second house/well source
60	5/10/22	Filtered water source.
52	7/12/22	Unfiltered water source
51	7/26/22	Unfiltered water source
27	6/7/22	Untreated water source
24	6/7/22	Treated water source
39	7/26/22	House- kitchen sink
46	7/26/22	Barn- old hose on hydrant
42	7/26/22	First house/well source
43	7/26/22	Second house/well source

Table 6. Water samples collected from the same participant with the sample number, date, and water source notes.

For participants whose well water was not used for drinking water in the kitchen, the water sample was collected from another water source that is connected to the well water (Table 7). Water samples that were not collected from the kitchen sink includes sample 9 from the laundry room, sample 67 from an outside faucet, sample 59 from an outside water spicket, sample 51 from an outside hydrant, and sample 46 collected from an old barn hose on a hydrant (Table 7). These samples were still included in the analysis of water quality and the connection to region, well factors, and climate data.

Sample Number	Location
9	Laundry Room
67	Outside faucet
59	Outside water spicket
51	Outside hydrant
46	Old barn hose on hydrant

Table 7. Water samples that were collected in locations other than the point of use (kitchen sink).

3.3.3 Private drinking water well quality analysis

Twenty-six out of the 67 (39%) well water samples were positive for *E. coli* total coliforms (fluorescent blue colonies on MI plate) and thus had concentrations higher than the recommended maximum contaminant level defined by the EPA (Figure 5). The Western region of Maryland had the largest percentage of samples positive for total coliforms with eight out of nine (89%) water samples positive for total coliforms (Figure 6). All regions except for the Northern region had well water samples with total coliforms present (Figure 6).

Seven out of the 67 (10%) water samples were positive for *E. coli* and thus had concentrations higher than the recommended maximum contaminant level defined by the EPA (Figure 5). The Western region of Maryland had the largest percentage of *E. coli* positive samples with five out of nine (56%) water samples being positive for *E. coli* (Figure 6). The only other regions that had positive *E. coli* samples were Central (n=1; 13%) and Southern (n=1; 9%) (Figure 6). The Northern region was the only region to not have any microbial contamination present in the farm private drinking water well samples.

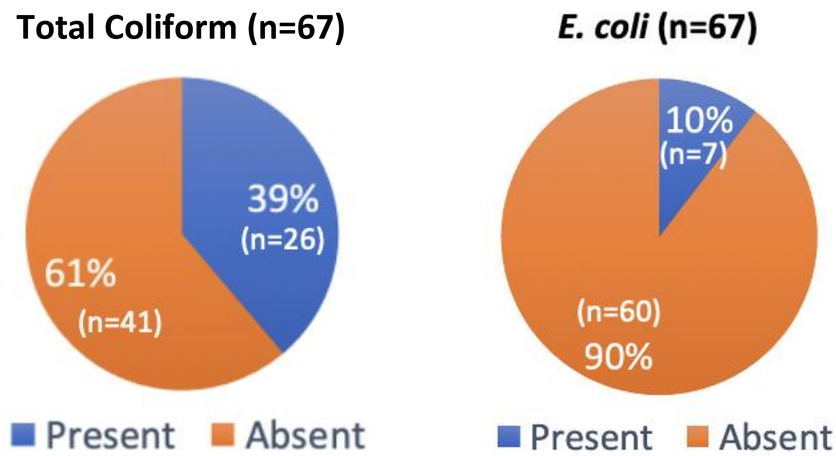

Figure 5. The percentage and number of total coliforms and E. coli positive samples (n=67).

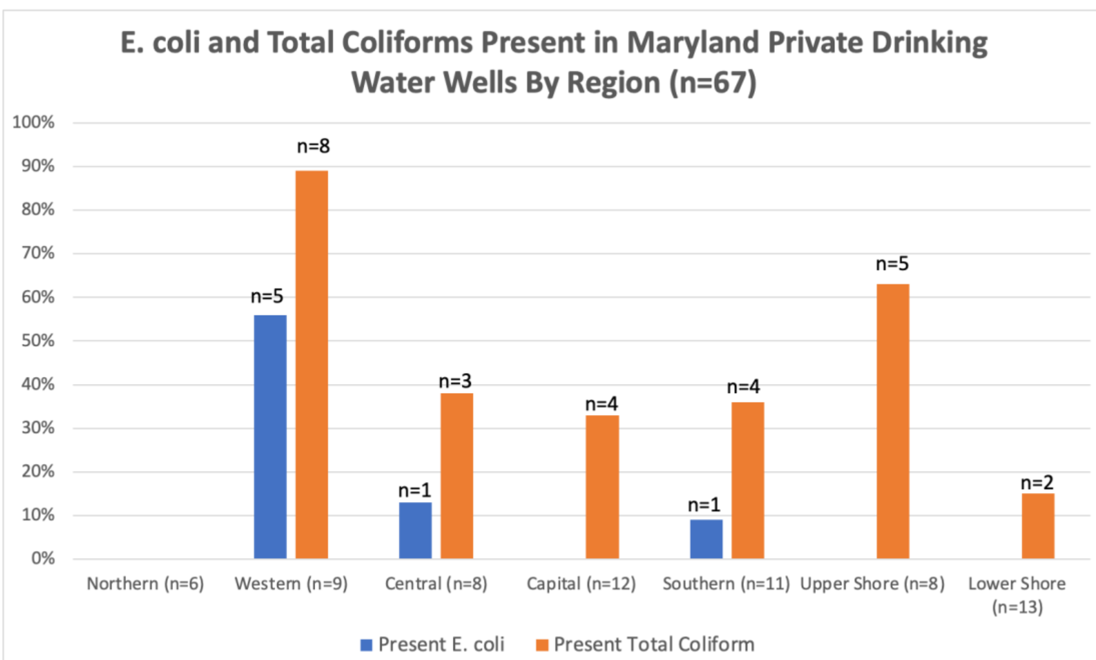

Figure 6. The percentage and number of total coliforms and E. coli positive samples by regions (n=67).

3.3.4 Participant survey data analysis

When looking at all the responses together (n=67) a large percentage of the wells were drilled (n=61; 92%), were over 25 years in age (n=39; 60%), currently considered to be in good condition (n=57; 85%), and no repairs or well management (n=40; 63%) (Table 8). When comparing the five options for well depth, the largest percentage of participants had deeper wells greater than 251 feet (n=21; 41%). Ninety-six percent of participants (n=64) currently know where the drinking water wellhead is located and for 91% of the participants (n= 59) the wellhead cover is plastic (Table 8). A large percentage of the homes plumbing is less than 50 years old (n=53; 82%), and the plumbing pipes are made from multiple types (n=31; 48%) (Table 8). 63% of the participants have previously tested their well water, 28% of participants (n=27) have tested for total coliforms and 23% of participants (n=22) have tested for nitrates (Table 8). Lastly, 64% of participants (n=42) have observed issues with the well water quality, 69% of participants (n=45) have serviced the well and water system and 57% of participants (n=36) have installed some type of water treatment device (Table 8).

In addition to providing the response number and percentage for the 14 survey questions, Table 8 also provides the number and percent of responses that were positive for total coliforms and *E. coli*. For the samples that were positive for total coliforms and *E. coli* respectively, the well was considered to be in good condition (96%, 86%), a large percent was drilled wells (85%, 71%), over 25 years old (52%, 67%), observed issues with their well water quality (65%, 86%), no well repairs or management (58%, 67%), serviced the well and water system (62%, 86%), and installed a treatment device (58%, 71%) (Table 8). Of the water samples that we collected from, there were more samples positive for total coliforms from deeper wells greater than 251 feet (42%) (Table 8). More samples were positive for *E. coli* from well depths of 30 feet (29%), 76-150 feet (29%), and 150-250 feet (29%) (Table 8). This was determined by evaluating how participants responded and specifically analyzing the responses from participants with positive samples of total coliforms and *E. coli*. Lastly, more total coliforms and *E. coli* were present in water samples that have previously been tested prior to this study (65%, 71%) (Table 8).

Characteristics	**Category**	**Response Number (%)**	**Positive TC (%)**	**Positive *E. coli* (%)**
Well Type	Drilled	61 (92%)	22 (85%)	5 (71%)
	Other	5 (8%)	4 (15%)	2 (29%)
	Total	66 (100%)	26 (100%)	7 (100%)
Well Age	<25 years	26 (40%)	12 (48%)	2 (33%)
	>25 years	39 (60%)	13 (52%)	4 (67%)
	Total	65 (100%)	25 (100%)	6 (100%)
Well Depth	30 ft	4 (8%)	4 (17%)	2 (29%)
	31-75 ft	2 (4%)	0 (0%)	0 (0%)
	76-150 ft	12 (24%)	6 (25%)	2 (29%)
	150-250 ft	12 (24%)	4 (17%)	2 (29%)
	>251 ft	21 (41%)	10 (42%)	1 (14%)
	Total	51 (100%)	24 (100%)	7 (100%)
Current Well Condition	Good	57 (85%)	25 (96%)	6 (86%)
	Ok	10 (15%)	1 (4%)	1 (14%)

	Total	67 (100%)	26 (100%)	7 (100%)
Do you know the drinking water wellhead location	Yes	64 (96%)	25 (96%)	7 (100%)
	No	3 (4%)	1 (4%)	0 (0%)
	Total	67 (100%)	26 (100%)	7 (100%)
Type of wellhead cover	Plastic	59 (91%)	22 (85%)	5 (71%)
	Other	6 (9%)	4 (15%)	2 (29%)
	Total	65 (100%)	26 (100%)	7 (100%)
Well repairs or well management	Yes	23 (37%)	10 (42%)	2 (33%)
	No	40 (63%)	14 (58%)	4 (67%)
	Total	63 (100%)	24 (100%)	6 (100%)
Age of homes plumbing	<50 years	53 (82%)	22 (88%)	6 (86%)
	>51 years	12 (18%)	3 (12%)	1 (14%)
	Total	65 (100%)	25 (100%)	7 (100%)
Type of plumbing pipes	Copper	10 (15%)	5 (19%)	0 (0%)
	PEX	1 (2%)	0 (0%)	0 (0%)
	PVC	23 (35%)	10 (38%)	2 (29%)
	Multiple type	31 (48%)	11 (42%)	5 (71%)
	Total	65 (100%)	26 (100%)	7 (100%)
Tested drinking water well quality	Yes	42 (63%)	17 (65%)	5 (71%)
	No	25 (37%)	9 (35%)	2 (29%)
	Total	67 (100%)	26 (100%)	7 (100%)
Drinking water well tested for	Total coliform	27 (28%)	11 (25%)	4 (27%)
	Nitrate	22 (23%)	10 (23%)	3 (20%)
	Other	12 (13%)	3 (7%)	1 (7%)
	pH	20 (21%)	12 (27%)	4 (27%)

	TDS	14 (15%)	8 (18%)	3 (20%)
	Total	95 (100%)	44 (100%)	15 (100%)
Observed issues with water quality	Yes	42 (64%)	17 (65%)	6 (86%)
	No	24 (36%)	9 (35%)	1 (14%)
	Total	66 (100%)	26 (100%)	7 (100%)
Serviced well/water system	Yes	45 (69%)	16 (62%)	6 (86%)
	No	20 (31%)	10 (38%)	1 (14%)
	Total	65 (100%)	26 (100%)	7 (100%)
Installed water treatment device	Yes	36 (57%)	15 (58%)	5 (71%)
	No	27 (43%)	11 (42%)	2 (29%)
	Total	63 (100%)	26 (100%)	7 (100%)

Table 8. The number and percentage of survey responses for 14 survey questions focusing on well factors.

3.3.5 Climate data analysis

Approximately half (n=35; 52%) of the farm private drinking water well samples were collected in an ambient temperature of 65-74 F, with the second largest percentage of samples collected in ambient temperatures of 75-84 F (n=20; 30%) (Table 9). Water samples with *E. coli* present were found in ambient temperatures of 65-74 F (n=5; 71%) and 75-84 F (n=2; 29%) (Table 9). A large percentage (n=17; 65%) of the water samples with total coliforms present were found in ambient temperatures of 65-74 F (Table 9).

The Fisher's Exact test depicts that ambient temperature is a significant factor for total coliforms (p=0.05) (Table 9). The ambient temperature is not a significant factor for *E. coli* (p=0.91) (Table 9). A p value <0.05 from the two-sided Pr≤P was considered significant.

Variable	**TC Present (n, %)**	**TC Absent (n, %)**	***p*-value (TC)**	**E. coli Present (n, %)**	***E. coli* Absent (n, %)**	***p*-value (*E. coli*)**
<u>Ambient Temperature</u>			0.05			0.91
55-64 F	4 (15%)	4 (10%)		0 (0%)	8 (13%)	
65-74 F	17 (65%)	18 (44%)		5 (71%)	30 (50%)	
75-84 F	3 (12%)	17 (41%)		2 (29%)	18 (30%)	
85-94 F	2 (8%)	2 (5%)		0 (0%)	4 (7%)	
Total	26	41		7	60	

Table 9. Analysis of the presence or absence of total coliforms and E. coli based on ambient temperature at the time that water samples were collected. Significant: p<0.05.

The individual precipitation values without being collapsed into categories indicates that a large percentage of Maryland farm private drinking water wells that have total coliforms present had 0 inches of precipitation in the last 24 hours (n=14; 54%) (Table 10). Zero inches of precipitation in the last 7 days were recorded in 23% of samples and 3.17 inches of precipitation in the last 7 days were recorded in 19% of samples that had total coliforms present (Table 10). The largest percentage of water samples that had total coliforms present had 3.4 inches (n=5; 19%) of rain in the last 14 days of sampling (Table 10). For *E. coli* there was not one specific precipitation value that had a larger percentage of samples positive for *E. coli* in the last 24 hours, last 7 days, and last 14 days of samples (Table 10). The p-values from the Fisher's

Exact Test were statistically significant for precipitation in the last 24 hours (*p*=0.03) and precipitation in the last 14 days (*p*=0.05) for total coliforms (Table 10). The p-values from the Fisher's Exact Test were statistically significant for precipitation in the last 24 hours (*p*<0.01), the last 7 days (p=0.01), and the last 14 days (p=0.02) (Table 10). The only p-value not significant was for precipitation in the last 7 days for total coliforms (*p*=0.09) (Table 10).

Variable	**TC Present (n, %)**	**TC Absent (n, %)**	***p*-value (TC)**	***E. coli* Present (n, %)**	***E. coli* Absent (n, %)**	***p*-value (*E. coli*)**
Precipitation last 24 hours			0.03			<0.01
0 in.	14 (54%)	26 (63%)		2 (29%)	38 (63%)	
0.02 in.	1 (4%)	0 (0%)		0 (0%)	1 (2%)	
0.07 in.	0 (0%)	6 (15%)		0 (0%)	6 (10%)	
0.18 in.	1 (4%)	0 (0%)		0 (0%)	1 (2%)	
0.21 in.	0 (0%)	1 (2%)		0 (0%)	1 (2%)	
0.22 in.	3 (12%)	1 (2%)		0 (0%)	4 (7%)	
0.26 in.	3 (12%)	6 (15%)		1 (14%)	8 (13%)	
0.56 in.	2 (8%)	1 (2%)		2 (29%)	1 (2%)	
0.62 in.	2 (8%)	0 (0%)		2 (29%)	0 (0%)	
Total	26	41		7	60	
Precipitation last 7 days			0.09			0.01
0 in.	6 (23%)	4 (10%)		2 (29%)	8 (13%)	
0.08 in.	3 (12%)	7 (17%)		1 (14%)	9 (15%)	
0.26 in.	1 (4%)	6 (15%)		0 (0%)	7 (12%)	
0.31 in.	3 (12%)	1 (2%)		0 (0%)	4 (7%)	
0.36 in.	1 (4%)	0 (0%)		0 (0%)	1 (2%)	

0.37 in.	0 (0%)	2 (5%)		0 (0%)	2 (3%)	
0.39 in.	2 (8%)	5 (12%)		0 (0%)	7 (12%)	
0.41 in.	0 (0%)	5 (12%)		0 (0%)	5 (8%)	
0.48 in.	0 (0%)	1 (2%)		0 (0%)	1 (2%)	
0.55 in.	1 (4%)	3 (7%)		0 (0%)	4 (7%)	
0.58 in.	2 (8%)	1 (2%)		2 (29%)	1 (2%)	
1.77 in.	2 (8%)	0 (0%)		2 (29%)	0 (0%)	
3.17 in.	5 (19%)	4 (10%)		0 (0%)	9 (15%)	
4.21 in.	0 (0%)	2 (5%)		0 (0%)	2 (3%)	
Total	26	41		7	60	
Precipitation last 14 days			0.05			0.02
0.47 in.	0 (0%)	2 (5%)		0 (0%)	2 (3%)	
0.55 in.	0 (0%)	1 (2%)		0 (0%)	1 (2%)	
0.58 in.	2 (8%)	1 (2%)		2 (29%)	1 (2%)	
0.77 in.	4 (15%)	3 (7%)		2 (29%)	5 (8%)	
1.13 in.	2 (8%)	1 (2%)		0 (0%)	3 (5%)	
1.46 in.	1 (4%)	0 (0%)		0 (0%)	1 (2%)	
1.54 in.	3 (12%)	1 (2%)		0 (0%)	4 (7%)	
1.64 in.	1 (4%)	0 (0%)		0 (0%)	1 (2%)	
1.65 in.	0 (0%)	5 (12%)		0 (0%)	5 (8%)	
1.66 in.	1 (4%)	2 (5%)		0 (0%)	3 (5%)	
1.72 in.	0 (0%)	1 (2%)		0 (0%)	1 (2%)	
1.91 in.	3 (12%)	7 (17%)		1 (14%)	9 (15%)	
2.25 in.	0 (0%)	6 (15%)		0 (0%)	6 (10%)	
2.61 in.	2 (8%)	5 (12%)		0 (0%)	7 (12%)	
2.63 in.	2 (8%)	0 (0%)		2 (29%)	0 (0%)	
3.4 in.	5 (19%)	4 (10%)		0 (0%)	9 (15%)	
4.95 in.	0 (0%)	2 (5%)		0 (0%)	2 (3%)	
Total	26	41		7	60	

Table 10. Analysis of the presence or absence of total coliforms and E. coli based on precipitation. Significant: p<0.05.

When the precipitation values were collapsed into categories and analyzed in smaller groups it was recorded that most of the samples positive for total coliforms had precipitation values between 0-0.07 inches in the last 24 hours (n=15; 57%) (Table 11). Most of the water samples positive for total coliforms experienced less than 1.4 inches of precipitation in the last 7 days prior to sampling (n=19; 73%) and greater than 1.4 inches of rain in the last 14 days prior to sampling (n=18; 69%) (Table 11). After conducting the Fisher's Exact Test, the p-values were not significant for precipitation in the last 24 hours (p=0.08), precipitation in the last 7 days (p=0.34), and precipitation in the last 14 days (p=0.38) for total coliforms (Table 11).

Variable	**TC Present (n, %)**	**TC Absent (n, %)**	***p*-value (TC)**
Precipitation last 24 hours (organized data)			0.08
0-0.07 in.	15 (57%)	32 (76%)	
0.18-0.26 in.	7 (27%)	8 (20%)	
0.56-0.62 in.	4 (15%)	1 (2%)	
Total	26	41	
Precipitation last 7 days (organized data)			0.34
< 1.4 in.	19 (73%)	35 (85%)	
> 1.4 in.	7 (27%)	6 (15%)	
Total	26	41	
Precipitation last 14 days (organized data)			0.38
< 1.4 in.	8 (31%)	8 (20%)	

> 1.4 in.	18 (69%)	33 (80%)	
Total	26	41	

Table 11. Analysis of the presence or absence of total coliforms based on precipitation values collapsed into categories. Significant: p<0.05.

For the collapsed categories of precipitation data for *E. coli* most samples that were positive for *E. coli* had experienced 0.56-0.62 inches of precipitation in the last 24 hours (n=4; 57%) (Table 12). In the last 7 days all the samples positive for *E. coli* experienced less than 2.1 inches of precipitation (n=7; 100%) (Table 12). For precipitation in the last 14 days most of the samples positive for *E. coli* experienced less than 2.1 inches of precipitation (n=5; 71%) (Table 12). Upon completing the Fisher's Exact Test, it was determined that the p-value for precipitation in the last 7 days (*p*= 0.59) and precipitation in the last 14 days (*p*=0.70) were not statistically significant (Table 12). The *p*-value for precipitation in the last 24 hours was significant (*p*<0.01) (Table 12).

Variable	***E. coli* Present (n, %)**	***E. coli* Absent (n, %)**	***p*-value (E. coli)**
Precipitation last 24 hours (combined data)			<0.01
0-0.07 in.	2 (29%)	45 (75%)	
0.18-0.26 in.	1 (14%)	14 (23%)	
0.56-0.62 in.	4 (57%)	1 (2%)	
Total	7	60	
Precipitation last 7 days (combined data)			0.59
< 2.1 in.	7 (100%)	49 (82%)	
> 2.1 in.	0 (0%)	11 (18%)	
Total	7	60	

Precipitation last 14 days (combined data)			0.70
< 2.1 in.	5 (71%)	36 (60%)	
> 2.1 in.	2 (29%)	24 (40%)	
Total	7	60	

Table 12. Analysis of the presence or absence of E. coli based on precipitation values collapsed into categories. Significant: p<0.05.

3.3.6 Significance of well water factors, region, and survey data

The Fisher's Exact Test was conducted for each of the well factors survey questions and demographic questions. Table 13 lists three of these survey questions and the p-values associated with them. The p-value for region is significant for total coliforms (p<0.01) and is significant for *E. coli* (p<0.01) (Table 13). The p-value for county is significant for total coliforms (p=0.03) and is not significant for *E. coli* (p=0.10) (Table 13). The p-value for water samples that previously tested their drinking water well for pH is significant for total coliforms (p<0.01) but not significant for *E. coli* (p=0.11) (Table 13).

The other well factors and demographic factors were not significant when compared to the presence of total coliforms and *E. coli*. A p value <0.05 from the two-sided Pr≤P was considered significant.

Characteristic & Category (n)	**Positive *E. coli* (n, %)**	***p*-value (*E. coli*)**	**Positive Total Coliform (n, %)**	***p*-value (Total Coliform)**
Region		<0.01		<0.01
Capital (n=12)	0 (0%)		4 (15%)	

Central (n=8)	1 (14%)	3 (12%)	
Lower Shore (n=13)	0 (0%)	2 (8%)	
Northern (n=6)	0 (0%)	0 (0%)	
Southern (n=11)	1 (14%)	4 (15%)	
Upper Shore (n=8)	0 (0%)	5 (19%)	
Western (n=9)	5 (71%)	8 (31%)	
Total (n= 67)	7 (100%)	26 (100%)	
<u>County</u>	0.10		0.03
Allegany (n= 2)	2 (29%)	2 (8%)	
Anne Arundel (n=2)	0 (0%)	0 (0%)	
Baltimore (n=1)	0 (0%)	0 (0%)	
Carroll (n=2)	0 (0%)	0 (0%)	
Cecil (n=8)	0 (0%)	1 (4%)	
Charles (n=1)	0 (0%)	3 (12%)	
Dorchester (n=1)	0 (0%)	0 (0%)	
Frederick (n=4)	1 (14%)	0 (0%)	
Harford (n=3)	0 (0%)	0 (0%)	
Kent (n=1)	0 (0%)	1 (4%)	
Montgomery (n=4)	0 (0%)	3 (12%)	
Prince Georges (n=2)	0 (0%)	1 (4%)	
Queen Annes (n=3)	0 (0%)	1 (4%)	
Somerset (n=5)	0 (0%)	2 (8)	
St. Mary’s (n=11)	1 (14%)	4 (15%)	
Talbot (n=3)	0 (0%)	2 (8%)	
Washington (n=7)	3 (43%)	6 (23%)	
Wicomico (n=3)	0 (0%)	0 (0%)	
Worcester (n=4)	0 (0%)	0 (0%)	
Total (n= 67)	7 (100%)	26 (100%)	

Previously tested drinking water well for pH	0.11		<0.01
Yes (n=20)	4 (100%)	12 (86%)	
No (n=19)	0 (0%)	2 (14%)	
Total (n= 39)	4 (100%)	14 (100%)	

Table 13. Survey factors (region, county, previous testing of pH) statistical analysis from Fisher's Exact Test. Significant: $p<0.05$.

3.4 Discussion

3.4.1 The presence of microbial contaminants in Maryland farm private wells

With total coliforms (39%) and *E. coli* (10%) being detected there is a risk of contamination in Maryland farm private drinking water wells (Figure 5). These results can be compared to the results from Murray et al., 2018 which found total coliforms present in 25% of the well samples and *E. coli* in 3% of the 118 well water samples collected in Maryland [5]. The differences in the percentage of total coliforms and *E. coli* present could be because this thesis was collecting samples from a smaller sample size compared to Murray et al., 2018. Another difference is that this thesis was collecting from seven different regions and 19 counties in Maryland and Murray et al., 2018 focused on 4 counties [5]. Therefore, this study may have had differences in the percent of total coliform and *E. coli* present due to their being a difference in the geography and geology of the land where each sample was collected. Since the water samples were collected from different regions with different environmental conditions it is understandable that the total coliform and *E. coli* presence percentage would be slightly higher in this thesis. With only three regions having positive samples for *E. coli* and the Western region having more than half of the positive samples (56%), region is an important factor (Figure 6). Western region was also found to have the highest number of total coliform samples (89%) (Figure 6), again depicting this idea that region is playing a role in contamination. With region being an important factor of microbial contamination, this shows the importance of geology in Maryland.

The Maryland Department of Natural Resources provides information on the geology of six provinces in Maryland which make up the seven regions that we collected from [44]. The provinces of Appalachian Plateau, Ridge and Valley, Blue Ridge, and Piedmont are in fractures and consolidated rock consisting of consolidated sedimentary, igneous, and metamorphic rock [44]. Ridge and Valley and Piedmont provinces typically consist of limestone aquifers [44]. The coastal plain province on the other hand consists of unconsolidated material [44]. The Western region is part of the Ridge and Valley Province and consists of limestone, consolidated rock, and fractures [44]. Based on White et al., 2021 wells made of limestone were at a greater risk of contamination [12]. If the well did consist of limestone in the Western region of Maryland it follows the findings of White et al., 2021 for why there was higher presence of total coliforms and *E. coli*. Fractured material which the Western region is potentially located on, can cause greater transport of contaminants through the water source [45]. The central region also had one positive sample of *E. coli* and is part of the Piedmont province which also consist of limestone, consolidated rock, and fractures [44]. The southern region also had a positive sample however this region is part of the Atlantic coastal plain which is the unconsolidated material and consist of sand and gravel material [44].

While we did not collect data from the participants about the type of bedrock, rock material, and geological factors we can get a preliminary understanding of geological differences from the Maryland Department of Natural Resources Geological Survey.

3.4.2 Participant survey responses and significant factors

The participant survey data provided us with a better understanding of current well maintenance, type, and conditions for Maryland farms with private drinking water wells (Table 8). When looing specifically at well depth, White et al., 2021 discovered that shallow wells could introduce more contaminants however this does not mean that deep wells prevent contamination [12]. Of the samples that were positive for total coliforms, a larger percentage was found in the deeper well over 251 feet (42%) showing there is not a protective effect of deeper wells (Table 8). Total coliforms were still able to be introduced into the well water. This represents that for total coliform contamination, deep wells were more contaminated compared to shallow wells. Going back to the idea of geology, this may also be related to whether there is fractured rock present which could transport the contaminant more readily [45]. *E. coli* was present in a variety of depth conditions and there was not one specific depth where we saw an increase. There is a risk in both shallow and deep wells for the presence of *E. coli*. More research should be conducted to determine if the wells present at deeper wells had fractured rock present or limestone if it is in the Western or Central region.

There were only three factors that were statistically significant for the presence of total coliforms and one factor statistically significant for the presence of *E. coli* (Table 13). While the remaining survey questions provided an understanding of well depth, type, and condition in Maryland farms, they were not significant factors for the presence of total coliforms and *E. coli* (Table 13). Previously testing for pH was also a statistically significant factor for the presence of total coliforms ($p<0.01$) (Table 13). This means that previously testing for pH could be a potential factor that leads to the presence of total coliforms in Maryland farm private drinking water wells. pH is one of the recommended tests for private well water and having an incorrect pH level could cause harm to pipes and introduce heavy metals [13]. It is currently unclear why this is considered significant; however, Procopio et al., 2017 did find that pH of 5 to 6.99 had greater risk of total coliform presence [34]. While we did not collect the pH for the Maryland participants water, if the participants that previously tested for pH have had concerns with pH in the past or had pH in the range of 5 to 6.99 as seen in New Jersey it could have been a factor to the presence of total coliforms [34]. More research on the connection between pH testing and total coliform presence should be conducted.

Another significant factor for total coliforms presence was the county ($p=0.03$) (Table 13). This means that county could be a potential factor that leads to the presence of total coliforms in Maryland farm private drinking water wells. A concern with this analysis is that many of the 19 counties only have about 2-11 water samples collected, therefore the sample size from each county is small. Since there were not many samples collected from each individual county and there were also very few positive total coliform samples from each county, ranging from 1-6, the results could have been skewed (Table 13). However, given what is known about geological differences between the regions, we can also expect there to be differences between county therefore county could still be a significant factor. The p-value for region was also significant for both total coliforms (p<0.01) and *E. coli* (p<0.01) (Table 13). While again we did have a small sample size and a small number of samples collected from each individual region which could have impacted the p-value in the Fisher's Exact Test, the results did show an obvious difference in positive samples among regions (Figure 6; Table 13).

3.4.3 Climate data significant factors

Previous data from White et al., 2021, shows that dry conditions and hot temperatures are more likely to lead to presence of microbial contamination due to the potential of the ground cracking [12, 29]. This led me to hypothesize that warmer temperatures would be associated with higher rates of total coliforms and *E. coli*. However, our results show that a larger percentage of samples positive for total coliforms (65%) and *E. coli* (71%) had an ambient temperature ranging between 65-74 F at the time of collection (Table 9). This is cooler temperatures than I initially expected. It was also discovered that ambient temperature is a significant factor for total coliform concentration (p=0.05) (Table 9). This means that total coliform presence can be impacted by the temperature. There are currently no studies available that provide information as to why cooler temperatures in the 60's and 70's would lead to increase rates of total coliforms. A previous study in North Carolina found an increase in total coliforms and *E. coli* in private wells located close to hot lagoons where the air temperature was 90 F [48]. For this thesis it is possible that although the temperature was lower, it could have potentially been more humid and drier, and this could have provided an ideal environment to introduce total coliforms.

Previous data in other geographical locations show that precipitation allows *E. coli* to travel far into water, increasing risk of contamination in water sources [31, 11]. Procopio et al., 2017 found that total coliforms were more likely to be present in areas that experienced over 1.4 inches or more of precipitation and *E. coli* was more likely to be present in areas that experienced over 2.1 inches of precipitation over a 10-day period [34]. Because of these findings, I hypothesized that increased precipitation would lead to greater presence of total coliforms and *E. coli*. The results for this thesis found that when looking at precipitation in the last 24 hours and the last 7 days there were more samples positive for total coliforms that experienced lower rates of rain (Table 11). However, when looking at precipitation over 14 days a larger percentage of samples were positive for total coliforms that experienced over 1.4 inches of rain (Table 11). These results, similar to Procopio et al., 2017 show that when looking at cumulative precipitation for a longer period, when there is an increased precipitation levels there is a higher risk of total coliforms [34]. However, when looking at shorter periods the opposite observation was noted where more positive total coliform samples were associated with lower precipitation values (Table 11). Cumulative precipitation for 14-day time period could be because this is a longer period of time for rainfall to carry contaminants to the well water and accumulate during this time. For the shorter time periods where less precipitation occurred, this could mean that it does not take a large precipitation event to lead to the presence of total coliforms.

The results for precipitation and its effect on *E. coli* also shows that smaller precipitation events could lead to the presence of total coliforms and *E. coli* (Table 12). Precipitation for the last 24 hours was a significant factor for *E. coli* concentration ($p<0.01$) meaning that precipitation could impact the presence of *E. coli* in private well water (Table 12). There was also a higher rate of positive samples for precipitation between 0.56-0.62 inches which is the highest range of precipitation that was recorded for precipitation in the last 24 hours (Table 12). This again depicts the idea that a small precipitation event in a short timeframe can potentially introduce *E. coli* into private drinking water wells.

When analyzing the precipitation values individually as they were recorded for each sample, the Fisher's Exact Test provided significant values for all precipitation factors except for precipitation in the last 7 days for total coliform concentration (Table 10). This significant value, however, was most likely because there were many categories, but small number of samples evaluated in each group (Table 10). For example, there were only 7 positive *E. coli* samples and 26 positive total coliform samples, so when analyzing these groups, the small number of samples in the analysis could have skewed the results (Table 10). This is why for the final results and discussion I focused on the collapsed categories of precipitation values.

3.5 Project limitations

A limitation of this thesis is that we did not collect water samples from a uniform number between the seven regions of Maryland. The goal was to collect a total of 75 water samples in total but there was not a set number for how many participants or water samples we would collect from each region. Instead, there was a range of water samples collected from each region. The largest number of samples was collected from the Lower Shore (n=13; 19%) and the smallest number of samples was collected from the Northern region (n=6; 9%) (Table 4). There is also a range in how many counties from each region samples were collected from, ranging from one to four counties for each region. This makes the comparison of water quality results more difficult and not as accurate compared to if the sample size was uniform across the seven regions. Conducting this study again with a more uniform sample size would provide a more accurate comparison. However, even with the non-uniform sample size from each region we were still able to get a preliminary understanding of the risk of total coliform and *E. coli* contamination for the seven regions.

Another limitation in terms of collection of water samples from each region is the small number of samples collected in each region and in total. This may limit the statistical significance of the results, limit the comparisons that can be made, and limit the conclusions that can be made for each region and for Maryland farms in general. A larger sample size would provide a better understanding of the risk of total coliforms and *E. coli* contamination in Maryland farm private drinking water wells and offer a more representative result of this risk. Conducting this study again with a larger sample size would provide an opportunity to represent more of the Maryland farms with private drinking water wells and conduct more comparisons between the county and region. It would also determine if the results that we recorded for the presence of total coliforms and *E. coli* with the small sampling size would still occur with a larger sampling size that better represents the Maryland farming population in each region. However, even with the small number of water samples collected the results still provided a starting point to better understanding the risk of contamination and the role that factors, specifically region, play in the presence of total coliforms and *E. coli*.

Another limitation is related to this being a convenience sample where any participant was able to express their interest and then be a part of the study. A convenience sample may not provide an accurate and complete representation of Maryland farms. If the study was conducted with guidelines of which participants and samples would be selected to participate in the study, the samples may provide a better representation with more diverse demographic backgrounds and more diverse well factors that better represent what is observed in Maryland. A study with a better representation allows for us to make more assumptions of what the results for these participants mean for farmers throughout Maryland.

A limitation of the collection protocol is the risk of experiencing contamination when collecting the water samples. This is because participants and Agricultural Agents collected the water samples on their own rather than a member of the lab collecting the well water sample. Contamination could occur if participants touched the inside of the collection bottle or if some other material entered the bottle while collecting the water sample. Water samples could also be impacted if they were not shipped or delivered on ice and kept cold during the transportation from the farm to the lab. We tried to minimize this risk by providing instruction sheets to each participant and Agricultural Agent with details on how to collect and deliver the water samples.

Even though the results depict that there were three well and region factors that are statistically significant- region, county, and previously testing for pH- it is possible that the small sample size could have impacted the results for county. As mentioned in *Section 3.4.2,* there were 19 counties to collect from and there was a very small number of samples collected from each county. With the small number of samples collected, there was then a small number of samples positive for total coliforms and *E. coli* for each individual county. Therefore, the smaller sample size may not be as accurate. It is known that the larger the sample size the more accurate the statistical analysis will be [47]. Conducting this study again with larger sample size in total and larger and uniform sample size from each county and region would provide a more statistically accurate result. We could also make comparisons to see if the statistical significance still occurs with a larger sample size. However, this study still did provide an understanding of factors that could impact contamination concentration and during the statistical analysis I collapsed survey categories so that there were not as many variables being studied.

A limitation of the results is that we did not include questions in the participant survey that focused on geological data, bedrock type or if it is consolidated or unconsolidated well. Procopio et al., 2017 and White et al., 2021 both found differences between consolidated and unconsolidated wells [12, 34]. In the thesis results, it was discovered that Western region had higher risk of total coliforms and *E. coli.* Using the Maryland Department of Natural Resources: Maryland Geological Survey I was able to make assumptions of the material that the well would be surrounded by for that specific region. Conducting this study again with these factors would provide us with the opportunity to also confirm the geological material with what was used from the Maryland Geological Survey. This is also important given the fact that although we are assuming that limestone is present in the Western region, it can also have sandstone and shale present so understanding the official geology of the land is important [46]. This information would also give us another opportunity to better understand the well characteristics throughout Maryland.

3.6 Public health implications of research

Previous research has shown that approximately 40% to 58% of private well water has a contaminant present in their water that is over the SDWA standard [49, 51, 52, 6]. Individuals that have access to public water systems for drinking water are notified if their water source becomes contaminated and is over the maximum contaminant level [6]. Individuals that own private wells, however, are not notified and therefore contamination can go unnoticed and undetected if not properly tested. The lack of data, resources, and education for private well owners are a public health concern [6]. Therefore, understanding the importance of well water testing and the factors that could introduce contamination is crucial. Increased rates of testing and education of private well owners can help better detect contamination and try to limit the potential for negative health complications such as diarrhea and nausea [9] or the potential exposure to the harmful strain, *E. coli O157:H7* [14]. Since total coliforms act as an indicator for presence of other microbial contaminants [10], testing would limit negative health complications that could arise from the presence of other harmful contaminants.

This research impacts the approximately 350,000 Maryland homes that have private wells as their primary source of drinking water [7]. On a large scale, this research could be utilized by the 43 million people in the United States that have a private drinking water well [41]. This research provides data on Maryland farm private well water quality for 67 farms in seven regions of Maryland. It provides a glimpse of the risks of contamination of total coliforms and *E. coli* for Maryland farm private drinking water wells by utilizing the results from this subset of farms with private drinking water wells. It provides an insight into which regions of Maryland are at a greater risk of experiencing contamination and shows that region, precipitation, ambient temperature, and previously testing for pH can be significant factors for presence of total coliforms and *E. coli*. This provides a better understanding of what conditions and factors will put Maryland private well owners at greatest risk of contamination thus providing Maryland well owners with data to make more informed decisions.

Prior studies in Maryland have not taken into consideration well factors and climate factors impact on the presence of total coliforms and *E. coli* on private drinking water wells. This thesis increases our understanding of contamination risk in Maryland farm private wells while putting special focus on the factors that play a role in this risk of contamination of a primary water source for many.

3.7 Conclusions

In conclusion, this thesis investigated the risk of total coliforms and *E. coli* being present in private Maryland farm drinking water wells. It was determined that there is a risk of total coliforms and *E. coli* contamination, particularly for farms located in the Western region of Maryland. This thesis was the first to find a connection between the presence of total coliforms and *E. coli* and region in Maryland. Region is a significant factor that could impact the presence of total coliforms and *E. coli*. This could be due to the differences in geology between the regions. Precipitation was also considered a significant factor for *E. coli* concentration with their being higher rates of contamination with lower levels of precipitation within 24 hours of collection. This goes against what I initially hypothesized but shows that a large among of precipitation is not needed to introduce *E. coli* into well water. A small precipitation event of 0.56-0.62 inches of rain appears sufficient to potentially introduce *E. coli* into the environment.

Ambient temperature was found to be a significant factor that could impact the presence of total coliforms in well water and there was higher risk of contamination for temperatures of 65-74 F. This shows that ambient temperature does impact water quality. This is the first study in Maryland to find that ambient temperature is a factor for total coliforms presence in well water. Previous testing of pH and county were also statistically significant factors for total coliform concentration. More research with a larger sample size from each county is needed to ensure that county is a significant factor. However, this data provided a starting point to better understand the difference in contamination among counties in Maryland. While this thesis depicts that region is significant, it is now important to see if the counties within these regions differ in contamination.

Future research should be done with a larger sample size in the 19 counties and seven regions of Maryland to see how county impacts concentration of microbial contamination and to see if it remains significant. pH for each water sample should also be tested to see if there is correlation between pH levels and contamination. Lastly, surveys should also include questions about geology to conclude that the geology is the primary reason that region is a factor. This thesis depicts the importance of consistent well water testing, education, and resources for owners of private wells to ensure that the well water is safe to consume. This is especially important for farmers that live in areas with higher risk of contamination such as Western Maryland.

Appendices

Appendix A. TDS results for Maryland farm private drinking water well samples (n=67)

Sample Number	Total Dissolved Solids (TDS)
1	423
2	164
3	140
4	240
5	34
6	417
8	255
9	165
10	95
11	184
12	186
13	111
14	197
15	26
16	179
17	438
18	625
19	160
20	302
21	256
22	21
23	115
24	374
25	250
26	158
27	347
28	885

29	331
31	99
32	66
34	75
35	39
36	54
37	38
38	125
39	105
40	100
41	105
42	96
43	84
44	119
45	107
46	110
48	103
51	152
52	183
56	123
57	109
58	114
59	208
60	213
61	109
64	126
65	40
67	140
68	112
70	89
71	47
72	293

73	408
74	67
75	273
76	142
77	34
79	60
80	93
83	89

Appendix B. Climate data for precipitation and ambient temperature for Maryland farm private drinking water well samples (n=67)

Sample Number	Precipitation last 24 hours (in.)	Precipitation last 7 days (in.)	Precipitation last 14 days (in.)	Ambient temperature (F)
1	0	0.39	2.61	86
2	0	0.55	1.66	73
3	0	0.55	1.66	76
4	0	0.39	2.61	72
5	0	0.39	2.61	82
6	0	0.39	2.61	86
8	0	0.55	1.66	79
9	0.22	0.31	1.54	65
10	0.18	0.26	1.46	65
11	0.22	0.31	1.54	68
12	0.22	0.31	1.54	68
13	0.22	0.31	1.54	68
14	0	0.39	2.61	85
15	0	0.39	2.61	76
16	0	0.39	2.61	88
17	0.07	0.41	1.65	67
18	0.07	0.41	1.65	67
19	0.07	0.41	1.65	66
20	0.07	0.41	1.65	63
21	0.07	0.48	1.72	71
22	0.07	0.41	1.65	69
23	0	0	0.77	71
24	0	0	0.77	74
25	0.62	1.77	2.63	73
26	0	0	0.77	73
27	0	0	0.77	74
28	0.62	1.77	2.63	73
29	0	0	0.77	73
31	0	0	0.77	73

32	0	0	0.77	75
34	0	0	1.13	70
35	0	0	1.13	70
36	0	0	1.13	74
37	0	3.17	3.4	57
38	0	3.17	3.4	60
39	0	0.37	0.47	76
40	0.26	0.08	1.91	77
41	0	3.17	3.4	66
42	0.26	0.08	1.91	76
43	0.26	0.08	1.91	76
44	0	3.17	3.4	71
45	0.26	0.08	1.91	78
46	0	0.37	0.47	77
48	0.26	0.08	1.91	78
51	0.26	0.08	1.91	73
52	0	4.21	4.95	74
56	0	3.17	3.4	61
57	0	3.17	3.4	61
58	0	3.17	3.4	64
59	0	0.08	1.91	80
60	0	3.17	3.4	56
61	0	3.17	3.4	64
64	0	0.26	2.25	70
65	0	0.26	2.25	70
67	0	0.26	2.25	75
68	0	0.26	2.25	70
70	0	0.26	2.25	68
71	0	0.26	2.25	68
72	0.56	0.58	0.58	74
73	0.56	0.58	0.58	74
74	0.21	0.55	0.55	84

75	0.56	0.58	0.58	77
76	0.02	0.36	1.64	73
77	0	4.21	4.95	76
79	0.26	0.08	1.91	82
80	0.26	0.08	1.91	80
83	0.26	0.08	1.91	80

Appendix C. Selected well factor survey questions and new combined responses

Survey Question Number	Question	Original Response Options	New Combined Responses for Analysis
3	What type of well do you use for drinking purposes?	Hand dug Drilled Do not know Other	Drilled Other
4	What is the age of the well?	Less than 10 years 11-25 years 26-50 years 51+ years Do not know	≤ 25 years ≥ 26 years
5	What is the depth of your well?	Less than 30 feet 31-75 feet 76-150 feet 150-250 feet 251+ feet Do not know	< 30 feet 31-75 feet
6	How would you describe the condition of your well?	Excellent Good OK Fair Poor	Good (& Excellent) OK (& Fair)
7	Do you know where your drinking water wellhead is located?	Yes No	Yes No
8	What type of cover does your wellhead have?	Plastic or metal well cap Concrete Wood Do not know None Other	Plastic Other
9	Have you had any well repairs or other well management done in the past 10 years?	Yes No Not sure	Yes No
10	What is the age of your homes plumbing?	Less than 30 years 31-50 years 51-75 years 76-100 years 100+ years Do not know	< 50 years > 51 years
11	What type of plumbing pipe is in your home? Select all that apply	Copper Lead PVC PEX Do not know Multiple types	Copper Lead PVC PEX Multiple Types
12	Have you tested your drinking water well quality?	Yes, within the last 5 years Yes, within the last 10 years Tested over 10 years ago	Yes No

		Tested when I bought the house No	
14	What did you have your drinking water well tested for? Select all that apply.	Total coliform bacteria Nitrate Total dissolved solids pH Other	Yes or No for each option separately
15	Have you observed any issues with your water quality? Select all that apply.	No Scale or residue on dishes and fixtures Blue staining on tubs or other fixtures Rust or black staining on fixtures Cloudy or colored water Pin hole leaks in pipe Appliances using water do not last as expected Odors Off taste Salty taste Someone in house experienced gastrointestinal illness Other	Yes No
16	What type of service to your well and water system have you had? Select all that apply.	None New pump Repaired well casing or wellhead Replacement well Disinfected the well Other Installed a water filter or filtration system	Yes (Service done) No
17	Which of the following treatment devices are installed on your drinking water well? Select all that apply.	Carbon filter Water softener Acid neutralizing filter Ultraviolet light Sediment filter Chlorination system Aeration system Reverse osmosis (RO) system Distillation unit Anion exchange system Small faucet filter Iron removal system Have treat equipment, but unsure what it is Other Do not know	Yes (treatment) No

None

Appendix D. Water collection sampling instructions provided to each participant with their sample collection kit

SCHOOL OF
PUBLIC HEALTH
MARYLAND INSTITUTE FOR APPLIED ENVIRONMENTAL HEALTH

UNIVERSITY OF
MARYLAND
EXTENSION

SAMPLING INSTRUCTIONS:

You MUST collect your water samples only on the day that you intend to drop them off.

If you have questions about sampling procedures, please call Dr. Rachel Rosenberg Goldstein at 301-314-1588 or 301-405-5509, or email her at rerosenb@umd.edu.

General Notes:

- **Wash your hands thoroughly before collecting water samples.**
- **Do not remove the cap from the sample bottle until you are wearing gloves and ready to collect the sample.**
- **Do not touch the inside of the cap or mouth of either bottle, even with gloved hands.**

Farm Well Sampling Protocol Video: https://rb.gy/cqeh5k

Instructions:

1. Choose a cold water faucet in the kitchen (if possible, select a stationary, non-swivel type faucet). Be sure that the sink is clean, and that all dishes and other items are removed in order to minimize splashing.
    a. If well water is not used for drinking water in the kitchen, water can be collected from an outside water hydrant connected to the well water source.
2. Remove all screens, filters, aerators, etc. from faucet and allow water to run at least 5 minutes to flush out the pipes.
3. Adjust the water flow to a pencil thin stream.
4. Take the larger sample bottle and carefully remove the cap, taking care not to touch the inside of the cap or sample bottle. Do not remove the cap from more than one bottle at a time. Do not put the cap down while collecting the sample.
5. Fill the sample bottle completely.
6. After filling the bottle, tighten the cap securely.
7. Record your name, sample location (kitchen or outside water hydrant), and the sample collection time and date on the bottles.
8. Take the smaller sample bottle. Repeat steps 4 through 7 with this bottle.

Store samples in a refrigerator or cooler if not delivered to the Extension office or Extension agent immediately.

Samples MUST be collected the same day they are provided to the Extension Office or Extension agent.

Appendix E. Survey questions provided to participants

Maryland Farm Well Water Quality Survey

Q1 The following survey is designed to provide University of Maryland faculty and Extension Educators more information about drinking water wells located on farms in Maryland, and to develop education programming to assist farmers in ensuring safe, good quality drinking water. We appreciate your time and feedback by completing this survey. We will use this information to develop well care and water quality educational programs. Your personal identifiable information is strictly confidential and will not be shared with anyone or any entity. By completing this survey, you indicate that you are at least 18 years old and understand the purpose of this survey and freely and voluntarily choose to participate. More information on consent process is available at:

Q2 Would you like to participate in the study?

○ No (1)

○ Yes (2)

Skip To: Q26 If Would you like to participate in the study? = No

Drinking Water Well and Home Information

Q2 In what zip code is your farm located?

____

Q3 What type of well do you use for drinking purposes?

○ Hand dug (1)

○ Drilled (2)

○ Do not know (3)

○ Other (4) ____

Q4 What is the age of the well?

- ◯ Less than 10 years (1)
- ◯ 11-25 years (2)
- ◯ 26- 50 years (3)
- ◯ 51 + years (4)
- ◯ Do not know (5)

Q5 What is the depth of your well?

- ◯ Less than 30 feet (1)
- ◯ 31 - 75 feet (2)
- ◯ 76 - 150 feet (3)
- ◯ 150 - 250 feet (4)
- ◯ 251 + feet (5)
- ◯ Do not know (6)

Q6 How would you describe the condition of your well?

- ◯ Excellent (1)
- ◯ Good (2)
- ◯ OK (3)
- ◯ Fair (4)
- ◯ Poor (5)

Q7 Do you know where your drinking water wellhead is located?

- Yes (1)
- No (2)

Q8 What type of cover does your wellhead have?

- Plastic or metal well cap (1)
- Concrete (2)
- Wood (3)
- Do not know (4)
- None (6)
- Other (5) ____________________

Q9 Have you had any well repairs or other well management done in the past 10 years?

- Yes (1)
- No (2)
- Not sure (3)

Q10 What is the age of your home's plumbing?

- Less than 30 years (1)
- 31 - 50 years (2)
- 51 - 75 years (3)
- 76 - 100 years (4)
- 100 + years (5)
- Do not know (6)

Q11 What type of plumbing pipe is in your home? Select all that apply.

- ☐ Copper (1)
- ☐ Lead (2)
- ☐ PVC (3)
- ☐ PEX (4)
- ☐ Do not know (5)
- ☐ Multiple types (6)

Water Quality Information

Q12 Have you tested your drinking water well quality?

- ○ Yes, within the last 5 years (1)
- ○ Yes, within the last 10 years (2)
- ○ Tested over 10 years ago (3)
- ○ Tested when I bought the house (4)
- ○ No (5)
- ○ Click to write Choice 6 (6)

Skip To: Q14 If Have you tested your drinking water well quality? = Yes, within the last 5 years
Skip To: Q14 If Have you tested your drinking water well quality? = Yes, within the last 10 years
Skip To: Q14 If Have you tested your drinking water well quality? = Tested over 10 years ago

Q13 Why have you not tested your drinking water well quality?

○ Did not know I needed to test (1)

○ Do not know what to test for (2)

○ Do not know where to have my water tested (3)

○ Other, please describe (4) ____________________

Skip To: Q15 If Why have you not tested your drinking water well quality? = Did not know I needed to test
Skip To: Q15 If Why have you not tested your drinking water well quality? = Do not know what to test for
Skip To: Q15 If Why have you not tested your drinking water well quality? = Do not know where to have my water tested
Skip To: Q15 If Why have you not tested your drinking water well quality? = Other, please describe

Q14 What did you have your drinking water well tested for? Select all that apply.

▢ Total Coliform bacteria (1)

▢ Nitrate (2)

▢ Total dissolved solids (3)

▢ pH (4)

▢ Other, please describe (5) ____________________

Q15 Have you observed any issues with your water quality? Select all that apply.

- ▢ No (1)
- ▢ Scale or residue on dishes and fixtures (2)
- ▢ Blue staining on tubs or other fixtures (3)
- ▢ Rust or black staining on fixtures (4)
- ▢ Cloudy or colored water (5)
- ▢ Pin hole leaks in pipe (6)
- ▢ Appliances using water do not last as expected (7)
- ▢ Odors (8)
- ▢ Off taste (12)
- ▢ Salty taste (9)
- ▢ Someone in the house has experienced gastrointestinal illness (10)
- ▢ Other, please describe (11) ____

Q16 What type of service to your well and water system have you had? Select all that apply.

- ▢ None (7)
- ▢ New pump (1)
- ▢ Repaired well casing or wellhead (2)
- ▢ replacement well (3)

- [ ] Disinfected the well (4)
- [ ] Other, please describe (5) ________________________________
- [ ] Installed a water filter or filtration system (6)

Q17 Which, of the following water treatment devices are installed on your drinking water well? Select all that apply.

- [ ] Carbon filter (1)
- [ ] Water softener (4)
- [ ] Acid neutralizing filter (5)
- [ ] Ultraviolet light (6)
- [ ] Sediment filter (7)
- [ ] Chlorination system (8)
- [ ] Aeration system (9)
- [ ] Reverse osmosis (RO) system (10)
- [ ] Distillation unit (11)
- [ ] Anion exchange system (nitrate removal) (12)
- [ ] Small faucet filter (Brita, etc.) (13)
- [ ] Iron removal system (greensand or other oxidizing filter) (14)
- [ ] Have treat equipment, but unsure what it is (15)
- [ ] Other, please describe (16)

☐ Do not know (17)

☐ None (18)

Preference on Receiving Educational Information

Q18 Would you be interested in attending educational events on the following topics:

	Yes (1)	Maybe (2)	No (3)
Groundwater and aquifers (7)	☐	☐	☐
Home well systems (8)	☐	☐	☐
How to protect your well (9)	☐	☐	☐
Sources of contamination (11)	☐	☐	☐
Water quality parameters and associated health risks (12)	☐	☐	☐
Water quality testing (13)	☐	☐	☐
Treatment options (14)	☐	☐	☐

Q19 What time of day would you prefer to attend a webinar or other educational event?

○ Before noon (1)

○ Afternoons (2)

○ Evenings (3)

Q20 In what ways would you like to receive educational information? Check all that apply.

- ☐ Fact sheet or other printed materials (1)
- ☐ Webinars (2)
- ☐ Videos (3)
- ☐ Telephone (4)
- ☐ In person workshop (5)
- ☐ Other (6) ____

Q21 Is there additional information would you like receive on wells and water quality?

____

Demographic Information

Q23 What is your ethnicity?

- Hispanic or Latino (1)
- Non Hispanic or Latino (2)
- Prefer not to answer (3)

Q24 Which category best describes your race? Check all that apply.

- American Indian or Alaskan Native (1)
- Asian (2)
- Black or African-American (3)
- Native Hawaiian or Other Pacific Islander (4)
- White (5)
- Other Race (6)
- Two or More Races (7)
- I prefer not to answer (8)

Q25 What is your age?

- 18-29 (1)
- 30-49 (2)
- 50-69 (3)
- 70-89 (4)
- Over 90 (5)
- I prefer not to answer (6)

Q26 What is your sex?

- ◯ Male (1)
- ◯ Female (2)
- ◯ Other (3)
- ◯ I prefer not to answer (4)

Q27 What is the highest level of education you have completed?

- ◯ less than high school (1)
- ◯ High school graduate/GED (2)
- ◯ Some college or Associate's Degree or Certificate (3)
- ◯ Bachelor Degree (4)
- ◯ Graduate or Professional Degree (5)
- ◯ I prefer not to answer (6)

Q28 If interested in participating in a farm drinking water well testing program, please provide your name and email address

____

Thank you for your feedback! Please be sure to click on the arrow to right to submit your responses.

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